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CONTENTS

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Determination of the mass diffusion coefficient of wood by thin-layer drying kinetics

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Abstract. The aim of this paper is to present the method of using thin layer drying under controlled conditions, to obtain variable moisture diffusion coefficient expression of wood drying. The obtained relationship parameters can then be used for a larger sample drying process simulation. This paper includes the theoretical study of changes of moisture content determination in wood in response to high temperature of the drying air (105 °C). A 1-D diffusion model with a variable concentration-dependent diffusion coefficient is considered. This problem is solved, using the differential scheme. Paper described theory and experimental results of wood drying by the high temperature at 105 °C. For studies have selected five types of wood: oak (*Quercus robur*), beech (*Fagus silvatica*), spruce (*Picea abies*), scots pine (*Pinus silvestris*), and larch (*Larix decidua*). Experimental measurements and modelling results are given.

Key words: wood pieces, oven, natural convection, mass diffusion coefficient.

INTRODUCTION

The aim of this paper is to present the methodology of determination of the mass diffusion coefficient of wood by thin-layer drying kinetics and show some results of this measurement. Using a wood measurement of a small wood sample in drying process, it is possible to obtain information on the diffusion coefficient depending on the moisture concentration in the sample. Wood heat treatment is a compulsory technological process for gluing materials, plywood, building materials and so on. This process is energy-intensive, complex and long-lasting, and therefore it is necessary to scientifically justify it. The usual drying methods are based on low-temperature convective drying and the drying process lasts long time (Simpson, 1983). To reduce the drying time without decreasing the quality of wood, the drying temperature of product is above the boiling point of water. This paper describes the experiments and experimental results of wood drying at 105 °C.

This research is focused on the most important wood used as a timber or in the industry in European conditions. The tested samples are made from spruce (*Picea abies*), scots pine (*Pinus silvestris*), larch (*Larix decidua*), oak (*Quercus robur*) and beech (*Fagus silvatica*).

There are some research studies that are limited to 'catching' the drying curves (Hua et al., 2016; Anisimov et al., 2017). One of the basic drying parameters is the diffusion coefficient. Knowing its value and using Fick's second law is possible to predict moisture concentration change in timeand get the moisture distribution in the sample during drying. The several fundamental multiphase models has been compiler and shown how these models can be applied to the wood drying process (Ciegis & Starikovicius, 2002). The mass transfer process is studied in four widely used Central African tropical woods using a climatic chamber permanently maintained at 59% air relative humidity (Simo et al., 2016). This study shows that diffusion coefficient correlate with the density of wood by an exponential function.

Mass diffusion coefficients for pine, oak, spruce, beech and larch are calculated at initial samples moisture 14% wet basis (Aboltins et al., 2017a) drying at a temperature105 °C. These results show, that average diffusion coefficients in first 10 drying hours are higher more that 20% than average diffusion coefficients in all drying time - 24 hours. For example, for spruce this difference reaches 21%, for larch 25% and oak only 16%.These results show that water diffusion coefficient depends on the water concentration in wood sample.

The main objective of this article was to using thin-layer drying kinetics of wood for determination of coefficient of diffusion. This paper includes the theoretical study of changes of moisture content determination in wood in response to high temperature of the drying air. The aim of this research was to investigate theoretical background of wood drying by high temperatures and determination of changed diffusion coefficients.

MATERIALS AND METHODS

The tested samples are made from five types of wood: oak (*Quercus robur*), beech (*Fagus silvatica*), spruce (Picea abies), scots pine (*Pinus silvestris*), and larch (*Larix decidua*).

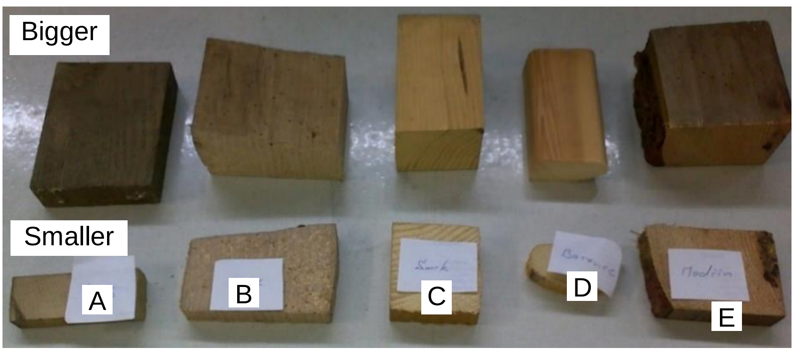

Figure 1. Bigger and smaller samples of wood used for drying measurement. Bigger samples on upper row and smaller samples on bottom row: A – oak (*Quercus robur*); B – beech (*Fagus silvatica*); C – spruce (*Picea abies*); D – scots pine (*Pinus silvestris*); E – larch (*Larix decidua*).

To be able to study different way of drying up the water from the wood, two samples of each type of wood were examined (larger and smaller length) (Fig. 1). Small samples are cut from the large sample perpendicular to the fibres direction. The wood density depends not only on moisture content of the wood but also on growth conditions. The exact dimensions and properties of tested samples are measured and presented in Tables 1 and 2. The length of the sample is measured in the direction of the fibres.

Table 1. The characteristics of bigger wood samples used for drying measurements

Type of wood	Weight (g)	Density (kg m^{-3})	Sample profile (mm x mm)	Length (mm)
Oak	229.35	725	93 x 33	103
Beech	367.18	720	100 x 60	85
Spruce	190.96	450	67 x 68	93
Pine	100.00	535	55 x 34	100
Larch	295.21	590	88 x 65	88

Table 2. The characteristics of smaller wood samples used for drying measurements

Type of wood	Weight (g)	Density (kg m^{-3})	Sample profile (mm x mm)	Length (mm)
Oak	46.33	725	93 x 33	21
Beech	110.99	720	100 x 60	25
Spruce	48.90	450	67 x 68	24
Pine	18.39	535	55 x 34	18
Larch	79.17	590	88 x 65	23

The determination of wood moisture was carried out by gravimetric method, that is direct method and the results are very accurate. There was used for drying of samples of wood the electric oven Memmert UNB with automatic control of temperature and natural flow of air inside the chamber. Samples were weighed on the digital laboratory balance KERN-440-35N with maximum load weight 400 g and with resolution 0.01 g. The experiments lasted 30 hours, measurements were made every 1.25 hours. No charges in samples weight after 24 hours were observed. This indicates that the water in the samples has been removed. Wood moisture is determined on a wet basis.

Using the experimental data with natural convective drying method in the laboratory conditions under high temperature 105 °C (near the boiling point of water) there were calculated the theoretical drying coefficients, useful for description and modelling of the drying process, calculated theoretical results of moisture removal and compared with experimental results obtained from the measurements. The results of drying of different samples dimensions and small mass of wood pieces are compared. The obtained results of this research are parameters, which can be used for the future research work and for improvement of the whole drying process. This destructive method of measurement can be also used for laboratory control of another method of measurement, e.g. non-destructive sensor tests, based on other physical principles (capacitive or resistive) sensors (Papez & Kic, 2013).

Mathematical model with changing coefficient of diffusion

In order to determinate the effective moisture diffusion in solid wood is used mass maintenance law usually presented in the following form:

$$\frac{\partial \tilde{c}}{\partial t} = div(D grad \tilde{c}) \qquad (1)$$

D – coefficient of diffusion ($m^2 s^{-1}$), $\tilde{c}(x, y, z, t)$ – concentration of moisture in wood in wet basis (g 100^{-1}g^{-1}), x,y,z – space coordinates (m), t – time (s).

Since the diffusion of vapours in wood fibre direction is several times greater than in radial and tangential ones (Aboltins et al., 1999) and surface of fibre direction of samples is greater that surfaces of radial and tangential directions, we choose 1-dimentional model with $D_x$in fibre direction (diffusion in a plane sheet $\tilde{c}(x,y,z,t) \approx c(x,t)$.

$$\frac{\partial c}{\partial t} = \frac{\partial}{\partial x}\left(D_x \frac{\partial c}{\partial x}\right) \tag{2}$$

There is case, where diffusion occurs through all surfaces of samples and is possible to assume that the diffusion coefficient D_x depending from concentration c i.e. $D_x = D(c)$. For modelling assumed that in the moment $t = 0$ concentration of moisture in sample of wood is constant c_s. The water vapour concentration on surfaces is constant $c(x,t) = 0$ during drying time. The diffusion process in this case can considered as symmetrical situation and get mathematical problem:

$$\frac{\partial c}{\partial t} = \frac{\partial}{\partial x}\left(D(c) \frac{\partial c}{\partial x}\right) \quad -l < x < l, \qquad t > 0 \tag{3}$$

$$c|_{t=0} = c_s \tag{4}$$

$$c|_{x=-l} = \ c|_{x=l} = 0 \tag{5}$$

where $2l$ – islength of sample in x direction.

Choose the simplest and widely used for small concentrations linear (Fox et al., 1968; Rowland, 1984) relationship $D(c) = a + b \cdot c(x,t)$ and mathematical problem (3)–(5) transforms as (6)–(8):

$$\frac{\partial c}{\partial t} = a\frac{\partial^2 c}{\partial x^2} + b\frac{\partial}{\partial x}\left(c \frac{\partial c}{\partial x}\right) \quad -l < x < l, \qquad t > 0 \tag{6}$$

$$c|_{t=0} = c_s \tag{7}$$

$$c|_{x=-l} = \ c|_{x=l} = 0 \tag{8}$$

Analytical solution to the problem (6)–(8) does not exist. Moreover, not know the coefficients a and b of expression of $D(c)$. These expression's coefficients a and b we define from experimental data. For solving (6)–(8) we can use difference schemes (Samarskii 1988; Aboltins & Morozovs, 2003).

If take condition (5) as $c|_{x=-l} = c|_{x=l} = c_0$ and use substitution $u(x,t) = c(x,t) - c_0$ is obtained Cauchy problem (6')–(8')

$$\frac{\partial u}{\partial t} = (a - b \cdot c_0)\frac{\partial^2 u}{\partial x^2} + b\frac{\partial}{\partial x}\left(u \frac{\partial u}{\partial x}\right) \qquad -l < x < l, \qquad t > 0 \tag{6'}$$

$$u|_{t=0} = c_s - \ c_0 \tag{7'}$$

$$u|_{x=-l} = u|_{x=l} = 0 \tag{8'}$$

As shown the systems (6)–(8) and (6')–(8') differ only with constant coefficients.

RESULTS AND DISCUSSION

For solving (6) – (8) we discretize the region of space-time where we want to obtain a solution. For discretization and numerical calculation have been taken domain $\Omega = \big((x,t)\colon 0 \le x \le 2l,\ 0 \le t \le T\big)$. We have put a bound on time T because in practise we only solve a problem up until a finite time. Discretizing means defining a lattice of points in this space-time region by

$$x_i = ih, \qquad t_j = j\tau, \quad i = 0,1,\dots,N, \qquad j = 0,1,\dots,K,$$

where the fixed numbers h and τ are the spatial and temporal step sizes, respectively. Here, $h=2l/N$ and $\tau=1/K$. The integer N is the number of subintervals in $0 \le x \le 2l$ and K is the number of time steps to be taken. Using forward difference approximation for the first and second derivates (Samarskii 1988; Aboltins & Morozovs, 2003) Cauchy problem can solve as difference problem (9)–(11):

$$\frac{c_i^{j+1} - c_i^j}{\tau} = a\frac{c_{i-1}^j - 2c_i^j + c_{i+1}^j}{h^2} + b\frac{c_{i+1}^j \frac{\partial c}{\partial x} - c_{i-1}^j \frac{\partial c}{\partial x}}{2h}, \tag{9}$$

$$i = 1,2,\dots,N-1; \qquad j = 0,1,\dots,K$$

$$c_i^0 = c_s, \qquad i = 0,1,2,\dots,N \tag{10}$$

$$c_0^j = c_N^j, \qquad j = 1,2,\dots,K \tag{11}$$

Solve c_i^{j+1} from (9),

$$c_i^{j+1} = c_i^j + \frac{a\tau}{h^2}\left(c_{i-1}^j - 2c_i^j + c_{i+1}^j\right) + \\ + \frac{b\tau}{2h^2}\left(\left(c_{i+1}^j\right)^2 - c_i^j\left(c_{i-1}^j + c_{i+1}^j\right) + \left(c_{i-1}^j\right)^2\right), \tag{12}$$

$$i = 1,2,\dots,N-1; \qquad j = 0,1,2,\dots,K$$

The difference Eq. (12) gives the approximate solution at the node (x_i, t_{j+1}) in terms of approximations at three earlier nodes.

To solve the problem (10) – (12) need to determine the values a and b for each tree type. These values can be determined from experimental data of small samples of each type of wood. This determination methodology is similar to the drying rate determination (Aboltins, 2013). Assume that $D_x = const$. If M_t denotes the amount of diffusing moisture which has come out from the material at time t, and M_∞ the corresponding quantity after infinite time, then (Crank, 1956):

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2\pi^2} \exp\left(-\frac{D_x(2n+1)^2\pi^2 t}{4l^2}\right) \tag{13}$$

The first we must estimate D_x. Looking at the series (13), it converges very fast. The first member ($n = 0$) of series (13) is selected for estimation of approximated D_x, taking into account inaccuracy of this assumption at the beginning of drying process (Aboltins et al., 2017b):

$$\frac{8}{\pi^2} \cdot \exp\left(-\frac{D_x \cdot \pi^2 t}{4l^2}\right) = 1 - \frac{M_t}{M_\infty} \qquad (14)$$

Since only smaller samples (Fig. 1) are used in the experiment, it is assumed that thickness of the samples has small effect on the diffusion coefficient and it is not taken into account. Right-hand side of the Eq. (14) known (experimental data at time $= t_k$, $k = 1,2,\ldots,k_o$) and is possible to express coefficient of diffusion $D_x = D_x^k$ for each $t = t_k$ and $M_t = M_{t_k}$:

$$D_x = -\frac{4l^2 ln\left(\frac{\pi^2 (M_\infty - M_t)}{8 \cdot M_\infty}\right)}{\pi^2 \cdot t} \qquad (15)$$

Computed coefficient values corresponding to the literature considered (Shubin, 1990; Simo et al., 2016). The calculation results for four types of wood shown Figs 2–5:

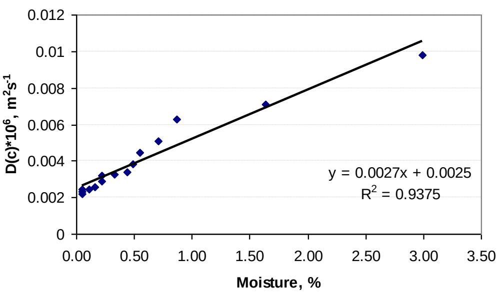

Figure 2. Water diffusion coefficient depending from moisture concentration in sample of pine.

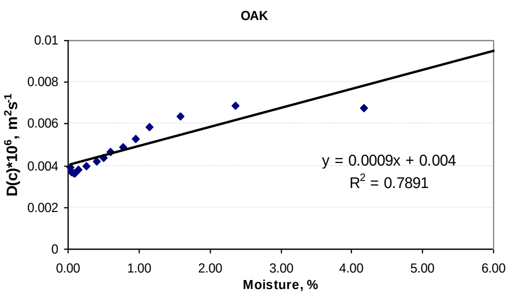

Figure 3. Water diffusion coefficient depending from moisture concentration in oak sample.

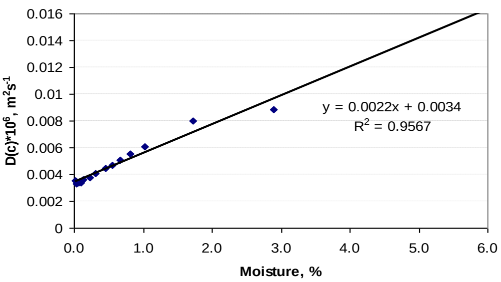

Figure 4. Water diffusion coefficient depending from moisture concentration in larch sample.

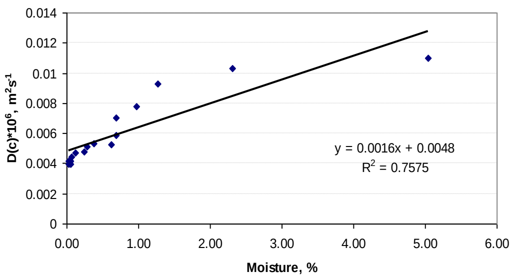

Figure 5. Water diffusion coefficient depending from moisture concentration in spruce sample.

Using the experimental data processing (Figs 2–5) it is possible to determine the expressions $D(c) = a + b \cdot c(x,t)$ constants a and b for each tree species examined. Using problem's (6)–(8) numerical solution (10)–(12) and calculated diffusion coefficients for oak $D(c) = (0.004 + 0.0009 \cdot c(x,t)) \cdot 10^{-6}$ and spruce $D(c) = (0.0048 + 0.0016 \cdot c(x,t)) \cdot 10^{-6}$samples (Fig. 3, Fig. 5) the moisture concentration distribution inside longer samples is possible calculated. Results for 0.1 m length samples are shown at Fig. 6.

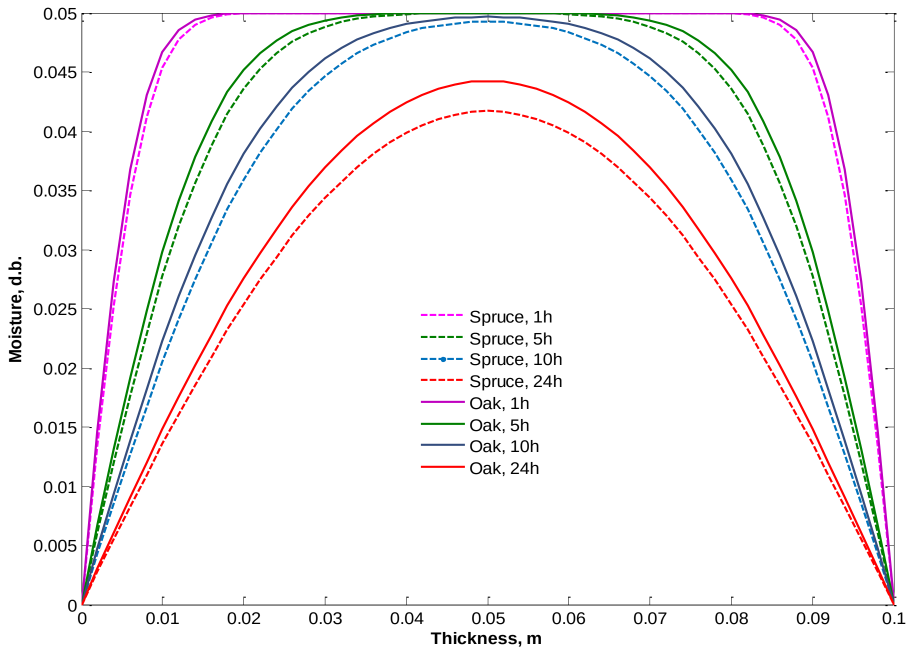

Figure 6. Comparison of moisture content distribution in oak and pine samples at different drying time.

It should be noted that in this case it is considered that the water removal takes place only in the longitudinal direction. Since the drying occurred at 105 °C, it is then assumed, that moisture concentration on surface is zero Eq. (11). Of course, in real situation at lower temperatures, we must take into account equilibrium moisture content and capillarity at higher wood moisture. It will also produce amore complicated mathematical model with more unknown parameters.

The effect of moisture concentration on the diffusion coefficient of spruce wood is almost 2 times higher than of oak wood, which could be explained by different wood densities. As density of oak is higher as spruce wood moisture diffusion goes quicker in spruce. It means the spruce wood dry quicker as oak wood (Fig. 6).

CONCLUSIONS

The proposed methodology, originally used for determination of drying coefficient of wood and can be used to determinate diffusion coefficient depending from concentration.

Using offered difference scheme is possible calculate moisture distribution in solid body at different drying time.

Using the proposed methodology, more accurate results will be achieved if the cross-sectional area / perpendicular to the direction of the capillary / is greater than the lateral surface area, or if the side surfaces are isolated.

It is necessary to realize different measurements in variable wood moisture range in order to specify changes of moisture diffusivity.

REFERENCES

Aboltins, A., Papez, J. & Kic, P. 2017a. Wood drying in high air temperature.*Proceedings of International scientific conference 'Engineering for rural development', Latvia University of Agriculture, Jelgava*, 1364–1368.

Aboltins, A., Rubina, T. & Jotautiene, E. 2017b. Diffusion coefficient estimation difficulties at the beginning of drying experiment *Proceedings of International Scientific conference 'Engineering for rural development', Latvia University of Agriculture, Jelgava,* 1327–1332.

Aboltins, A. 2013. Theoretical study of material drying coefficient; *Proceedings of International scientific conference 'Engineering for rural development', Latvia University of Agriculture, Jelgava,* 153–158.

Aboltins, A., Buikis, A., Cepitis, J., Kalis, H. & Reinfelds, A. 1999. Diffusion and Chemical Attachment of Substances with Simple Molecular Structure in Wood. *Progress in Industrial mathematics at ECMI98*, Edited by L. Arkeryd, J. Bergh, P. Brenner and R. Pettersson, B.G. Teubner Stuttgart –Leipzig, 188–195.

Aboltins, A. & Morozovs, A. 2003. Solid wood impregnation process with acetic anhydride computer modelling, *Fourth Nordic-Baltic agrometrics conference, Conference proceedings* Edit. U.Olsson, J.Seek Uppsala, 1–8.

Anisimov, P., Onuchin, E. & Vishnevskaja, M. 2017. Modeling Pine and Birch Whole Tree Drying in Bunches in the Cutting Area *Croat. journal for eng*. **38**(1),11–17.

Ciegis, R. & Starikovicius, V. 2002 Mathematical modeling of wood drying *process Mathematical modeling and analysis* **7**(2), 177–190.

Crank, J. 1956.*The mathematics of diffusion*. Oxford, Clarendon Press, 347 pp.

Fox, D., Labes, M. & Weissberger, A. 1968. Physics and chemistry of the organic solid state Moscow, Mir, 475 pp. (in Russian).

Hua, J., Ju, L., Cai, L. & Shi, S.Q. 2016. Modeling the air drying rate of Chinese larch lumber *Bioresources* **11**(3), 5931–5940.

Holan, J. 2008. Against what the timber must be protected. *All about wood in interiorand exterior* **8**(2), pp. 128–132 (in Czech).

Papez, J. & Kic, P. 2013. Wood Moisture of Rural Timber Constructions. *Agronomy Research* **11**(2), 505–512.

Rowland, S. 1984. *Water in polymers*, Moscow, Mir, 555 pp. (in Russian)

Samarskii, A.A. 1988. *The theory of difference schemes*, Nauka, Moscow (in Russian).

Simpson, W.T. 1983. Drying wood: a review-part I, *Drying technology* **2**(2), 235–264.

Simo, T.M., Remond, R., Rogaume, Y., Zoulalian, A. & Perre, P. 2016 Characterization of sorption behaviour and mass transfer properties of four central Africa tropical woods: ayous, sapele, frake, lotofa., *Ciencia y tecnologia* **18**(1), 207–226.

Shubin, G.S. 1990. *Wood drying and thermal treatment*, Moscow, Lesnaja Promishlennost, 336 pp. (in Russian).

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Effects of selected process parameters on the compaction of carob powder

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Abstract. The effects of important process parameters on mechanical response during the densification of an industrial food powder were investigated and important phenomena described using the power rule. The factors studied had highly significant effects on mechanical response. The effects of the models in predicting the behaviour of the system were also highly significant. The findings are of relevance to processing and handling of food powders.

Key words: applied pressure, strain, deformation, compressibility, bulk density.

INTRODUCTION

Important stages during compression or compaction of food powders include particle reorganisation, elimination of fluid components and deformation of solid elements to achieve compact forms (Faborode & O'Callaghan, 1986; Faborode, 1989). These are accompanied by formation of bond bridges and relaxation of the compressed matrix when the applied load is disengaged (Pietsch, 2005; Imole et al., 2014). Several material or equipment and process factors influence mechanical response within the matrix (Laskowski et al., 2005; Wongsiriamnuay & Tippayawong, 2015), some of which interact considerably with one another (Halliday & Smith, 1997). Careful selection of study factors can minimize the size and likelihood of unexplained events (Halliday & Smith, 1997). Uniaxial compression of solid particulates is significantly influenced by the depth of product charge (Divišová et al., 2014) but the effects vary as the capacitive dimensions of the product compression device. The aspect ratio relates depths of charge to diameters of compression gadgets and provides basis for generalising observed effects which may are considered when modelling powder compaction systems. Energy expended during mechanical compaction of food powders is significantly absorbed by the biomaterial as strain (Tomas, 2004; Suescun-Florez et al., 2015). Energy absorption, however, cannot be a function, only, of stress intensity but also a function of the rate at which strain occurs (Russell et al., 2014), which is of direct importance to process efficiency. In uniaxial compression of food materials, the time rate of deformation may be employed to vary the rate of occurrence of strain (Bagley etal., 1988). In this study, the effects of applied pressure, deformation rate and equipment aspect ratio on mechanical response during the compaction of carob powder were investigated.

MATERIALS AND METHODS

Carob (*Ceratonia siliqua* L.) powder, purchased in Czech Republic, was used for this study. The material had average particle size of 0.5 mm and initial bulk density of 711.69 ± 26.55 kg m^{-3}.

The compression device used is of the form shown in Fig. 1. It consists essentially of a cylindrical die, a base plug and a piston. The die bore diameter was 25 mm and wall thickness was about 40 mm. The base plug is of solid steel, 95 mm in diameter and stepped inwards diametrically, 25 mm from its top, 35 mm from its circumference. The smaller end of the base plug fits closely in the die. A solid piston of 25 mm diameter, 200 mm long compresses the material in the die. The device was mounted on the ZDM 50 universal test rig and loaded compressively through a hemispherical disc.

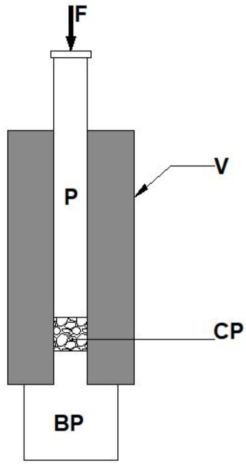

Figure 1. Schematic view of the product compression device showing the base plug, BP, compressed powder, CP, applied load, F, piston, P and cylindrical vessel, V.

Measures of the test powder corresponding to some aspect ratios were fed into the die. The piston was inserted and the device engaged with the load source. Pressure was gradually applied on the material until a maximum value was attained. Two pressure levels were selected as maxima for this study, that is 50 and 100 MPa. The loads were applied at three rates of deformation – 5.5, 10 and 14.5 mm min^{-1}. Three aspect ratios (0.5, 1.0 and 1.5) were also used, being ratios of the depths of material in the die to its bore diameter. These constituted 18 treatments and, in three repetitions, 54 experimental runs – a full factorial concept in a completely randomised design. The tests were conducted under a laboratory condition of 20 °C. Data acquisition was done using the TiraTest software developed by TIRA GmbH, Schalkau, Germany. Sample masses were acquired using the Kern 440–35N (Kern & Sohn GmbH, Stuttgart, Germany) top-loading type weighing balance.

For any treatment condition, a degree of deformation of the compressed material is attained. The highest value of deformation during each test was defined as δ_c (mm). For an established initial depth of product, δ_o (mm) in the compression chamber, the amount of strain induced, ϵ (–), was determined using Eq. 1.

$$\epsilon = \frac{\delta_c}{\delta_o} \qquad (1)$$

The rate of strain was obtained as the ratio of this strain to the time required to effect the indicated deformation. The initial bulk density, ρ_b (kg m^{-3}) of the batch of carob powder used was determined as the mass of the sample of carob powder to the known volume of a cylinder it fills, freely (Mohsenin, 1986). The initial volume of compressed material, v (mm^3) may be determined as (Eq. 2).

$$v = \frac{\pi D^2}{4} \times \delta_O \quad (2)$$

Upon compression, given an internal die diameter, D (mm), compressed material volume, v_c (mm) and a known product mass, m_s (g), the bulk density of the compressed material, ρ_c (kg m^{-3}) may be determined using Eq. 3.

$$\rho_c = \frac{m_s}{v_c} \quad (3)$$

The volume of the compressed product, v_c (mm^3) was calculated using Eq. 4.

$$v_c = \frac{\pi D^2}{4} \times \delta_f \quad (4)$$

where δ_f (mm) – final height of the compressed material in the compression chamber.

The energy required for an indicated amount of deformation may be determined as the area below the force – deformation curve. This was computed numerically by applying the trapezoidal rule (Eq. 5).

$$E = \sum_{n=0}^{n=i-1} \left[\left(\frac{F_{n+1} + F_n}{2} \right) \times (\delta_{n+1} - \delta_n) \right] \quad (5)$$

where n – subdivisions of the deformation axis or incremental deformation, as logged by the test equipment (Akangbe & Herak, 2017); F_N – force required for a deformation, δ_n (mm). With respect to the volume of material compressed, specific energy demand, E_v (J mm^{-3}) may be calculated as follows (Eq. 6).

$$E_v = {}^{E}\!/_{v} \quad (6)$$

The specific power requirement, $\dot{E}$ is therefore the time rate of expenditure of this energy, determinable using Eq. 7.

$$\dot{E} = {}^{E_v}\!/_{t} \quad (7)$$

where t – deformation time, s.

Numerical computations and graphical plots were done in MS Excel. Test data were subjected to the analysis of variance using the completely randomised design model in Genstat. Means were compared using Duncan's multiple range test.

RESULTS AND DISCUSSION

The main effects of pressure, deformation rate and aspect ratio on all mechanical response parameters were highly significant ($P < 0.001$), except for the effect of the levels of pressure considered on the rate of strain ($P = 0.493$) and those of the time rate of deformation on specific energy ($P = 0.08$). Significant effects were also observed at the first levels of interaction of the factors studied (Table 1). For instance, except for the effects on deformation and strain, which were not significant ($P = 0.387$ and $P = 0.194$, respectively) the interaction of the time rate of deformation and aspect ratio had highly significant effects ($P < 0.0029$) on all mechanical response parameters. Except for the rate of strain, no significant effects on response parameters were attributable to the second level of interaction of the parameters studied.

Table 1. Effects of deformation rate and aspect ratio on response variables

Response parameters	Source of variation						
	p	r_d	r_a	$p \times r_d$	$p \times r_a$	$r_d \times r_a$	$p \times r_d \times r_a$
Deformation, δ	0.001**	0.001**	0.001**	0.011*	0.001**	0.387^{ns}	0.028*
Strain, ϵ	0.001**	0.001**	0.001**	0.066^{ns}	0.001**	0.194^{ns}	0.158^{ns}
Strain rate, $\dot{\in}$	0.493^{ns}	0.001**	0.001**	0.901^{ns}	0.658^{ns}	0.001**	0.884^{ns}
Sp. energy, E_v	0.001**	0.08^{ns}	0.001**	0.003	0.001**	0.001**	0.001**
Sp. power, $\dot{E}$	0.001**	0.001**	0.001**	0.001**	0.001**	0.001**	0.349^{ns}
BDCM, ρ_C	0.001**	0.001**	0.001**	0.329^{ns}	0.317^{ns}	0.029*	0.277^{ns}
Gain, G_ρ	0.001**	0.001**	0.001**	0.329^{ns}	0.317^{ns}	0.029*	0.277^{ns}

ns = not significant at the 5% level; * = significant (at the 5% level); ** = highly significant (at the 1% level).

Figure two shows the force–deformation details of compressed powder given a maximum applied pressure of 100 MPa. The profiles are curvilineal, typical of biomaterials (Blahovec, 1982; Yan & Barbosa-Cánovas, 1997). Packing improved with incremental application of compressive load (Raji & Favier, 2004). Higher amount of deformation was occasioned by an increase in pressure from 50–100 MPa (Table 2). The higher the pressure, the higher will be the deformation that may be achieved. Higher rates of deformation resulted in increased deformation. These effects were, however, similar at deformation rates of 10 and 14.5 mm min^{-1} (Table 3). As the aspect ratio increased, deformation also increased (Table 4) and was significantly higher with every increase in the aspect ratio.

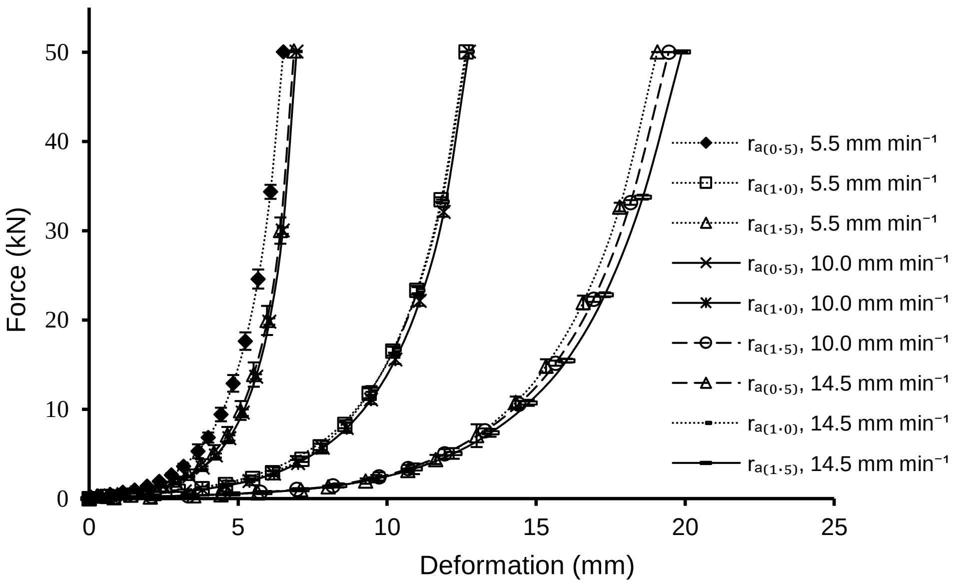

Figure 2. Force – deformation profiles of carob powder at applied pressure of 100 MPa, aspect ratios (r_a) of 0.5, 1.0 and 1.5 and deformation rates of 5.5, 10 and 14.5 mm min^{-1}.

More strain was induced at higher pressure (Table 2) and at higher rates of deformation (Table 3); although similar amounts of strain were induced at 10 and 14.5 mm min^{-1}. The highest amount of strain was obtained with the least aspect ratio (Table 4).

Table 2. Main effects of applied pressure on mechanical response parameters

	Applied pressure, p (MPa)	
Response Parameters	50	100
Deformation, δ (mm)	10.5^{b}	11.4^{a}
Strain, ϵ (-)	0.427^{b}	0.465^{a}
Strain rate, $\dot{\in}$ (S^{-1})	0.0045^{a}	0.0081^{a}
Specific energy, E_v (MJm^{-3})	4.70^{b}	4.71^{a}
Power, $\dot{E}$ ($kJm^{-3}S^{-1}$)	49.7^{b}	83.2^{a}
Bulk density of compressed material, ρ_C (kgm^{-3})	1246.0^{b}	1337.0^{a}
Gain in bulk density, G_ρ (%)	75.1^{b}	87.9^{a}

Mean values are compared row-wise. Similar alphabets indicate homogeneous subsets. Significant effects are valid at the 5% level of significance.

Table 3. Effects of the time rate of deformation on mechanical response

	Deformation rate, r_d (mm min^{-1})		
Response Parameters	5.5	10	14.5
Deformation, δ (mm)	11.69^{b}	12.11^{a}	12.21^{a}
Strain, ϵ *(-)*	0.4728^{b}	0.4909^{a}	0.4968^{a}
Strain rate, $\dot{\in}$ (S^{-1})	0.0045^{c}	0.0081^{b}	0.0117^{a}
Specific energy, E_v (MJm^{-3})	6.924^{a}	6.794^{a}	6.924^{a}
Power, $\dot{E}$ ($kJm^{-3}S^{-1}$)	65.6^{a}	108.8^{b}	162.3^{a}
Bulk density of compressed material, ρ_C (kgm^{-3})	1361^{b}	1429^{a}	1416^{a}
Gain in bulk density, G_ρ (%)	90.8^{b}	100.3^{a}	98.5^{a}

Mean values are compared row-wise. Similar alphabets indicate homogeneous subsets. Significant effects are valid at the 5% level of significance.

Table 4. Effects of aspect ratio on mechanical response

	Aspect ratio, r_a (-)		
Response Parameters	0.5	1.0	1.5
Deformation, δ (mm)	6.46^{c}	11.67^{b}	17.88^{a}
Strain, ϵ (-)	0.5170^{a}	0.4667^{c}	0.4767^{b}
Strain rate, $\dot{\in}$ (S^{-1})	0.0132^{a}	0.0066^{b}	0.0044^{c}
Specific energy, E_v (MJm^{-3})	7.35^{a}	6.85^{b}	6.44^{c}
Power, $\dot{E}$ ($kJm^{-3}S^{-1}$)	183.3^{a}	95.2^{b}	58.4^{c}
Bulk density of compressed material, ρ_C (kgm^{-3})	1534^{a}	1374^{b}	1299^{c}
Gain in bulk density, G_ρ (%)	115.0^{a}	92.6^{b}	82.1^{c}

Mean values are compared row-wise. Similar alphabets indicate homogeneous subsets. Significant effects are valid at the 5% level of significance.

Volumetric strain – estimated as axial strain in constrained configurations – may be expressed in direct proportion to increments of pressure (Raji & Favier, 2004). Void capacity is lost progressively, and is strain dependent; there is evidence that this effect is pulsatile and accounts for two notable phases during compaction (Ozbay & Cabalar, 2016).

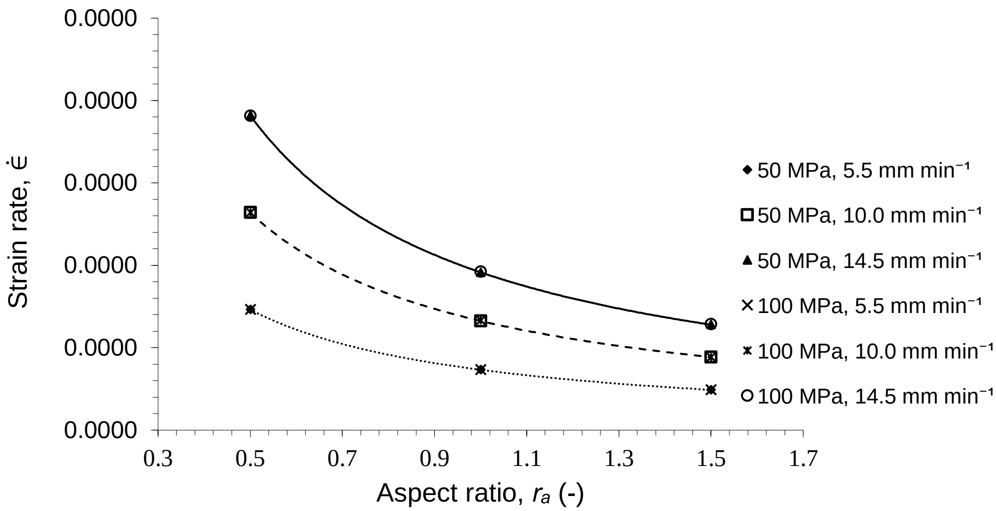

Figure 3. Rates of strain in the compressed powder as functions of the aspect ratio at 50 and 100 MPa and deformation rates of 5.5, 10.0 and 14.5 mm min^{-1}.

Non-recoverable strain is a consistent component of induced strain (O'Dogherty, 1989). Higher rates of strain were occasioned at higher deformation rates and lower aspect ratios (Tables 3 and 4). This relationship is shown in Fig. 3. The rate of deformation is an important determinant of the rate of strain. High rates of strain have been associated with stiffer compacts (Sarumi & Al-Hassani, 1991). The strain rate, $\dot{\epsilon}$ (s^{-1}) may be expressed as a power function of the equipment's aspect ratio, r_a (-), at every level of deformation (Table 5).

$$\dot{\epsilon} = k r_a^n \qquad (8)$$

where k – proportionality constant, s^{-1}; n – exponential constant, (-).

Table 5. Estimated parameters of strain rate and specific power as functions of the aspect ratio

Pressure (MPa)	Deformation rate (mm min^{-1})	Strain rate, $\dot{\epsilon}$ (s^{-1})			Specific power, $\dot{E}$ (Jm^{-3}s^{-1})		
		k (s^{-1})	n (-)	R^2	k (s^{-1})	n (-)	R^2
50	5.5	0.0037	-0.9990	1.0000	38.7910	-1.0680	0.9928
	10.0	0.0066	-0.9930	1.0000	66.3140	-1.0880	0.9968
	14.5	0.0096	-0.9960	1.0000	96.5020	-1.1380	0.9967
100	5.5	0.0037	-0.9980	1.0000	64.8650	-1.1430	0.9990
	10.0	0.0066	-0.9930	1.0000	112.1500	-0.9400	0.9970
	14.5	0.0096	-0.9900	1.0000	167.8900	-0.9460	0.9906

Increasing the applied pressure raises energy expenditure (Table 2), as does lowering the aspect ratio (Table 4). Energy demand decreased per unit volume of material compressed as aspect ratio became larger. The time rate of expenditure of energy increased as deformation rate increased (Table 3) and as the aspect ratio was lowered. Specific power requirement is therefore lower at lower rates of deformation and higher aspect ratios, indicating patterns for energy efficiency during powder compaction. Similar results were reported for related densification systems (Akangbe et al., 2018). There are indications (Mittal & Puri, 2005) that rate dependent compression response is non-linear. Considerable amount of the energy required for compaction is absorbed as plastic deformation (Kulig et al., 2015), translocation of product particles being the dominant initial activity. Fluid pressure builds up within the matrix and is lost, repeatedly over the span of strain. Two positions are reported on fluid pressure build up and loss during uniaxial compression of food materials namely, repeated (Ozbay & Cabalar, 2016) and progressive (Stasiak et al., 2012). A fourth element of energy is responsible for relaxation in the matrix after compaction. Specific power requirement was observed to be a power function of the aspect ratio (Eq. 9), at all rates of deformation (Fig. 4)

$$\dot{E} = kr_a^n \tag{9}$$

where $\dot{E}$ – time rate of expenditure of energy, $\mathrm{Jm^{-3}s^{-1}}$; r_a – aspect ratio, (-); k – constant of proportionality, $\mathrm{s^{-1}}$; n – exponential constant, (-). Parameters of this equation are presented in Table 5, for the different rates of deformation considered. The power law has been used to describe similar relationships such as that between the ratio of in-die to relaxed material density and aspect ratio (O'Dogherty & Wheeler, 1984; Kronbergs et al., 2013).

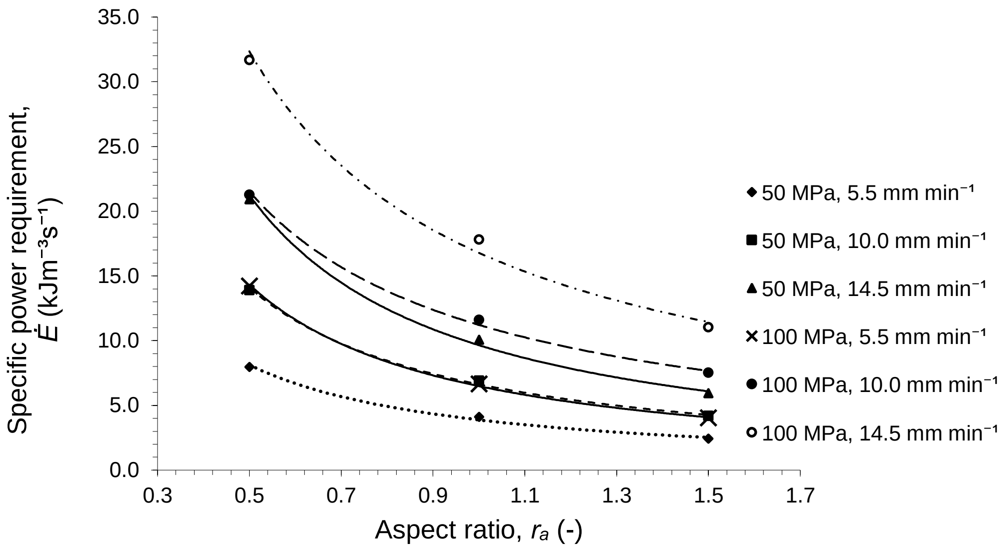

Figure 4. Specific power requirement as a function of the aspect ratio, 50 and 100 MPa of applied pressure and deformation rates of 5.5, 10.0 and 14.5 mm $\mathrm{min^{-1}}$.

The degree of compaction of carob powder was similar at deformation rates of 14.5 and 10.0 mm $\mathrm{min^{-1}}$ but higher than bulk density achieved at 5.5 mm $\mathrm{min^{-1}}$. Applied pressure and aspect ratio were better determinants of gain in bulk density than the time rate of deformation. Bulk density was significantly higher in material compressed at 100 MPa than those compressed at 50 MPa and represented 88% gain at applied pressure of 100 MPa over initial values, compared to gains of 75% at 50 MPa.

Given associated bulk moduli (Johnson et al., 2013), applied pressure considerably determines achievable deformation, with positive correlation to energy requirement for resulting compacts (Adapa et al., 2013). Powder compacted at the least aspect ratio (Table 4) had the highest value of bulk density, which was 1,534 kg m^{-3}. Gains in bulk density increased significantly as aspect ratio was lowered.

CONCLUSIONS

Effects of applied pressure, deformation rate and aspect ratio on mechanical response during the compaction of carob powder were investigated. Main effects of each factor had highly significant effect on mechanical response. Strain rate and specific power requirement were found to be power functions of the aspect ratio at all rates of deformation investigated. Whereas achievable degrees of compaction are higher at lower aspect ratios, energy use efficiency improves with larger aspect ratios. These findings are of direct relevance to processing of food powders into preferred product forms as well as their handling, transport and storage.

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REFERENCES

Adapa, P., Tabil, L. & Schoenau, G. 2013. A Comprehensive analysis of the factors affecting densification of barley, canola, oat and wheat straw grinds. *CSBE/SCGAB 2011 Annual Conference* (Vol. 11, p. Paper No. CSBE11-513). Winnipeg, Manitoba.

Akangbe, O. L., Adamovsky, R. & Mosna, F. 2018. Optimising cold compressive recovery of oil from the seeds of Sesame (*Sesamum indicum* L.). *Agronomy Research* **1**6(3), 634–645.

Akangbe, O.L. & Herak, D. 2017. Mechanical behaviour of bulk seeds of some leguminous crops under compression loading. *Scientia Agriculturae Bohemica* **48**(4), 238–244.

Bagley, E.B., Christianson, D.D. & Martindale, J.A. 1988. Uniaxial compression of a hard wheat flour dough: Data analysis using the upper convected Maxwell model. *Journal of Texture Studies* **19**(3), 289–305.

Blahovec, J. 1982. Stlačování upravené žitné slámy [Compaction of modified rye straw]. *Zemědělská Technika* **28**(2), 65–75 (in Czech).

Divišová, M., Herák, D., Kabutey, A., Šleger, V., Sigalingging, R. & Svatoňová, T. 2014. Deformation curve characteristics of rapeseeds and sunflower seeds under compression loading. *Scientia Agriculturae Bohemica* **45**(3), 180–186.

Faborode, M.O. 1989. Moisture effects in the compaction of fibrous agricultural residues. *Biological Wastes* **28**(1), 61–71.

Faborode, M.O. & O'Callaghan, J.R. 1986. Theoretical analysis of the compression of fibrous agricultural materials. *Journal of Agricultural Engineering Research* **35**(3), 175–191.

Halliday, P.J. & Smith, A.C. 1997. Compaction and flow of potato starch and potato granules. *Food Science and Technology International* **3**, 333–342.

Imole, O.I., Paulick, M., Magnanimo, V., Morgeneyer, M., Montes, B.E.C., Ramaioli, M., Kwade, A. & Luding, S. 2014. Slow stress relaxation behavior of cohesive powders. *Powder Technology* **293**, 82–93. http://dx.doi.org/10.1016/j.powtec.2015.12.023

Johnson, P., Cenkowski, S. & Paliwal, J. 2013. Compaction and relaxation characteristics of single compacts produced from distiller's spent grain. *Journal of Food Engineering* **116**(2), 260–266. Retrieved from http://dx.doi.org/10.1016/j.jfoodeng.2012.11.025

Kronbergs, A., Kronbergs, E. & Repsa, E. 2013. Evaluation of reed canary grass shredding and compacting properties. *Agronomy Research* **11**(1), 61–66.

Kulig, R., Łysiak, G. & Skonecki, S. 2015. Prediction of pelleting outcomes based on moisture versus strain hysteresis during the loading of individual pea seeds. *Biosystems Engineering* **129**, 226–236. Retrieved from http://dx.doi.org/10.1016/j.biosystemseng.2014.10.013

Laskowski, J., Łysiak, G. & Skonecki, S. 2005. Material properties in grinding and agglomeration. In J. Horabik & J. Laskowsk (Eds.), *Mechanical properties of granular agro-materials and food powders for industrial practice, Part II* (pp. 47–86). Lublin: Institute of Agrophysics PAS.

Mittal, B. & Puri, V.M. 2005. Rate-dependent elasto-viscoplastic constitutive model for industrial powders. Part 1: Parameter quantification. *Particulate Science and Technology* **23**(3), 249–264.

Mohsenin, N.N. 1986. *Physical properties of plant and animal materials. Vol. I: Structure, physical characteristics and mechanical properties*. New York: Gordon and Breach Science Publishers, pp. 66–76.

O'Dogherty, M.J. 1989. A review of the mechanical behaviour of straw when compressed to high densities. *Journal of Agricultural Engineering Research* **44**, 241–265.

O'Dogherty, M.J. & Wheeler, J.A. 1984. Compression of straw to high densities in closed cylindrical dies. *Journal of Agricultural Engineering Research* **29**(1), 61–72.

Ozbay, A. & Cabalar, A.F. 2016. Effects of triaxial confining pressure and strain rate on stick-slip behavior of a dry granular material. *Granular Matter* **18**(3), 1–9.

Pietsch, W. 2005. *Agglomeration in Industry: Occurrence and Applications*. Weinheim, Germany: WILEY-VCH.

Raji, A.O. & Favier, J.F. 2004. Model for the deformation in agricultural and food particulate materials under bulk compressive loading using discrete element method. I : Theory, model development and validation. *Journal of Food Engineering* **64**, 359–371.

Russell, A., Müller, P. & Tomas, J. 2014. Quasi-static diametrical compression of characteristic elastic-plastic granules: Energetic aspects at contact. *Chemical Engineering Science* **114**, 70–84. Retrieved from http://dx.doi.org/10.1016/j.ces.2014.04.016

Sarumi, M.A. & Al-Hassani, S.T.S. 1991. High and low strain rate properties of powders for continuum analysis. *Powder Technology* **65**, 51–59.

Stasiak, M., Skiba, K., Molenda, M., Tys, J. & Mościcki, L. 2012. The mechanical parameters of rapeseed cake. *Energy Sources, Part A: Recovery, Utilization and Environmental Effects* **34**(13), 1196–1205.

Suescun-Florez, E., Kashuk, S., Iskander, M. & Bless, S. 2015. Predicting the Uniaxial Compressive Response of Granular Media over a Wide Range of Strain Rates Using the Strain Energy Density Concept. *Journal of Dynamic Behavior of Materials* **1**(3), 330–346.

Tomas, J. 2004. Product design of cohesive powders - Mechanical properties, compression and flow behavior. *Chemical Engineering and Technology* **27**(6), 605–618.

Wongsiriamnuay, T. & Tippayawong, N. 2015. Effect of densification parameters on the properties of maize residue pellets. *Biosystems Engineering* **139**, 111–120. Retrieved from http://dx.doi.org/10.1016/j.biosystemseng.2015.08.009

Yan, H. & Barbosa-Cánovas, G.V. 1997. Compression characteristics of agglomerated food powders: Effect of agglomerate size and water activity Caracter & iacute; sticas de la compresi & oacute ; n de. *Food Science and Technology International* **3**, 351–359.

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Effects of experimental warming on peroxidase, nitrate reductase and glutamine synthetase activities in wheat

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Abstract. Given the effects of climate change and its significant consequences on plant productivity, it is necessary to evaluate the enzymatic responses of the most important crops such as wheat (*Triticum durum* L.). We examined the response of foliar peroxidase activity, nitrate reductase, and glutamine synthetase to experimental increments of temperature (+2 °C) under field conditions following conventional agricultural practices. Foliar samples, in both control and warming treatments were taken during growth, tillering and flowering phenophases to test the peroxidase activity. Similarly, nitrate reductase, glutamine synthetase activities, and glutamine content were measured during the heading phenophase. Due to the effects of experimental warming, peroxidase activity significantly increased. The nitrate reductase activity was also higher in the warming treatment, suggesting a high nitrogen metabolism efficiency. Whereas, the increase observed in the glutamine synthetase activity, and consequently the glutamine content, evidenced a biochemical signal of an early senescence due to the effect of warming.

Key words: *Triticum*, warming, nitrogen metabolism, Yaqui Valley.

INTRODUCTION

Warming is one of the most stressing factors for plant productivity (Asseng et al., 2017). Every year, a significant amount of land is abandoned because species do not tolerate heat-stress conditions and, when they show some tolerance, their genetic potential is not fully expressed (Argentel-Martínez et al., 2017). Warming is a common stress in agricultural ecosystems that is often ignored by producers (Siebers et al., 2017) but it has a significant impact on agricultural yield reductions (Xin et al., 2016).

The most common warming effects on plant physiology, consist in modifications of growth patterns and affectations to phenology, however all physiological process are closely tied to cellular metabolism (Ramani et al., 2017; Garatuza-Payan et al., 2018). A single heat wave can alter the activity of the enzymatic system that modulates both, the synthesis and the degradation processes of macromolecules needed for plant yield (Mphande, et al., 2016). Under such stressing conditions, plants might turn on various enzymatic and non-enzymatic mechanisms for tissue and organ protection, to guarantee seed formation, as a final phase of the biological cycle (Baxter et al., 2014).

For example, the peroxidase participates in the antioxidative defense system, when reactive oxygen species are synthesized following stress. The importance of the study of nitrate reductase and glutamine synthetase activities lies on the variation of nitrogen use efficiency (NUE), mainly at the most critical wheat phenophases such as heading and flowering, when considerable amounts of nitrogen are necessary to ensure a good fertilization and seed formation (Majláth et al., 2016). The N required for the synthesis of proteins that accumulate in wheat pollen comes mainly from remobilization of N previously assimilated and accumulated into the leaves. N remobilization occurs through the phloem (and occurs actively) mostly in the form of aminoacids (Zhang & Du, 2016). In this way, N remobilization efficiency from leaves becomes the main factor of grain protein concentration at the end of the crop cycle. This process is affected by abiotic stresses such as heat. Despite of the importance of this theme, there are not abundant reports about its regulation. It is known that remobilization processes are regulated by internal factors (biochemical and molecular), with an important role in nitrogen metabolism, highlighting the role of glutamine synthethase (GS) family (Tian et al., 2016), and by external factors (abiotic stresses) such as heat, drought and salinity (Hoffman et al., 2016).

In the Yaqui Valley, Sonora, one of the main wheat production sites of México and across the world, increments of about 2 °C are expected in the next 50 years (Navarro-Estupiñan et al., 2018), likely bringing profound effects on wheat yields as suggested by modelling synthesis studies (Mendoza-Ochoa et al., 2018). An important step for the adoption of agronomical strategies to adapt to climate change consist in carrying detailed studies about the enzymes involved in plant protection and nitrogen metabolism. Such information will allow to explain the variability of physiological and agronomic responses to climate change scenarios in commercial varieties. In this context, the present study aimed to evaluate the peroxidase, nitrate reductase and glutamine synthetase activity in the commercial crystalline wheat variety CIRNO C2008, under experimental warming conditions in the field, in the Yaqui Valley, Sonora, Mexico.

MATERIALS AND METHODS

Experimental area location

The experiment was carried out during the crop cycle of December 2016 to April 2017, under field conditions, at the Technology Transfer Experimental Center (CETT-910) of the Instituto Tecnológico de Sonora (ITSON), located at 27°22'0.4"N and 109°54'50.6"W (UTM: 607393.24 m E; 3027508.34 m N). During the cropping cycle mean daily temperatures were in the range of 8° to 24 °C with the lowest temperatures occurring during December and January. Accumulated rainfall was less than 25 mm with two events occurring in February. The wind speed did not exceed 3 m s^{-1}. The experiment was established on a vertisol soil with 25 years of crop management. Soil characteristics previous to sowing are shown in Table 1.

Table 1. Soil characteristics and mineral composition

pH	M.O (%)	CE (dS m^{-1})	Cations (meq L^{-1})				Anions (meq L^{-1})			
			K	Na	Ca	Mg	SO_4	CO_3	HCO_3	Cl
7.1	1.7	1.96	1.13	8.7	7.8	5.23	4.59	0.85	3.34	5.7

Treatments and temperature control

Two treatments were established: T1: increase of 2 °C with respect to the ambient temperature of the crop canopy (Warming treatment); and T2: ambient temperature of the crop canopy (Control treatment) as described previously by Garatuza-Payan et al. (2018). Shortly, treatments were distributed following a completely randomized experimental arrangement. To increase the crop canopy temperature, a T-FACE system designed by Kimball (2015) was mounted. Six 1000 W thermal radiators were used per plot (model FTE-1000, 245 mm long x 60 mm wide, built by Mor Electric Company Heating Association Inc. Comstock Park, MI, EEU.). The radiators were deployed on five triangular equilateral structures of 5.2 m on each side (Fig. 1). Two radiators were installed on each side of the triangular structures forming a regular hexagon that effectively raised the temperature in a 3 m diameter circle on each plot. To control the temperature, infrared temperature sensors were installed (ITSR Apogee Instruments Inc., Logan, UT, USA) in both control and warmed plots. The ITSR sensors were coupled to a data logger (CR1000 Campbell Sci, Inc. Logan, UT, USA) that send a voltage signal to an interface (MAI-05V, Avatar Instruments) which, in turn, translates the signal from volts to milliamps into a dimmer (Attenuator A1P-24-30-S05, Avatar Instruments). This dimmer controlled the current delivered to heaters, so that the amount of emitted heat increases or decreases depending on the temperature difference between the warmed and control plots, through the proportional integrative and derivative routine described by Kimball (2015).

Figure 1. Aerial image of de experimental site where triangular structures were placed containing six thermal radiators each one.

Plant material and Agronomic management

CIRNO C2008 wheat variety was used as experimental model, which is classified as a crystalline or hard wheat (*Triticum durum* L.). It originated from a segregating selection of interbreeding SOOTY-9 / RASCON-37 // CAMAYO, carried out in the International Center for the Improvement of Maize and Wheat (CIMMyT). This variety was released for crop extension since 2008 (Figueroa-López et al., 2010) and still maintains genetic stability of yield components (Argentel-Martínez et al., 2018).

The sowing was carried out with a SUB-24 drill on December 8, 2016, in a vertisol soil (Bockheim et al., 2014), forming three rows for each furrow, using a seed density of 170 kg ha^{-1}. Background fertilization was carried out applying 250 kg ha^{-1} of urea + 100 kg ha^{-1} of monoammonium phosphate (MAP), 11-52-00. Three irrigations were applied with an average water depth of 14 cm and irrigation intervals of 25 days. Two additional nitrogen applications of 50 kg ha^{-1} of urea were applied before the second and third irrigations (growth and heading phenophases). A slight presence of aphids (*Schizaphis graminum*) was observed during tillering phenophase using the Muralla Max (ia Imidacloprid + Betaciflutrin) pesticide for control, at a dose of 0.20 L ha^{-1}. Weed control was done manually before irrigations.

Peroxidase activity (POD)

Peroxidase activity was measured according to the continuous method similar to the one described by Maehly & Chance (1954). The enzyme extract was obtained with a sample of 0.25 g of leaf fragments sampled from 15 plants per repetition at each treatment, which were homogenized in a mortar, with 5 mL of Tris-HCl buffer pH 7.4. The homogenate was centrifuged at 12,900 rpm during 15 min and the supernatant was used to determine POD activity. The oxidative substrate was made of Guaiacol (2-Methoxyphenol, Catechol monomethyl ether, Pyrocatechol monomethyl ether) (0.5 mL) and hydrogen peroxide at 30% (0.1 mL). The POD extract (0.1 mL) was mixed with the oxidative substrate (0.25 mL) to measure absorbance at 470 nm. This reaction was used to define enzymatic activity units (UEA) where one unit was the change of 0.01 in the absorbance reading for one minute and is reported by per gram of fresh weight. This assay was done during growth, tillering and heading phenophases, when 51% of plants showed similar characteristics.

Nitrate reductase activity (NR)

The Jaworsky (1971) methodology was used for NR measurement during the heading phenophase. In a final volume of 2 mL of the reagents, 0.8 mL of phosphate buffer (100 mM of K_2HPO_4 / KH_2PO_4, pH 7.5), 0.2 mL of 100 mM KNO_3, 0.2 mL of 10 mM cysteine, 0.2 mL of 2 mM NADH, were mixed together with 0.6 mL of enzymatic extract (see above). The NR activity was stopped by the addition of 0.1 mL of 1 M zinc acetate after 30-min incubation of samples in the dark at 30 °C. Subsequently, to eliminate the precipitate formed after the addition of zinc acetate, the samples were centrifuged at 12,000 rpm for 15 min. The NR activity data were expressed in (µmol NO_2^- g FW^{-1} h^{-1}).

Activity of glutamine synthetase (GS)

The extraction of the GS was carried out following the method of O'Neal & Joy (1973) during heading phenophase. For this, an amount of 0.5 g of fresh plant material (a sample from fragments of 15 plants by repetition at each treatment) was homogenized with 5 mL of 0.2 M HEPES buffer, pH 7.9. The homogenate was centrifuged at 16,000 rpm for 20 min, and the obtained supernatant was used to measure GS activity. The enzymatic activity was determined by the method described by Slawky & Rodier (1988). The extracted material was centrifuged at 11,000 rpm, for 10 min., then a 50 μL sample was taken from the resulting supernatant to quantify the inorganic phosphorus (Pi) from the enzymatic use of ATP which was determined using the colorimetric method of vanadomolydophosphoric (Hogue et al., 1970), at a wavelength of 430 nm and against a standard curve of K_2HPO_4 (5–100 μM) and it was expressed in μmol GS s^{-1} g^{-1} FW. To determinate glutamate concentrations (mg g^{-1} FW), a blank per omission of the enzymatic extract and glutamate was used, where the corresponding volumes were replaced by the HEPES buffer.

Statistical analysis

For POD activity data comparison, we implemented a two way analysis of variance using a factorial arrangement, based on a linear model of fixed effects, taking as sources of variation the phenophases (growth, tillering and heading) and treatments (Warming and Control) (Fisher, 1937). Treatment means were compared by the Tukey multiple comparison test for $p < 0.01$ (Tukey, 1960). Statistical indicators: coefficient of determination without adjustment (R^2) was determined for the isolated factors and the phenophase-treatment interaction. The coefficient of variation (CV) was also determined.

For the NR, GS activities the mean of a total of 6 repetitions from each treatment and its standard deviation were determined, which were compared by a theoretical distribution of probabilities for continuous quantitative variables of t-Student for $p < 0.01$. For all analyzes the statistical package ESTATISTICA, version for Windows (StatSoft, 2014) was used.

RESULTS AND DISCUSSION

Peroxidase activity (POD)

Peroxidase activity increased significantly from the tillering phenophase in the warming (Fig. 2) compared to the control treatment. This result suggests that during the initial growth phenophase the warming treatment did not affect the enzymatic activity. This result could be related to the protective mechanisms of macromolecules reserve to avoid biological oxidations during its mobilization towards the reproductive organs (Yoneyama et al., 2016).

Highly significant differences in POD activity were obtained between the phenophases of warmed plants and such increase was more than double (Fig. 2). The observed increase in control plants during the heading phenophase, denotes the beginning of biological oxidation processes in plants induced by stress (Djanaguiraman et al., 2018). On the other hand, a high POD activity indicates the arrival of adverse conditions that affect cellular dynamics (Rached-Kanouni & Alatou, 2013). The variability found in POD activity was explained up to 61% by the warming effect, while a 23% was explained due to changes in phenophases. There was also a significant interaction (warming treatments x phenophases, $p = 0.05$), although its contribution to total POD variability was only 15%. POD plays an important role in the symplastic hydrogen peroxide (H_2O_2) level regulation by its conversion to H_2O together with the regeneration of $NADP^+$ (Kumar et al., 2015). Several studies have shown that POD activity increases during exposure to high temperatures in rice and maize (Xin et al., 2016; Matos-Trujillo et al., 2017).

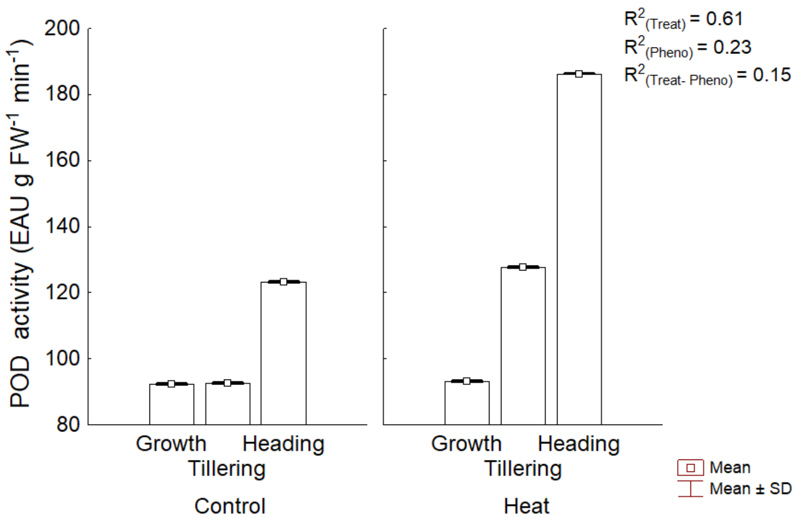

Figure 2. Peroxidase activity during Growth, Tillering and Heading phenophases. R^2: Determination coefficient without adjustment for: Treat (treatments) Pheno (phenophases) and Treat-Pheno (interaction). SD: Standard deviation.

There are also evidences that in corn (*Zea mays*) exposed to drought and saline stress the peroxidase activity increases significantly causing the formation of superoxide radicals (O_2^-) (Mittler, 2017). The superoxide radical and its reduced product $H_2O_2^-$ are potentially toxic when combined with a hydroxyl radical (OH^-), and are also known as reactive oxygen species (ROS) (Zhang et al., 2017; Hasanuzzaman et al., 2018). The plant species that show drought and salinity stress tolerance have an efficient active system (Goyal & Asthir, 2016) that avoid oxidative damage as a function of peroxidase activity (Argentel-Martínez et al., 2017).

Nitrate reductase activity during heading phenophase

The NR activity varied significantly between treatments, showing the highest activity in the warming treatment (Fig. 3). This result demonstrates that increased heat did not negatively affect plant capacity for assimilation and reduction of nitrogen and actually promoted NR activity. A high NR activity in plants indicate faster transformation kinetics of oxidases to reduced forms of nitrogen, therefore, to the formation of proteins. The NR is the first enzyme involved in $N\text{-}NO_3$ assimilation and it is essential for plant growth. In most herbaceous plants, reduction of $N\text{-}NO_3$ occurs in both the root and the shoot (Han et al., 2017). More particularly, $N\text{-}NO_3$ reduction occurs in the cytosol and the resulting NO_2^- is transported to the interior of chloroplasts, where it is reduced to NH_3 by NR. When there is a supply of N-NH_4^+, and with the participation of α-oxoglutarate, N is directly assimilated into chloroplasts to form glutamate through the activity of glutamine synthetase (GS) which has been localized in both roots and leaves in multiple species of cereals (Tian et al., 2016).

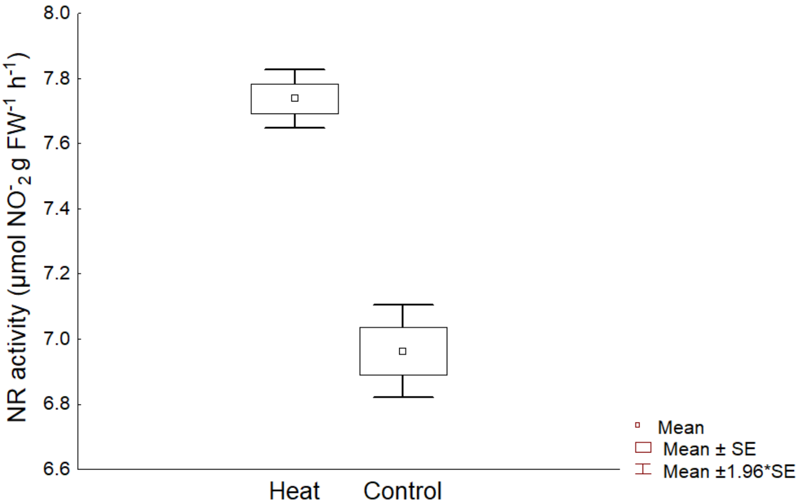

Figure 3. Nitrate reductase activity in wheat in the Control and heat treatments. SE: standard error.

Glutamine synthetase activity in the heading phenophase

The GS activity also significantly increased under warming conditions (Fig. 4, a), and this response led to the increase of glutamine content (Fig. 4, b), a metabolite that is generally the main substrate of remobilization of protein nitrogen during reproductive stage in cereals (Yoneyama, et al., 2016). These results verify that under warming, the observed early senescence occurs through protein metabolic translocation, which suggests a reduction of the biological crop cycle (Zandalinas et al., 2018). The GS activity is directly involved with the ureids and asparagine biosynthesis and with some other reactions of normal cell metabolism. In addition, it may have a close relationship with the self NR synthesis, so the differences in activity of (GS) can be directly or indirectly correlated to the NO_3^- assimilation efficiency of roots (Yang et al., 2017). The increase in GS activity generally forces an imbalance of glutamine / glutamate ratio, also causing a reduction in the glutamate content. Glutamate is the most abundant amino acid in wheat plants phloem during the vegetative stage, while glutamine is the predominant amino acid during reproductive stages, mainly during grain filling. This behavior suggest that GS would act as a repressor of nitrogen compounds transport in vegetative stages and also, as a promoter during reproductive stages. The promoter effect would have a positive consequence in protein accumulation in grains (Kaur & Kaur, 2017). The GS activity is an appropriate parameter to reveal the degradation of reserve protein and the end of the nitrogen assimilation processes in leaves. From this increase in enzymatic activity, the nitrogen mobilization, mainly from amino acid origin, is high and rapid, cascading in foliar senescence (Fernando et al., 2017).

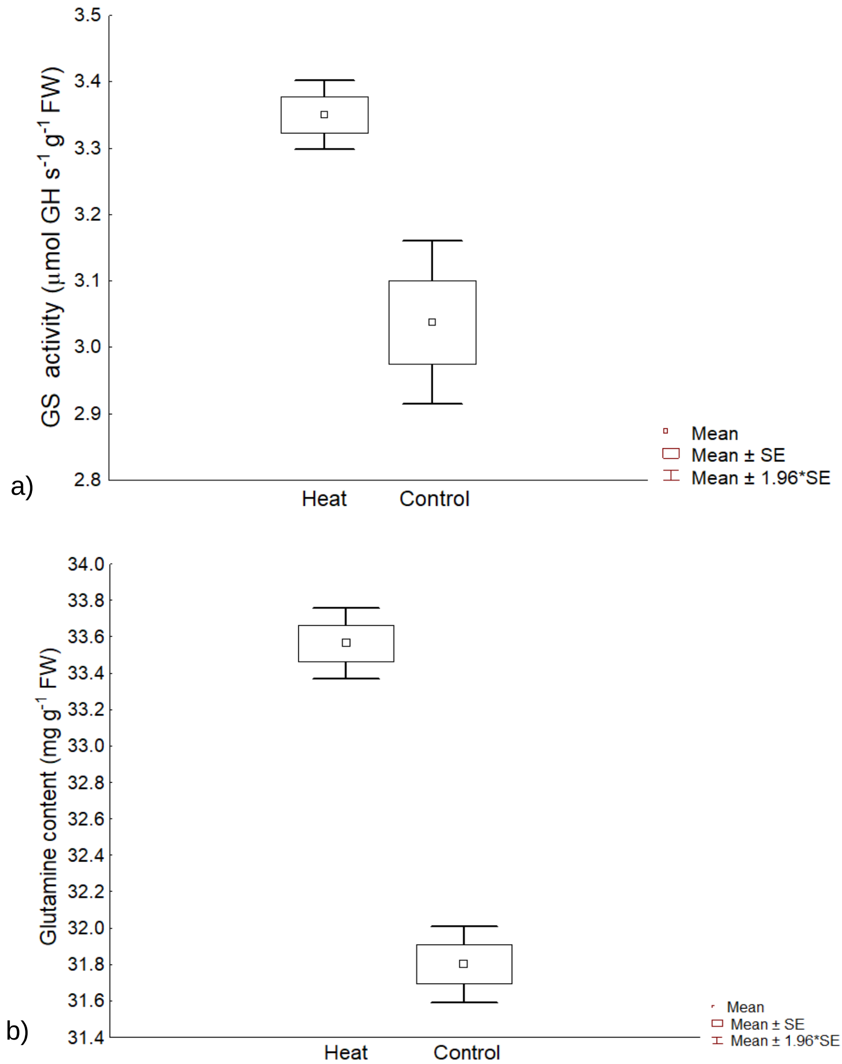

Figure 4. a) Glutamine synthetase activity and b) glutamine content during heading phenophase.

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REFERENCES

Argentel-Martínez, L., Garatuza-Payán, J.G., Armendáriz-Ontiveros, M.M.A., Yépez-González, E.A., Garibaldi-Chávez, J.M.G., Ortiz-Enríquez, J.E. & González-Aguilera, J.G. 2018. Physiological and agronomic characters of the CIRNO C2008 crystalline wheat variety confirm its genetic stability. *Agrociencia* **52**(3), 419–435.

Argentel-Martínez, L., Garatuza-Payán, J., Armendáriz-Ontiveros, M.M., Yépez-González, E.A., Arredondo Moreno, J.T. & González Aguilera, J. 2017. Thermal stress in wheat cultivation. Physiological, biochemical and agronomic implications. *Cultivos Tropicales* **38**(1), 57–67 (in Spanish).

Asseng, S., Cammarano, D., Basso, B., Chung, U., Alderman, P.D., Sonder, K. & Lobell, D.B. 2017. Hot spots of wheat yield decline with rising temperatures. *Global change biology* **23**(6), 2464–2472.

Baxter, A., Mittler, R. & Suzuki, N. 2014. "ROS as key players in plant stress signalling." *Journal of Experimental Botany* **65**(5), 1229–1240.

Bockheim, J.G., Gennadiyev, A.N., Hartemink, A.E. & Brevik, E.C. 2014. Soil-forming factors and Soil Taxonomy. *Geoderma* **226**, 231–237.

Djanaguiraman, M., Boyle, D.L., Welti, R., Jagadish, S.V.K. & Prasad, P.V.V. 2018. Decreased photosynthetic rate under high temperature in wheat is due to lipid desaturation, oxidation, acylation, and damage of organelles. *BMC plant biology* **18**(1), 55.

Fernando, N., Hirotsu, N., Panozzo, J., Tausz, M., Norton, R.M. & Seneweera, S. 2017. Lower grain nitrogen content of wheat at elevated CO_2 can be improved through post-anthesis NH_4^+ supplement. *Journal of Cereal Science* **74**, 79–85.

Figueroa-López, P., Félix-Fuentes, J.L., Fuentes-Dávila, G., Vallenzuela-Herrera, V., Chávez-Villalba, G. & Mendoza-Lugo, J.A. 2010. CIRNO C-2008, new variety of crystalline wheat with high potential yield for the state of Sonora. *Revista Mexicana de Ciencias* Agrícolas **1**(5), 739–744 (in Spanish).

Fisher, R.A. 1937. The design of experiments. Oliver and Boyd; Edinburgh; London, 260 pp.

Garatuza-Payan, J., Argentel-Martinez, L., Yepez, E.A. & Arredondo, T. 2018. Initial response of phenology and yield components of wheat (*Triticum durum L.*, CIRNO C2008) under experimental warming field conditions in the Yaqui Valley. *Peer J* **6**, e5064.

Goyal, M. & Asthir, B. 2016. Role of Sulphydral Compounds on Antioxidant Defense Mechanism under High Temperature Stress in Wheat. *Indian Journal of Agricultural Biochemistry* **29**(1), 17–22.

Han, R., Yang, D., Hassani, D., Wang, X., Liu, X. & Huang, D. 2017. Evaluation of the yield, nitrogen use efficiency and adaptation in pakchoi cultivars under organic and conventional management systems during two successive seasons. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science* **67**(1), 85–93.

Hasanuzzaman, M., Oku, H., Nahar, K., Bhuyan, M.B., Al Mahmud, J., Baluska, F. & Fujita, M. 2018. Nitric oxide-induced salt stress tolerance in plants: ROS metabolism, signaling, and molecular interactions. *Plant Biotechnology Reports* **12**(2), 77–92.

Hoffman, B., Szira, F., Monostori, I. Vágújfalvi, A. & Hoffman, S. 2016. Productivity of winter wheat (Triticum aestivum L.) cultivars depending on N fertilization. Berichte aus dem Julius Kühn-Institut **185**, 16.

Hogue, E., Wilcow, G. & Cantliffe, D.J. 1970. Effect of soil P on phosphate fraction in tomato leaves. *J. Am. Soc. Hortic. Sci*. **95**,174–176.

Jaworsky, E.G. 1971. Nitrate reductase assay in intact plants tissues. *Biochem. Biophys. Res. Comm*. **43**, 1274–1279.

Kaur, A. & Kaur, P. 2017. Effect of elevated temperature regimes on phenological development of wheat (*Triticum aestivum L*.). *Agricultural Research Journal* **54**(1), 132–135.

Kimball, B.A. 2015. Using canopy resistance for infrared heater control when warming open-field plots. *Agronomy Journal* **107**(3), 1105–1112.

Kumar, R.R., Sharma, S.K., Goswami, S., Verma, P., Singh, K., Dixit, N. & Rai, R.D. 2015. Salicylic acid alleviates the heat stress-induced oxidative damage of starch biosynthesis pathway by modulating the expression of heat-stable genes and proteins in wheat (*Triticum aestivum*). *Acta physiologiae plantarum* **37**(8), 1–12.

Maehly, A.C. & Chance, B. 1954. The assay of catalases and peroxidases. *Methods of biochemical analysis* **1**, 357–424.
Majláth, I., Darko, E., Palla, B., Nagy, Z., Janda, T. & Szalai, G. 2016. Reduced light and moderate water deficiency sustain nitrogen assimilation and sucrose degradation at low temperature in durum wheat. *Journal of plant physiology* **191**, 149–158.
Matos-Trujillo, M., Díaz-Solares, M., Samaniego-Fernández, L.M., Cortegaza-Ávila, L., Pérez-Milian, J.R., Pellón-Guzmán, Y. & Pérez-Pérez, J. 2017. Expression of the enzyme peroxidase in plants of Saccharum sp. hybrid inoculated with Xanthomonas albilineans Ashby (Dowson). *Pastos y Forrajes* **40**(3), 195–200 (in Spanish).
Mendoza-Ochoa, I.M., Asseng, A., Kassie, B.T., Xiong, W., Robertson, R., Luz pequeño, D.N., Sonder, K., Reynolds, M., Barbar, M.A., Molero Milan, A. & Hoogenboom, G. 2018. Climate change impact on Mexico wheat production. *Agricultural and Forest Meteorology* **263**, 373–387.
Mittler, R, 2017. ROS are good. *Trends in plant science* **22**(1), 11–19.
Mphande, W., Nicolas, M.E., Seneweera, S. & Bahrami, H. 2016. Dynamics and contribution of stem water-soluble carbohydrates to grain yield in two wheat lines contrasted under Drought and elevated CO_2 conditions. *J. Plant Physology* **214**(2), 1037–1058.
Navarro-Estupiñan, J., Robles-Morua, A., Vivoni, E.R., Zepeda, J.E., Montoya, J.A. & Verduzco, V.S. 2018. Observed trends and future projections of extreme heat events in Sonora, Mexico. *International Journal of Climatology*. doi.org/10.1002/joc.5719
O´Neal, D. & Joy, K.W. 1973. Glutamine synthetase of pea leaves. I. Purification, stabilization and pH óptima. *Arch. Biochem. Biophysics* **159**, 113–122.
Rached-Kanouni, M. & Alatou, D. 2013. Change in activity of antioxidative enzymes in leaves of Acacia retinodes, Biota orientalis and Casuarina equisetifolia under heat stress condition. *Eur. Sci. J.* **9** (24), 402–410.
Ramani, H.R., Mandavia, M.K., Dave, R.A., Bambharolia, R.P., Silungwe, H. & Garaniya, N.H. 2017. Biochemical and physiological constituents and their correlation in wheat (*Triticum aestivum L.*) genotypes under high temperature at different development stages. *International Journal of Plant Physiology and Biochemistry* **9**(1), 1–8.
Siebers, M.H., Slattery, R.A., Yendrek, C.R., Locke, A.M., Drag, D., Ainsworth, E.A. & Ort, D.R. 2017. Simulated heat waves during maize reproductive stages alter reproductive growth but have no lasting effect when applied during vegetative stages. *Agriculture, Ecosystems & Environment* **240**, 162–170.
Slawky, G. & Rodier, M. 1988. Biosynthetically active glutamine synthetase in the marine diatom *Phaeodactylum tricornutum:* optimization of the forward–reaction assay. *Mar. Bol.* **97**, 269–274.
StatSoft, Inc, 2014. STATISTICA (data analysis software system). Version 12. http://www.statsoft.com.
Tian, Z., Li, Y., Liang, Z., Guo, H., Cai, J., Jiang, D. & Dai, T. 2016. Genetic improvement of nitrogen uptake and utilization of winter wheat in the Yangtze River Basin of China. *Field Crops Research* **196**, 251–260.
Tukey, J.W.A. 1960. A survey of sampling from contaminated distributions. p. 448–485. In: Ingram Olkin, ed. Contribution to probability and statistics: Essays in honor to Harold Hotelling. Stanford University Press. USA
Xin, C., Wang, X., Cai, J., Zhou, Q., Liu, F., Dai, T. & Jiang, D. 2016. Changes of transcriptome and proteome are associated with the enhanced post-anthesis high temperature tolerance induced by pre-anthesis heat priming in wheat. *Plant growth regulation* **79**(2), 135–145.

Yang, X., Lu, Y., Ding, Y., Yin, X., Raza, S. & Tong, Y.A. 2017. Optimizing nitrogen fertilization: A key to improving nitrogen-use efficiency and minimizing nitrate leaching losses in an intensive wheat/maize rotation (2008–2014). *Field Crops Research* **206**, 1–10.

Yoneyama, T., Tanno, F., Tatsumi, J. & Mae, T. 2016. Whole-plant dynamic system of nitrogen use for vegetative growth and grain filling in rice plants (Oryza sativa L.) as revealed through the production of 350 grains from a germinated seed over 150 days: a review and synthesis. *Frontiers in Plant Science* **7**, 1151.

Zandalinas, S.I., Mittler, R., Balfagón, D., Arbona, V. & Gómez-Cadenas, A. 2018. Plant adaptations to the combination of drought and high temperatures. *Physiologia Plantarum*. **162**(1), 2–12

Zhang, M., Ma, D., Ma, G., Wang, C., Xie, X. & Kang, G. 2017. Responses of glutamine synthetase activity and gene expression to nitrogen levels in winter wheat cultivars with different grain protein content. *Journal of Cereal Science* **74**, 187–193.

Zhang, R. & Du, S. 2016. Properties of nitrogen fertilization are decisive in determining the effects of elevated atmospheric CO_2 on the activity of nitrate reductase in plants. *Plant signaling & behavior* **11**(4), 893–904.

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Theoretical study on sieving of potato heap elements in spiral separator

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Abstract. One of the principal problems in the harvesting of potatoes is the cleaning of the lifted heap from soil and plant impurities. The low quality in the performance of this harvesting work process operation is the main cause of the withdrawal from fields of considerable amounts of fertile soil. In order to facilitate choosing the optimal parameters for the new design of the spiral potato heap cleaning unit, a mathematical model of sieving the soil impurities through its cleaning spirals has been developed. The obtained analytical expressions have been solved with the use of a PC and the results have been used for plotting the graphic relations between the parameters of the examined process of a variable-mass body's motion on the surface of a spiral, which have made it possible to select the optimal design and kinematic parameters of the spiral cleaning unit under consideration. The obtained graphic relations indicate that the rate of sieving in the spiral separator depends on many factors, including the initial mass, the design dimensions (spiral's radius, helix angle etc.), the frictional properties of the surface, the angular parameters of the position of the potato heap elements on the spiral's surface, and the angular velocity of the rotational motion of the spiral roll itself.

Key words: cleaning, digging, mathematical model, potato, spiral roll.

INTRODUCTION

The industrial production of potatoes is among the energy and material intensive sectors in the agricultural industry, in view of the fact that just with regard to its energy costs per unit of output product it exceeds the production of grain crops 4 to 5 times (Vasilenko, 1996). The most undeveloped area in the mechanized technologies of potato production is its harvesting, in particular, the achievement of the standard quality in the output product. Therefore, in the further development and improvement of the tools for potato harvesters and the optimization of their parameters it is necessary, first of all, not only to provide for reducing their material and energy intensity, but substantially raise the quality of the potato tubers lifted from the soil as well (Misener & McLeod, 1989;

Peters, 1997; Bishop et al., 2012; Ichiki et al., 2013; Guo & Campanella, 2017; Wang et al., 2017).

The accomplished theoretical and experimental investigations (Bulgakov et al., 2018a) as well as the numerous trials of various types of potato harvesters (Petrov, 2004) have proved that the high quality of the cleaning of potato tubers from soil and plant impurities is achievable, when a considerable amount of the soil and other heap components (haulm residues, rootstock, hardened soil bodies, stones etc.) is separated from the tubers in the process of digging the potato bed or immediately after their lifting. In that case a significant mass of the dug heap will not be fed into the harvester together with the potato tubers. However, the widely used elevators, the units for loosening and breaking the dug bed installed immediately after the passive and active vibrational type lifting tools, screens, clod smashers, while demonstrating sufficient transporting capacity, have relatively low separating performance. As a result of that, considerable quantities of soil impurities and plant residues are still fed into the potato harvesters and reach their other separating tools.

At the same time, the various kinds of cleaning tools in the majority of potato harvesters, such as agitators, open-web elevators, grading screens as well as drum and rotary separators mostly perform what is known as passive or without power driven separation' of impurities (Petrov, 2004). That implies that despite the fact that these tools can vibrate, be equipped with various actuators (for example, rotary-vane, auger etc.), perform other types of forced motions, the removal of impurities in them is not directly intended and enforced. Even though various cleaning forces, which have different lines of action and magnitudes, are applied to potato tuber bodies as well as to the accompanying impurities, in the majority of cases the up-to-date separators of impurities from potato heaps do not establish the conditions for exactly their positive capture and forced evacuation from the separation zone. Therefore, in the well-known separation facilities (Feller et al., 1987), the impurities are not always suitable for their efficient separation, especially in the conditions of wet soil, which heavily clogs almost all separating spaces. When the dug heap contains rootstock and other plant residues (dry and green), they close the said separating gaps even more. Moreover, the currently known potato heap separators can feature such sieving gaps, which do not rule out the entrapment and damaging of potato tuber bodies.

The design and process schematic model of the discussed spiral unit (Bulgakov et al., 2018b) for cleaning the potato heap from impurities is presented in Fig. 1.

In this potato heap cleaning unit (Fig. 1) with driven cantilevered spirals 5, its cleaning surface features large-area sieving gaps formed by the spacing between the own turns of the spirals 5 and the spacing between the coils of the adjacent spirals 5. Overall, such a considerable increase of the effective separating area (i.e. the total area of all sieving gaps) relative to the total area of the whole cleaning surface not only provides a marked increase in the throughput capacity of the cleaning machine under consideration, but also facilitates the improvement of the quality indicators of its performance. For example, the fast and efficient sieving of the soil and plant impurities that fall down through the horizontal cleaning surface outside from the cleaning unit results in the increase of the time of contact between the potato tubers themselves and the turns of the spirals 5, which improves the cleaning of their side surfaces from the stuck soil. Moreover, the absence of a shaft inside any of the spirals 5 ensures the unobstructed passing down of all impurities input with the heap and also eliminates the wrapping of plant residues, which would be possible in case the drive shafts resided inside the spirals 5. The hollow interior space inside each spiral 5 enables raising significantly its capacity of positively transporting all the mass of the soil and plant impurities that have fallen here towards the output (cantilever) end of the spiral 5 and discharge it through the open end to the field surface. At the same time, the bodies of potato tubers cannot enter the interior spaces of the spirals 5.

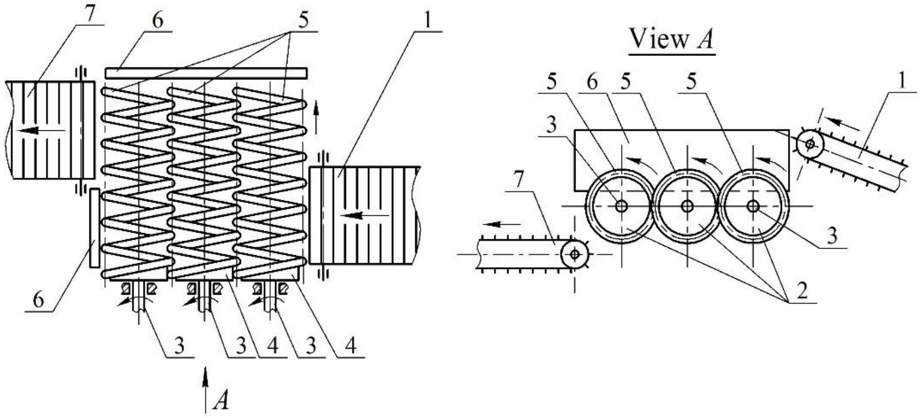

Figure 1. Schematic model of the potato heap separator of improved design: a – top view, b – side view; 1 – feeding conveyor; 2 – cantilevered spiral springs of cleaning rolls; 3 – power axle; cleaning roll; 4 – hub; 5 – spirals; 6 – protective apron; 7 – discharge conveyor.

In order to prevent the caking of the soil on each cleaning roll 2 and avoid complete sealing of the gaps between the spirals 5 with wet soil, the spirals are positioned with certain overlapping, i.e. the turns of the spiral 5 of each roll 2 partially enter the gaps between the turns of the spiral of the adjacent roll 2.

The work process of the spiral cleaning unit under consideration progresses as follows. The mass of the heap of the potato tubers dug out from the soil together with a considerable quantity of soil and plant impurities is fed by the conveyor 1 and arrives to the separating surface formed by the spirals 5 rotating in the same sense. Due to that rotation, the said mass of the potato heap is further transported along the coils of the spirals 5 (i.e. along their centrelines) and during that motion smaller soil impurities immediately spill through the separating gaps between the coils of the spirals 5. When the soil mass enters in greater quantities, it can fall down, but be accumulated in the interior spaces of the spirals 5, which will result in the situation, where the bottom inside surfaces of the spirals 5 are also unable to sieve the soil impurities down and outside of the cleaning unit at their full capacity. But the coil of each spiral 5 will transport the impurities already contained inside the spiral, which have been detained in the interior space of the spiral, and they will be positively transported to its free cantilever end and in the same way always, without exception fall down and outside of the cleaning unit. The arrangement of cleaning rolls 2 so that their spirals 5 overlap provides for the spirals 5 mutually cleaning each other from the stuck wet soil.

The undertaken experimental laboratory and field studies of the spiral separator (Bulgakov et al., 2017) mounted on the test unit modelling a single-row potato-digger have shown the high efficiency of its operation.

The research objective of this study was to determine theoretically the optimal design and kinematic parameters of the potato heap cleaning unit that ensure the efficient evacuation of soil impurities.

MATERIALS AND METHODS

It is obvious that the satisfactory progress of the separation process, i.e. the sieving of the elements of the potato heap in the spiral separator to a considerable extent depends on the dimensions of the heap elements - soil impurities fed to the spiral cleaning unit (Krause & Minkin, 2005). For that reason, an important factor in the said process is the reduction of the size of the said heap elements resulting from their displacement on the working surface of the spiral cleaning unit (Holland-Batt, 1989).

In view of the fact that the soil clods of different size input together with the potato tubers are the principal component of the potato heap lifted from the soil, the research into the process of sieving the potato heap elements, i.e. the soil impurities, which is equivalent to reducing the mass of the soil clod moving along the working surface of the spiral cleaning unit as a result of its dynamic (force) interaction with the said spiral surface is of considerable interest. As a consequence of the said interaction, the mass separated from the soil clod sifts without fail through the gaps in the working spirals and thus the continuously fed stream of the heap with potato tubers is overall cleaned from the accompanying mass of soil impurities.

In view of the aforesaid, it is reasonable to give consideration to a single soil clod moving on the separating spiral surface of the spiral cleaning unit as a variable mass body under the effect of the system of forces arising as a result of the interaction between the said body and the working surface of the spiral cleaning unit. For that purpose, the first step is to generate an equivalent schematic model, in which the conditions described above are represented (Fig. 2). This aim can be achieved by considering two cleaning spirals. In this case, the equivalent schematic model contains two driven spiral springs 1 and 2 installed as cantilevers at points D and D_1, their longitudinal axes being parallel to each other, which are in rotational motion at same angular velocities of ω and in the same sense. The spiral springs 1 and 2 are shaped like cylinders with the same radii of R, their coils have the same helix pitches of S and the same hands of helix (right-handed looking from the cantilever end of the spiral, which is shown by the arrows) and the springs are positioned so that they overlap. The helix angle (angle between the plane of the spiral's cross-section and the helical line) of spirals 1 and 2 is the same and is equal to γ. The centres of the spiral springs 1 and 2 are designated by the points O and O_1 respectively.

The particle (arbitrary-shape clod) of soil designated M in the equivalent schematic model is considered to be a variable-mass body, which at an arbitrary instant of time t is situated on some turn of spiral 1, and is in contact with the turn at the point K.

In order to investigate the process of motion of the said body M on the surface of the spiral cleaning unit, the principles of the dynamics of the variable-mass body movement are to be applied. For the purpose of generating the differential equation of the variable-mass body movement, it is necessary to choose the three-dimensional Cartesian coordinate system $xOyz$. The point of origin of the system of coordinates $xOyz$ (point O) is located on the longitudinal axis of the spiral 1, the axis Oz is aligned along the longitudinal axis of the spiral 1, the axes Ox and Oy are situated in the plane of cross-section of the spiral 1, in which case the axis *Ox* is directed horizontally to the right and perpendicular to the axis *Oz*, the axis *Oy* is directed vertically up and perpendicular to plane *zOx*. The described system of coordinates is assumed absolute (fixed) and the movement of the above-mentioned variable-mass body *M* is assumed to take place in this system of coordinates.

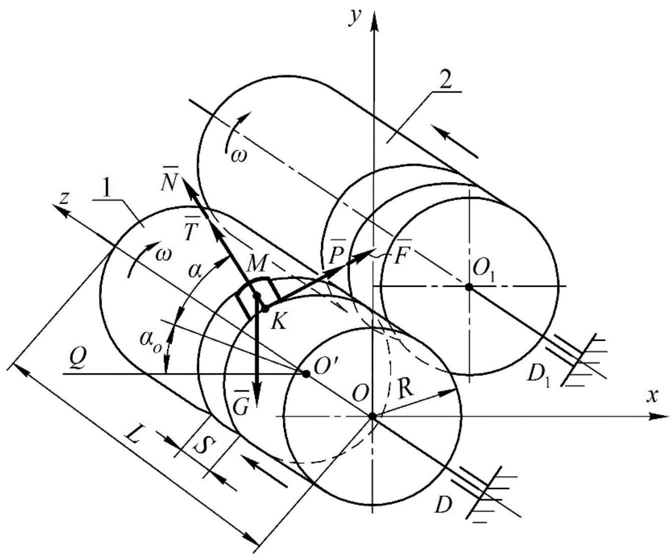

Figure 2. Equivalent schematic model of interaction between soil particle from potato heap with surface of spiral cleaning unit: 1 – first cleaning spiral; 2 – next cleaning spiral.

Apart from the coordinates *x*, *y* and *z*, which define the position of the variable-mass body *M* on the cleaning surface, taking into account the fact that the spiral 1 has a shape of cylinder, additional parameters are introduced – the angles α_0 and α, which define the position of the variable-mass body *M* in the cross-section of the spiral 1. The said cross-section passes through the point *K* of contact between the soil clod *M* and the turn of the spiral 1 and crosses the axis of the spiral 1 at the point O′.

Hence, α_0 is the angle that indicates the initial (at $t = 0$) position of the point *K* of contact between the soil clod *M* and the turn of the spiral 1 in the cross-section under consideration. Then, the angle $\alpha_0 + \alpha$ indicates the position of the point of contact *K* of the soil clod *M* at an arbitrary instant of time t. The angle α_0 is measured from the horizontal half line *O′Q*, which is parallel to the axis *Ox*, in the same direction, in which the spiral 1 rotates (clockwise). The angle α is measured in the same direction after measuring angle α_0. Under the given conditions, if $t = 0$, then also $\alpha = 0$.

RESULTS AND DISCUSSION

It is assumed that the length of the spiral 1 is equal to *L*. Also, the current value of the mass of the body under consideration *M* is assumed to be a function of time, i.e. $m = m(t)$, its initial value being equal to m_0.

The next step is to designate the forces acting on the variable-mass body *M*. They are, first of all: $\bar{G}$ – weight force of the variable-mass body. The force is applied at the body's centre of mass, directed vertically downwards and has the following magnitude:

$$G = m(t) \cdot g, \tag{1}$$

where g – acceleration of gravity; $\bar{F}$ – force of friction. It is directed at a tangent to the surface of the spiral and along the helical line (at an angle of γ with the plane of cross-section of the spiral), applied at the point of contact *K*, is vectored opposite to the relative velocity of the body *M* and, as is known, has the following magnitude:

$$F = f \cdot N, \tag{2}$$

where f – coefficient of sliding friction of the body on the surface of the spiral; $\bar{N}$ – normal reaction of the working surface of the spiral; $\bar{T}$ – acceleration force of the oscillatory motion of the spiral, which arises due to the deflection of the longitudinal axis of the spiral caused by the weight of the potato heap fed onto the cleaning surface.

The magnitude of the force *T* can be determined as follows.

As a result of the deflection of the longitudinal axis of the spiral 1, the centre of each cross-section of the spiral becomes displaced from the axis *Oz* by a certain distance varying from zero at the beginning of the spiral (point *O*) to a certain maximum distance at the cantilever end of the spiral. The magnitude of this displacement at a specific cross-section will be designated *A*. Thus, when the spiral rotates about the axis *Oz* at an angular velocity of ω, its deflected longitudinal axis circumscribes in space a certain surface of revolution, the cross-sections of which (perpendicular to the axis *Oz*) are concentric circles with their centres lying on the axis *Oz*. In the earlier mentioned specific cross-section the radius of such a circle is equal to *A*. Therefore, the centre of the said cross-section moves on the above-mentioned circle with a radius of *A*. In view of the smallness of the *A* value, it can be assumed that the centre of the cross-section oscillates during the operation of the unit following the sinusoidal law with an amplitude of *A* about the axis *Oz* along the diameter of the said concentric circle with a radius of *A*.

Accordingly, it can be assumed that the law of the oscillatory motion of the cleaning unit's spiral in its radial direction can be represented by the following expression:

$$l = -A\sin(\alpha_0 + \omega t), \tag{3}$$

where l – deflections of the cross-section centre from the axis Oz at an arbitrary instant of time t; A – amplitude of oscillation; ω – angular velocity of the spiral's rotation (it is assumed to be pre-set and have a fixed value).

By taking the first derivative of the expression (3), the following is obtained:

$$\dot{l} = -A \cdot \omega \cdot \cos(\alpha_0 + \omega t). \tag{4}$$

The acceleration of the spiral's oscillatory motion is equal to the first derivative of the expression (4) or the second derivative of the expression (3):

$$\ddot{l} = A \cdot \omega^2 \sin(\alpha_0 + \omega t). \tag{5}$$

Hence, the value of the force T is finally determined by the following formula:

$$T = m(t) \cdot \ddot{l} = m(t)\omega^2 \cdot A \cdot \sin(\alpha_0 + \omega t). \tag{6}$$

The force T at an arbitrary instant of time t is vectored normally to the spiral's helical line.

In order to generate the differential equation of motion of the variable-mass body M, the principle of the linear momentum of a particle in its differential form (Bulgakov et al., 2018c) is to be used:

$$\frac{d}{dt}\left(m\bar{V}\right)=\sum_{k=1}^{n}\bar{F}_k, \tag{7}$$

where m – mass of the particle (in this particular case the mass of the body M), $m = m(t)$ in the general case; $\bar{V}$ – velocity of the particle, $\bar{V} = \bar{V}(t)$ in the general case; $\sum_{k=1}^{n}\bar{F}_k$ – vector sum of the forces acting on the particle (body M) at an arbitrary instant of time t.

After substituting all above-listed forces into the expression (7), the following is obtained:

$$\frac{d}{dt}\left[m(t)\cdot\bar{V}(t)\right]=\bar{G}+\bar{N}+\bar{F}+\bar{T} \tag{8}$$

or

$$m\frac{d\bar{V}}{dt}+\bar{V}\frac{dm}{dt}=\bar{G}+\bar{N}+\bar{F}+\bar{T} \tag{9}$$

As the mass of the body on the surface of the spiral cleaning unit with the passage of time t decreases, the following can be stated:

$$\frac{dm}{dt}<0 \tag{10}$$

Taking into account (10), the expression (9) can be written in the following form:

$$m\frac{d\bar{V}}{dt}=\bar{G}+\bar{N}+\bar{F}+\bar{T}-\bar{V}\frac{dm}{dt}. \tag{11}$$

The last member of the sum in the expression (11), that is $-\bar{V}\frac{dm}{dt}$, represents the reactive force $\bar{P}$ generated by the change (in this case, decrease) of the body's mass. As a result of the mass decrease, this force $\bar{P}$ is vectored together with the displacement of the body and serves to increase its acceleration. Since the variable-mass body M moves on the cylindrical surface, the said force $\bar{P}$ is also directed at a tangent to that surface, which is shown in Fig. 2.

Thus, the expression (11) is the differential equation of motion of the variable-mass body – particle M (soil clod), on the surface of the spiral of the cleaning unit in the vector form.

The next step is to write the differential equation (11) in its projections on the axes of the Cartesian coordinate system $xOyz$.

Prior to doing that, it is to be noted that, since the projections of the vector of velocity $\bar{V}$ on the coordinate axes Ox, Oy and Oz are equal to $\dot{x}$, $\dot{y}$ and $\dot{z}$ respectively, the projections of the vector of force $\bar{V}\frac{dm}{dt}$ on the mentioned coordinate axes are equal to $\dot{x}\frac{dm}{dt}$, $\dot{y}\frac{dm}{dt}$ and $\dot{z}\frac{dm}{dt}$ respectively. Taking into account also the earlier statement about the direction of the friction force vector $\bar{F}$, it can be concluded that the projection of the said vector on the plane of cross-section of the spiral is equal to $\bar{F}\cos\gamma$, while on the axis *Oz* it is equal to $-F\sin\gamma$.

In view of the last remark as well as the obtained schematic model of forces (Fig. 2), the vector equation (11) in the projections on the assumed coordinate axes is resolved into the following system of differential equations:

$$\left.\begin{aligned}
&m\ddot{x} = -N\cos(\alpha_0+\alpha) + F\cos\gamma\cdot\sin(\alpha_0+\alpha) - \\
&-T\cos(\alpha_0+\alpha) - \dot{x}\frac{dm}{dt}, \\
&m\ddot{y} = N\sin(\alpha_0+\alpha) + F\cos\gamma\cdot\cos(\alpha_0+\alpha) + \\
&+T\sin(\alpha_0+\alpha) - \dot{y}\frac{dm}{dt} - mg, \\
&m\ddot{z} = -F\sin\gamma - \dot{z}\frac{dm}{dt}.
\end{aligned}\right\} \qquad (12)$$

Taking into account the fact that in the general case the variable-mass body (soil clod) can move (slip) relative to the cylindrical surface of the spiral, the value of angle α is equal to:

$$\alpha = (\omega t + \varphi), \qquad (13)$$

where φ – angle, through which the variable-mass body can move in the cross-section of the spiral relative to its surface.

It is obvious that the value of the angle φ depends on many factors, especially taking into account the oscillatory motion of the spiral due to the existence of eccentricity resulting from the deflection of the longitudinal axis of the spiral. The said angle φ can change both with regard to its value and its sign as a consequence of the change of the normal reaction *N*, and, accordingly, the force of friction $F = f \cdot N$, also under the action of the weight force *G*. There are grounds for suggesting that the angle φ in the most general case is a random variable.

Taking into consideration the expressions (1), (2) and (6) as well as the above statements about the angle α, which has a value of $\alpha = (\omega t + \varphi)$, the system of differential equations (12) can be transformed into the following representation:

$$\left.\begin{aligned}
&m\ddot{x} = -N\cos(\alpha_0+\omega t+\varphi) + fN\cos\gamma\cdot\sin(\alpha_0+\omega t+\varphi) - \\
&-m\cdot\omega^2\cdot A\cdot\sin(\alpha_0+\omega t)\cdot\cos(\alpha_0+\omega t+\varphi) - \dot{x}\frac{dm}{dt}, \\
&m\ddot{y} = N\sin(\alpha_0+\omega t+\varphi) + fN\cos\gamma\cdot\cos(\alpha_0+\omega t+\varphi) + \\
&+m\cdot\omega^2\cdot A\cdot\sin(\alpha_0+\alpha)\cdot\sin(\alpha_0+\omega t+\varphi) - \dot{y}\frac{dm}{dt} - mg, \\
&m\ddot{z} = -fN\sin\gamma - \dot{z}\frac{dm}{dt}.
\end{aligned}\right\} \qquad (14)$$

Thus, the system of differential equations (14) of the motion of the variable-mass body *M* (soil clod) in the most general view has been obtained. The determination of the angle φ for this system involves considerable difficulties for the reasons indicated earlier.

It ought to be noted that the obtained system of differential equations (14) has been generated for describing the motion of a single (isolated) soil clod.

However, considering the fact that the discussed soil clod is inside the heap stream, i.e. surrounded by other elements of the heap, the mass (and, accordingly, the inertia) of which in total considerably exceeds the mass of the single soil clod under consideration, also, the total force of friction of which again considerably exceeds the force of friction of the said soil clod, it can be assumed in a first approximation that this soil clod slips in the cross-section of the spiral insignificantly and, therefore, it can be assumed within the practically sufficient accuracy that $\varphi \approx 0$. That results in the assumption that the angle α is equal to $\alpha = \omega t$, which to some extent simplifies the system of differential equations (14). Hence, after assuming the conditions stipulating that $\varphi = 0$, the following system of differential equations of motion is obtained:

$$\left.\begin{aligned}
&m\ddot{x} = -N\cos(\alpha_0 + \omega t) + f N \cos\gamma \cdot \sin(\alpha_0 + \omega t) - \\
&-m\cdot\omega^2\cdot A\cdot\sin(\alpha_0 + \omega t)\cdot\cos(\alpha_0 + \omega t) - \dot{x}\frac{dm}{dt}, \\
&m\ddot{y} = N\sin(\alpha_0 + \omega t) + f N\cos\gamma\cdot\cos(\alpha_0 + \omega t) + \\
&+m\cdot\omega^2 A\sin^2(\alpha_0 + \omega t) - \dot{y}\frac{dm}{dt} - mg, \\
&m\ddot{z} = -f N \sin\gamma - \dot{z}\frac{dm}{dt}.
\end{aligned}\right\} \tag{15}$$

Since the rate of the soil clod mass reduction with the passage of time, i.e. the value $\frac{dm}{dt}$ is of prime importance at this stage, it has to be determined with the use of the system of differential equations (15).

For that purpose, the following is to be done. First, from the first two equations of the system (15) the expressions for the derivative $\frac{dm}{dt}$ are found and, after equating them to each other and then making a number of simple transformations, the expression for determining the normal reaction *N* is obtained in the following form:

$$N = \frac{B}{C}, \tag{16}$$

where

$$\begin{aligned}
B = m\Bigg[&\frac{\ddot{x}}{\dot{x}} - \frac{\ddot{y}}{\dot{y}} + \frac{\omega^2 A}{2\dot{x}}\sin 2(\alpha_0 + \omega t) + \\
&+\frac{\omega^2\cdot A}{\dot{y}}\sin^2(\alpha_0 + \omega t) - \frac{g}{\dot{y}}\Bigg],
\end{aligned} \tag{17}$$

and

$$C = \frac{-\cos(\alpha_0 + \omega t) + f\cos\gamma \cdot \sin(\alpha_0 + \omega t)}{\dot{x}} - \\ - \frac{\sin(\alpha_0 + \omega t) + f\cos\gamma \cdot \cos(\alpha_0 + \omega t)}{\dot{y}}. \tag{18}$$

The value of $\frac{dm}{dt}$ is also to be found from the third equation of the system (15). It is equal to:

$$\frac{dm}{dt} = -\frac{m\ddot{z} + fN \cdot \sin\gamma}{\dot{z}}. \tag{19}$$

After that, the value of the normal reaction N in accordance with the earlier obtained expression (16) can be substituted into the formula (19). The following is obtained:

$$\frac{dm}{dt} = -\frac{m\ddot{z} + f \cdot \frac{B}{C}\sin\gamma}{\dot{z}}, \tag{20}$$

where B and C are determined with the use of the expressions (17) and (18) respectively.

Considering the earlier made assumption about the sufficiently small displacement (slipping) of the soil clod M in the cross-section of the spiral relative to its surface, it can be assumed within the practically acceptable accuracy that the movement of the body on the surface of the spiral is described in this way. In view of the fact that the displacement of the soil clod M is effected under the action of the turn of the spiral, the equation of which is a helical line equation in the parametric form, it can be represented, according to (Ye et al., 2017), as follows:

$$\left.\begin{aligned} x &= -R \cdot \cos(\alpha_0 + \omega t), \\ y &= R \cdot \sin(\alpha_0 + \omega t), \\ z &= \frac{S}{2\pi} \cdot (\alpha_0 + \omega t), \end{aligned}\right\} \tag{21}$$

Hence, it is possible to determine the projections of the velocity and acceleration of the soil clod M onto the axes x, y and z of the Cartesian coordinate system.

At the beginning, the projections of the velocity of the said body M on the respective coordinate axes in the assumed coordinate system $xOyz$ are to be determined. For that purpose, the equations (21) are differentiated with respect to the time t. The result is as follows:

$$\left.\begin{aligned} \dot{x} &= \omega R \cdot \sin(\alpha_0 + \omega t), \\ \dot{y} &= \omega R \cdot \cos(\alpha_0 + \omega t), \\ \dot{z} &= \frac{S}{2\pi} \cdot \omega. \end{aligned}\right\} \tag{22}$$

Further, after differentiating the components of the expression (22) with respect to the time t, it becomes possible to obtain the values of the projections of the spiral turn acceleration on the respective coordinate axes:

$$\left.\begin{aligned} \ddot{x} &= R\omega^2 \cdot \cos\left(\alpha_0 + \omega t\right), \\ \ddot{y} &= -R\omega^2 \cdot \sin\left(\alpha_0 + \omega t\right), \\ \ddot{z} &= 0. \end{aligned}\right\} \tag{23}$$

In view of the assumption that the body *M* moves on the working surface without slipping, which assumption, as indicated earlier, can be made within the practically sufficient accuracy, there are good reasons to conclude that the acceleration of the body *M* is equal to the acceleration of that turn of the spiral, on which the body resides at the current instant of time.

Substituting the obtained values (22) and (23) into the expressions (17) and (18) respectively, the following is arrived at:

$$\begin{aligned} B = m\Bigg[& \frac{\omega}{\tan\left(\alpha_0 + \omega t\right)} + \omega \cdot \tan\left(\alpha_0 + \omega t\right) + \\ & + \frac{\omega \cdot A \cdot \cos\left(\alpha_0 + \omega t\right)}{R} + \frac{\omega \cdot A \cdot \sin^2\left(\alpha_0 + \omega t\right)}{R \cdot \cos\left(\alpha_0 + \omega t\right)} - \\ & - \frac{g}{\omega \cdot R \cdot \cos\left(\alpha_0 + \omega t\right)} \Bigg], \end{aligned} \tag{24}$$

and

$$\begin{aligned} C = & \frac{-\cos\left(\alpha_0 + \omega t\right) + f\cos\gamma \cdot \sin\left(\alpha_0 + \omega t\right)}{\omega \cdot R \cdot \sin\left(\alpha_0 + \omega t\right)} - \\ & - \frac{\sin\left(\alpha_0 + \omega t\right) + f\cos\gamma \cdot \cos\left(\alpha_0 + \omega t\right)}{\omega \cdot R \cdot \cos\left(\alpha_0 + \omega t\right)}. \end{aligned} \tag{25}$$

Further, substituting the values of $\dot{z}$ and $\ddot{z}$ found in (22) and (23) into the equation (20), the following is finally obtained:

$$\frac{dm}{dt} = -\frac{2\pi f \cdot \frac{B}{C} \cdot \sin\gamma}{S \cdot \omega} \tag{26}$$

where the values of *B* and *C* are determined in accordance with the expressions (24) and (25) respectively.

Thus, the differential equation of the variation (in this case decreasing) of the mass of the soil clod *M* with the time *t* under the action of the forces shown in the equivalent schematic model (Fig. 2) taking into account the design and kinematic parameters of the spiral potato heap cleaning unit has been obtained.

Solving and analysing the solution of the obtained differential equation enables finding the rational design and kinematic parameters of the cleaning unit that ensure the efficient reduction of the mass of the soil clods arriving together with the potato heap lifted from the soil and fed onto the top cleaning surface of the spiral cleaning unit.

The differential equation (26) is nonlinear. It can be solved only with the use of numerical methods and the assistance of a PC.

In order to perform the numerical computation, the first step is to write down the initial conditions required for solving the problem.

At $t = 0$:

$m = m_0$;

$\alpha_0 = \dfrac{\pi}{4}$;

$x = x_0 = -R\cos\alpha_0$;

$y = y_0 = R\sin\alpha_0$;

$z = z_0 = \dfrac{S}{2\pi}\alpha_0$;

$\dot{x} = \dot{x}_0 = \omega R \cdot \sin\alpha_0$;

$\dot{y} = \dot{y}_0 = \omega R \cdot \cos\alpha_0$;

$\dot{z} = \dot{z}_0 = \dfrac{S}{2\pi} \cdot \omega \cdot$

For the PC-assisted numerical computation, the design and kinematic parameters of the spiral potato heap cleaning unit of our design that has been tested in production conditions and subjected to laboratory and field laboratory experimental investigations have been used. The main parameters of the potato heap cleaning unit input in the PC-assisted numerical computation have been as follows:

$m_0 = 0.2$ kg; $R = 0.15$ m; $S = 0.035$ m; $\gamma = 20°$; $\omega = 10, 20, 30, 40, 50$ rad·s^{-1}; $f = 0.5$; $A = 0.005$ m.

On the basis of the results of solving the differential equation (26) on a PC with the use of the specially developed programme in the Mat Lab environment, the diagram of the function $m = m(t)$ presented in Fig. 3 has been plotted.

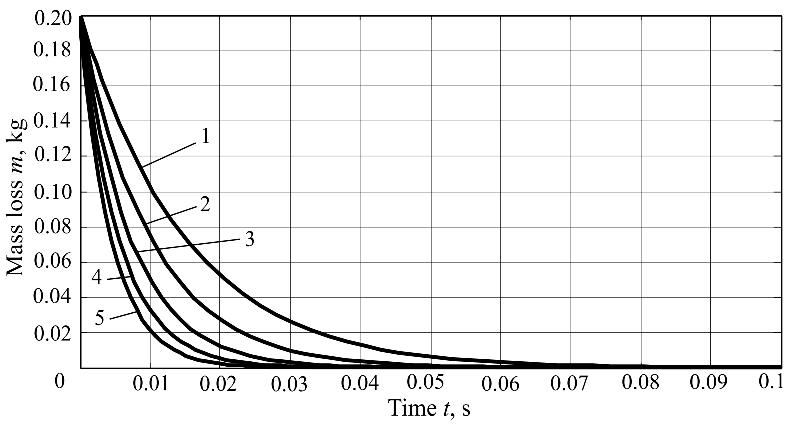

Figure 3. Reduction of soil clod mass $m = m(t)$ as function of time t at different values of spiral roll's angular velocity ω: 1 – $\omega = 10$ rad·s^{-1}; 2 – $\omega = 20$ rad·s^{-1}; 3 – $\omega = 30$ rad·s^{-1}; 4 – $\omega = 40$ rad·s^{-1}; 5 – $\omega = 50$ rad·s^{-1}.

As is seen from the graphs shown in Fig. 3, when the angular velocity ω of rotation of the spiral roll increases, the mass m_0 of the soil clod fed to the top working surface is reduced at a higher rate. That is, at $\omega = 10$ rad·s^{-1} the mass of the soil clod decreases from its initial value of $m_0 = 0.2$ kg to zero in 0.07 s, at $\omega = 20$ rad·s^{-1} the soil clod mass falls from a value of m_0 = 0.2 kg to zero in 0.05 s, at ω = 30 rad·s^{-1} – in 0.035 s, at ω = 40 rad·s^{-1} – in 0.030 s, at ω = 50 rad·s^{-1} – in 0.025 s. Therefore, it is obvious that the separating capacity (rate of separation) of the spiral cleaning unit increases together with the increase of the angular velocity ω of rotation of the spiral roll.

At the same time, the value $\Delta m(t) = m_0 - m(t)$ represents the mass of the soil sieved through the surface of the spiral separator at an arbitrary instant of time t.

In Fig. 4 and Fig. 5, the diagrams that represent the three-dimensional relations between the time t of the separation of a soil clod with a mass of m_0 and the radius of the spiral R and the amplitude of the oscillations of the spiral A, as well as the radius of the spiral R and the helix angle of the spiral γ respectively are shown.

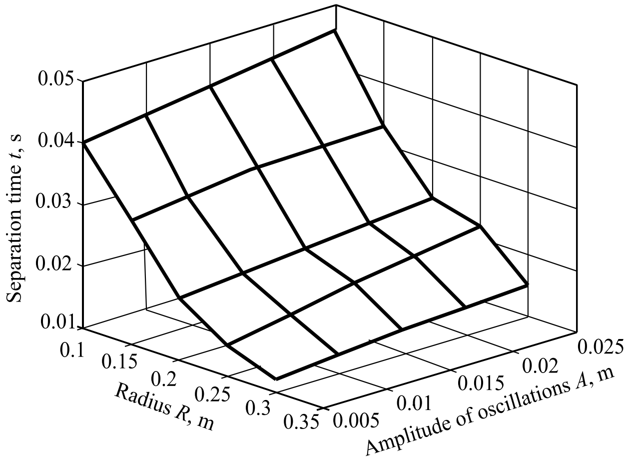

Figure 4. Relation between time t of separation of soil clod with mass m_0 and radius R of spiral and amplitude A of oscillations of spiral.

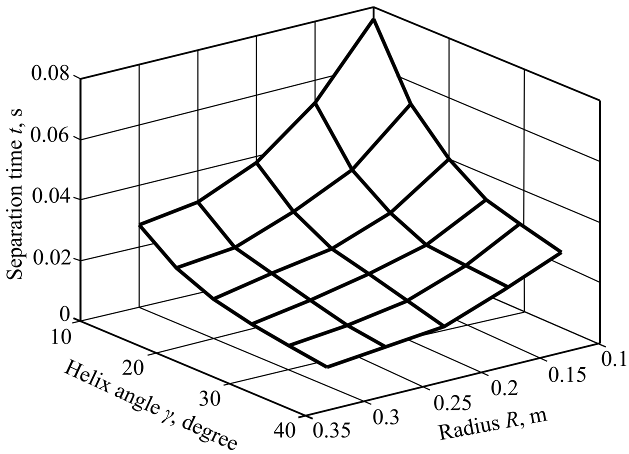

Figure 5. Relation between time t of separation of soil clod with mass m_0 and radius of spiral R and helix angle of spiral γ.

It can be concluded from the graph shown in Fig. 4 that with the increase of the spiral roll's radius R from 0.10 m to 0.30 m the time of the complete disintegration of the soil clod and, accordingly, its final sieving out is reduced from 0.04 s to 0.01 s, which gives evidence of the substantial effect that the radius R of the spiral has on the disintegration of the soil arriving to the working surface of the cleaning unit. At the same time, the increase of the amplitude of the oscillatory motion of the spiral from 0.005 m to 0.025 m has only an insignificant effect on the process of breaking down the soil clod and, accordingly, on the separation rate.

As is seen from the graph shown in Fig. 5, with the increase of the helix angle γ of the helical line of the spiral from 10° to 30° the time t of the separation of the soil clod under consideration at a spiral radius of R = 0.28 m decreases from 0.028 s to 0.005 s, at R = 0.10 m – from 0.075 s to 0.026 s. Hence, a greater value of helix angle γ in the spiral of the spiral roll contributes to the improvement of the separating capacity of the spiral cleaning unit.

In Fig. 6 – Fig. 8, the diagrams of the relations between the residual mass m on the surface of the cleaning roll and the radius R of the spiral, its helix angle γ and the amplitude A of the oscillations of the spiral in the process of the unit's operation are presented.

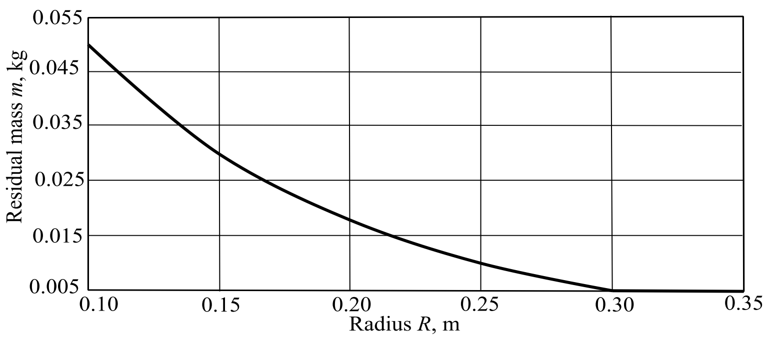

Figure 6. Relation between residual mass $m = m(R)$ and radius R of spiral.

As is evident from the graph plotted in Fig. 6, the increase of the radius R of the spiral to 0.30 m ensures the virtual absence of the residual mass m in the soil clod residing on the surface of the spiral cleaning unit.

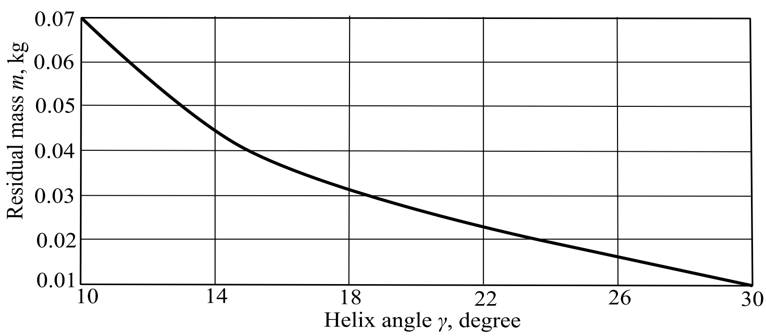

Figure 7. Relation between residual mass $m = m(\gamma)$ and helix angle γ of spiral.

The diagram shown in Fig. 7 indicates that the increase of the spiral's helix angle γ similarly provides for the virtual absence of the residual mass m of the soil clod on the surface of the cleaning roll.

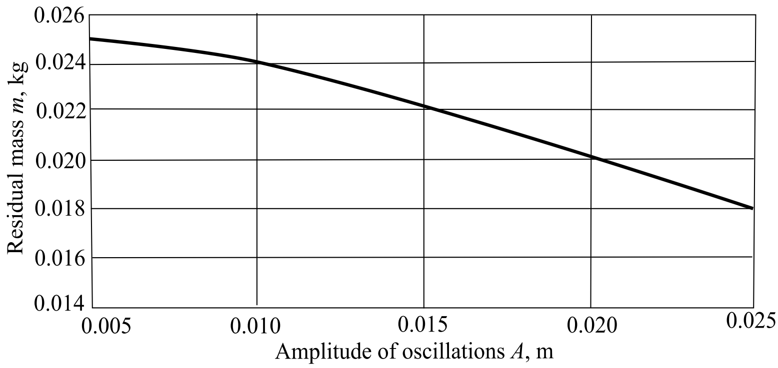

Figure 8. Relation between residual mass $m = m(A)$ and amplitude A of oscillations of spiral.

However, the variation of the amplitude A of the oscillations of the spiral does not, as is obvious from the graph presented in Fig. 8, produce any substantial effect on the reduction of the residual mass m of the soil clod fed onto the surface of the spiral roll in the spiral cleaning unit.

CONCLUSIONS

1. A mathematical model of the process of sieving the elements of the potato heap lifted from the soil in the spiral cleaning unit has been generated.

2. The differential equation of the process of gradually reducing the mass of the soil clod fed to the working surface of the spiral cleaning unit considering it as a function of the time of the soil clod's movement on the spiral of the cleaning roll has been generated taking into account the design and kinematic parameters of the cleaning unit.

3. The PC-assisted calculations carried out in the Mat Lab environment have shown that the increase of the angular velocity of rotation of the spiral roll results in the considerable decrease of the time, in which the process of reducing the mass of the investigated soil clod is completed. That is, when the angular velocity changes from 10 to 50 rad·s^{-1}, the time of complete sieving out decreases from 0.07 to 0.025 s.

4. The increase of the spiral's radius R also leads to the significant reduction of the time t spent for the complete sieving out of a single soil clod. Thus, when the spiral's radius R increases from 0.10 m to 0.30 m, the time of complete sieving out becomes reduced from 0.04 s to 0.01 s.

5. The increase of the cleaning spiral's helix angle γ has a substantial effect on the process of the complete sieving out of soil clods. When the said helix angle increases from 10° to 30°, the time spent for the complete sieving out of the soil at R = 0.10 m is reduced from 0.75 s to 0.026 s, at R = 0.28 m – from 0.028 s to 0.005 s.

6. It has also been established that the change of the spiral's radius R and its helix angle γ has a substantial effect on the residual mass of the soil clod. The amplitude A of

the oscillatory motion of the spiral has an effect of no significance on the residual mass of the soil clod.

7. Therefore, the rational design and kinematic parameters of the spiral cleaning unit that provide for the high-rate separation of the potato heap have been theoretically substantiated.

REFERENCES

Bishop, C., Rees, D., Cheema, M.U.A., Harper, G. & Stroud, G. 2012. *Potatoes*. Crop Post-Harvest: Science and Technology: Perishables. Book Chapter, pp. 179–189.

Bulgakov, V., Ivanovs, S., Adamchuk, V. & Ihnatiev, Y. 2017. Investigation of the parameters of the experimental spiral potato heap separator on the quality of work. *Agronomy Research* **15**(1), 44–54.

Bulgakov, V., Nikolaenko, S., Adamchuk, V., Ruzhylo, Z. & Olt, J. 2018a. Theory of retaining potato bodies during operation of spiral separator. *Agronomy Research* **16**(1), 41–51, DOI: 10.15159/AR.18.036.

Bulgakov, V., Nikolaenko, S., Adamchuk, V., Ruzhylo, Z. & Olt, J. 2018b. Theory of impact interaction between potato bodies and rebounding conveyor. *Agronomy Research* **16**(1), 52–64, DOI: 10.15159/AR.18.037.

Bulgakov, V., Nikolaenko, S., Adamchuk, V., Ruzhylo, Z. & Olt, J. 2018c. Mathematical model of cleaning potatoes on surface of spiral separator. *Agronomy Research* **16**(4), 1590–1606, DOI: 10.15159/AR.18.173.

Feller, R., Margolin, E., Hetzroni, A. & Galili, N. 1987. Impingement angle and product interference effects on clod separation. *Transactions of the American Society of Agricultural Engineers* **30**(2), 357–360.

Guo, W. & Campanella, O.H. 2017. A relaxation model based on the application of fractional calculus for describing the viscoelastic behavior of potato tubers. *Transactions of the ASABE* **60**(1), 259–264.

Holland-Batt, A.B.1989. Spiral separation: theory and simulation. *Transactions of the Institution of Mining and Metallurgy*, Section C: Mineral Processing and Extractive Metallurgy, **98**, 46–60.

Ichiki, H., Nguyen Van, N. & Yoshinaga, K. 2013. Stone-clod separation and its application to potato in Hokkaido. *Engineering in AgricultureEnvironment and Food* **6**(2), 77–85.

Krause, F. & Minkin, A. 2005. Research on shaftless screw conveyors. *Bulk Solids Handling* **25**(2), 92–100.

Misener, G.C. & McLeod, C.D. 1989. Resource efficient approach to potato-stone-clod separation. *AMA, Agricultural Mechanization in Asia, Africa and Latin America* **20**(2), 33–36.

Peters, R. 1997. Damage of potato tubers: A Review. *Potato Research* **39**(Spec. Issue), 479–484.

Petrov, G. 2004. *Potato harvesting machines*. Mashinostroenije, Moskow, 320 pp (in Russian).

Vasilenko, P.M. 1996. *Introduction to agricultural mechanics*. Kiev, Agricultural Education, 252 pp.

Wang, X., Sun, J., Xu, Y., Li, X. & Cheng, P. 2017. Design and experiment of potato cleaning and sorting machine. *Nongye Jixie Xuebao/Transactions of the Chinese Society for Agricultural Machinery* **48**(10), 316–322 and 279.

Ye, G., Ma, L., Huang, G., Yu, Y., Sun, X. & Wu, L. 2017. Dynamic and optimization analysis of spiral separator. Meitan Xuebao/*Journal of the China Coal Society* **42**, 479–485, DOI: 10.13225/j.cnki.jccs.2017.0271.

Agronomy Research **17**(1), 49–59, 2019
https://doi.org/10.15159/AR.19.001

Assessment of a low-cost solar water heating systems in farrowing facilities

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Abstract. The objective of this study was to develop a prototype solar heater using alternative materials and then to compare its thermal efficiency against that of two other commercial solar heating systems when heating the floor of piglet housing. To evaluate the thermal heaters, temperature sensors were installed in the inlet and outlet of each floor and the thermal reservoir. The results showed good performance, however the thermal efficiency of the alternative heater was lower than the conventional systems. However, due to the construction of this solar collector with alternative materials its cost was relatively low and its operation is easier than the other conventional heater, therefore this heater is a good alternative to use for small livestock producer.

Key words: swine, solar energy, reusable materials, prototype.

INTRODUCTION

Pig farming is an activity that requires a lot of dedication from the breeder to achieve good productivity levels and, consequently, satisfactory economic results. External environmental factors and the microclimate inside the pig houses have direct and indirect effects on pig production, with temperatures outside the zone of thermal comfort leading to reduced productivity, with consequent economic damage to the operation (Pandorfi et al., 2015).

The productive and reproductive performance of pigs depends on the management system employed, which involves the system chosen for breeding, nutrition, health and facilities. These facilities, which require the larger part of initial fixed investment, are built on the basis of cost and management efficiency, with the comfort of the animal sometimes being neglected (Tolon & Nääs, 2005).

In the case of the maternity stage, this problem is heightened by the co-existence within it of two categories with very different environmental requirements. On the one hand there is the sow which must be cooled, and on the other hand, piglets, which must be heated. The range of temperatures for thermal comfort of the piglets during the first days of life is between 32 and 34 °C, whereas for the mother the within the band of 16 to 21 °C (Renaudeauet al., 2003; Johnson et al., 2009). Thus, to uphold good animal welfare by maintaining both the piglets and sows in thermal comfort presents the producer with a major problem. He is required, in a small physical space, to provide two different microenvironments or otherwise the performance of both the pigs and the piglets will be compromised (Pandorfi et al., 2005, Morello et al., 2018).

In general, the supplemental heating of piglets in maternity and nursery phase requires significant energy on the farm. Thus, there is a need for further research to minimize consumption without harming animal welfare or damaging the environment.

Alternative solar heating systems have started to be implemented in agricultural, greenhouse and animal facilities over the last few years, with the objective to save energy consumption Aiming at the possibility of replacing conventional systems with alternative systems, some prototypes of low-cost systems solar heating have been developed and studied. Studies show that rather than conductors, non-conductive alternative materials may well be used in solar heater operation (Kudish et al., 2002).

Several researchers have been working in the search for methodologies used in heating farrowing shed floors for piglets, seeking greater energy efficiency, welfare and productive performance of these animals (Kudish et al., 2002; Silva et al., 2005; Sarubbi et al., 2010; Fernandes et al., 2011; Seok Mun et al., 2015; Tamvakidis et al., 2015). This work will consider solar-energy as a water heating alternative, as this could have great applicability in agriculture and could be used to heat farrowing areas in the maternity on a pig farm.

The objective of this study was to evaluate the thermal efficiency and economic characteristics of an alternative solar heater prototype by comparing it with two other commercial solar heaters in order to determinate the feasibility of use of this kind the heaters for small livestock producers. The application of the systems is in the heat of thermal floors usually present inside farrowing shelters for piglets.

MATERIALS AND METHODS

Construction and installation of solar collectors

The entire study was conducted at the Federal University of Lavras, in the gantry of the Department of Engineering, latitude 21° 14” S longitude 45° 00” W and altitude 920 m, with climate, according to the Köppen classification, classified as Cwa (humid temperate with dry winter and rainy summer).

For this, we built a solar water heater prototype using alternative materials (ASWH) and compared it with a conventional solar water heater (CSWH).

The conventional solar water heater 1 (CSWH1) had a solar collector plate of PVC, painted matt black, and a thermal reservoir fabricated from high density polyethylene and coated with thermal interface material.

The conventional solar water heater 2 (CSWH2) had a solar collector of glass plate, made of extruded aluminum, with internal fins painted in matte black to absorb radiation and transfer it to internal piping. The thermal reservoir had components of internal cylinder, pipes manufactured with stainless steel, and rigid expanded polyurethane. More details can be seen in Fig. 1.

The prototype of solar water heaters manufactured with alternative materials (ASWH) was built with PVC pipes and connections (1/2” diameter), PET bottles and milk cartons (Tetra Pak®). In this prototype, the PET bottles were intended to protect the interior of the collector from external interference, such as winds and changes in air temperature. 60 bottles of polyethylene terephthalate (PET) transparent 2 liters were used. For this the cap and bottom of each bottle were removed. Tetra Pak® boxes were opened at the top and bottom, leaving them flattened. To maintain the standard in all the boxes, a cutting jig was used. The thermal properties of these material are show in the Table 1 (Scheirs et al., 2003).

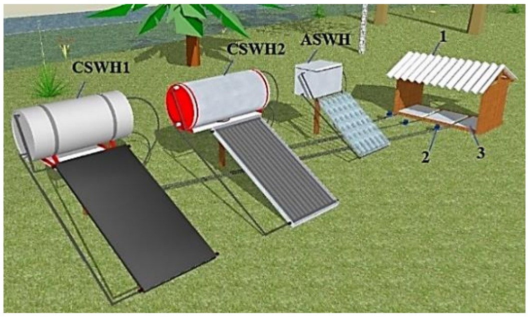

Figure 1. Assembly diagram of conventional solar heaters (CSWH1 and CSWH2) and alternative (ASWH). Legend: 1 – Protective Shelter; 2 – Water pump; and 3 – Heated cement floor.

Table 1. Average efficiency of thermal reservoirs tested in this study

Thermal Properties	PET bottles	Milk cartons
Specific heat J K^{-1} kg^{-1}	1,200–1,350	1,340–1,400
Thermal conductivity W m^{-1} K^{-1}	0.15–0.40	0.078–0.760

They were then folded in order to take advantage of the side creases of the package and two cuts were made on top, diagonally, to make it possible to fit the internal curvature of the PET bottle, also giving support to the box, and keeping it straight and in contact with the PVC pipe. Tetra Pak® boxes were painted matte black to absorb heat which is retained within the bottles to then be transferred to the water through the PVC pipes which were also painted matte black. In the ASWH assembly process, we used 10 columns with PVC pipes for hot water with 6 bottles in each column, the last bottle, cut just below the upper nozzle (Fig. 2, a).

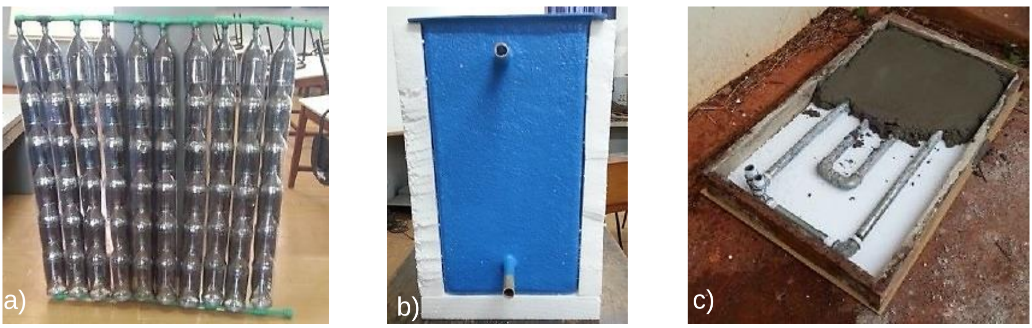

Figure 2. (a) Alternative solar heater prototype mounted and (b) the process of building alternative hot water tank, and (c) cement floors.

In an alternative construction of the hot water reservoir, a 50 liter fiberglass water tank was used, covered with polystyrene plates (3.0 cm) Silver Tape and a self-adhesive asphalt and aluminum blanket (0.25 cm) to protect the Polystyrene boards from the weather (Fig. 2, b). Four 20 mm holes were made in the reservoir, two holes being for circulation of water from the thermal reservoir to the solar collector and the other two holes for water circulation from the heat reservoir to the floor.

To test the three water heating systems three concrete floors (1:3 mix) have been built in the dimensions of 74.0 cm long, 46.0 cm wide by 7.0 cm high (Fig. 2, c). The floors were based on the same design characteristics used on floors present in a pig maternity. To reduce the heat dissipation at the base of the floors, we used 30 mm polystyrene boards. A 20 mm galvanized steel pipe was placed on each floor, forming a coil, to evenly distribute heat from the water inside the floor. The floors were left in places with shade and covered with plastic sheeting, and were daily moistened to prevent cracks.

Instrumentation and data collection

To test and evaluate the heating efficiency of the two water heating systems four thermocouple sensors (K type, precision ± 1.0 °C) with digital display were used in each system, being allocated to the entrance and exit of the heat tank and to the entrance and exit of the floor. The floors were placed inside a wooden shed with fibro-cement tile cover to prevent direct incidence of solar radiation on the floor surface. We used a small low-flow water pump (mod. ZC-T40, voltage 12 V and 1.05 A) in each system for recirculating hot water. To control the pump drive in each system, a digital controller was used - thermostat (Tholz® and Mod. 601 N) designed for solar heating applications, which operated to control water flow through the temperature differential between the entrance to the floor (Tep) and the thermal reservoir (Ter). So every time the gradient between Tep and Ter was higher than 5.0 °C, the water pump was activated.

Water temperature data (input - Ter and output - Tsr of the tank and input - Tep and output - Tsp of the floor, sensors 1, 2, 3 and 4, respectively), surface temperature of the floor and climatic data were collected for 10 non-consecutive days in July during the hottest time of day (9:00 to 16:00 h), the interval between collections being fifteen minutes (Fig. 3). We used an infrared laser digital thermometer (Instrutemp®, mod. ITTI 550 and precision ± 2.0 °C) for measuring the temperature of the floor surface, which were collected at nine equidistant points.

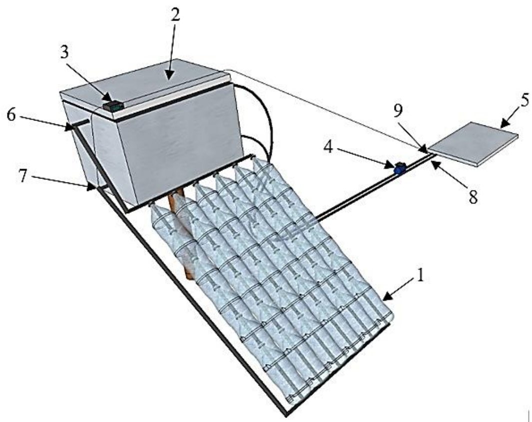

Figure 3. Scheme for installation of temperature sensors Legend: 1 – Solar Collector; 2 – Water reservoir (boiler); 3 – Controller (thermostat); 4 – Water pump; 5 – Floor; 6 – Sensor 1 (Ter); 7 – Sensor 2 (Tsr); 8 – Sensor 3 (Tep); 9 – Sensor 4 (Tsp).
Source: the authors.

Environmental climate data (air temperature, relative humidity, irradiance, wind speed, and wind direction) were collected at the meteorological station of the National Institute for Space Research (INPE) located about three hundred meters from the place of study.

Mathematical model for calculation of efficiency

With the water temperature data (input and output of the thermal reservoir and entrance and floor exit) surface temperature of the floor and climatic data from this study, we calculated the amount of heat (Q_u, kcal) required to heat a given volume of water using equation 1, as suggested by Siqueira et al. (2008) and Sprenger et al. (2007).

$$Q_u = m \cdot Cp \cdot \Delta t \qquad (1)$$

where m – volume of water to be heated (kg); C – Specific heat of water (kcal kg^{-1} °C); Δt – variation in temperature between water and environment (°C).

The heating efficiency (η) of each collector was calculated by Eq. 2 (Struckmann, 2008):

$$\eta = \frac{Q_u}{A \cdot I} \qquad (2)$$

where η – instantaneous thermal efficiency of the collector; Q_u – useful energy gain (kcal); I – intensity of solar radiation, incident on the aperture plane of the solar collector (kcal m^{-2}); A – area of the solar collector (m^2).

Experimental design and statistical analysis

The experiment was conducted following a randomized block design (RBD) with a factorial scheme of 3 x 8 (3 treatments and 8 blocks). Measurements were made for 10 days, and every day was considered as a block. Replicates were performed every 15 min for each treatment. The mean response variable inlet water temperature and output of the thermal reservoir and entrance and exit to floor, surface temperature of the floor and microenvironment climate data were compared by Tukey test ($P < 0.05$). All statistical analysis was performed using the statistical program Statistical Analysis System (SAS, 1992).

RESULTS AND DISCUSSION

During the trial period the average air temperature remained at 21.9 ± 3.7 °C, with an average relative humidity of 60.4 ± 5.0%, solar radiation of 391.2 ± 207.8 W m^{-2} and air velocity of 1.7 ± 0.8 m s^{-1}.

Fig. 4 shows, the inlet water temperature behavior in the hottest hours of the day of (T_{er}) and outlet (T_{sr}) of the thermal reservoir. It was found that CSWH1 and ASWH systems showed no significant differences in the average values of T_{er} ($p < 0.05$, Tukey). The results show that during the day the behavior of T_{er} in these two systems is similar throughout the study period, with small variations (Fig. 4, a). The largest values of T_{er} were observed in the CSWH2 system (40.05 ± 3.2 °C), where the average value of T_{er} remained above 40 °C most of the time, and the mean variation of T_{er} in the CSWH2 system from the others was 4.8 °C.

It was found that all systems showed significant differences in the mean values of T_{er} ($p < 0.05$, Tukey). In ASWH and CSWH1 systems, there was a growing trend in values of T_{sr} which remained a stable trend between 13:00 and 15:30 (Fig. 4, b). The highest average value of T_{sr} was observed in the CSWH2 system (43.6 ± 2.6 °C), followed by CSWH1 (35.6 ± 3.7 °C) and ASWH (33.4 ± 3.5 °C).

Bortoletto et al. (2012), evaluating a solar heating system with an alternative thermal reservoir and comparing with a conventional system, found mean temperature of incoming water in the reservoir 55.9 and 81.5 °C respectively.

Pereira et al. (2000), evaluating a solar collector constructed of alternative materials and compared with a conventional system found mean water temperature in the reservoir of alternative and conventional systems of 35.4 °C and 45.4 °C respectively.

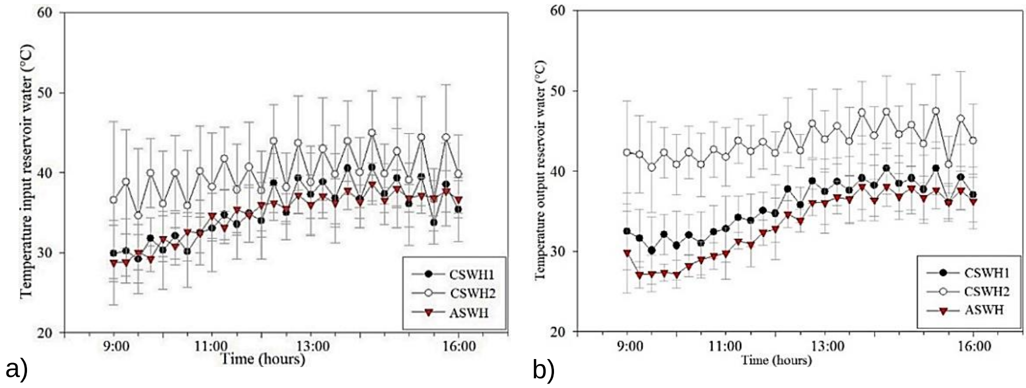

Figure 4. Mean and standard deviation of temperature (a) input (T_{er}) and (b) output (T_{sr}) reservoir water (in °C) during the collection period.

Fig. 5 shows the inlet water temperature (T_{ep}) and outlet (T_{sp}) from the floor. The mean of T_{ep} in the tested treatments showed significant differences ($p > 0.05$, Tukey). In CSWH2 system, it presented higher mean values of T_{ep}, reaching values greater than 45 °C after 13:00. The system presented CSWH1 T_{ep} value slightly larger than the ASWH system. The average value of T_{ep} CSWH2 the system was 43.5 ± 2.5 °C, followed by CSWH1 systems (35.1 ± 2.8 °C) and asthma (33.8 ± 2.5 °C).

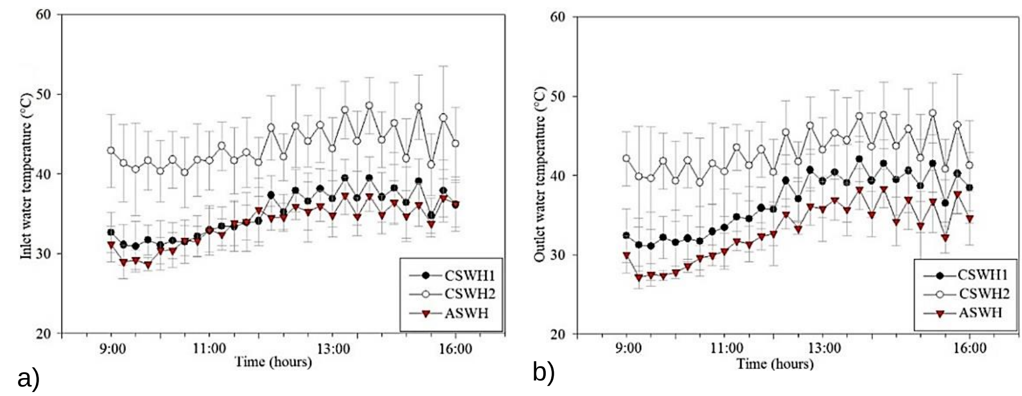

Figure 5. Mean and standard deviation of temperature (a) input (T_{ep}) and (b) output (T_{sp}) floor (°C) during the collection period.

There was a significant difference between the mean values of T_{sp} for the evaluated systems ($p > 0.05$, Tukey). The results show a trend similar to the behavior of T_{sr} of

CSWH1 and ASWH systems during the study period, although the ASWH system presented a lower mean (33.4 ± 4.5 °C) in view of the difference of the constructive characteristics of each system. This is due to the quality of materials with low thermal insulation used in the thermal reservoir. The mean T_{sp} of the CSWH2 system had different behavior when compared to other systems evaluated in this study (Fig. 5, b).

The fluctuation in the average values of T_{er} T_{sr} T_{ep} and T_{sp} over the study period was due to the variation in the activation of the water pump by the controller (thermostat) that was activated every time the difference T_{ep} and T_{er} was greater than 5.0 °C. This difference is probably related to heat loss in the pipes leading the water from the heat reservoir to the floor, and in this case better insulation on these tubes is recommended.

The results show that early in the day, T_{er} and T_{ep} of ASWH and CSWH1 systems were close to the average values of the air temperature. Probably the thermal insulation of these systems is not sufficient to minimize heat loss during the night.

The comparison between treatments shown in Fig. 6 is the mean result of floor surface temperature (T_s) of each rated heating system. According to the results, significant differences were found between the mean T_s in all heating systems, by Tukey test, considering a nominal value of 5% probability. The mean and the standard deviation of the floor surface temperature for CSWH1, CSWH2 and ASWH systems were 33.2 ± 2.8 °C, 36.5 ± 2.7 °C and 31.4 ± 2.9 °C respectively. In general, one can say that for the thermal comfort zone for piglets in the farrowing phase, the three treatments are recommended, but it should be remembered that these data are daily averages.

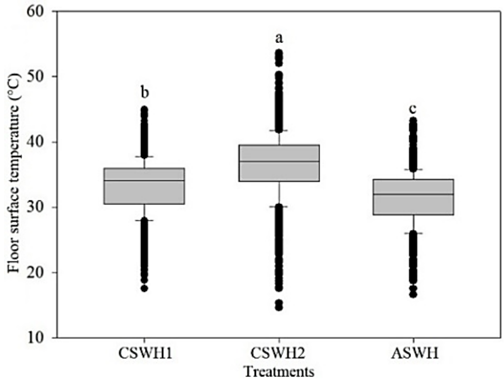

Figure 6. Analysis of variance between the temperature values of the floor surface in this study. Means followed by letter are not statistically different from each other by the Tukey test at 5% probability.

Fig. 7 illustrates the behavior of T_s in each rated heating system. Heat distributions were verified in different shades of colors on the surface of the floors in the evaluated systems. The regions at the top and bottom are regions with lower values of T_s, considered cooler, which correspond to the location in and out of the protective shelter. These regions may be cooler by air currents and by the fact that the heat does not heat those locations, due to the positioning of the water pipe being situated in the central part of the floor. The light tones correspond to the heat emitted by the pipe water in these locations. This may be an indication that, to increase the thermal efficiency of the shelter, one can recommend the use of two sources of heating.

Quiniou et al. (1999), evaluating different heating systems for pigs, states that heated farrowing sheds with underfloor heating by circulating water through a hybrid system (solar panels, biogas, LPG and electricity gas) had the highest average temperatures and the largest gain of weight of piglets, providing better thermal comfort for the piglets and higher revenues from the sale of piglets.

To keep sows and piglets in their thermal comfort zones in conventional maternities is challenging, even in thermally controlled environments due to the comfortable temperature of the sows being situated in the range of 22 °C (Sulzbach et al., 2016), while for the piglets it is within the range 29–34 °C (Lossec et al., 1998; Renaudeau, 2001; Sulzbach et al., 2016). While in sows, heat stress has adverse behavioral, and physiological productivity effects (Lossec et al., 1998; Quiniou, et al., 1999; Renaudeau, 2001; Penereiro et al., 2016; Sulzbach et al., 2016), in piglets a drop in body temperature of 2 °C to 4 °C during birth, if not provided with a source of enough heat, means a hypothermic condition may occur, reducing strength and milk intake, and eventually can mean death (Lossec et al., 1998).

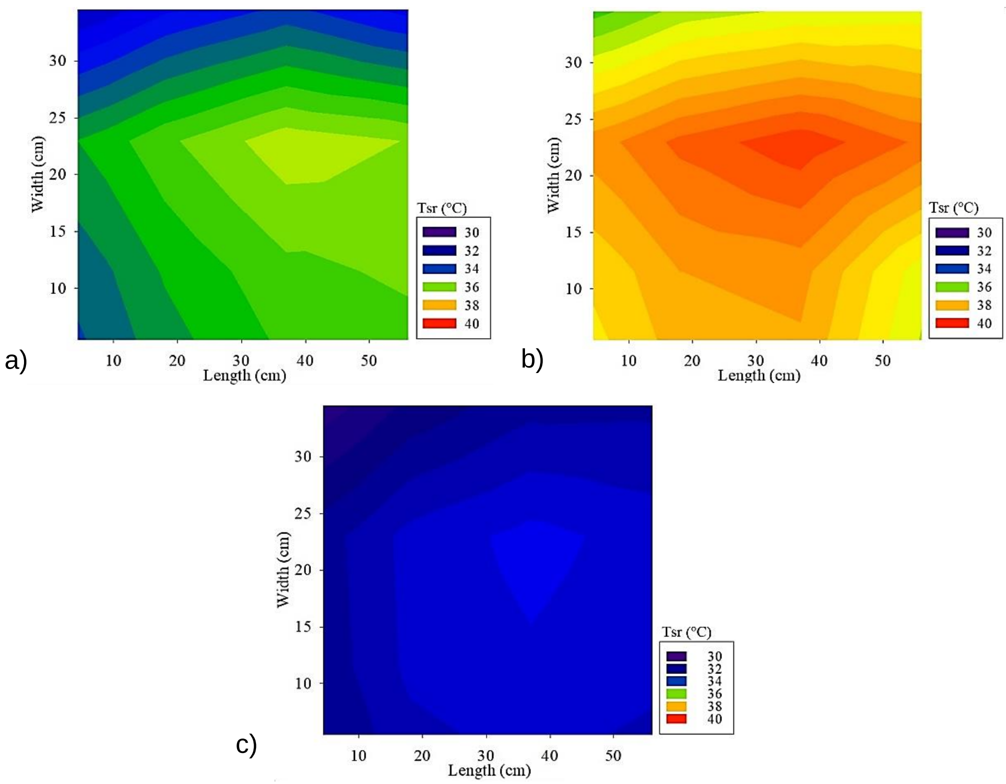

Figure 7. Mean surface temperature values (T_{sr}, °C) of the floors heated by different heating systems: a) CSWH1, b) CSWH2 and c) ASWH.

Fig. 8 shows the variation of the efficiency of the solar collectors in function during the evaluated period. It is noticed that as the solar radiation rises, the temperature gradient of inlet water temperature and outlet reservoir increases, increasing the efficiency of the collector. According to Incropera et al. (2011), this is due to the retention of heat inside these two surfaces, because the glass allows the passage of visible light waves and is opaque infrared radiation, so the radiation emitted by the absorber plate does not pass through the glass thereby increasing its temperature.

As can be observed in Fig. 8, it is possible to verify the influence caused by the wind in determining the thermal efficiency of each collector studied. As expected, the more intense winds significantly affect the ASWH and CSWH1 collector yields when compared to CSWH2. The use of the glass cover in the CSWH2 collector, which causes the accumulation of radiation emitted by the surface of the solar collector, maintaining for a longer time the thermal energy inside the pipes that exchange this energy with the water, avoiding the energy loss due to the action of the wind to the environment.

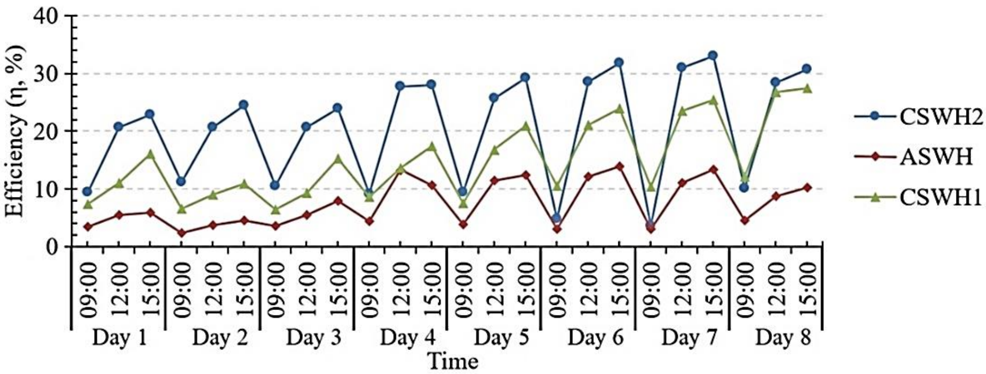

Figure 8. Efficiency of the thermal reservoirs tested during the time of study.

With the results obtained in each heating system in Table 2, where the inlet (T_{er}) and outlet (T_{sr}) water temperatures in the reservoir are explicitly listed, it is possible to find cumulative amount of heat (Q_{ac}), collector area (A_c), mean solar radiation (ΣI) and average efficiencies of each system (η) calculated by Eq. 2. According to Table 2, it can be seen that the variation between T_{er} and T_{sr} in the CSWH2 system is greater. The amount of heat (Q_{ac}) and collector area in CSWH1 and CSWH2 system are virtually equal. However, for the same amount of solar radiation (ΣI) incident in the experimental area, the CSWH2 system efficiency is higher, but when the efficiency is compared with others researchs like made by Seok Mun et al. (2015), who found The average was 64.8% and others systems as develomented by Tamvakidis et al. (2015), founded the alternative systems could have generated between 50–70% the energy that required the farrowing during the warm periods.

Table 2. Average efficiency of thermal reservoirs tested in this study

	System		
	CSWH1	CSWH2	ASWH
T_{er} (°C)	33.4	37.0	31.4
T_{sr}(°C)	36.7	43.7	33.0
Q_{ac} (Kw)	0.34	0.33	0.25
A_c (m²)	1.73	1.70	1.30
ΣI (kW m^{-2})	0.391	0.391	0.391
η(%)	10.9	22.5	7.0
Total value (R$)	1,618.31	2,452.58	891.36

Legend: inlet water temperature (T_{er}) and outlet (T_{sr}) the reservoir, amount of accumulated heat (Q_{ac}), Collector area (A_c), the sum of mean solar radiation (ΣI) and average efficiencies of each system (η).

Probably, this greatest value in heating efficiency is due not only to the type of material used in this system, it is also due to the protective glass on the solar collector that this system has and which provides a better absorption of solar radiation, where the heat of the sun, picked up by the collector of the CSWH2 system is transferred to water circulating within its copper pipes.

As can be seen from Table 2, the ASWH system did not show good efficiency, however, when comparing the total cost of the heating efficiency of each system, we can conclude that the ASWH system is an alternative for replacing the conventional system and decreasing final cost, and the maintenance can be performed with few tools and in situ. Another alternative for increasing the heating efficiency of this system is to increase the area of the solar collector.

According Penereiro et al. (2016), assessing the heating efficiency of a solar heater constructed of alternative materials (PVC) and comparing with a conventional system (metal) mentions that thermal efficiency in the conventional metal solar heater is higher (14.4%) than that inferred in the solar heater of PVC.

CONCLUSIONS

Due to the weather conditions recorded at this stage, the solar heating systems that were more suitable in function of thermal efficiency were the conventional solar water heater with solar glass plate collector (CSWH2), followed by conventional solar water heater with solar collector of PVC (CSWH1) had the best results, follow by the solar water heater manufactured with alternative materials (ASWH).

According to the technical and economic analysis, it was verified that the lowest efficiency cost for the period studied was for the CSWH1 (US$/% 14.99) system, followed by the CSWH2 system (US$/% 27.25) and ASWH system (US$/% 31.83), respectively.

The results showed that the alternative system has a thermal efficiency lower than the conventional, however the construction of solar collectors using alternative materials stands out for its strong social and environmental nature, due to the construction cost being relatively low and the operation easy and it can be constructed and used by livestock producers for heating purposes.

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REFERENCES

Bortoletto, D. & Pezzuto, C. 2012. Performance Analysis of a Low Cost Solar Heater: Alternative Thermal Reservoir. *XVII Encontro de Iniciação Científica*, Brasil, 1–6 (in Portuguese).

Fernandes, H., Moreira, F., Longui Rinaldi, P. & Siqueira, W. 2011. Effect of heating and cooling of floors in the performance of lactating mothers and piglets. *Revista Ceres* **58**, 701–709 (in Portuguese, English abstr.).

Incropera, F.P., Bergman, T.L., Lavine, A.S.; DeWitt, D.P. 2011. Fundamentals of Heat and Mass Transfer. 7th ed, Rio de Janeiro: LTC.

Johnson, A. & Marchant-Forde, J. 2009. Welfare of Pigs in the Farrowing Environment. Dordrecht: Springer Netherlands, pp. 141–188.

Kudish, A., Evseev, E., Walter, G. & Leukefeld, T. 2002. Simulation study of a solar collector with a selective caoted polymeric double walled absorber plate. *Energy Conversionand management* **43**, 651–671.

Lossec, G., Herpin, P. & Le Dividich, J. 1998. Thermoregulatory Responses of the Newborn Pig during Experimentally Induced Hypothermia and Rewarming. Experimental Physiology 83, 667–678.

Morello, G.M., Lay Jr., D.C., Rodrigues, L.H.A., Richert, B.T. & Marchant-Forde, J.N. 2018. Microenvironments in swine farrowing rooms: the thermal, lighting, and acoustic environments of sows and piglets. *Scientia Agricola* **75**(1), 1–11.

Pandorfi, H., Silva, I., Moura, D. & Sevegnani, K. 2015. Análise de imagemaplicada ao estudo do comportamento de leitões em abrigo escamoteador. *Engenharia Agrícola* **24**, 274–284 (in Portuguese, English abstr.)

Pandorfi, H., Silva, I., Moura, D. & Sevegnani, K. 2005. Microclima de abrigos escamoteadores para leitões submetidos a diferentes sistemas de aquecimento no período de inverno. *Revista Brasileira de Engenharia Agrícola e Ambiental* **9**, 99–106 (in Portuguese, English abstr.).

Penereiro, J. 2016. Measures of thermal efficiencies in solar heaters: a complementary alternative for the teaching of physics concepts for engineering students. *Revista Brasileira de Ensino de Ciência e Tecnologia* **9**, 99–122 (in Portuguese).

Pereira, R., Freitas, A., Torres, C. & Costa, N. 2000. Development of an alternative solar collector using recyclable materials. *Mundo & Vida* **2**, 57–60 (in Portuguese).

Quiniou, N. & Noblet, J. 1999. Influence of High Ambient Temperatures on Performance of Multiparous Lactating Sows. *Journal of Animal Science* **77**, 2124–2134.

Renaudeau, D., Noblet, J. & Dourmad, J. 2003. Effect of Ambient Temperature on Mammary Gland Metabolism in Lactating Sows. *Journal of Animal Science* **81**, 217–231.

Renaudeau, D. & Noblet, J. 2001. Effects of Exposure to High Ambient 440 Temperature and Dietary Protein Level on Sow Milk Production and Performance of Piglets. *Journal of Animal Science* **79**, 1540–1548.

SAS INSTITUTE. SAS/STAT software: changes and enhancements, release 6.07. Cary: Statistical Analysis System Institute. Chapter 16: The MIXED procedure, 1992.

Sarubbi, J., Rossi, L., Moura, D., Oliveira, R. & David, E. 2010. Electrical energy use in different heating systems for weaned piglets. *Engenharia na Agricultura* **30**, 1003–1011 (in Portuguese, English abstr.).

Scheirs, L & Long, T. 2003. *Modern Polyesters: Chemistry and Technology of Polyesters and Copolyesters*: Wiley & Sons, 733 pp.

Seok Mun, H., Tabasum, S., Manirul, Md., Keum-Joo, P. & Chul-Ju, Y. 2015. Retrofitting of a pig nursery with solar heating system to evaluate its ability to save energy and reduce environmental pollution. *Engineering in Agriculture, Environment and Food* **8**(4), 235–240.

Silva, I., Pandorfi, H.& Piedade, S. 2005. Use of animal precision production in the behavior evaluation of lactation piglets submitted to different heating systems. *Revista Brasileira de Zootecnia* **34**, 220–229 (in Portuguese, English abstr.).

Siqueira, D., Gonçalves, C., Besteti, Vieira, M. & Damasceno, J. 2008. Analysis and Performance the LCSH System. *12th Brazilian Congress of Thermal Engineering and Sciences,* Brasil, 14–24.

Sprenger, R. 2007. Application of closed system in low cost solar water heater for thermal insulated residential tanks: design and commissioning of a pilot testing system. Master Dissertation. Universidade Federal do Paraná, Brasil (in Portuguese, English abstr.).

Struckmann, F. 2008. Analysis of a Flat-plate Solar Collector. Project Report, Heat and Mass Transport, Dept. of Energy Sciences, Faculty of Engineering, Lund University, 2008.

Sulzbach, J. 2016. Conception and evaluation of different heating systems for newborns pigs. Master Dissertation. Universidade Tecnológica Federal do Paraná, Brasil (in Portuguese, English abstr.).

Tamvakidis, S., Firfirisa, V., Martzopoulou, A., Fragos, V. & Kotsopoulos T. 2015. Performance evaluation of a hybrid solar heating system for farrowinghouses. *Energy and Buildings* 97, 162–174.

Tolon, Y.B., Nääs, I.A. 2005. Evaluation of types of ventilation in swine maternity. *Eng. Agrícola* **25**(3), 565–574 (in Portuguese, English abstr.).

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Environmental and economic assessment of sustainability in Mediterranean wheat production

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Abstract. This study proposes the application of the Life Cycle Assessment (LCA) and economic evaluations conducted on different cereal production systems in semi-arid environments. Two different crop management systems of durum wheat were analysed by distinguishing Continuous Cropping (CC) and Crop Rotation (CR) with vetch, that are conducted through two different levels of crop intensification (in terms of tillage and fertilization strategies): Conventional (Conv) and Conservative (Cons). The resulting four scenarios were examined using LCA methodology to assess the environmental impacts, and the Production Cost (PC) analysis to estimate the economic results. Overall, the findings of this research provide an opportunity to identify sustainable crops management strategies.

Key words: crop management systems, life cycle assessment (LCA), cost production analysis.

INTRODUCTION

Among crops for food use, cereals represent one of the most important groups in the world agricultural economy since they play a fundamental role both in human nutrition, with the production of starchy grains for the flours used in numerous food preparations, and in animal feed used as fodder.

In terms of harvested area, grain is the most cultivated cereal in the world mainly as soft wheat (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum* ssp. durum). Today, wheat cultivation represents, overall, the first crops in terms of the arable surface in the world, accounting 220.11 million of hectares, and the third (after sugarcane and maize) in terms of production quantity, with 749.46 million tonnes (Faostat, 2016). Asia and Europe intercept around 77% of the worldwide production; in particular, India, China, Russian Federation, France, Ukraine, Pakistan, and Germany are in the first positions, by covering 50% of production. In Europe, the cultivation of wheat represents, overall, the most important agricultural production with 62.52 million hectares and 250.13 million tons (Faostat, 2016).

It is undoubted that, in coming decades, the request of land for food, fibre, and fuel will continue to increase rapidly. In this sense, the search for an equilibrium between agricultural harvests to meet demand from a growing world population and minimizing environmental impacts represents an everlasting challenge in sustainable agricultural production.

Agricultural practices are responsible both to sustain and to degrade the environment (MEA, 2005); therefore, it is increasingly urgent to reaffirm the necessity to preserving soil, water, biodiversity and ecosystems and improving resilience to climate change in a long-term perspective. Starting from the American Agronomy Society's definition of sustainable agriculture (Weil, 1990), in the last thirty years, this concept, while remaining substantially unchanged, it has enriched itself with contents on practical solutions for the achievement of its objectives (De Luca et al., 2015). According to several scholars, the need for eco-compatible crops management is increasingly growing, particularly in regards to agronomic practices, soil processing, and fertilisation strategies (Montemurro et al., 2008; Rabbinge & Bindraban, 2012; Bindraban et al., 2015).

Among all the sustainable practices, conservation agriculture (CA) is widely recognized to be able to cause positive outcomes on organic matter, soil proprieties, and to reduce environmental risks (Montemurro & Maiorana, 2015), and at the same time, to sustain yield and crop quality. CA considers the coexistence of three main elements related to minimize soil disturbance, maintenance of permanent soil covers, and crop rotations (Gonzalez-Sanchez et al., 2015). According to Montemurro (Montemurro et al., 2008; Montemurro & Maiorana, 2014), continuous cropping systems in some Mediterranean areas, like Southern Italy, are still widespread, especially for cereal production; for these contexts, or similar, the contribution of CA practices has been largely studied (Montemurro & Maiorana, 2015), but to the authors' best knowledge, specific analysis to measure environmental impact indicators are few implemented. Therefore, just as the effects of the use of chemical inputs, loss of crop and livestock genetic diversity through the spread of monocultures have been studied with greater reliability, in the meantime it needs verify the real effects of sustainable practices applied for meeting agricultural productivity and environmental goals.

To this aim, the use of innovative methods for assessment of sustainability can represent a powerful tool to increase the knowledge about new paradigms of agricultural production processes (Sands & Podmore, 2000). Life Cycle Assessment (LCA) method (Rebitzer et al., 2004; Guinée et al., 2011; Falcone et al., 2017; 2018) allows to investigate the sustainability issues through a conceptual model, based on the deepening of all impacts that a product or a service generates during its whole life cycle, related to all sustainability components, from design to disposal of the used product. Based on a standardized framework, the LCA represents, then, the tool more appropriate to compare transparently in different methods to achieve the same product, in order to choose the most sustainable (Brankatschk & Finkbeiner, 2015). However, an assessment of sustainability cannot be distinct from the profitability evaluation, a factor that mainly affects decision making in business activity, in the absence of specific constraints.

Despite several studies exist on impacts of cereal crops evaluated by means of LCA, the specific issue on modeling crop rotations is very topical, as confirmed recently by Brankatschk (2019), who tries to give an answer to the numerous open questions related to this topic. To confirm this, carrying out a survey on the main web search engines of scientific literature (Scopus, ScienceDirect, Google Scholar, etc.), it emerges as few works exist and, therefore, that this issue needs to be further explored.

However, among the published article existing, Nemecek et al. (2007), performed one of the first case studies on LCA applied to crop rotations, with the aim to underline the environmental impacts linked to the introduction of grain legumes in the crop rotation systems. Later, results of applications carried out by Hayer et al. (2010), Kulshreshtha et al. (2013), Naudin et al. (2014) and Nemecek et al. (2015) suggested the intercropping of a legume crop in rotations in order to reduce environmental burdens, while Brankatschk & Finkbeiner (2014; 2015) and Goglio et al. (2018) have focused on methodological aspects related to the LCA modeling of crop rotations. In this context, the issue of environmental impacts of CA techniques has been treated as a problem in its own right and there is a very limited number of studies that have deepened it (Sparrevik et al., 2012); furthermore, none specific LCA analysis exists on crop rotation systems with conservative agricultural practices.

In particular, this study aimed to evaluate the environmental impacts of four wheat management systems with LCA methodology that by now is widespread, increasingly, in agro-food studies (Abeliotis et al., 2013; Cerutti et al., 2014; Notarnicola et. al, 2015). Furthermore, an economic appraisal was performed through a Production Cost (PC) analysis (Gresta et al., 2014; Bernardi et al., 2017; Stillitano et al., 2017a), in order to compare the results of the different crop scenarios in terms of profitability performances.

MATERIALS AND METHODS

Experimental design

Data used in this research were obtained from a multidisciplinary three-year field experiment conducted in Foggia (Southern Italy) in collaboration with the Agricultural Research Council. Details of these experimental trials are available on Montemurro and Maiorana (2014). The experimental design involved four scenarios: the cultivation of durum wheat (W) – *Triticum durum* Desf. – grown in Continuous Cropping Conventional (CCConv) and Continuous Cropping Conservative (CCCons); and wheat in alternation with vetch (V) – *Vicia sativa* L. – conducted with Crop Rotation Conventional (CRConv) and Crop Rotation Conservative (CRCons) techniques. In particular, conventional management entailed soil tillage at 40–50 cm depth with mineral nitrogen fertilization, while conservative management required soil tillage at 15–20 cm depth with organic-mineral nitrogen fertilization.

For each scenario examined, quantitative data of the three-year of experimentation are related to hectare and are adapted to farm context in the Apulia region (South Italy). To assess the environmental and economic sustainability the typologies and quantities of inputs and outputs were directly observed (as primary data) for the establishment of a single inventory, useful for further elaborations. Inventory data were monetized considering the current market prices, to determine the economic results of different crop scenarios.

Environmental analysis

The environmental dimension of sustainability was analysed using the LCA methodology according to ISO norms (ISO, 2006a; 2006b), ‘an objective process to evaluate the environmental burdens associated to a product, a process, or an activity by identifying energy and materials usage and environmental releases, and to evaluate opportunities to achieve environmental improvements’ (SETAC, 1991). Four steps are necessary to implement a rigorous LCA study (ISO, 2006a). Primarily, the phase of goal and scope definition should be carried out, including a clear statement on the specification of Functional Unit (FU) – i.e. the measurement unit to which all inputs and outputs data are related – as well as of system boundaries, data quality, limitations and procedures of allocation.

In this research, a system boundary from the 'cradle to gate farm' was chosen and in order to incorporate the interactions within the scenarios, all inputs and outputs of the three-years of experimentation were considered (Fig. 1).

In order to appraisal the environmental impacts of different soil management systems, the hectare of land was chosen as FU. This choice allows to evaluate the potential environmental impact in an area per time, but it would lead the decision-makers to undertake critical actions for the economic sustainability of the individual farms. In this sense, the use of a functional unit related to the mass, allows connecting directly the impacts generated from the cultivation processes to the product for which such impacts were generated. However, in the case of complex cultivation systems, like rotations, cannot be used this type of functional unit, because of the different yield per hectare of the crops which succeed.

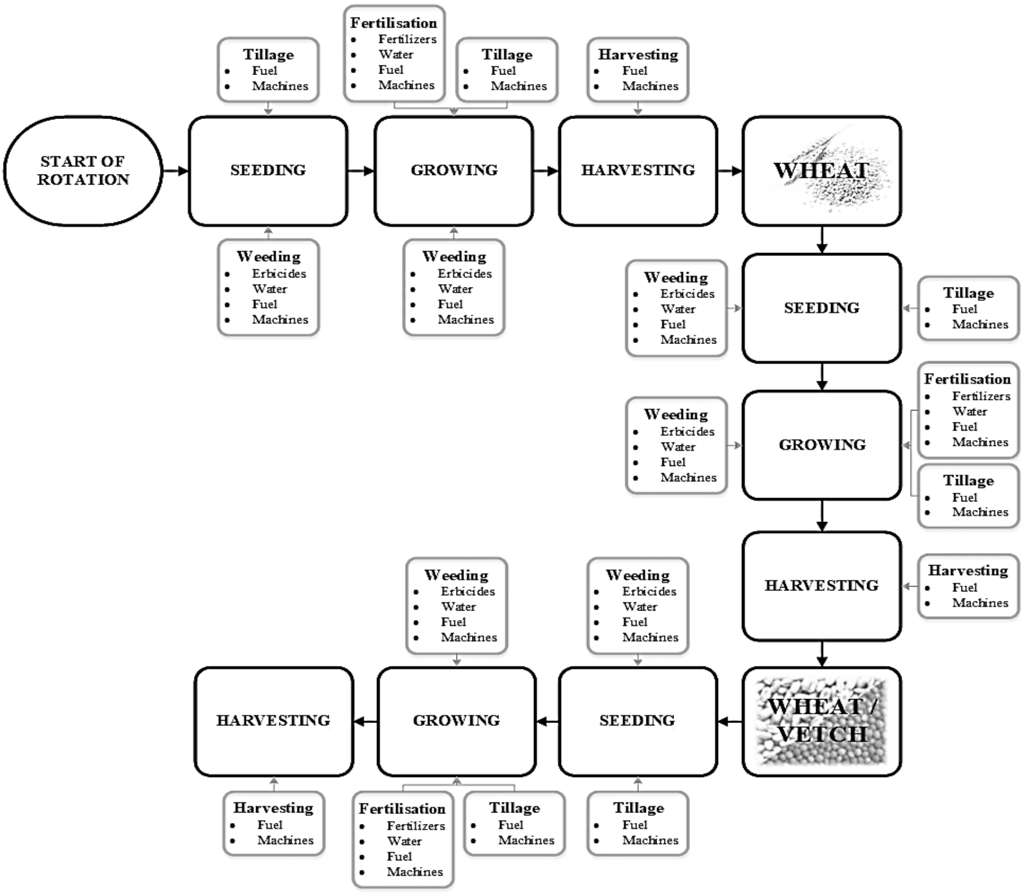

Figure 1. Production systems analysed with the detail of input per unitary operation and growing phase.

The use of a ‘financial type’ functional unit could be a good choice, combining different aspects related both to produced mass and to the economic value of this; however, this choice is strongly influenced by the performances agricultural markets. Furthermore, the use of a financial functional unit may cause double accounting problems in the economic sustainability evaluation phase.

In terms of data quality, primary data on quality and quantities of inputs really used in the experimentations were considered for the environmental and economic assessment. Data were converted to productive scale multiplying quantity used in the experimental parcel for the quotient of ratio ‘hectare/experimental parcel surface’.

In particular, data on the foreground processes as seeds, fuels, fertilisers, herbicides and, water consumption were obtained directly from on-field trials and were collected during three years for each experimental hypothesis (Table 1). Referring to the load and fate of fertilisers due to their dispersion, the assessment of the emissions was carried out by using Brentrup et al. (2000) and Nemecek & Kägi (2007). To quantify the fuel combustion emissions, the suggestions by Nemecek & Kägi (2007) were followed, while for the emissions related to the dispersion and fate of the pesticides, the assumptions of Margni et al. (2002) were used. Data on background processes as the production phases of fuel, fertilisers and, pesticides were found in the Ecoinvent V.3.0 database, the most used Life Cycle Inventory dataset in the world. None allocation procedure were considered.

The compilation of Life Cycle Inventory (LCI) was performed by the collection of primary, secondary and tertiary data related to background and foreground processes. The calculation of incoming and outgoing flows (e.g. energy, materials, and emissions) was carried out in function of the FU chosen, as well as the validation of data quality. Three-year average data were used, considering a triangular distribution for primary data, in order to assess the uncertainty of Life Cycle Impact Assessment (LCIA) results.

The LCIA represents the third step and consists of quantifying potential environmental impacts, through the selection of impact categories and, for all of them, relevant indicators and characterization models.

The elaborations were performed using SimaPro 8.1 software and choosing as impact assessment method the ReCiPe midpoint (Pré, 2014), one of the method most appropriate for the evaluation of agricultural production processes thanks to the number of indicators closely related to cultivation processes. For example: Marine and Freshwater eutrophication and Terrestrial acidification, influenced by the use of fertilizers; Marine, Freshwater and Terrestrial Ecotoxicity as well as Human Toxicity, influenced by the dispersion of pesticides and herbicides; Agricultural and Urban land occupation as well as Natural land transformation, influenced by the type of crop cultivated. The impacts assessment phase only includes the results of characterisation.

The final phase is the interpretation of results that highlight the hotspots of life cycle analysed and it allows formulating suggestions to improve the production process. Uncertainty analysis was performed by applying the Monte Carlo sampling technique (Sonnemann et al., 2003). In particular, the analysis was carried out using SimaPro software, running 1,000 iterations (Niero et al., 2015). For secondary data, the distribution considered in the Ecoinvent database was held (Niero et al., 2014). LCIA and Interpretation phases will be discussed in the results section.

Table 1. Components of inventory (inputs and outputs for each scenario analysed)

Description	Input/output	Scenarios							
				CRConv			CRCons		
		CCConv	CCCons	1st year Wheat	2nd year Vetch	3rd year Wheat	1st year Wheat	2nd year Vetch	3rd year Wheat
Deep tillage	Diesel L ha^{-1}	22 ± 1.5	-	22 ± 1.5	22 ± 0.35	-	-	22 ± 0.35	-
Surface tillage			9 ± 0.8	-	-	-	9 ± 1.2	-	-
Harrowing		11 ± 1							
Rotary tillage		11 ± 0.85							
Seeding		6 ± 2							
	Seed kg ha^{-1}	200 ± 1.5	200 ± 1.5	200 ± 1.5	130 ± 0.5	200 ± 1.5	200 ± 1.5	130 ± 0.5	200 ± 1.5
Fertilisation	Diesel L ha^{-1}	2.8 ± 0.3							
	Diammonium phosphate (N18%-$P_2O_5$46%) kg ha^{-1}	200 ± 20		200 ± 20		200 ± 10			
	Urea (N 46%) kg ha^{-1}	150 ± 10	-	150 ± 10	-	100 ± 5	-	-	-
	Azoslow (N 29%) kg ha^{-1}	-	250 ± 15	-	-	-	250 ± 15	-	150 ± 10
	Nitrogen-phosphorus (N9%-$P_2O_5$24%) kg ha^{-1}	-	300 ± 10	-	-	-	300 ± 10	-	300 ± 10
	Triple superphosphate ($P_2O_5$46%) kg ha^{-1}	-	-	-	200 ± 25	-	-	200 ± 25	-
Weeding	Diesel l ha^{-1}	3.13 ± 0.2							
	Erbitox® E30 (MCPA 230 g L^{-1}) L ha^{-1}	2.5 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	-	2.5 ± 0.2	2.5 ± 0.2	-	2.5 ± 0.2
Harvesting	Diesel L ha^{-1}	21.0 ± 2.5	21.0 ± 2.5	21.0 ± 2.5	27.0 ± 3.0	21.0 ± 2.5	21.0 ± 2.5	27.0 ± 3.0	21.0 ± 2.5
Waste	HDPE kg ha^{-1}	0.7 ± 0.06	1.1 ± 0.05	0.7 ± 0.06	0.4 ± 0.05	0.6 ± 0.03	1.1 ± 0.05	0.4 ± 0.05	0.96 ± 0.04
Yield (1st year)	t ha^{-1}	3.29 ± 0.3	2.6 ± 0.3	3.3 ± 0.3	-	-	2.67 ± 0.3	-	-
Yield (2nd year)		2.62 ± 0.25	2.17 ± 0.25	-	2.00 ± 0.25	-	-	1.68 ± 0.22	-
Yield (3th year)		2.88 ± 0.2	2.5 ± 0.2	-	-	3.2 ± 0.3	-	-	2.74 ± 0.3

Economic analysis

In order to highlight the economic performance of the four production systems for durum wheat under study, the total production costs and revenues, referred to 1 ha of cultivated surface, were evaluated as in other studies (Pardo et al., 2009; Sartori et al., 2005; Sgroi et al., 2014; Vach et al., 2016). The economic analysis was conducted using data from the field trials concerning all inputs, machinery, and labour involved in agriculture operations, from pre-seeding to harvesting, and assuming the real wheat crop management performed widely by local farmers. For the latter purpose, the farm data have been collected through face-to-face interviews with farmers and privileged stakeholders of the durum wheat supply chain. Then, correction coefficients were calculated to convert the experimental data into real data. Input prices and all cost items refer to the year 2015.

The production cost analysis was carried out by dividing the total cost into its variable and fixed components. The first component depends on the activity undertaken, while the second one is linked with the farm structure (Peris Moll and Juliá Igual, 2006). In this study, variable costs included all inputs used in the production process such as seeds, fertilizers, pesticides, fuel and lubricants consumption of machinery ownership (specific costs), the rental cost of machinery for harvesting, labour cost needed during field operations and interests on advance capital.

Within the fixed costs, ownership costs of machinery and equipment, and land investments (i.e. depreciation, insurance, repairs, and maintenance), interests on capital goods, taxes, rent for land use and administration overheads were taken into account (Bernardi et al., 2017; Stillitano et al., 2017b; Strano et al., 2017; Stillitano et al., 2018). This analysis allowed to investigate those activities more affecting the production and profitability, in order to reduce production costs and improve profit margins, as suggested by Mohamad et al. (2013), Tudisca et al. (2013), Testa et al. (2014) and Iotti & Bonazzi (2015).

Specifically, regarding inputs cost, it was calculated taking into consideration each environmental input considered in LCA inventory and pricing them according to the current market. Rental cost of machinery for harvesting operation was evaluated considering current tariffs charged by firms hire. Labour cost was assessed in terms of opportunity cost and was equalised to the employment of casual workers for manual and mechanical operations by assuming local current hourly wage as the basis (Strano et al., 2015; Stillitano et al., 2016). Interests on advance capital and capital good were determined by applying an interest rate equal to 4.5% and 2%, respectively. To evaluate the rental cost for land use, the local rental prices were considered. Administration overheads were estimated as 5% of the Gross Production Value (GPV), which corresponds to the annual total revenues. In particular, total revenues (TR) for each production system took account of both the market value of the crop, obtained by multiplying the yield for its market price, and the EU Agricultural Policy subsidies. The average selling prices of durum wheat and common vetch were referred to 2017 crop year and provided by the Italian public institution for the monitoring of agricultural market (ISMEA, 2017). According to Pardo et al. (2009) and (2014), the main purpose of such analysis was to consent right understanding of the importance of each combined crop management system, i.e. in terms of cropping systems, soil tillage, and fertilization techniques, to final farm profitability. Therefore, to compare the profitability of the durum wheat production under the different crop management strategies investigated, specific economic indicators were used, such as gross margin (GM) and net profit (NP) (Tatlidil et al., 2005; Banaeian et al., 2011; Nave et al., 2013; Tudisca et al., 2014). Gross margin was calculated by subtracting the variable costs to the total revenues (TR), while the net profit was determined as the difference between GPV and total production cost (Table 2).

Table 2. Economic indicators used

	Description			
	TR	Total Revenues	GPV + EU subsidies	€ ha^{-1}
Economic	TC	Total Production Cost *	Sc + Ch + T + S + W + I + Lp	€ ha^{-1}
Indicators	NP	Net Profit	TR - TC	€ ha^{-1}
	GM	Gross Margin	TR - (Sc + W + I)	€ ha^{-1}
	EC	Energetic Cost	(Fuel cost + Fertilization cost) / Sc	%

* Sc: Specific costs; Ch: Depreciation, maintenance, and insurance charges; T: Taxes; S: Salaries; W: Wages; I: Interest; Lp: Land profit.

RESULTS AND DISCUSSION

Life Cycle Assessment results

Results of impact characterization are described grouping the different impact categories according to the areas of protection where they have a negative impact, in a cause-and-effect perspective. In this sense, the connection between the impact category and the areas of protection was performed according to the ReCiPe Method (Goedkoop et al., 2013). The first six impact categories listed in Table 3, are related to Human Health protection area, except for Climate Change which has environmental effects also on the Ecosystems area. Comparing the four experimental scenarios, CRConv shows better performances in five out of six impact categories. In particular, expressing impacts on Human Toxicity in kg 1.4-Dichlorobenzene equivalent, the CRConv emits 1,061.20 kg, versus the worst scenario (CCCons) that emits 1,303.29 kg. The same ranking can be observed for Ionising Radiation, Photochemical oxidant formation, Particulate matter formation, and Climate Change, where the CRConv is on average better than CCCons about 23.7%. For this area of protection, Crop Rotation (CR) scenarios result generally better than Continuous Crop (CC) scenarios, excluding for Ionising Radiation where the second better scenario was CCConv. This result can be explained by a greater amount of fertilizers used in conservative scenarios. In particular, the higher impacts in human health area of protection can be attributable to fertilizers production (Fig. 2). The only impact category where CRCons represents the low pollutant scenarios was Ozone Depletion due to the higher impact in this category of nitrogen fertilizer.

As already mentioned, Climate Change represents a bridge category in the ReCiPe method, because are attributed to it negative effects both on Human Health and on the Ecosystems areas (Table 3). In this category, the CRConv scenario gets the best performance, followed by the CRCons scenario, penalized by a large use of fertilizers. For the terrestrial ecotoxicity indicator as well, the best scenario was CRConv, but the highest percentage of impact can be attributed to seeding, which represents in each scenario about 80% (Fig. 2), in particular, due to the seed production. In relation to terrestrial acidification, the better scenario was CRCons, thanks to the lower emission of slow-release fertilisers used in conservative scenarios. For Agricultural Land Occupation too, the CRCons gained the best performance, though the difference with CRConv scenario is not considerable. It is significant to underline that this impact category by herself does not represent a comprehensive indicator of land use, providing only the surface occupied during the life cycle. The same consideration should be applied to Urban Land Occupation and Natural Land Transformation. In the first case, the scenario CRConv have lower impacts because of the smaller quantity of fertilizers used, input which represents the highest contributor in this impact category due to the fertilizers production plant; for the second indicator the best performance of CRCons scenario can be attributed to tillage operations, as already seen for Agricultural Land Occupation. The different tillage techniques in terms of land transformation and/or land occupation derive from different quantities of inputs utilized, and not from different types of land use. A comprehensive land use assessment must consider a broader set of specific indicators (e.g., soil erosion, biotic production, etc.).

Table 3. Life Cycle Impact Assessment through ReCiPe Midpoint method

Impact Category	Unit	CCConv	CCCons	CRConv	CRCons
Ozone Depletion	kg CFC-11 eq	6.64E-04	6.04E-04	4.72E-04	***4.33E-04***
Human Toxicity	kg 1,4-DB eq	1.23E+03	1.30E+03	***1.06E+03***	1.11E+03
Ionising Radiation	kBq U235 eq	4.50E+02	5.47E+02	***4.49E+02***	5.12E+02
Photochemical Oxidant Formation	kg NMVOC	2.32E+01	2.63E+01	***2.01E+01***	2.23E+01
Particulate Matter Formation	kg PM10 eq	1.76E+01	1.81E+01	***1.38E+01***	1.41E+01
Climate Change	kg CO_2 eq	4.54E+03	4.88E+03	***3.44E+03***	3.67E+03
Terrestrial Ecotoxicity	kg 1,4-DB eq	3.53E+00	3.64E+00	***3.03E+00***	3.10E+00
Terrestrial Acidification	kg SO_2 eq	7.94E+01	7.56E+01	5.58E+01	***5.20E+01***
Agricultural Land Occupation	m^2a	3.10E+04	3.10E+04	3.09E+04	***3.09E+04***
Urban Land Occupation	m^2a	3.61E+01	4.54E+01	***3.47E+01***	4.09E+01
Natural Land Transformation	m^2	1.48E+00	1.39E+00	1.07E+00	***1.01E+00***
Marine Ecotoxicity	kg 1,4-DB eq	3.08E+01	3.30E+01	***3.06E+01***	3.21E+01
Freshwater Ecotoxicity	kg 1,4-DB eq	2.02E+01	1.74E+01	1.30E+01	***1.18E+01***
Marine Eutrophication	kg N eq	***8.76E-01***	1.11E+00	1.49E+00	1.65E+00
Freshwater Eutrophication	kg P eq	2.80E+01	3.08E+01	***2.49E+01***	2.68E+01
Fossil Depletion	kg oil eq	1.72E+03	1.57E+03	1.22E+03	***1.13E+03***
Metal Depletion	kg Fe eq	4.73E+02	4.53E+02	3.94E+02	***3.84E+02***
Water Depletion	m^3	***6.51E+01***	7.17E+01	8.55E+01	8.98E+01

In relation to the categories of Marine Ecotoxicity and Freshwater Eutrophication, the best results are obtained by CRConv while, in Marine Eutrophication and Freshwater Ecotoxicity categories, the best results are obtained respectively by CCConv and CRCons scenarios. It should be noted that while for the Marine Ecotoxicity, Freshwater Ecotoxicity and Freshwater Eutrophication indicators the main hotspot is the fertilisation, for the Marine Eutrophication indicator, the impacts have to be attributed also to seeding, and in particular to fertilisers used for seed production.

The last three categories are related to resources depletion and are mainly interested by fertilisation, seeding and tillage operations. In relation to Fossil and Metal Depletion, the CRCons scenario has the better performance thanks to the lower impact in fertilisation and tillage operations, while for Water depletion CCConv gains the best performance thanks to lower impacts linked to the fertilisers production. In relation to Fossil and Metal

Depletion must be underlined the influence of machinery, diesel (Lovarelli & Bacenetti, 2017; Lovarelli et al., 2017) and urea production (Bacenetti et al., 2016) (Fig. 2).

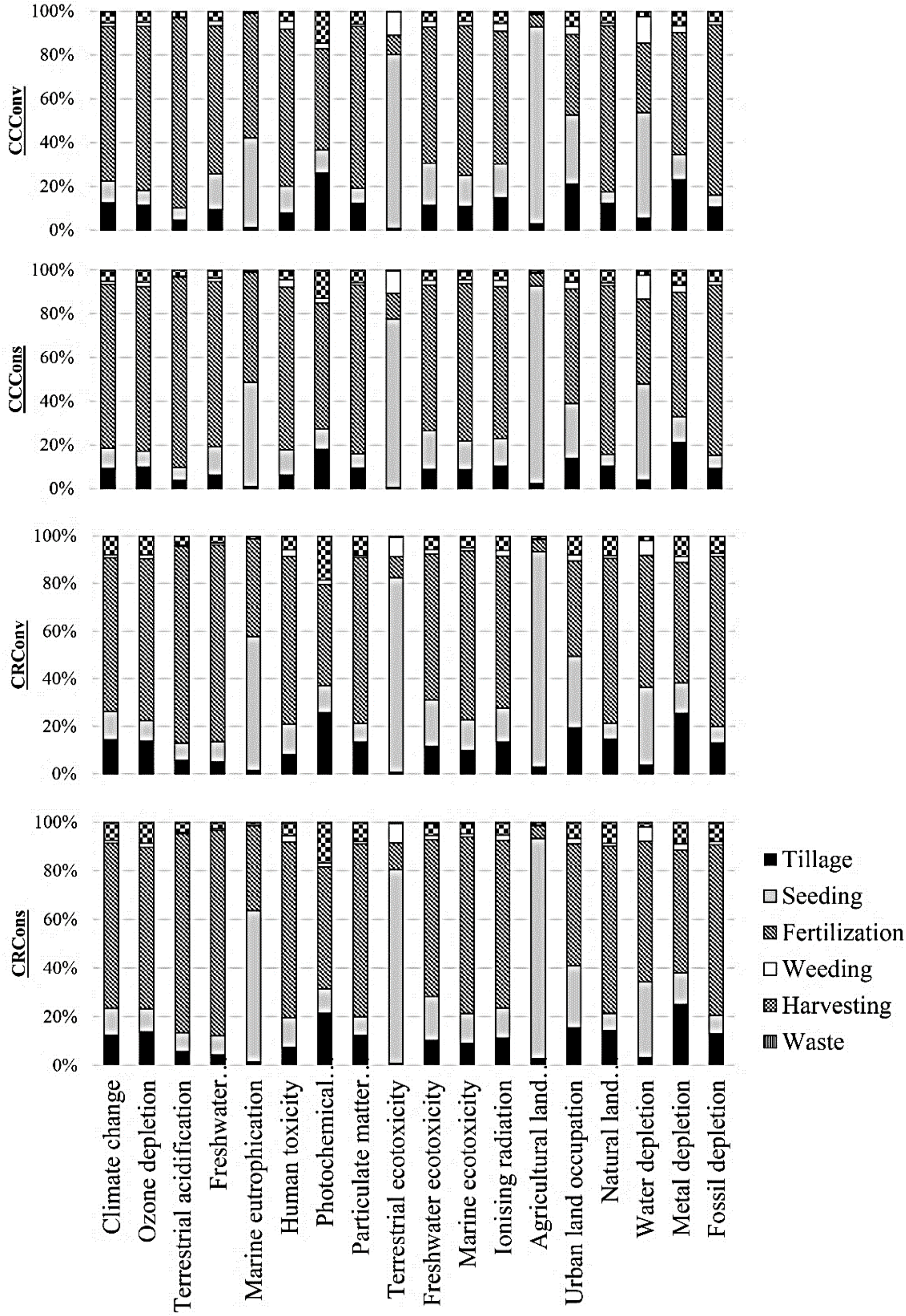

Figure 2. Environmental impacts per scenario per agronomic operation.

More in detail, by analysing LCA results, for all scenarios considered and for each agronomic operation (Figs 2 and 3), it is shown that the fertilisation, which account, on average, for 68.8% on each impact category, represents the crop operation most impactful for all environmental indicators, according to Schneider et al. (2010). This is not true for Agricultural Land Occupation, Marine Eutrophication and Terrestrial Ecotoxicity indicators. For the latter, the most impactful operation is represented by planting operation, in particular, by seed production that weights, on average, 87.9% on Agricultural Land Occupation, 63.5% on Marine Eutrophication and, 80% on Terrestrial Ecotoxicity.

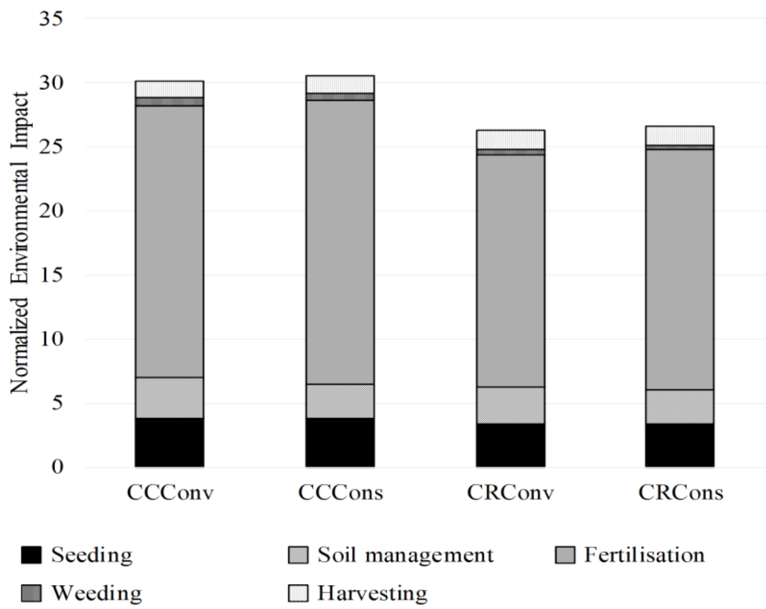

Figure 3. LCA results for agronomic operation.

In cropping rotation scenarios, the introduction of vetch allows avoiding a large amount of environmental impact, approximately 36% less, due to the reduction of fertilizers, especially thanks to nitrogen fixation process, according to Nemecek et al. (2008) and Ryan et al (2008). However, although in absolute values, these scenarios have a diesel consumption lower than continuous crop ones, in percentage terms, fossil fuels represent the second most important impact factor (in respect to a decrease of fertilization influence on impacts), mainly due to the soil management and harvesting operations.

The uncertainty analysis performed through 1000-runs Monte Carlo simulation (Table 4) allowed analysing the toughness of results for each Impact Assessment Category, by considering the probability distribution of LCI parameters.

In particular, two different insights were carried out in order to assess the robustness of inventory. A preliminary a Monte Carlo analysis has been carried out for each scenario by considering only the distribution of primary data; a subsequently Monte Carlo analysis has been performed considering both primary and secondary data distribution.

Table 4. The coefficient of variation (%) of results depending on the distribution of primary (P) and secondary (P+S) data

Impact Category	CCConv		CCCons		CRConv		CRCons	
	P	P+S	P	P+S	P	P+S	P	P+S
Ozone Depletion	1.94	23.84	1.07	23.25	0.90	23.17	1.12	24.74
Human Toxicity	1.96	286.57	0.97	268.98	1.27	259.00	1.39	305.03
Ionising Radiation	1.64	106.67	0.98	108.73	1.49	95.67	1.62	139.13
Photochemical Oxidant Formation	1.38	4.30	1.04	5.23	1.05	5.27	1.09	4.97
Particulate Matter Formation	1.50	3.21	0.92	3.38	0.99	6.29	1.06	10.54
Climate Change	1.83	6.80	1.00	5.96	0.91	5.24	1.11	6.08
Terrestrial Ecotoxicity	0.47	13.48	0.41	12.93	0.36	12.90	0.38	13.97
Terrestrial Acidification	2.16	2.65	1.30	2.30	1.03	7.33	1.26	12.42
Agricultural land Occupation	0.37	7.65	0.33	7.89	0.27	6.98	0.27	8.81
Urban Land Occupation	1.70	8.95	1.18	9.58	1.24	9.45	1.35	8.51
Natural Land transformation	1.99	49.00	1.09	47.21	0.94	51.28	1.15	55.94
Marine Ecotoxicity	1.88	51.32	0.95	49.27	1.63	40.53	1.69	43.08
Marine Eutrophication	1.32	6.01	0.80	7.03	0.42	8.95	0.73	7.57
Freshwater Eutrophication	1.81	51.59	0.95	41.43	1.86	21.67	1.98	27.07
Freshwater Ecotoxicity	1.73	63.23	0.90	58.88	1.17	54.94	1.28	58.97
Fossil Depletion	2.00	14.34	1.10	11.71	0.95	10.41	1.16	12.44
Metal Depletion	1.81	30.51	1.13	30.09	1.16	22.59	1.19	27.69
Water Depletion	0.85	184.77	0.52	179.20	2.06	134.62	2.07	149.12

The uncertainty assessment considering only primary data underlined a good significance level of results with a coefficient of variation, on average, lower than 1.5%. Considering also the distribution of secondary data, the uncertainty of results for Ozone Depletion, Natural Land Transformation, Marine Ecotoxicity, Freshwater Eutrophication, Freshwater Ecotoxicity, and Metal Depletion categories was found to be appreciable. Instead, for Human Toxicity, Ionising Radiation and, Water Depletion categories, the uncertainty was found to be very high.

These results depend on the quality of some inventory data that possess, intrinsically, a high degree of uncertainty, being modeled on the average values of a given process.

Economic performances of different scenarios

In Table 5, the results of economic indicators obtained from PC analysis are reported. The results show that the cropping rotation scenarios record the best performance in terms of both total production cost and energetic cost compared to the continuous cropping ones. In particular, CRCons scenario has the lowest TC and EC indicators with 1,829.46 € ha^{-1} and 78.33%, respectively, followed by the CRConv with 1,887.14 € ha^{-1} (+3.2%) and 79.90% (+0.7%). This is mostly caused by the lower cost related to the inputs employed in crop rotation, which accounts for around 49% to the total production cost for both scenarios with rotation. Within the input costs (or specific costs), the main contributors are the fertilization and fuel costs estimated at 48.42% and 29.91%, respectively, for the CRCons scenario, while these amount to 45.22% and 33.68% for the CRConv scenario. Likewise, Vach et al. (2016) found the highest fuel consumption in all crops cultivated under conventional tillage.

Table 5. Results of economic analysis

Scenarios	TC, € ha^{-1}	EC, %	TR, € ha^{-1}	NP, € ha^{-1}	GM, € ha^{-1}
CCConv	2,101.90	80.38	3,084.60	982.70	1,554.01
CCCons	2,192.45	82.64	2,719.80	527.35	1,067.29
CRConv	1,887.14	78.90	2,732.19	845.05	1,368.43
CRCons	1,829.46	78.33	2,415.00	585.54	1,080.49

However, in terms of benefit indicators, the CCConv scenario achieves the best performance. In particular, it is observed for TR, NP and GM indicators the higher values equal to a three-year total amount of 3,084.60 € ha^{-1}, 982.70 € ha^{-1} and 1,270.18 € ha^{-1}, respectively. This scenario is able to maximize the remuneration of entrepreneur. Therefore, it represents the most profitable crop management system. The high value of TR indicator can be explained, on one hand, by the higher wheat yield equal to 8,790.00 kg ha^{-1}, on the other hand by the amount of European subsidies, e.g. 325 € ha^{-1} $year^{-1}$ for wheat against 181 € ha^{-1} $year^{-1}$ for vetch in crop rotation. Conversely, in terms of TR, the CRCons scenario shows the worst performance with an amount equal to 2,415.00 € ha^{-1}, mainly due to the lower total production yield equal to 7,090.00 kg ha^{-1} of which 5,410.00 kg ha^{-1} are wheat. However, it should be noted that, in CRCons scenario, an increase of grain yield equal to 2.6% is reached, likely due to the capability of leguminous crops to increase soil fertility.

This latter result endorses the findings by Montemurro & Maiorana (2015), who highlighted a possible improvement of wheat yield, mainly when in rotation with leguminous crops compared to the continuous cropping. Similarly, Calzarano et al. (2018) confirmed that under conservation agriculture higher yields are reached, also due to the improved nutrient availability achieved by including fava beans in the rotation.

For the remaining scenarios, the values of TR ranging from 2,732.19 € ha^{-1} (CRConv) to 2,719.80 € ha^{-1} (CCCons). The CCCons scenario reaches the worst performance in terms of NP and GM indicators estimated at 46.3% and 31.3% lower than the best scenario (CCConv).

CONCLUSIONS

In modern agricultural crop production, get to maintain farm income and reduce environmental impacts is a prerequisite for sustainable agriculture. To obtain useful recommendations for farmers in selecting crop management systems that combine environmental and economic benefits, key measurement methods are needed. In this work, LCA and production cost analysis were used in order to assess the environmental and economic performance of different cereal production methods. The environmental results highlighted that the crop rotation with conventional management (CRConv) obtain better performances in almost all impact categories. The economic finding showed that CRCons scenario reached the lowest total production costs, followed by CRConv, although in terms of benefit indicators the CCConv scenario achieves the best performance. An evaluation in the long period should highlight some benefits of conservation agriculture in environmental and economic terms. The growing interest towards the vegetable proteins could cause an increase of leguminous grain price encouraging crop rotations.

REFERENCES

Abeliotis, K., Detsis, V. & Pappia, C. 2013. Life cycle assessment of bean production in the Prespa National Park Greece. *Journal of Cleaner Production* **41**, 89–96.

Bacenetti, J., Lovarelli, D. & Fiala, M. 2016. Mechanisation of organic fertiliser spreading, choice of fertiliser and crop residue management as solutions for maize environmental impact mitigation. *European Journal of Agronomy* **79**, 107–118.

Banaeian, N., Omid, M. & Ahmadi, H. 2011. Energy and economic analysis of greenhouse strawberry production in Tehran province of Iran. *Energy Conversion and Management* **52**(2), 1020–1025.

Bernardi, B., Tous, J., Benalia, S., Abenavoli, L.M., Zimbalatti, G., Stillitano, T. & De Luca, A.I. 2017. The assessment of hazelnut mechanical harvesting productivity. *Agronomy Research* **15**(4), 1491–1497.

Bindraban, P.S., Dimkpa, C., Nagarajan, L., Roy, A. & Rabbinge, R. 2015. Revisiting fertilisers and fertilisation strategies for improved nutrient uptake by plants. *Biology and Fertility of Soils* **51**(8), 897–911.

Brankatschk, G. & Finkbeiner, M. 2015. Modeling crop rotation in agricultural LCAs – Challenges and potential solutions. *Agricultural Systems* **138**, 66–76.

Brankatschk, G. & Finkbeiner, M. 2014. From wheat to beet – challenges and potential solutions of modeling crop rotation systems in LCA. In: Schenck, R. & Huizenga, D., (eds) *Proceedings of the 9th International Conference on Life Cycle Assessment in the Agri-Food Sector*. 8-10 October 2014 - San Francisco.

Brankatschk, G. 2019. Modeling Crop Rotations and Co-Products in Agricultural Life Cycle Assessments. Springer Vieweg, Wiesbaden, Germany.

Brentrup, F., Küsters, J., Lammel, J. & Kuhlmann, H. 2000. Methods to estimate on-field nitrogen emission from crop production as an input to LCA studies in the agricultural sector. *International Journal of Life Cycle Assessment* **5**(6), 349–357.

Calzarano, F., Stagnari, F., D'egidio, S., Pagnani, G., Galieni, A., Di Marco, S., Metruccio, E.G. & Pisante, M. 2018. Durum wheat quality, yield and sanitary status under conservation agriculture. *Agriculture (Switzerland)* **8**(9), art. no. 140.

Cerutti, A.K., Beccaro, G.L., Bruun, S., Bosco, S., Donno, D., Notarnicola, B. & Bounous, G. 2014. Life cycle assessment application in the fruit sector: State of the art and recommendations for environmental declarations of fruit products. *Journal of Cleaner Production* **73**, 125–135.

De Luca, A.I., Molari, G., Seddaiu, G., Toscano, A., Bombino, G., Ledda, L., Milani, M., Vittuari, M. 2015. Multidisciplinary and innovative methodologies for sustainable management in agricultural systems. *Environmental Engineering and Management Journal* **14**(7), 1571–1581.

Falcone, G., De Luca, A.I., Stillitano, T., Iofrida, N., Strano, A., Piscopo, A., Branca, M.L. & Gulisano, G. 2017. Shelf life extension to reduce food losses: The case of Mozzarella Cheese. *Chemical Engineering Transactions* **57**, 1849–1854.

Falcone, G., Lovarelli, D. & Bacenetti, J. 2018. Electricity generation from anaerobic digestion in Italy: Environmental consequences related to the changing of economic subsidies. *Chemical Engineering Transactions* **67**, 475–480.

Faostat. 2016. Crop production. *http://faostat3.fao.org/download/Q/QC/E* (accessed 30/10/2018)

Goedkoop, M., Heijungs, R., Huijbregts, M.A.J., De Schryver, A., Struijs, J. & Van Zelm, R. 2013. ReCiPe 2008. A life cycle impact assessment method which comprises harmonised category indicators at the midpoint and the endpoint level. First edition (Version 1.08) – Report I: Characterisation. RIVM report

Goglio, P., Brankatschk, G., Knudsen, M.T., Williams, A.G. & Nemecek, T., 2018. Addressing crop interactions within cropping systems in LCA. *International Journal of Life Cycle Assessment* **23**, 1735–1743.
Gonzalez-Sanchez, E.J., Veroz-Gonzalez, O., Blanco-Roldan, G.L., Marquez-Garcia, F., Carbonell-Bojollo, R. 2015. A renewed view of conservation agriculture and its evolution over the last decade in Spain. *Soil Till. Res*. **146**, 204–212.
Gresta, F., De Luca, A.I., Strano, A., Falcone, G., Santonoceto, C., Anastasi, U. & Gulisano, G. 2014. Economic and environmental sustainability analysis of guar (Cyamopsis tetragonoloba L) farming process in a Mediterranean area: two case studies. *Italian Journal of Agronomy* **9**, 20–24.
Guinée, J.B., Heijungs, R., Huppes, G., Zamagni, A., Masoni, P., Buonamici, R., Ekvall, T. & Rydberg, T. 2011. Life Cycle Assessment: Past, Present, and Future. *Environmental Science Technology* **45**, 90–96.
Hayer, F., Bonnin, E., Carrouée, B., Gaillard, G., Nemecek, T., Schneider, A. & Vivier, C. 2013. Designing sustainable crop rotations using Life Cycle Assessment of crop combinations. In: Darnhofer, I. & Grötzer, M., (eds) *Proceedings of the 9th European IFSA Symposium*. Universität für Bodenkultur, Vienna, 903–911.
International Organization for Standardization (ISO) 2006a ISO Norm 14040:2006 Environmental management – Life Cycle Assessment – Principles and framework. Geneva CH.
International Organization for Standardization (ISO) 2006b ISO Norm 14044:2006 Environmental management – Life Cycle Assessment – Requirements and guidelines. Geneva CH.
Iotti, M. & Bonazzi, G. 2015. Profitability and Financial Sustainability Analysis in Italian Aquaculture Firms by Application of Economic and Financial Margins. *Am. J. Agric. Biol. Sci*. **10**(1), 18–34.
ISMEA. 2017. http://www.ismeamercati.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/8082 (accessed 30/08/2017)
Kulshreshtha, S., MacWilliam, S. & Wismer, M. 2013. Economic and environmental assessment of pulse rotations in canadian prairies. *19th International Farm Management Congress Vol.1* July 21st – 26th, 2013.
Lovarelli, D. & Bacenetti, J. 2017. Bridging the gap between reliable data collection and the environmental impact for mechanised field operations. *Biosystems Engineering* **160**, 109–123.
Lovarelli, D., Bacenetti, J. & Fiala, M. 2017. Effect of local conditions and machinery characteristics on the environmental impacts of primary soil tillage. *Journal of Cleaner Production* **140**(2), 479–491.
Margni, M., Rossier, D., Crettaz, P. & Jolliet, O. 2002. Life cycle impact assessment of pesticides on human health and ecosystems. *Agriculture Ecosystems and Environment* **93**, 379–392.
Millennium Ecosystem Assessment (MEA). 2005. Ecosystems and Human Well-being: Synthesis. Island Press, Washington, DC, pp. 1–24.
Mohamad, R.S., Bteich, M.R., Cardone, G. & Marchini, A. 2013. Economic analysis in organic olive farms: the case of the ancient olive trees in the rural parkland in Apulia. *New Medit* **4**, 55–61.
Montemurro, F. & Maiorana, M. 2015. Agronomic practices at low environmental impact for Durum wheat in Mediterranean conditions. *Journal of Plant Nutrition* **38**, 624–638.
Montemurro, F. & Maiorana, M. 2014. Cropping systems tillage and fertilization strategies for durum wheat performance and soil properties. *International Journal of Plant Production* **8**(1), 51–75.
Montemurro, F., Maiorana, M., Convertini, G. & Fornaro, F. 2008. Cropping systems: the role of continuous cropping crop rotation leguminous crops and catch crop in Mediterranean conditions, pp. 141–171. In: Berklian YU (ed) Crop Rotation. Nova Science Pub Inc Hauppauge NY USA.

Naudin, C., van der Werf, H.M.G., Jeuffroy, M-H. & Corre-Hellou, G. 2014. Life cycle assessment applied to pea-wheat intercrops: A new method for handling the impacts of co-products. *Journal of Cleaner Production* **73**, 80–87.
Nave, S., Jacquet, F. & Jeuffroy, M.H. 2013. Why wheat farmers could reduce chemical inputs: evidence from social, economic, and agronomic analysis. *Agronomy for Sustainable Development* **33**(4), 795–807.
Nemecek, T. & Kägi, T. 2007. Life Cycle Inventories of Agricultural Production Systems. Final report Ecoinvent v2.0 N°15 Swiss Centre for Life Cycle Inventories Dübendorf CH, pp. 1–360.
Nemecek, T., Frick, C., Dubois, D. & Gaillard., G. 2007. Comparing farming systems at crop rotation level by LCA. In: Geerken, T., Mattson, B., Olsson, P. & Johansson, E., (eds.) *Proceedings of the International Conference on LCA in Foods*, Gothenburg. SIK, VITO, Gothenburg, pp. 65–69.
Nemecek, T., Schneider, A., Hayer, F., Bonnin, E., Carrouée, B., Vivier, C. 2015. Designing eco-efficient crop rotations using life cycle assessment of crop combinations. *European Journal of Agronomy* **65**, 40–51.
Nemecek, T., von Richthofen, J.S., Dubois, G., Casta, P., Charles, R. & Pahl, H. 2008. Environmental impacts of introducing grain legumes into European crop rotations. *European Journal of Agronomy* **28**, 380–393.
Niero, M., Ingvordsen, C.H., Jørgensen, RB. & Hauschild, M.Z. 2015. How to manage uncertainty in future Life Cycle Assessment (LCA) scenarios addressing the effect of climate change in crop production. *Journal of Cleaner Production* **107**, 693–706.
Niero, M., Pizzol, M., Bruun, H.G. & Thomsen, M. 2014. Comparative life cycle assessment of wastewater treatment in Denmark including sensitivity and uncertainty analysis. *Journal of Cleaner Production* **68**, 25–35
Notarnicola, B., Salomone, R., Petti, L., Renzulli, P.A., Roma, R. & Cerutti, A.K. 2015. Life Cycle Assessment in the Agri-food Sector Case Studies, Methodological Issues and Best Practices. Springer International Publishing Switzerland, pp. 1–420.
Pardo, G., Aibar, J., Cavero, J. & Zaragoza, C. 2009. Economic evaluation of cereal cropping systems under semiarid conditions: minimum input, organic and conventional. *Sci. Agric. (Piracicaba, Braz.)* **66**(5), 615–621.
Pardo, G., Perea, F., Martínez Y. & Urbano, J.M. 2014. Economic profitability analysis of rainfed organic farming in SW Spain. *Outlook on Agriculture* **43**(2), 115–122.
Peris Moll, E.M. & Juliá Igual, J.F. 2006. Production costs of the organic Clementine crop in the region of Valencia (Spain). *Span. J. Agric. Res*. **4**(1), 17–25.
Pré Consultants. 2014. SimaPro Database Manual – Methods library. The Netherlands.
Rabbinge, R. & Bindraban, P.S. 2012. Making More Food Available: Promoting Sustainable Agricultural Production. *Journal of Integrative Agriculture* **11**(1), 1–8.
Rebitzer, G., Ekvall, T., Frischknecht, R., Hunkeler, D., Norris, G., Rydberg, T., Schmidt, W.P., Suh, S., Weidema, B.P. & Pennington, D.W. 2004. Life cycle assessment Part 1: Framework, goal and scope definition, inventory analysis, and applications. *Environment International* **30**(5), 701–720.
Ryan, J., Pala, M., Masri, S., Singh, M. & Harris, H. 2008. Rainfed wheat-based rotations under Mediterranean conditions: Crop sequences nitrogen fertilization and stubble grazing in relation to grain and straw quality. *European Journal of Agronomy* **28**, 112–118.
Sands, G.R. & Podmore, T.H. 2000. A generalized environmental sustainability index for agricultural systems. *Agriculture Ecosystems & Environment* **79**, 29–41.
Sartori, L., Basso, B., Bertocco, M. & Oliviero, G. 2005. Energy Use and Economic Evaluation of a Three Year Crop Rotation for Conservation and Organic Farming in NE Italy. *Biosystems Engineering* **91**(2), 245–256.

Schneider, A., Flénet, F., Dumans, P., Bonnin, E., De Chezelles, E., Jeuffroy, M.H., Hayer, F., Nemecek, T. & Carrouée, B. 2010. Diversifying cereal crop rotations notably with peas and rapeseed – Recent experiment and study data. *OCL – Oleagineux Corps Gras Lipides* **17**(5), 301–311 (in French with abstract in English).

Sgroi, F., Di Trapani, A.M., Testa, R. & Tudisca, S. 2014. Economic sustainability of early potato production in the Mediterranean area. *American Journal of Applied Sciences* **11**(9), 1598–1603.

Society of Environmental Toxicology and Chemistry [SETAC]. 1991. A technical framework for [product] life-cycle assessments. Held in Smuggler's Notch Vermont USA.

Sonnemann, G.W., Schuhmacher, M. & Castells, F. 2003. Uncertainty assessment by a Monte Carlo simulation in a life cycle inventory of electricity produced by a waste incinerator. *Journal of Cleaner Production* **11**, 279–292.

Sparrevik, M., Field, J.L., Martinsen, V., Breedveld, G.D. & Cornelissen, G. 2012. Life Cycle Assessment to Evaluate the Environmental Impact of Biochar Implementation in Conservation Agriculture in Zambia. *Environmental Science & Technology* **47**(3**)**, 1206–1215.

Stillitano, T., De Luca, A.I., Falcone, G., Spada, E., Gulisano, G. & Strano, A. 2016. Economic profitability assessment of Mediterranean olive growing systems. *Bulg. J. Agric. Sci.* **22**(4), 517–526.

Stillitano, T., Falcone, G., Spada, E., De Luca, A.I., Grillone, N., Strano, A. & Gulisano, G. 2017a. An economic sustainability assessment of 'Fichi di Cosenza' PDO production compared with other profitable permanent crops. *Acta Horticulturae* **1173**, 395–400.

Stillitano, T., De Luca, A.I., Iofrida, N., Falcone, G., Spada, E. & Gulisano, G. 2017b. Economic analysis of olive oil production systems in southern Italy. *Quality - Access to Success* **18**(157), 107–112.

Stillitano, T., Falcone, G., De Luca, A.I., Spada, E., Gulisano, G. & Strano, A. 2018. Long-term feasibility of Mediterranean olive production systems. *Acta Horticulturae* **1199**, 203–208.

Strano, A., Stillitano, T., De Luca, A.I., Falcone, G. & Gulisano, G., 2015. Profitability analysis of small-scale Beekeeping firms by using Life Cycle Costing (LCC) methodology. *American Journal of Agricultural and Biological Sciences* **10**(3), 116–127.

Tatlidil, F.F., Kiral, T., Gündoğmuş, E., Fidan, H. & Aktürk, D. 2005. The effect of crop losses during pre-harvest and harvest periods on production costs in tomato production in the Ayaş and Nallihan districts of Ankara province. *Turkish Journal of Agriculture and Forestry* **29**(6), 499–509.

Testa, R., Di Trapani, A.M., Sgroi, F. & Tudisca, S. 2014. Economic analysis of process innovations in the management of olive farms. *Am. J. Appl. Sci*. **11**(9), 1486–1491.

Tudisca, S., Di Trapani, A.M., Sgroi, F. & Testa, R. 2013. The cost advantage of Sicilian wine farms. *Am. J. Appl. Sci*. **10**(12), 1529–1536.

Tudisca, S., Di Trapani, A.M., Sgroi, F. & Testa, R. 2014. Organic farming and economic sustainability: the case of Sicilian Durum Wheat. *Quality - Access to Success* **15**(138), 93–96.

Vach, M., Strašil, Z. & Javůrek, M. 2016. Economic efficiency of selected crops cultivated under different technology of soil tillage. *Scientia Agriculturae Bohemica* **47**(1), 40–46.

Weil, R.R. 1990. Defining and Using the Concept of Sustainable Agriculture. *J. Agron. Educ.* **19**(2), 126–130.

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Studies on combining ability in tomato (*Solanum lycopersicum* L.)

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Abstract. The present study was done seven elite tomato lines (*Solamun lycopersicum* L.) of determinate and indeterminate growth with good yield potential and good combining ability, using diallel fashion without reciprocals to produce 21 F_1s. General combining ability (GCA) and Specific combining ability (SCA) analysis were conducted, with Diallel-SAS, assessing six yield and component traits. Results showed highly significant differences ($p \leq 0.01$) among genotypes, as well as in GCA and SCA effects in all the characteristics that was assessed, with the exception of Days to First Cut. The results revealed that variance contribution to the yield attributed to the crossings had more non-additive effects (SCA) than additive effects (GCA). Furthermore, Line D4 had the greatest effect on yield in terms of GCA, as well in AFW (Average Fruit Weight), NFP (Number of Fruits per Plant) and PD (Polar Diameter) followed by D3 and K3. These lines can be used as donor parent in future tomato-breeding program. Hybrids K3×D4, R1×Y53, D3×IR13 and F3×Y53 had the highest level of SCA, with average yields of 93 t ha^{-1}. These potential hybrids could be exploited at commercial level after critical testing.

Key words: Combining ability, genetic effects, tomato, yield.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the vegetable crop with the highest demand and the greatest economic value in the world. Tomato trade and production have particular importance in tropical, subtropical and mild regions of the world, for both, fresh and processing markets (Meena et al., 2017). Mexico ranks 11th in global tomato production, with 3,055,861 tons and a total planted surface area of 48,394 hectares in 2017(SAGARPA-SIAP, 2015).

Parental selection in any plant-breeding program is one of the most important decisions that breeders must make (Broem & Miranda, 2005). Current biometric techniques enable us to select the right parental lines for hybrid seed production. The technique of diallel analysis developed and illustrated by Jinks & Hayman (1953) provides a guide to assess the relative reproductive potential of the parents. This technique is widely used to identify good parents. Based on the information gathered about the combining ability and genetic effects, were can combine the selected lines to exploit their hybrid vigor through the accumulation of non-additive genetic effects or by using cultivars that have accumulated additive genetic effects (Saleem et al., 2013).

The information about the General Combining Ability (GCA) and the Specific Combining Ability (SCA) is of great importance in breeding programs, in order to select the right parents for hybrids development. GCA indicates the average effect that a line gives to its crosses; measured as the general mean deviation. GCA is an expression of the additive genetic effects. SCA refers to a deviation from the foreseen behavior due to the general combining abilities of the parents, reflecting non-additive genetic effects (Sprague & Tatum, 1942). The combining ability is an effective tool that provides useful genetic information to select parents for the development of hybrid (Chezhian et al., 2000).

Development of hybrids and varieties for better yield and quality traits requires identification of good specific and general combiners. Combining ability studies provide reliable information for selection of parents for hybrid combination by revealing the nature and magnitude of gene actions involved in expression of quantitative traits (Agarwal et al., 2017).

In any hybridization program, recognition of the best combination of two (or more) parental genotypes to maximize variance within related breeding populations, and as a result the chance of recognizing superior transgressive segregants in the segregating populations, are the most critical challenge to plant breeders. Since the combining ability was introduced in 1942, it has been widely adopted in plant breeding to compare performances of lines in hybrid combinations (Fasahat et al., 2016).

Saleem et al. (2013) were able to identify tomato lines with good general combining ability, and therefore they are suitable for breeding high-yield tomato genotypes. On the other hand, López et al. (2012), Gabriel et al. (2013), Kumar et al. (2013) found high positive GCA values in the yield variables of tomato genotypes, leading to the selection of those genotypes with the highest yields.

The purpose of this research work was to select elite tomato lines with different growth habits (determinate and indeterminate) to develop the right hybrids to grow in open field, by identifying parental lines with good combining ability through diallel analysis.

MATERIALS AND METHODS

Genetic material

Seven lines of tomato was used as determinate (K3, Y53) and indeterminate (R1, F3, D4, D3, IR13) growth and its twenty-first direct potential F1 crosses, according to a diallel design involving the parents (Method 2 by Griffing (1956)). These lines came from the plant breeding department of 'Universidad Autónoma Agraria Antonio Narro' (UAAAN) and they were selected due to their yield potential ant their tolerance to *Fusarium oxysporum* f.sp. *lycopersici*.

Seeds of the evaluated genotypes was deposited in 200-cell polystyrene boxes filled with commercial Peat-Moss substrate (one seed per cavity).

Seedlings of 40 days old was planted five plants of each line in four-litter pots under greenhouse conditions following the regular agronomic management given to tomato crops (Ha, 2015). Was obtained twenty-one F_1 crosses using the methodology of Argerich & Gaviola's (1995).

Experiment initiation

Twenty-eight genotypes (progenitors and F1 crosses) was transplanted at open field after the seedlings developed three to four true leaves and reached 25 cm in height.

The experimental field belongs to UAAAN and it is located in Saltillo, Coahuila, at 25°21'18.74"N at 101°1'48.45" O, height 1,780 mamsl, 417 mm of annual rainfall; mean monthly temperature of 12 and18 °C and semi-arid climate (INEGI 2012).

Assessed variables

Four central plants was evaluated with full competition per treatment and a repetition, recording the values of the number of days until the first cut (DFC), equatorial diameter (ED), polar diameter (PD): five fruits per cut of each parent and crosses which were determined equatorial and polar diameter with the help using a vernier caliper gauge; number of fruits per plant (NFP) sum of fruits harvested in four cuts; average fruit weight (AFW): weight of the fruits of the central plants divided by the total number of fruits; fruit weight per plant (FWP) and yield (Y): the yield of fruit of four central plants was determined, extrapolated to tons per hectare (t ha^{-1}) considering a planting density of 33,300 plants per hectare.

Genetic model

In order to calculate the general and specific combining ability was used method 2 model I by Griffing (1956), including parents and direct crosses, with [p(p + 1)]/2 combinations. Was used the following analytical model for the combining ability: $X_{ijk} = \mu + g_i + g_j + s_{ij} + b_k + (gb)_{ijk} + 1/bc\sum\sum e_{ijk}$.

Where: X_{ijk} = observed phenotypic value; μ = general experimental mean;g_i y g_j = GCA effect of the parents, s_{ij} = SCA effect in crossesi x j ($s_{ij} = s_{ji}$);b_k = effect of k block; $(gb)_{ijk}$ = effect of the interaction between genotype ij and k block; 1/bc $\sum\sum e_{ijk}$ = residual effect of ijk.

Statistical analysis

Diallel-SAS procedure (Zhang & Kang, 2003) of the SAS Version 9.0 statistical program was used for the analysis of the genetic effects of GCA and SCA. Was also conducted a variance analysis and a mean comparison Tukey's test, p = 0.05 to assess the yield component variables.

The experimental unit included six plants in 2 m-long furrows, with 90 cm between furrows and 30 cm between plants, in a randomized block experimental design with three replicates.

RESULTS AND DISCUSSION

Diallel analysis

Found highly significant differences ($p \leq 0.01$) was observed in the genotypes, as well as in the GCA and SCA effects for all the assessed traits; except for Days to the First Harvest (Table 1). The variance contribution to the yield attributed to crosses included more non-additive effects (SCA) than additive effects (GCA) (67.3% y 32.7% respectively).The proportions for the effects of SCA and GCA were calculated based on the sum of squares with respect to the proportion they occupy when the crosses are partitioned into these effects.

Table 1. Mean squares of diallel analysis from seven parental tomato plants and their twenty-one crosses to assess their yield traits

Source of variation	Degrees of freedom	Days to first cut	Equatorial diameter (mm)	Polar diameter (mm)	Fruit average weight (g)	Number of fruits per plant	Fruit weight per plant (g)	Yield (t ha^{-1})
Repetitions	2	8.36	0.78	0.90	1,425.95	51.15	0.12	131.83
Genotypes	27	7.85	1.87**	0.63**	707.65**	110.74**	1.85**	2,024.44**
GCA	6	14.82	2.47**	1.29**	1,131.57**	121.24**	2.73**	2,984.59**
SCA	21	5.86	1.70**	0.45**	586.53**	107.73**	1.60**	1,750.11**
Error	54	11.66	0.25	0.12	169.48	15.22	0.14	160.06
Total	110	48.57	7.09	3.41	4,021.19	406.11	6.46	7,051.04
C.V.		4.17	8.85	6.71	14.85	15.82	17.58	17.60

*, ** significant at 5% and 1% level, respectively.

Statistical difference observed in the genotypes can be due to parental genetic diversity, leading to the identification of crosses with significant yields, which is indispensable to measure the traits' behavior through their hybrids. Therefore, the significance of these genotypes validates the mean square subdivision of the specific and the general combining abilities.

The importance of non-additive and additive genetic effects related to the equatorial diameter, polar diameter, average fruit weight, number of fruits per plant, fruit weight per plant and tomato yield traits was shown by the statistical difference as revealed in GCA and SCA effects. Akram et al. (2012), Saleem et al. (2013) and Aisyah et al. (2016) reported similar results by using different parents and environments.

Yield's variance contribution attributed to genotypes coming mostly from non-additive effects shows the relative importance of these effects in yield expression. The superiority of non-additive effects may be due to the dominance deviation variance, as genetic background of the resulting heterosis expression by the hybrid combination between parents with great genetic diversity. Souza et al. (2012) found high heterotic responses for fruit yield and the number of fruits per plant, with values up to 49.7% and 47.1%, respectively, in fifteen tomato genotypes at Itatiba, Sao Paulo, Brasil. Martínez et al. (2016) assessed the heterotic behavior of 40 crosses from 10 S5 lines from tomato collections. In terms of fruit yield, mean heterosis varied from 21.8 to 111.2%, while fruit average weight was 13 to 80.7%.

Non-additive type of effects were greater than additive effects for fruit weight per plant, number of fruits per plant and average fruit weight. These values are an important tool in the selection of the best breeding method. However, the strategy of breeding by selection, hybridization or, breeding by selection followed by hybridization, depends on the rate of additive variance and the dominance of the studied population, versus total genetic variation (Reyes et al., 2004).

Effects of general combining ability (GCA)

Genotypes D4, R1 and D3 showed the highest negative effects without significant differences in Days to the First Cut. On the other hand, IR13 and R1 showed the highest positive effects with highly significant differences ($p \leq 0.01$) for equatorial diameter. Regarding the number of fruits per plant, lines D4 and K3 showed the highest positive values, with significant differences. Line D4 had the highest positive effects and highly significant differences ($p \leq 0.01$) in terms of polar diameter, average fruit weight, fruit weight per plant, number of fruits per plant and yield (Table 2).

Table 2. Effects of the general combining ability (GCA) of seven tomato lines on yield traits

Genotypes	Assessed variables						
	Days to first cut	Equatorial diameter (mm)	Polar diameter (mm)	Average fruit weight (g)	Number of fruits per plant	Fruit weight per plant (g)	Yield (t ha^{-1})
K3	0.02	-0.30**	-0.11	-4.07	1.59*	0.07	2.49
R1	-0.31	0.18*	-0.06	0.25	-1.03	-0.06	-2.31
F3	0.61	-0.06	0.02	-3.10	-0.66	-0.15*	-5.18*
Y53	0.61	-0.10	-0.09**	3.77	-0.55	0.02	0.87
D4	-0.75	-0.31**	0.47**	11.39**	3.03**	0.54**	17.89**
D3	-1.05	0.04	-0.16	0.74	1.11	0.10	3.34
IR13	0.87	0.55**	-0.06	-8.97**	-3.48**	-0.51**	-17.10**

*, ** significant at 5% and 1% level, respectively.

The effects of general combining ability are a reflection of the parental ability to express a trait in their offspring. The values obtained for GCA effects show the parental potential to transfer some of their traits to their crosses. In this study, Line D4 seemed to be a very good source of germplasm for future breeding programs, due to the positive values found in the general combining ability related to polar diameter, average fruit weight, fruit weight per plant, number of fruits per plant and yield. López et al. (2012), Gabriel et al. (2013), Kumar et al. (2013) found high positive GCA values in yield variables of tomato genotypes. They selected the genotypes with the highest potential to start a breeding program.

Effects of the specific combining ability (SCA)

The effects of the specific combining ability represent deviations of the foreseen behavior according to a simple additive model; reflecting non-additive genetic effects.

Concerning the estimation of SCA (sij) effects on diallel crosses (Table 3), in days to the first cut, crosses K3×Y53 and R1×D4 showed the highest negative effects with values of -2.027 and -1.99 respectively, without significant differences. The results indicated that these hybrids are the earliest with less days to the first cut.

Hybrid F3×IR13 showed the highest positive values with highly significant differences ($p \leq 0.01$) on equatorial diameter and polar diameter variables. Crosses Y53×IR13, F3×D3 also had polar diameter values. Crosses K3×D4 and R1×Y53 had the highest positive values with highly significant differences ($p \leq 0.01$) in the number of fruits per plant and fruit weight per plant.

Crosses K3×D4, R1×Y53 and K3×D3 had the highest positive yield values (60.09, 43.54 and 18.97 respectively), in contrast with the highest negative values shown by crosses Y53×D3 and Y53×D4.

The specific combining ability reveals the best combination of crosses among genotypes for the development of hybrids having the desired traits. The results showed consistently good combinations that can express such traits.

Crosses with high yields can be the result of the additive effects of both parents, as well as the interaction of dominant alleles of one parent, with the recessive alleles of the other parent. Negative effects of the specific combining ability lead to superior parents to the development of inferior hybrids, or vice versa, due to complex interaction systems, in particular the supplementary systems, which are responsible for the expression of traits (Falconer, 1981).

Table 3. Effects of the specific combining ability (SCA) of twenty-one F1 tomato hybrids on yield traits

Crossing	Assessed Variables						
	Days to first cut	Equatorial diameter (mm)	Polar diameter (mm)	Average fruit weight (g)	Number of fruits per plant	Fruit weight per plant (g)	Yield (t ha^{-1})
K3×R1	0.56	0.29	0.10	11.66	-0.88	0.12	4.31
K3×F3	-0.69	0.42	0.14	5.80	-3.92	-0.21	-6.97
K3×Y53	-2.02	0.26	-0.10	-13.94	1.62	-0.24	-7.93
K3×D4	1.00	0.15	0.07	13.46	13.37**	1.82**	60.09**
K3×D3	1.30	0.50	0.09	15.91*	1.62	0.57**	18.97**
K3×1R13	-0.62	-0.44	0.27	4.38	-4.77*	-0.29	-9.84
R1×F3	-0.02	0.55*	0.16	7.24	2.03	0.35	11.56
R1×Y53	-1.69	0.54*	0.31	15.77*	9.25**	1.32**	43.54**
R1×D4	-1.99	0.21	0.07	-5.95	1.00	-0.11	-3.76
R1×D3	1.63	0.08	-0.14	-11.86	-3.07	-0.54**	-18.07**
R1×IR13	-0.28	-0.75**	0.40*	5.39	-2.48	-0.09	-3.04
F3×Y53	0.37	0.35	-0.14	0.20	6.22**	0.56**	18.62**
F3×D4	-1.25	-0.65**	-0.21	1.34	-1.37	-0.06	-2.09
F3×D3	-0.95	-0.26	0.54**	-25.94**	3.55	-0.47**	-15.75**
F3×IR13	1.78	0.62*	0.52**	-14.07	-1.18	-0.34	-11.49
Y53×D4	-0.25	0.58*	0.23	-0.40	-8.48**	-0.95**	-31.68**
Y53×D3	0.71	0.22	-0.10	-20.18**	-10.22**	-1.23**	-40.57**
Y53×IR13	-1.21	-1.28**	0.64**	10.93	-1.96	0.04	1.46
D4×D3	0.41	0.38	0.11	5.57	2.51	0.38*	12.77*
D4×IR13	-1.50	-1.37**	0.08	-16.12*	3.77	-0.15	-5.21
D3×IR13	-1.21	-0.48	-0.20	-3.09	10.03**	0.70**	23.11**

*, ** significant at 5% and 1% level, respectively.

Research on diallel crosses indicated that high yield simple crosses have at least one of the lines with high GCA (g_i) and the two lines produce high positive SCA (s_{ij}). On the other hand, simple crosses with low yield have both lines with low GCA and the two lines produce negative SCA effects (sij) of high absolute value (Reyes et al., 2004).

High SCA values in any variable indicate that hybridization will be the best method to make the best use of such variable. We can use genetic variance more efficiently by taking advantage of the dominance or epistatic effects. In that regard, Peña et al. (1998) affirm that when SCA is positive and shows a high value, both parents are good candidates to produce families, populations or lines in a breeding program, aiming towards specific targets.

Specific combining ability (SCA) is the manifestation of non-additive component of genetic variance and associated with interaction effects, which may be due to dominance and epistatic component of genetic variation that are non-fixable in nature (Basavaraj et al., 2015). Hence among 21 hybrids (Table 3) K3×D4, R1×Y53 and K3×D3 are identified as the good specific combiner with high per se yield.

Yield analysis

According to the results of this study, line Y53 had the highest yield value of 55 t ha^{-1} followed by D4 with 54.4 t ha^{-1} (Table 4). On the other hand, hybrids Y53×D4, F3×IR13 and Y53×D3 had the highest yield values, compared to the total number of assessed crosses, with 93.5, 69.8 and 68.6 t ha^{-1} respectively, and they were superior to the most productive parents (Y53, D4 y K3).

Table 4. Mean comparison of seven tomato lines and their 21 crosses for yield traits, using Tukey's test

	Assessed Variables			
Lines	Average fruit weight (g)	Number of fruits per plant	Fruit weight per plant (kg)	Yield (t ha^{-1})
K3	96.47 cd	17.33 eg	1.67 fh	53.50 eh
R1	111.47 ab	13.00 gh	1.45 fj	46.41 fk
F3	74.17 gj	21.00 ce	1.55 ei	49.81 ei
Y53	68.00 il	25.33 bc	1.72 dg	55.06 dg
D4	91.13 cf	18.67 ef	1.70 dh	54.41 dh
D3	95.97 dc	17.33 eg	1.66 eh	53.24 eh
IR13	16.70 a1	11.00 h	1.28 hl	41.09 hl
Crosses				
K3×R1	95.40 cd	18.33 ef	1.75 cf	55.97 cf
K3×F3	99.90 bc	17.33 eg	1.74 cg	55.59 cg
K3×Y53	93.29 ce	21.00 ce	1.96 be	62.70 be
K3×D4	70.10 hk	24.33 bd	1.71 dg	54.70 dg
K3×D3	64.17 jl	18.00 ef	1.15 il	36.97 il
K3×IR13	56.60 l	17.00 eg	0.96 l	30.78 l
R1×F3	121.06 a	12.00 h	1.45 fk	46.53 fk
R1×Y53	122.53 a	11.33 h	1.38 fk	44.41 fk
R1×D4	60.53 lk	18.33 fe	1.10 jl	35.29 jl
R1×D3	62.83 jl	18.33 fe	1.15 il	36.81 il
R1×IR13	116.80 a	11.33 h	1.33 hl	42.55 hl
F3×Y53	69.30 hl	20.33 de	1.41 fk	45.10 fk
F3×D4	96.47 cd	18.33 ef	1.76 cf	56.41 cf
F3×D3	81.87 eh	26.00 b	2.12 bd	67.73 bd
F3×IR13	80.20 fi	27.33 b	2.18 b	69.86 b
Y53×D4	85.23 dg	34.33 a	2.92 a	93.58 a
Y53×D3	77.67 gi	27.68 b	2.15 bc	68.69 bc
Y53×IR13	74.30 gj	25.68 b	1.91 be	61.14 be
D4×D3	77.33 gj	19.00 ef	1.43 fk	45.81 fk
D4×IR13	75.70 gj	19.33 e	1.46 fj	46.76 fj
D3×IR13	71.20 hk	14.68 fh	1.04 lk	33.25 kl

Different superscripts denote statistical significance at P = 0.05 by Tukey's post-hoc test.

Regarding the behavior of genotypes involved in fruit yield analysis, six genotypes exceeded the average tomato yield (56 t ha^{-1}) in Mexico. Chernet & Zibelo (2014) reported average yields of 56.07 t ha^{-1} in nine tomato varieties of Tigray, Ethiopia. In contrast, Nguyen & Nguyen (2015) reported 33.3 t ha^{-1} in Thai Nguyén, Vietnam.

With regard to these results Zewdie et al. (2000) mentioned that based on the ACG of parents can predict the contribution that each towards their progeny. This allows you to select plants that combine the superior characteristics of the parents, also predict the crosses with the greatest potential.

In this essay, one of the parental hybrid lines with the best yield, showed high GCA and high yield, meaning that genes with additive effects influenced the expression of this trait. It is worthwhile mentioning that this cross showed SCA negative values, despite having the best yield among assessed crosses. On the other hand, despite the cross with the best SCA was among high yield crosses, it was not the best among the assessed genotypes; may be because the parents had lower GCA values that affected yield.

Since both additive and non-additive variances were found to be important in the genetic control of all 4 yield component characters in the present study, the use of a population improvement method in the form of diallel selective mating (Jensen, 1970) or mass selection with concurrent random mating (Redden & Jensen, 1974) might lead to release of new varieties with higher yield in tomato (*Solanum lycopersicum* L.).

CONCLUSIONS

The estimations on general and specific combining abilities provided information on the breeding potential of seven tomato parents and their F1 crosses. These values represent an important tool in the selection of the breeding method. Genotypes D4, D3, K3 showed good yield potential and can be further utilized in future tomato-breeding program.

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REFERENCES

Agarwal, A., Sharma, U., Ranjan, R. & Nasim, M. 2017. Combining ability analysis for yield, quality, earliness, and yield-attributing traits in tomato. *International Journal of Vegetable Science* **23**(6), 1931–5260.

Aisyah, S.L., Wahyuni, S., Syukur, M. & Witono, J.R. 2016. The estimation of combining ability and heterosis effect for yield and yield components in tomato (*Solanum lycopersicum* Mill.) at lowland. *Ekin Journal of Crop Breeding and Genetics* **2**(1), 23–29.

Akram, F., Hossein, N., Hossein, A., Mirshamsi, K. & Navid, V. 2012. The estimate of combining ability and heterosis for yield and yield components in tomato (*Lycopersicon esculentum* Mill.). *Journal of Biodiversity and Environmental Sciences* **6**(17), 129–134.

Argerich, C.A. & Gaviola, J.C. 1995. Manual of production of horticultural seeds. Tomato. *Instituto Nacional de Tecnología Agropecuaria* ARG, 81 pp.

Basavaraj, L.B., Gasti, V.D., Rathod, V.D., Shantappa, T. & Shankarappa, K.S. 2015. Combining ability analysis for fruit yield and quality traits in tomato (*Solanum lycopersicum* L.). *Karnataka J. Agric. Sci*. **28**(4), 571–574.

Broem, A. & Miranda, G.V. 2005. Plant Breeding. *UFV, Vicosa*, pp. 525.

Chezhian, P., Babu, S. & Ganesan, J. 2000. Combining ability studies in eggplant (*Solanum melongena* L.). *Tropical Agricultural Research Journal* **12**, 394–397.

Chernet, S. & Zibelo, F. 2014. Evaluation of tomato varieties for fruit yield and yield components in western lowland of Tigray, Northern Ethiopia. *International Journal of Agricultural Research* **9**, 259–264.

Falconer, D.S. 1981. Introduction to quantitative genetics. 12ª impression. *Editorial CECSA*, MEX, 383 pp.

Fasahat, P., Rajabi, A., Rad, J.M. & Derera, J. 2016. Principles and utilization of combining ability in plant breeding. *Biometrics & Biostatistics International Journal* **4**(1), 00085.

Gabriel, J., López, E., Magne, J., Angulo, A., Luján, R., La Torre, J. & Crespo, M. 2013. Genetic basis of inheritance for morphological, agronomic and agro-industries characteristics in hybrid tomato *Solanum lycopersicum* L. (Mill). *Journal of the Selva Andina Biosphere* **4**, 37–46.

Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. *Australian Journal of Biological Sciences* **9**, 463–493.

Ha, T.M. 2015. Agronomic requirements and production methods of tomatoes in the red river delta of Vietnam. *Journal of Tropical Crop Science* **2**, 33–38.

INEGI (National Institute of Statistic and Geography). 2012. Statistical perspective Coahuila de Zaragoza. *National Institute of Statistic and Geography*, MEX, 98 pp.

Jensen, N.F. 1970. A diallel selective mating system for cereal breeding. *Crop Sciense* **10**, 629–635.

Jinks, J.L. & Hayman, B.I. 1953. The analysis of diallel crosses. *Maize Genetics Coop Newsletter* **27**, 48–54.

Kumar, R., Srivastava, K., Norang, P.S., Vasistha, N.K., Singh, R.K. & Singh, M.K. 2013. Combining ability analysis for yield and quality traits in tomato (*Solanum lycopersicum* L.). *Journal of Agricultural Science* **5**, 213–218.

López, B.A., Borrego, E.F., Zamora, V.V. & Guerra, Z.L. 2012. Estimation of general combining ability and specific combining ability in seven tomato (*Solanum lycopersicum* L.) lines. *Agraria* **9**(3), 87–95.

Martínez, V.E.A., Lobato, O.R., García, Z.J.J. & Reyes, L.D. 2016. Heterosis in crosses among bell pepper-shaped mexican native tomato (*Solanum lycopersicum* L.) lines and saladette type lines *Revista Fitotecnia Mexicana* **39**(1), 67–77.

Meena, R.K., Sanjay, K., Meena, M.L. & Shashank, V. 2017. Genetic variability, heritability and genetic advance for yield and quality attributes in tomato (*Solanum lycopersicum* L.). *Journal of Pharmacognosy and Phytochemistry* **7**, 1937–1939.

Nguyen, M.T. & Nguyen, T.M. 2015. Effect of plant density on growth and yield of tomato (*Solanum lycopersicum* L.) at Thai Nguyen, Vietnam. *International Journal of Plant & Soil Science* **7**(6), 357–361.

Peña, L.A., Molina, G.J.D., Cervantes, S.T., Márquez, S.F., Sahagún, C.J. & Ortiz, C.J. 1998. Heterosis between varieties of husk tomato (*Physalis ixocarpa* Brot.). *Revista Chapingo Serie Horticultura* **4**, 31–37.

Redden, R.J. & Jensen, N.F. 1974. Mass selection and mating system in cereals. *Crop Sciense* **14**, 345–350.

Reyes, L.D., Molina, G.J.D., Oropeza, R.M.A. & Moreno, P.E.C. 2004. Diallel crosses among maize inbred lines derived from the tuxpeño race. *Revista Fitotecnia Mexicana* **27**, 49–56.

SAGARPA-SIAP. 2014. Closure of agricultural production by crop. S*ecretariat of Agriculture, Livestock, Rural Development, Fisheries and Food-Agricultural and Fisheries Information Service*. Online document:http://www.siap.gob.mx/cierre-de-la-produccion-agricola-por-cultivo/.

Saleem, M.Y., Asghar, A., Iqbal, Q., Rahman, A.U. & Akram, M. 2013. Diallel analysis of yield and some yield components in tomato (*Solanum lycopersicum* L.). *Pakistan Journal of Botany* **45**(4), 1247–1250.

Souza, L.M., Paterniani, M.E., Melo, P.C.T. & Melo, A.M.T. 2012. Diallel cross among fresh market tomato inbreeding lines. *Horticultura Brasileira* **30**, 246–251.

Sprague, G. & Tatum, A. 1942. General vs specific combining ability in single crosses of corn. *Journal American Society Agronomy* **34**, 923–932.

Zewdie, Y., Bosland, P.W. & Steiner, R. 2000. Combining ability and heterosis for capsaicinoids in *Capsicum pubescens*. *Hort Sciencie* **36**, 1315–1317.

Zhang, Y. & Kang, M.S. 2003. Handbook of formulas and software for plant geneticists and breeders. Diallel-SAS: A program for Griffing´sdiallel methods. *Food products press and the haworth reference press*. NY, USA, pp. 1–99.

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Developing functional sterilised products technology using microwave-cooked semi-finished cod liver products

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Abstract. Cod liver which is extracted in significant amounts from fish during the process of gutting and harvesting by fishing vessels in the North Atlantic and Barents Sea is a very valuable raw material for producing 'natural' tinned foods that have not undergone any additional treatment and which do not have any additional ingredients, but its use is limited due to lipid instability in long-term storage in a frozen state. The authors advise that the production and use of pre-treated semi-finished products which are based on the use of cod liver is managed by means of a microwave-cooking process which results in a product that is more stable for frozen storage. Any semi-finished fish oil product that is extracted during microwave processing can also be used in food production.
The variants that can be included in using these semi-finished products are discussed here when it comes to the technology that is involved in functional multicomponent products (such as natural and paste tinned foods with the addition of sauces, vegetables, mushrooms, meat, and fish protein isolate).

Key words: tinned foods, cod liver, functional products, meat and fish products, polyunsaturated fatty acids.

Terms and abbreviations:
Generalised sensory score – a complex of sensory characteristics joined together into one value by considering their significance, and recalculated to percentage values according to (2);
MCSCL – Microwave cooked semi-finished cod liver product;
Full-grade protein – protein containing all essential amino acids in adequate quantities (the scores for all amino acid must be no less than 100%);
FPI – fish protein isolate;
PUFA – polyunsaturated fatty acids;
SCLO – Semi-finished cod liver oil product;
TG – triglycerides.

INTRODUCTION

Fish oils have a wide range of use both in terms of therapeutic and prophylactic purposes in the food industry, as well as in veterinary and medicinal fields. Fish oil is obtained during the processing either of fish liver (from the *Gadidae* family) or whole fish (herring, capelin, etc) (Rzhavskaya, 1976).

Fish oil is a rich source of ω-3 polyunsaturated fatty acids (PUFA), and fat-soluble vitamins (Petracci et al., 2009). It is known that fish oils from fishes which are caught in the northern seas are richer in valuable components than those from fish which live in warm water (Rzhavskaya, 1976).

Fatty acids of the ω-3 group have a very high biological value as well as therapeutic and prophylactic effectiveness against a series of diseases, especially cardio-vascular ones (Marventano et al., 2015). An optimum ratio between ω-3 and ω-6 is not absolutely clear at present, but nowadays researchers recommend increasing it to at least 1:4; some researchers refer to a range between 1:4 and 4:1 (Simopoulos, 2011).

Fish ('Food enrichment with Omega-3 fatty acids', 2013) and marine products (Patten et al., 2017) are a traditional and extremely well-known source of ω-3 PUFA.

Including PUFA (especially ω-3) in the composition of functional foods during their creation and production clearly holds a very high level of importance.

One of the main producers of the aforementioned valuable components is cod liver. The feature of its lipids is the high amount of PUFA (46.7–47.4%). The eicosapentaenoic (18.8–19.4%) and docosahexaenoic (19.1–19.6%) acids dominate amongst the polyenoic acids. Monounsaturated fatty acids form between 30–33% of cod liver oil, and almost half of them consist of oleic acid, while 25% are formed of palmitoleic (Konstantinova et al., 1997)

However, the hydrolytic and then oxidative processes occur even during long periods of frozen storage for products that are rich in ω-3 PUFA (Jacobsen, 2015), because free fatty acids are much less stable when it comes to oxidation than are triglycerides (TG).

It is possible to protect PUFA not only by storing the oil itself for a short time, but also by producing and storing the sterilised tinned foods from raw, chilled, or fresh-frozen cod liver (Gladyshev et al., 2009). It was experimentally proved that fatty acids are comparatively stable under high temperatures used during the sterilisation of tinned foods in the absence of oxygen (Karlsdottir, 2016). So producing delicious tinned cod liver is the most acceptable way of processing cod liver. One of the most popular tinned foods in the northern basin is 'natural cod liver', which can be produced from raw, chilled, or frozen liver.

The proportion of liver in the cod is known to be about 5% (Konstantinova et al., 1997). Considering that the total catch of cod in the northern basin alone is more than 500,000 tons, it is possible to calculate the total volume of extracted cod liver, which is around 25,000 tons. But it is impossible to immediately process this large volume of cod liver on the fishing vessels themselves or in the onshore factories, so the most part of caught liver goes to freezing. Thanks to this, the production of high quality tinned cod liver which comes from frozen raw materials is actually quite a demanding task, especially in light of increasing demand for such a product.

However, the frozen storage of cod liver results in the occurrence of negative processes which are related to lipid hydrolysis, followed by oxidation. It was noticed that the acid value significantly increases during frozen storage; moreover, the growth of peroxide value, the content of hydroxy acids, and aldehydes were all also noted. Sensory tests of natural tinned foods which came from frozen cod liver have confirmed a rancid taste and the dark colour of the product's surface due to oxidised lipids. Moreover, a significant quantity of free oil has been found in each tin which can result in quality being decreased. Another problem is related to any increase of the liquid content of the tinned foods (Volchenko et al., 2013).

It is possible to partially prevent such degradation in frozen cod liver using surface heating (blanching) before freezing, but the texture both of such semi-finished products and the resultant quality of tinned foods was poor (Grokhovsky & Volchenko, 2003). Therefore it is reasonable to use volume heating for this purpose.

It can be seen that the high quality of tinned foods that use such cod liver can only be provided by means of the utilisation of frozen storage at a temperature that is not higher than minus 18 °C and not for a period of more than one month. Therefore, the most valuable tinned foods that are in high demand can either be obtained from raw cod liver direct from the fishing vessels themselves, or from chilled cod liver with a storage time of four days, or from frozen liver with a very short storage time. All of this results in something of a headache for many producers, especially when taking into account the time it takes to ship frozen cod liver from possibly far-flung fishing areas to onshore canning facilities (Volchenko et al., 2013).

Tinned cod liver is traditionally consumed in small amounts due to its very high oil content (even despite the extremely high nutritional value of such oil), and the minimal content of protein and water with a complete absence of carbohydrates, so it is not a particularly good way of consuming it when it has been separated from the liver 'residue'. It can also be used as a raw material for producing a wide range of functional resources, mostly culinary (salads, fillings, etc). Despite its unique biochemical characteristics, tinned cod liver has a specific taste and flavour, one which serves to decrease the attractiveness of the product. So research that is directed towards the development of new forms of food items that can mask these specific characteristics are also of some importance.

All of these problems make it quite reasonable to conduct research that can be directed towards finding ways in which cod liver and its oil can be placed in frozen storage for extended periods of time, and for finding a rational use for this raw material when it comes to producing functional foods.

For that reason the goal of this research is to develop a form of technology that can be used in cod liver processing and which can preserve the quality of the cod liver in its frozen state for a longer period of time, and to find ways of using the obtained semi-finished products for the production of new, functional food items which have a high biological value.

MATERIALS AND METHODS

Preparing the raw materials

Blue whiting (*Micromestius poutassou*) was caught by the fishing company, JSC Murmansk Trawl Fleet OAO, in the central eastern Atlantic, subsequently being frozen and shipped to the port of Murmansk. It was stored at a temperature that was no higher than minus 18 °C. The vegetables, salt, and spices were purchased on the local market.

The chilled liver of Atlantic cod (*Gadus morhua*), which is shipped out by fishing companies all year round, was used as the source of the PUFA.

Producing fish protein isolates (FPI)

FPI was obtained by dissolving the muscle tissue of blue whiting in an alkaline solution, followed by sedimenting the protein in the isoelectric point and in a slightly acidic solution (Gholam, 2008). The temperature of the alkaline dissolution was 98 °C, while the pH level was 11.75. The isoelectric sedimentation was provided at a temperature of between 40–50 °C, while the pH was about five (the maximum for the sediment).

Choosing and preparing the reagents

All of the reagents that were used in this research were of an analytical grade (or higher). The standard samples of fatty acids were marked 'for chromatography'.

Analytical methods

The Kjeldahl method (FAO, 2003) uses Selecta Bloc Digest and Selecta Pro-Nitro modules (Spain). The nitrogen content that was obtained was recalculated to the raw protein value (*P*) using the following formula:

$$P = N \times K_P \quad (1)$$

where N – total nitrogen content; K_P – recalculation coefficient, and K_P = 6.25 for fish and some other products (Kondratyeva & Garkotina, 2014).

The lipid content in the samples of raw material, FPI, and foodstuffs which were produced from them (in the form of tinned foods) was determined by using a Selecta DET/GRAS extractor (Spain) by using the Soxhlet method (de Castro & Priego-Capote, 2010).

The fatty acid composition of lipids was determined by using the high-performance liquid chromatography method (HPLC), with an Agilent 1100 (USA) following the saponification of lipids with an alcohol solution of 2N KOH and pre-column derivatisation with bromophenacyl bromide and triethylamine (Gratzfeld-Huesgen, 1997).

The volume of secondary products from the lipid oxidation was estimated by means of the aldehydes content (by reaction with benzidine) which was recalculated to cinnamic aldehyde (Holm et al., 1957).

The acid value for lipids was determined after their extraction using a mixture of chloroform and ethyl alcohol by titration with 0.1M NaOH (phenolphthalein was used as an indicator). The peroxide value was determined by titration of the iodine displaced with peroxides from the potassium iodide with 0.01M sodium thiosulphate (Chakrabarty, 2003).

Sensory studies were carried out using the response scales (ISO 4121:2003) for the following characteristics: taste, odour, texture, state of product (large or small pieces, coarse or fine mince, etc), and colour. Tinned foods were checked for any absence of defects, and were opened and prepared prior to a sensory analysis being carried out. Every value was estimated using a five-point scale (between 1 to 5), considering the significance coefficients that were determined with the expert method. The result was a 'generalised sensory score' q, %, which was determined by the following formula:

$$q = \frac{\sum_{i=0}^{i=n}(B_i - B_{min}) \cdot K_{Si}}{\sum_{i=0}^{n} K_{Si} \cdot (B_{max} - B_{min})} \cdot 100 \quad (2)$$

where K_{Si} – the significance coefficient of i-th sensory value; B_i – the average mark by all tasters for the i-th sensory value; $B_{\min}$ – minimum possible mark from the scale (1); $B_{\max}$ – maximum possible mark from the scale (5); and n – number of sensory values in the scale.

Experimental planning and statistical data processing

The one-factor experimental design was also used. It contained five levels of factor variants. Statistical data processing (regression analysis) was carried out using an Oakdale DataFit 9.1. Common statistics were also used (involving a determination of confidence interval using the t criterion). All of the experiments were carried out in no less than three parallels.

Experimental order

The cod liver specimens were processed using a preliminary microwave treatment. The authors advise that such a treatment be used before freezing the cod liver in order to be able to prevent disadvantages arising – such as lipid oxidation during frozen storage and extra liquid oil from being collected in packaging tins – according to preliminary experiments. This method makes it possible to keep the partial integrity of the semi-finished product, and to make inactive lipolytic enzymes in the entire volume which would result in a slowing down of the hydrolytic and oxidative processes. It is also possible to select a lipid excess fraction following microwave cooking to be able to prevent any undesirable oil separation during the tinned food sterilisation process. Preliminary experiments have determined the most acceptable mode of microwave treatment for the liver, with a layer that is no thicker than 40 mm for 2.5 minutes at the specific power setting of 2000W kg^{-1} according to the inherent features of the microwave heating process (a thicker layer results in less uniform heating) and the sizes of cod liver being processed (less thickness requires additional cutting which results in a decreasingly impressive appearance). The microwave-cooked semi-finished cod liver product (abbreviated as MCSCL) was produced using this mode, and then was frozen and stored at a temperature of minus 18 °C in polymeric packaging for further research.

The tinned analogues of natural tinned cod liver were produced from the MCSCL. The sensory characteristics of this product have met the requirements that have been set out for the assortment of such tinned foods that are produced from chilled cod liver.

The semi-finished cod liver oil product (SCLO) which was separated during the microwave treatment was stored at a temperature that was no higher than plus 10 °C and for no more than a period of twelve months.

MCSCL (including frozen materials that were stored for a period of less than four months) was used in the production of tinned foods which are very similar to natural tinned cod liver in terms of composition and the method of production being used.

However, such a single-component form of production is not the only possible way in which processing can be handled, and is not even the most optimum way of doing it. Enriching such tinned foods with carbohydrates and proteins can be seen as being quite reasonable, by adding vegetables, mushrooms, tomato and sour-cream sauces, meat, and FPI.

The small assortment of tinned foods that are based on MCSCL with different sauces being added (sour-cream, and tomato and sour-cream), and with additional ingredients being added (champignons) was also developed. A new form of fish and vegetable tinned food was also developed which included minced MCSCL, vegetables (onion and carrot), champignons, and tomato and sour-cream sauce.

This brand new form of tinned food – tinned paste from cod liver and meat – has been developed during experimental research. The optimisation of its composition has been carried out during this research. Preliminary experiments proved that such tinned foods have excellent sensory characteristics despite the probably high cost of producing them. The optimum composition of such foods has been further developed following these preliminary experiments. The proportion of the main ingredients was around 60% despite different ratios being used. Other ingredients included vegetables (carrot and onion), and tomato and sour-cream sauce; their proportions have not been changed.

RESULTS AND DISCUSSION

Developing the technology involved in microwave-cooked, semi-finished cod liver products (MCSCL)

The hydrolytic and oxidative processes being used in the MCSCL have been researched. The objective characteristic of hydrolysis is the acid value, so it is this that has been determined in most experiments. Oxidative spoilage has been estimated by means of the content of the primary oxidation products (peroxides and hydroperoxides) and secondary products (aldehydes).

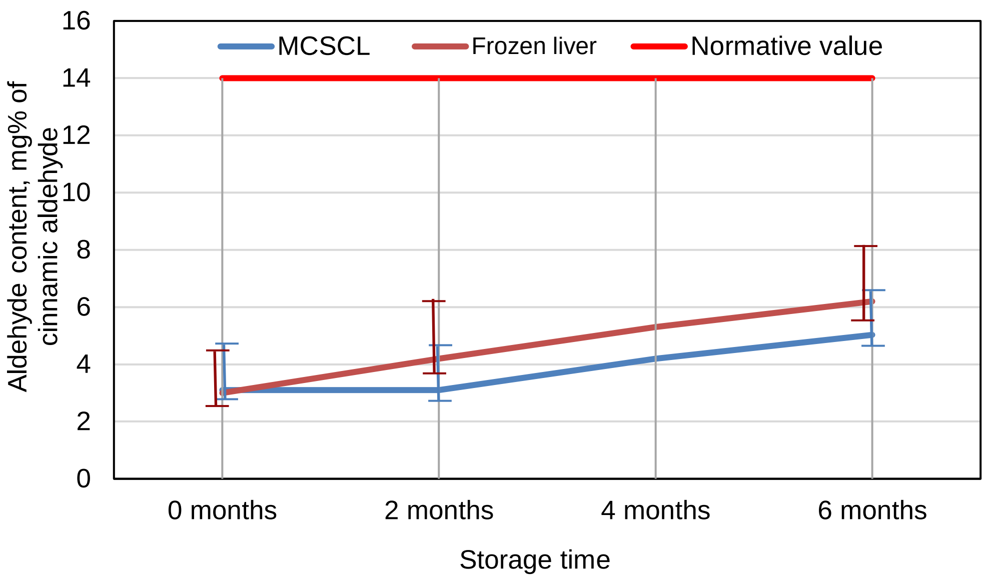
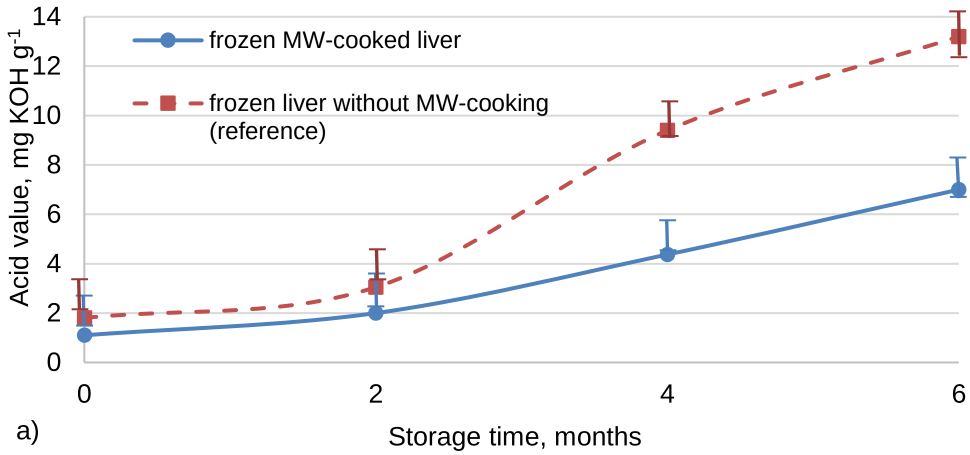


Figure 1. Dynamics for aldehyde content changes during the frozen storage of the cod liver.

The regression of aldehyde content on storage time was carried out. An equation for the total content of aldehydes was increasing by an insignificant amount during storage and did not exceed 7mg% of cinnamic aldehyde (Fig. 1), so the dynamics both for hydrolytic and primary oxidation products has been researched. The results for the comparative experiment in terms of storing frozen cod liver and MCSCL are shown in Fig. 2.



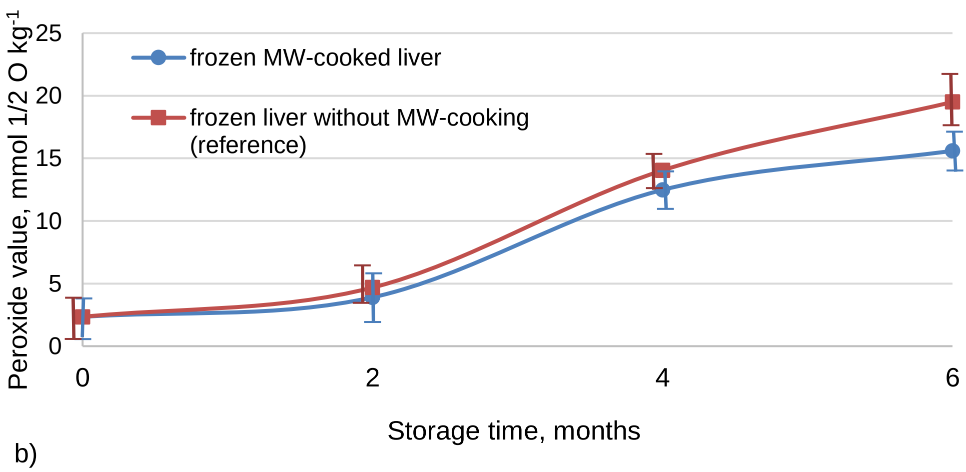


Figure 2. Dynamics for acid (a) and peroxide (b) values during frozen storage.

It can be seen that the use of the microwave method for cod liver treatment before being placed into frozen storage is rational in terms of slowing down the process of hydrolytic and oxidative spoilage in this raw material. Therefore the recommended shelf life at a temperature of no higher than 18 °C is no more than four months. A higher shelf life will result in undesirable acid values (6 mg KOH g and higher), the increase of the peroxide value (15 mmol 1/2O/kg and higher). This shelf life is also proven by microbiological tests (Volchenko et al., 2013), as shown on Fig. 3.

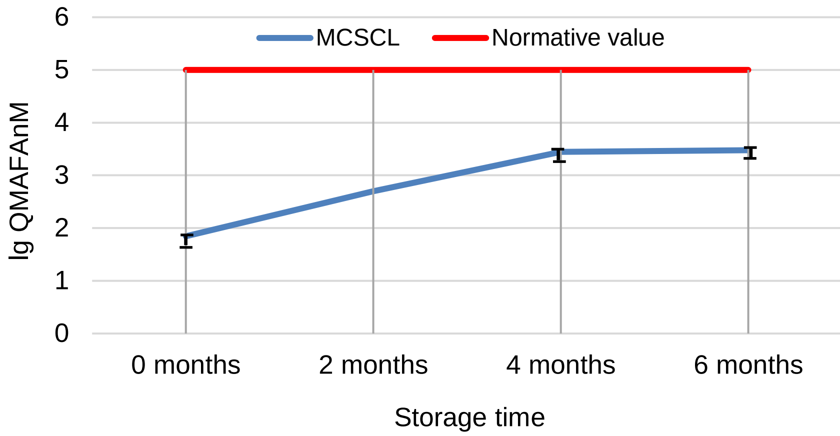


Figure 3. Dynamics of microbiological growth in the MCSCL.

Developing new forms of tinned foods with MCSCL

The experiment which involved optimisation has been carried out according to the plan shown in Table 1. The results of this experiment are shown in the same table.

It is reasonable to use the regression method to process the results of Table 1. The regression equation for quality level versus meat proportion is as follows:

Table 1. Plan for and results of experiments for optimising meat and cod liver ratios

Probe no	Meat (pork) proportion (of net mass), %	MCSCL proportion (of total mass), %	Generalised sensory score, %
1	20	40	93.9 ± 6.4
2	40	20	88.2 ± 7.7
3	30	30	86.1 ± 10.3
4	10	50	88.5 ± 8.5
5	25	35	92.6 ± 7.3

$$q = a \cdot x^3 + b \cdot x^2 + c \cdot x + d, \quad (3)$$

where a = 0.0038; b = -0.3; c = 7.0; and d = 45 – regression coefficients.

Analysis of the regression Eq. (3) shows that the best levels of quality can be found in the case of a meat proportion of 17.3% of the net mass of prepared tinned foods. Moreover, there is a clear trend for a new maximum to appear in which the proportion is above 35% meat content, but tinned foods of such a high meat content would be closer to meat pastes which are outside the scope of the current research.

It is reasonable to not just meat as raw materials, but also fish (in the form of FPI) because fish protein is more widely available on the market, while also being no worse – and often better – than meat protein.

The authors have developed FPI technology from the muscle tissue of blue whiting, which has protein that is full-grade in terms of its quality, meaning that it contains all of the amino acids (both essential and non-essential) that are required for the human organism (Derkach et al., 2017). The experiment has been carried out to determine the optimum composition of tinned paste which is produced from MCSCL with the addition of FPI. The plan for this experiment and the accompanying results are shown in Table 2.

The results of this experiment can be described by the same Eq. as (3), but with different regression coefficients: a = 0.0084; b = -0.443; c = 6.05; and d = 69.0.

The maximum generalised sensory score can be found in a 9.26% proportion of FPI, so it is reasonable to include between 9–10% of FPI in the composition.

Table 2. Plan for and results of experiments in optimising FPI and cod liver ratios

Probe no	FPI proportion (of net mass), %	MCSCL proportion (of total mass), %	Generalised sensory score, %
1	2	58	79 ± 10
2	5	55	90 ± 9
3	10	50	93 ± 8
4	20	40	80 ± 18
5	30	30	78 ± 16

Developing tinned food from SCLO

As SCLO is a by-product of the microwave technology that is involved in the production of MCSCL, it is reasonable to develop a complex of technologies for making use of it such as, for example, in tinned foods by enriching them with fatty acids which include PUFA. New groups of compositions in multicomponent tinned foods with SCLO have been developed for this very purpose. One such group includes tinned pastes that are based upon vegetable ingredients (carrot), and SCLO. The one-factor experiment for estimating the maximum acceptable proportion of SCLO in the product has been carried out in order to be able to optimise composition. The plan for and results of this experiment are shown in Table 3.

Table 3. Plan for and results of experiments for optimising SCLO proportions

Probe no	Carrot proportion (of net mass), %	SCLO proportion (of total mass), %	Generalised sensory score, %	Proportion of free liquid in the tin
1	35	35	53 ± 19	10.6
2	40	30	51 ± 25	8.9
3	50	20	58 ± 22	3.5
4	55	15	61 ± 22	1.3
5	60	10	67 ± 13	0.35

Data processing results are shown for the following regression equation for a generalised sensory score (q) versus SCLO proportion (x):

$$q = a + b \cdot x^2 + c \cdot x^3, \qquad (4)$$

where a = 71.8; b = -0.065; and c = 0.0014 – regression coefficients.

The equation adequately describes the generalised sensory score changes; regression coefficients are significant with a confidence level of 0.9 (higher confidence levels are not required for sensory tests as subjective methods).

The regression equation for the proportion of the free liquid part (ω_O) versus SCLO proportion is as follows:

$$\omega_O = d \cdot x^3 + f \cdot x^2 + g \cdot x + h \qquad (5)$$

where $d = -9.7 \cdot 10^{-4}$; f = 0.0694; g = -1.09; and h = 5.25 – regression coefficients.

The equation adequately describes the free liquid part's proportion changes; regression coefficients are significant with a confidence level of 0.9.

Therefore the acceptable quality of the product can be achieved in the case of an SCLO proportion that is no higher than 10%. A more precise choice of SCLO dosage value should be taken by means of objective reasoning. It is obvious that the free liquid part is practically absent if the SCLO proportion is less than 10%.

Characteristics of the finished products

The quality estimation for single and multicomponent tinned foods based on (or with the use of) MCSCL have been carried out. They include:

– 'Blanched cod liver' – single-compound MCSCL-based tinned foods using the technology involved in the processing of natural tinned cod liver;

– 'Liver and carrot paste' – multicomponent minced MCSCL-based tinned foods with the addition of carrot, tomato, and sour-cream sauce;

– 'Liver and mushroom paste' – multicomponent minced MCSCL-based tinned foods with the addition of champignons, carrot, tomato, and sour-cream sauce;

– 'Vegetable and mushroom paste' – multicomponent tinned foods which employ the use of minced cabbage, champignons, carrot, tomato, and sour-cream sauce with the addition of SCLO;

– 'Carrot and mushroom paste' – multicomponent tinned foods which employ the use of minced champignons, carrot, tomato, and sour-cream sauce with the addition of SCLO;

– 'Cod liver and meat paste' – multicomponent minced MCSCL-based tinned foods, with non-fatty pork, tomato, and sour-cream sauce;

– 'Cod liver and protein paste' – multicomponent minced MCSCL-based tinned foods with the addition of FPI, tomato, and sour-cream sauce.

Figs 4–5 show the profiles for the sensory evaluation of the tinned foods in question.

An analysis of the data shown in Fig. 4 shows that tinned foods that are produced using chilled cod liver (reference) are almost the same as tinned foods that are produced using MCSCL, according to characteristics such as oil colour, taste, and aroma. Such characteristics as surface colour, texture, and product state (which are not as important as the previous characteristics) are insignificantly higher for reference.

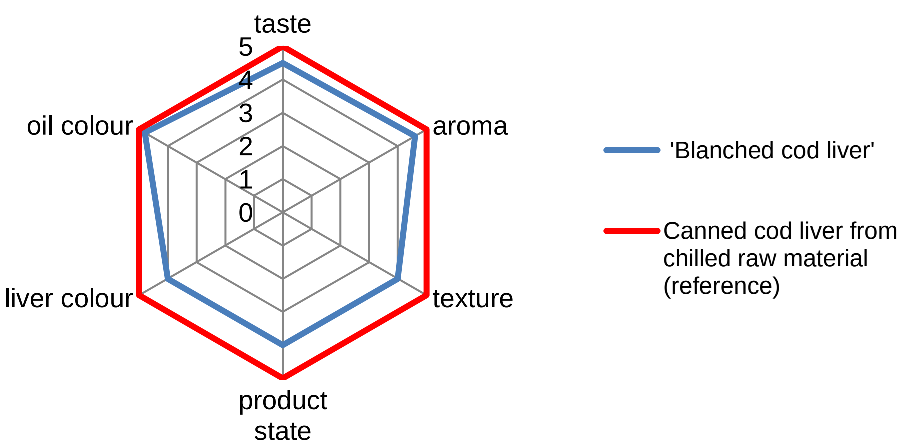


Figure 4. Comparative sensory evaluation of traditional tinned foods and MCSL-based tinned foods.

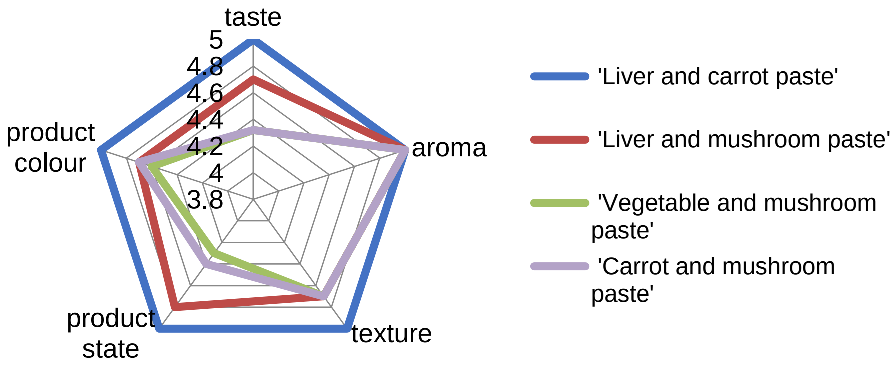


Figure 5. Comparative sensory evaluation of multicomponent MCSCL-based tinned foods, and with SCLO added.

When comparing the sensory characteristics of tinned pastes that are produced according to different recipes, it is possible to conclude that 'liver and carrot paste' dominates all other pastes.

Table 4 contains the separate characteristics of the chemical composition of the different tinned foods.

Table 4. Food value for tinned foods that contain MCSCL or SCLO

Tinned food	Content,%		Energy value, kcal
	Lipids	Proteins	
'Blanched cod liver'	63	5.0	587
'Liver and carrot paste'	11.6	4.5	122
'Liver and mushroom paste'	8.5	5.1	97
'Vegetable and mushroom paste'	14.0	6.2	151
'Carrot and mushroom paste'	13.7	5.5	146
'Cod liver and meat paste'	52.8	6.5	501
'Cod liver and protein paste'	56.1	7.4	535

The composition of fatty acids (Table 5) for semi-finished cod liver oil products (for comparison) and those for separate tinned foods has been determined in order to be able to evaluate the biological value of the selected tinned foods.

The results in this table show that tinned foods are rich in ω-3-PUFA, so MCSCL and SCLO can improve the ratios of ω-3:ω-6 in humans (which figures are considered to be increased to at least 1:4). Increasing the proportion of linoleic acid and decreasing the ω-3:ω-6 ratio can be explained by frying the ingredients with vegetable oil.

Physical, chemical, biochemical, and microbiological experiments have shown that tinned foods which are produced using MCSCL and SCLO can be characterised not only by good sensory marks, but also with high food and biological values.

Table 5. The composition of fatty acids

Fatty acid	Content, % from the total of fatty acids			
	SCLO	'Blanched cod liver'	'Vegetable and mushroom paste'	'Liver and mushroom paste'
Saturated, including:	14.65	16.02	16.0	14.47
Lauric	0.02	0.02	0.14	0.03
Tridecanoic	0.15	0.02	0.02	0.01
Myristic	3.06	2.93	3.06	2.98
Pentadecanoic	0.785	0.73	0.71	0.63
Palmitic	7.825	8.82	8.38	7.91
Margarine	0.825	0.86	0.75	0.62
Stearic	2.13	2.64	2.93	2.285
Monounsaturated, including:	54.52	51.26	50.2	53.47
Myristoleic	0.095	0.1	0.12	0.09
Palmitoleic	7.81	7.26	6.6	7.575
Heptadecenoic	0.555	0.6	0.44	0.445
Oleic	23.89	25.3	24.33	23.91
Gadoleic	15.345	12.38	13.04	14.93
Erucic	6.815	5.62	5.67	6.52
PUFA, including	30.83	32.72	33.8	32.06
Hexadecadienoic	0.51	0.38	0.42	0.54
Hexadecatrienoic	0.365	0.27	0.31	0.29
Linoleic	2.4	2.45	10.0	9.17
Linolenic	3.22	3.59	2.73	2.62
Octadecatetraenic	0.25	0.25	0.2	0.17
Eicosadienoic	0.27	0.27	0.21	0.21
Eicosatrienoic	0.675	0.62	0.54	0.54
Arachidonic	0.29	0.31	0.23	0.23
Eicosapentaenoic	9.17	9.81	7.65	7.75
Docosatetraenoic	0.445	0.39	0.37	0.39
Docosapentaenoic	1.075	0.81	0.8	0.82
Docosahexaenoic	12.165	13.57	10.34	9.33
Saturated to PUFA ratio	0.475	0.490	0.473	0.451
PUFA ω-3: ω-6 ratio (estimated)	3.88	4.57	1.47	1.47

CONCLUSIONS

The actuality and necessity of using very valuable raw materials – cod liver with lipids that are rich in ω-3 and ω-6 PUFA – can now be shown to be proven.

The technology involved in processing cod liver using microwave heating has already been developed, which made it possible to protect the quality of the produced semi-finished product in its frozen state over a long period of time.

The technology involved in microwave cooking for cod liver oil has also previously been developed. This technology made it possible to store the frozen MCSCL for a long period of time.

The most acceptable mode involved in microwave treatment of the cod liver has been obtained (the layer need be no thicker than 40mm, cooking time is 2.5 minutes, and specific power is 2,000W kg^{-1}).

The maximum shelf life for frozen MCSCL is recommended to be up to four months according to microbiological tests and lipid quality value changes during storage.

The available assortment of single-component sterilised tinned cod liver is extended by using MCSCL.

The series of new multicomponent functional tinned foods that can be based upon MCSCL and fish oil which is separated during production has been developed. These products contain ω-3 and ω-6 PUFA. Vegetables, mushrooms, lean pork, and FPI were also used in the production of such foodstuffs.

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REFERENCES

Chakrabarty, M.M. 2003. Chemistry and Technology of Oils and Fats. Allied Publishers Pvt. Ltd ISBN: 81-7764-495-5

Derkach, S.R., Grokhovsky, V.A., Kuranova, L.K. & Volchenko, V.I. 2017. Nutrient analysis of underutilized fish species for the production of protein food. *Foods and Raw Materials* **5**(2), 15–23.

de Castro, L. & Priego-Capote, F. 2010. Soxhlet extraction: Past and present panacea. *Journal of Chromatography A* **1217**, 2383–2389.

Food and Agriculture Organization of the United Nations (FAO). Food energy – methods of analysis and conversion factors. 2003. Available at: ftp://ftp.fao.org/docrep/fao/006/y5022e/y5022e00.pdf

Food Enrichment with Omega-3 Fatty Acids. 2013. Wood head Publishing, ISBN: 978-0-85709-428-5

Gholam, R.S. 2008. Evaluation and utilisation of fish protein isolate products. *MSc thesis* in food science. Reykjavik, 181 pp. Available at https://www.yumpu.com/en/document/read/50360955/evaluation-and-utilisation-of-fish-protein-isolate-products (last access 23.02.2019)

Gladyshev, M.I., Sushchik, N.N., Makhutova, O.N. & Kalachova, G.S. 2009. Content of essential polyunsaturated fatty acids in three canned fish species. *International Journal of Food Sciences and Nutrition* **60**(3), 224–230.

Gratzfeld-Huesgen, A. 1997. Analysis of hydrolyzed fatty acids in dietary fat using HPLC. *Agilent technologies*, Publication number 5966–0635E.

Grokhovsky, V.A. & Volchenko, V.I. 2003. Canned foods from frozen semi-finished liver. *Fisheries* **4**, 54–56 (in Russian).

Holm, U., Ekbom, K. & Wode, G. 1957. Determination of the extent of oxidation of fats. *Journal of American Oil Chemists Society* **34**, 606–609.

ISO 4121:2003 *Sensory analysis - Guidelines for the use of quantitative response scales*. ISO TC 34/ SC 12, Buenos Aires. 9 pp.

Jacobsen, C. 2015. Some strategies for the stabilization of long chain n-3 PUFA enriched foods: A review. *European Journal of Lipid Science and Technology* **117**(11), 1853–1866.

Karlsdottir, M., Arason, S., Thorarinsdottir, K., Nguyen, M.V. & Kristinsson, H. 2016. Lipid Degradation of Cod Liver During Frozen Storage as Influenced by Temperature, Packaging

Method, and Seasonal Variation. *Journal of Aquatic Food Product Technology* **25**(6), 802–810.
Kondratyeva, V.A. & Garkotina, V.H. 2014. *Report of validation/verification tests. Method of determining of total nitrogen, protein and raw protein content*. FGBU 'LMVL', St.Petersburg, 30 pp. (in Russian).
Konstantinova, L.L., Dvinin, Yu.F., Lyebskaya, T.K. & Kuzmina, V.I. 1997. *Technochemical characteristics of commercial fishes of the Northern Atlantic and surrounding seas*. PINRO, Murmansk, 183 pp. (in Russian).
Marventano, S., Kolacz, P., Castellano, S., Galvano, F., Buscemi, S., Mistretta, A. & Grosso, G. 2015. A review of recent evidence in human studies of n-3 and n-6 PUFA intake on cardiovascular disease, cancer, and depressive disorders: does the ratio really matter? *International Journal of Food Sciences and Nutrition* **66**(6), 611–622.
Patten, G.S., Sanguansri, L., Augustin, M.A., Abeywardena, M.Y., Bird, A.R., Patch, C.S. & Belobrajdic, D.P. 2017. Microencapsulated krill and tuna oil blend raises plasma long-chain n-3 polyunsaturated fatty acid levels compared to tuna oil with similar increases in ileal contractility in rats. *International Journal of Food Sciences and Nutrition* **68**(2), 201–209.
Petracci, M., Bianchi, M. & Cavani, C. 2009. Development of Rabbit Meat Products Fortified With n-3 Polyunsaturated Fatty Acids. *Nutrients* **1**(2), 111–118.
Rzhavskaya, F.M. 1976. *Oils of fishes and marine mammals*. Pishchevaya Promyshlennost' (Food Industry) Moscow, 470 pp. (in Russian).
Simopoulos, A.P. 2011. Evolutionary Aspects of Diet: The Omega-6/Omega-3 Ratio and the Brain. *Molecular Neurobiology* **44**, 203–215.
Volchenko, V.I., Kuranova, L.K. & Shveikina, K.S. 2013. Researching the influence of the storage time of blanched semi-product on the quality of the canned cod liver. In: *Science and Education: Materials of International Scientific and Practical Conference*. MSTU, Murmansk, pp. 1118–1120 (in Russian).

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Impact of rootstock on heavy metal bioaccumulation in apple plant grown near an industrial source in Obiliq, Kosovo

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Abstract. Food exposure to heavy metals such as Pb, Cd, Cr, Ni, As, Zn, Cu and Fe is considered a risk to human health. This study analyzes the level of heavy metals in soil and delicious apple tissues (fruit, leaf, shoot) in three different rootstocks: mm106, m26 and m9 grown in the Obiliq region (considered as a polluted region). The data obtained from the Obiliq areas are compared with those grown in reference clear area. Individual soil samples were collected from each plant to assess metal content in the immediate plant environment. Samples of soil, fruit, leaf and shoot have been analyzed for heavy metals (Pb, Cd, Ni, As, Zn, Cu, Cr and Fe) using atomic absorption spectrophotometry (AAS).
The results indicated that the average concentrations of Pb, Cd, Ni, As, Zn, Cu, Cr and Fe in soil of Obiliq areas were 2.03, 0.15, 6.99, 12.4, nd, 12.3, 4.68, 5.32 mg kg^{-1} d.w. respectively. The concentration of metals in the apple tissue increased with the increase of heavy metals in soil from polluted area. The accumulation ratios of heavy metals were calculated to assess the potential health risks. The mean concentrations of the heavy metals in the soil were in order of magnitude Ni > Zn > Cr > Cu > Fe > Pb > Cd > As while that in the fruits of apple were in order of magnitude Cr > Fe > Cu > Ni > Pb > Zn > Cd > As; in the leaves were Fe > Zn > Cu > Cr > Pb > Ni > Cd > As; in shoots were Zn > Fe > Cu > Pb > Ni > Cr > Cd > As.
Mobility of heavy metals and potentially hazardous in studied lands threatens the quality of apple fruit consumption, with a real risk that these elements (Cd, Pb, Ni and Cr) can enter the food chain.

Key words: pollution areas, apple, rootstock, accumulation, fruit consumption safety.

INTRODUCTION

Metals are present in the soil crust at different levels. Extraction of fuels, metallurgical minerals, municipal waste and excessive use of pesticides and fertilizers has affected accelerated release of metals and metals into various components of ecosystems (Arya & Roy, 2011). High concentrations of metals, either of natural origin or of anthropogenic activity, can become toxic to soil microflora. The toxicity of heavy metals depends on the type of metal element and its bioavailability on the soil. Some metals such as Zn, Cu are essential for plants with low concentrations, but become toxic in increasing concentrations, while some metals such as Pb, Cd, Cr have never been shown to be essential to the development of living organisms and are toxic even at very low concentrations (Todeschini et al., 2011).

Regardless of the source of heavy metals on the ground; higher concentrations of some metals degrade soil quality and reduce crop production, including poor quality products, pose risks to ecosystems and human and animal health (Blaylock & Huang, 2000). Heavy metals into the plant through the intake of water, which are then consumed by animals. The ingestion of these cplant and animal products is the main source of accumulation of heavy metals in humans because they are barely metabolised. Different plants exhibit different tolerance for heavy metals collection (Yang & Chu, 2011). In most cases, heavy metals are not easily absorbed from the ground because they have low mobility on the ground. Their absorption from the plant depends on pH, organic substances, water content, metal content and other elements in the rhizosphere.

Mining operation, grinding, concentrating ores and disposal of tailings, provide obvious sources of contamination in the environment, along with mine and mill wastewater. This will lead to the release and migration of heavy metals thus cause heavy metals pollution of soil near the mining area. Heavy metals can spread from soil to other ecosystem components, such as groundwater, plants, thus affecting human health through drinking water and food chain, so the evaluation of heavy metal pollution in soil is very important (Adamsa et al., 2004). Native plants are good indicators of ambient air quality and its growth. Plants absorb airborne anthropogenic pollutants, and their chemical composition may be a good indicator for contaminated areas when it is assessed against background values obtained for unpolluted vegetation. Optimum quantity of Mn, Zn, Ni, Cu and other elements is the main precondition for the proper growth and development of plants. However, high concentrations of these elements can have a negative and toxic effect on plants (Cairns, 1980). Seasonal fruits represent a source of nutrients and can contain toxic elements as well, which can cause the appearance of some chronic diseases in humans. Emission from anthropogenic pollution sources increases the concentration of pollutants in the environment, which poses a potential threat to fruit grown in polluted areas (Müller & Anke, 1994; Ramadan & Al-Ashkar, 2007). Consumption of contaminated food with heavy metals can be the cause of the reduction of immune defense and the high prevalence of the appearance of gastrointestinal cancer (Turkdogan et al., 2003).

This study analysed the heavy metal pollution in soil and apple (malus sp.) tissue of Obiliq area in Kosovo. The purpose of this research was to determine the distribution of selected heavy metals (Pb, Cd, As, Ni, Zn, Cu, Cr and Fe) in different fractional parts of agricultural land and apple tissues. This study was conducted in order to better understand the chemical fractionation and mobility of heavy metals selected in agricultural lands and their transfer to the food chain through apple fruit widely.

MATERIALS AND METHODS

Description of the Study area

Coal and lignite-mining, coal burning, industry, a nearby thermal power plant ‘Kosova’, traffic, and farming left ecotoxicological burdens in Kosovo industrial – rural region of Obiliq municipality, and surrounding regions. The newest data shows that lignite resources in Kosovo reach 15 billion tons (Rizaj et al., 2008). Lignite is the most important energy resource in Kosovo, providing about 87% of electric energy production in two thermo-power plants (6 units with 1,470 MW installed capacity). The discharges of liquid organic waste (measured as chemical oxygen demand, COD), from the industrial thermo-power complex in Obiliq, 5–10 tons per day, are, however, largely exceeded by the discharges of untreated urban sewage from the city of Prishtina: up to 700 tons per day. In addition to the huge modification of the landscape due to the open-cast mining and large dumps for overburden and solid wastes (ash and sludge), the Obiliq industries emit important quantities of air pollutants (dust, sulphur dioxide, and nitrogen oxides), and cause considerable pollution.

Figure 1. Area in this study – Obiliq, Kosovo.

Sample collection

Individual soil samples were collected from each plant to assess metal content in the immediate plant environment. Samples of fruit, leaf, shoot and soil have been analysed for eight heavy metals (Pb, Cd, As, Ni, Zn, Cu, Cr and Fe). Twenty soil samples and 90 apple samples were collected in the Obiliq region and reference area during September–November 2017 (Fig. 1). Three kinds of apple tree tissues were collected during the harvest period, including fruit, leaf and shoot. To reduce the effect of other agro environmental factors on the issue analysed, the experiment included a row of fruit trees with the same age (6 years), grafted on the same rootstock (mm106, m26, m9). Soil samples were taken from the surface layer (0–20 cm). All samples were sealed in polyethylene bags and transported to the laboratory within 6 h of collection. The soil samples were air-dried at room temperature, with impurities manually removed. Then, the soils were ground and sieved through 80 meshes (0.2 mm). For all samples, the decay and withered tissues were removed and the edible parts were washed with tap water to remove surface dirt. The edible parts of fruits or leaf were repeatedly rinsed with deionised water and dried at 60 °C to a constant weight.

Sample analysis

For the research needs of the metal content: Lead (Pb), Cadmium (Cd), Chromium (Cr), Nickel (Ni), Arsenic (As), Zinc (Zn), Copper (Cu), Iron (Fe) field samples were obtained in accordance with the respective protocols and consisted in sampling soil from apple planted surfaces within the respective localities. Samples received are labelled with all relevant data (locality, sampling date and other notes). Concentration of metals in soil samples was determined by the atomic absorption spectroscopy (AAS) of the Perkin-Elmer model 1200 mark. Work samples (2.0 gr of soil sample) were treated with a 1:3 aqueous regia mixture (4 mL HNO3 + 12 mL of concentrated HCl) in an electrical reso at a temperature of 200 °C for 60 minutes. Prior to mineralization with aqua regia, the organic matter was disintegrated with hydrogen peroxide concentrated (35% H_2O_2). Then the mineralized samples were mixed with distilled water and filtered with Whatman 0.45 μm filtration paper. The filter is placed on a volumetric balloon of 50 cm^3 and is levelled up to the mark with distilled water. Such samples are read with AAS and spectrophotometer. The AAS calibration is done with the standard reference material of 1,000 ppm (mg kg^{-1}) from which the respective metal standards are prepared.

Statistical analysis

The data were statistically analyzed using GraphPad Prism – version 7.05 and Microsoft Excel 2010 computer package. The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Concentrations of heavy metals Pb, Cd, Cr, Ni, As, Zn, Cu and Fe in the apple tissues varies depending on the type of rootstock. Table 1 shows the distribution values of the heavy metals in soils and the analyzed tissues (fruit, leaves and shoots) of the apple sp. with rootstock mm106, m26, m9 in the region of Obiliq. The average content in plants of each studied heavy metal was compared with the content of the same metal in the plants collected from the reference area, Table 2. Accumulation and distribution of heavy metals on soil depends on many different factors such as: chemical form of elements, pH, organic matter content, etc. Ph values in all analyzed soil samples were distributed from 5.8 to 7.5.

According to our results, the highest and lowest values of heavy metal accumulation in apple plant fruit with mm106 rootstock varied between 0.32–3.52 mg kg^{-1} d.w for Pb; 0.04–0.85 mg kg^{-1} d.w for Cd; 1.32–10.7 mg kg^{-1} d.w for Cr; 0.22–5.36 mg kg^{-1} d.w for Ni; **nd** mg kg^{-1} d.w for As; 0.39–3.06 mg kg^{-1} d.w for Zn; 0.54–5.35 mg kg^{-1} d.w for Cu; 1.27–7.32 mg kg^{-1} d.w for Fe.

Apple plant fruit with m26 rootstock varied between 0.27–4.16 mg kg^{-1} d.w for Pb; 0.04–1.03 mg kg^{-1} d.w for Cd; 1.42–11.1 mg kg^{-1} d.w for Cr; 0.36–4.75 mg kg^{-1} d.w for Ni; **nd** mg kg^{-1} d.w for As; 0.14–2.99 mg kg^{-1} d.w for Zn; 0.52–3.65 mg kg^{-1} d.w for Cu; 2.33–9.36 mg kg^{-1} d.w for Fe.

Apple plant fruit with m9 rootstock varied between 0.15–3.06 mg kg^{-1} d.w for Pb; 0.03–0.42 mg kg^{-1} d.w for Cd; 1.41–9.36 mg kg^{-1} d.w for Cr; 0.11–2.74 mg kg^{-1} d.w for Ni; **nd** – mg kg^{-1} d.w for As; 0.13–2.78 mg kg^{-1} d.w for Zn; 0.32–4.05 mg kg^{-1} d.w for Cu; 0.74–8.56 mg kg^{-1} d.w for Fe.

The results of the present study show that Pb, Cd and Fe were accumulated in the largest quantity of m26 rootstock, while Cr, Ni, Zn and Cu accumulated more in mm106 rootstock (Table 1). The m9 rootstocks have been found to be the lowest values of heavy elements compared to mm106 and m27. At the reference site, the amount of the heavy metals concentration was the lowest and the pollution rate was also very low (Table 2).

While significant differences in concentrations of metals in plant tissues mean that different apple rootstock had different abilities and capacities to take up and accumulate different metals. The average values of Pb, Cd, Cr, Ni, As, Zn, Cu, Fe in apple plant tissues with different rootstock in the Obiliq area are given in Figs 2, 3, 4.

Therefore, to determine the amount of accumulation of heavy metals in selected plant tissues, the transfer factor (TF) was determined. TF is an index for estimating the possible transfer of a metal from soil to plants versus the ability of fruit trees to accumulate a particular metal with respect to its concentration on the substrate of the earth (Adamsa et al., 2004).

Table 1. Heavy metal concentration (mg kg^{-1}) in soil, shoot, leaf and fruit of apple species depending on rootstocks type from Obiliq region

			Heavy metals (mg kg^{-1})							
Rootstock	Tissues	Level	Pb	Cd	Cr	Ni	As	Zn	Cu	Fe
M106	Shoot	Mean	5.19	0.18	1.46	2.53	0.01	52.1	7.39	19.4
		SD (±)	3.13	0.31	0.63	1.69	0.01	8.94	3.01	7.09
		CV (%)	60.3	172.2	43.1	66.7	100	17.1	40.7	36.5
	Leaf	Mean	2.21	0.01	3.03	0.71	0.05	46.1	11.8	51.1
		SD (±)	1.26	0.01	0.93	0.81	0.07	10.4	6.25	7.33
		CV (%)	57.1	100	30.6	114.1	140	22.5	52.9	14.3
	Fruit	Mean	1.41	0.23	5.47	1.96	Nd	1.49	2.54	4.31
		SD (±)	0.99	0.26	3.16	1.61		0.94	1.59	1.97
		CV (%)	70.2	113.1	57.7	82.1		63.1	62.5	45.7
M26	Shoot	Mean	3.98	0.13	1.35	1.74	0.01	43.1	9.11	33.3
		SD (±)	2.32	0.11	0.46	0.77	0.01	9.02	5.79	10.4
		CV (%)	58.3	84.6	34.1	44.2	100	20.9	63.5	31.2
	Leaf	Mean	2.47	0.01	2.63	0.44	0.02	24.2	15.2	63.1
		SD (±)	1.53	0.02	1.11	0.31	0.02	6.72	4.99	8.11
		CV (%)	40.4	200	42.2	70.4	100	27.7	32.8	12.8
	Fruit	Mean	1.56	0.29	3.95	1.36	Nd	1.22	2.36	5.02
		SD (±)	1.09	0.33	3.08	1.32		1.14	0.97	2.16
		CV (%)	69.8	113.7	77.9	97.1		93.4	41.1	43.1
M9	Shoot	Mean	3.49	0.09	0.94	2.29	Nd	39.2	8.83	28.1
		SD (±)	2.01	0.09	0.32	1.09		8.42	4.48	6.35
		CV (%)	57.5	100	34.1	47.5		21.4	50.7	22.5
	Leaf	Mean	1.77	0.003	1.59	0.61	Nd	15.3	10.5	45.8
		SD (±)	0.89	0.002	0.61	0.36		7.51	2.77	10.8
		CV (%)	50.2	66.6	38.3	59.1		49.1	26.3	23.5
	Fruit	Mean	1.12	0.14	4.23	0.88	Nd	0.88	1.22	3.29
		SD (±)	0.99	0.13	2.74	0.81		1.07	1.21	2.31
		CV (%)	88.3	92.8	64.7	92.1		121.5	99.1	70.2
Soil		Mean	2.03	0.15	6.99	12.4	Nd	12.3	4.68	5.32
		SD (±)	1.32	0.11	4.61	4.03		4.27	3.86	3.09
		CV (%)	65.1	73.3	65.9	32.5		34.7	82.4	58.1

Note: Values are expressed as means X and ± SD.

TF values are affected by several factors as: metal chemistry, type of soil, soil characteristics and also the plant species. On average, the transfer of heavy metals from soil to shoot, leaf and fruit of the apple with the rootstock mm106 was in the order:

TF=C(shoot)/C(soil) Zn (4.23) > Fe (3.64) > Pb (2.55) > Cu (1.57) > Cd (1.21) > Ni (0.22) > Cr (0.21) > As (nd).
TF=C(leaf)/C(shoot) As(5) > Fe (2.63) > Cr (2.07) > Cu (1.59) > Zn (0.88) > Pb (0.42) > Ni (0.28) > Cd (0.05).
TF=C(fruit)/C(leaf) Cd (23.1) > Ni (2.76) > Cr (1.81) > Pb (0.63) > Cu (0.21) > Fe (0.08) >Zn (0.03) > As(nd).

Transfer factor to apple tissues with rootstock m26 was in the order:
TF=C(shoot)/C(soil) Fe (6.25) > Zn (3.51) > Pb (1.96) > Cu (1.94) > Cd (0.86) > Cr (0.19) > Ni (0.14) > As (nd);
TF=C(leaf)/C(shoot) As (2) > Cr (1.94) > Fe (1.89) > Cu (1.66) > Pb (0.62) > Zn (0.56) > Ni (0.25) > Cd (0.07);
TF=C(fruit)/C(leaf) Cd (29.2) > Ni (3.09) > Cr (1.51) > Pb (0.63) > Cu (0.15) > Zn (0.05) > Fe (0.01).

Table 2. Heavy metal concentration (mg kg^{-1} d.w.) in soil, shoot, leaf and fruits of apple species depending on rootstocks type from Reference site

			Heavy metals (mg kg^{-1})							
Rootstock	Tissues	Level	Pb	Cd	Cr	Ni	As	Zn	Cu	Fe
M106	Shoot	Mean	3.23	0.23	0.26	3.76	nd	56.17	5.12	34.05
		SD (±)	1.09	0.17	0.18	0.54		10.3	0.91	4.16
		CV (%)	33.7	73.9	69.2	14.3		18.3	17.7	12.2
	Leaf	Mean	1.69	0.16	1.13	0.93	nd	23.2	8.55	79.14
		SD (±)	0.81	0.15	0.19	0.47		4.67	2.63	4.51
		CV (%)	47.9	93.7	16.8	50.5		20.1	30.7	5.69
	Fruit	Mean	0.51	0.009	1.27	0.28	nd	0.88	0.98	3.36
		SD (±)	0.34	0.001	0.63	0.07		0.44	0.32	1.79
		CV (%)	66.6	11.1	49.6	25		50	32.6	53.2
M26	Shoot	Mean	2.65	0.17	0.18	2.44	nd	48.02	6.31	41.03
		SD (±)	1.69	0.24	0.11	1.08		11.03	1.41	4.37
		CV (%)	63.7	141.1	61.1	44.2		22.9	22.3	10.6
	Leaf	Mean	1.38	0.19	1.06	0.41	nd	41.15	11.7	104.3
		SD (±)	0.7	0.16	0.43	0.33		6.66	3.92	9.55
		CV (%)	50.7	84.2	40.5	80.4		16.1	33.5	9.15
	Fruit	Mean	0.38	0.08	0.99	0.18	nd	0.74	0.78	3.23
		SD (±)	0.26	0.05	0.58	0.17		0.36	0.56	0.74
		CV (%)	68.4	62.5	58.5	94.4		48.6	71.7	22.9
M9	Shoot	Mean	2.12	0.13	0.42	3.33	nd	62.11	4.97	36.14
		SD (±)	1.05	0.09	0.14	1.38		12.17	1.65	6.09
		CV (%)	49.5	69.2	33.3	41.4		19.5	33.1	16.8
	Leaf	Mean	0.85	0.06	0.86	0.31	nd	29.2	9.84	94.9
		SD (±)	0.82	0.04	0.28	0.28		6.96	2.93	11.24
		CV (%)	96.4	66.6	32.5	90.3		23.8	29.7	11.8
	Fruit	Mean	0.31	0.003	0.49	0.19	nd	0.31	0.93	4.13
		SD (±)	0.21	0.001	1.37	0.27		0.13	0.87	2.59
		CV (%)	67.7	33.3	279.5	142.1		41.9	93.5	62.7
Soil		Mean	1.03	0.05	0.39	1.99	nd	4.72	1.92	9.22
		SD (±)	0.66	0.009	0.31	1.43		1.14	0.39	3.58
		CV (%)	64.1	18	79.4	71.8		24.1	20.3	38.8

Note: Values are expressed as means X and ± SD. nd- not detected.

Transfer factor to apple tissues with rootstock m9 was in the order: **TF=C(shoot)/C(soil)** Fe (5.28) > Zn (3.18) > Cu (1.88) > Pb (1.71) > Cd (0.61) > Ni (0.18) > Cr (0.13) > As (nd); **TF=C(leaf)/C(shoot)** Cr (1.69) > Fe (1.62) > Cu (1.18) > Pb (0.51) > Zn (0.39) > Ni (0.26) > Cd (0.03) > As (nd); **TF=C(fruit)/C(leaf)** Cd (46.6) > Cr (2.66) > Ni (1.44) > Pb (0.56) > Cu (0.11) > Fe (0.07) > Zn (0.05) > As (nd).

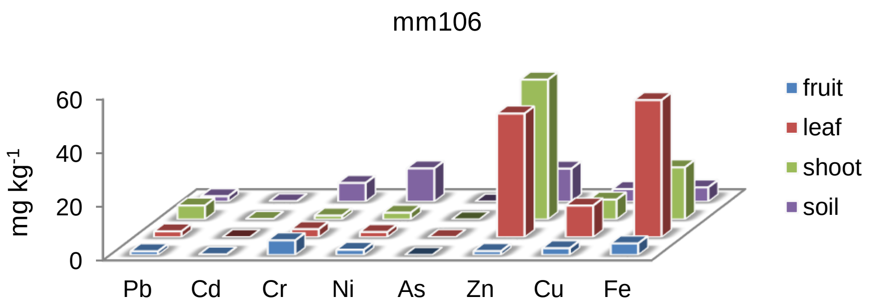

Figure 2. Heavy metals levels in soil and apple tissues with mm106 rootstock in Obiliq region.

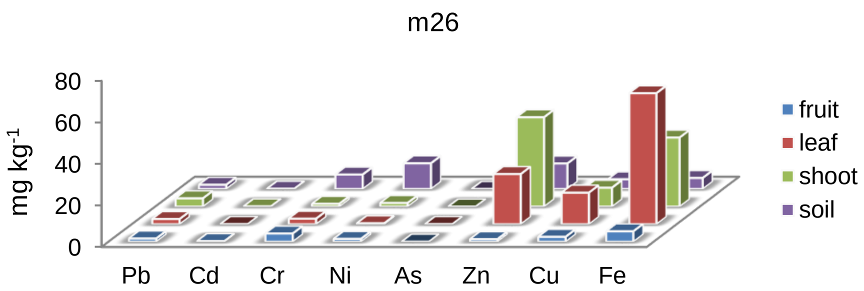

Figure 3. Heavy metals levels in soil and apple tissues with m26 rootstock in Obiliq region.

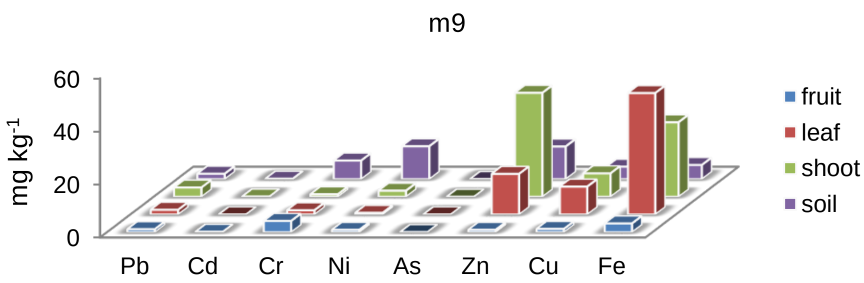

Figure 4. Heavy metals levels in soil and apple tissues with m9 rootstock in Obiliq region.

In this study, we analysed the level of heavy metals in soil and plant tissues of apple tree with different rootstock grown near the industrial area. Our main objective was to evaluate the availability of heavy metals from soil to fruit, leaves and shoot of apple tree. During the study, we have chosen some types of rootstock, and we have analysed their role in the transfer of heavy metals from soil to tissues of apple tree.

Our study analyzes showed that concentrations of heavy metals (Pb, Cd, Cr, Ni, As, Cu, Zn and Fe) in the analyzed tissue samples were higher in areas near the industrial zone of Obiliq region (Table 1) compared to control area (Table 2). Concentrations of heavy metals varied between different rootstocks due to their different absorption capacity and the level of soil pollution and atmospheric pollution (Roba et al., 2016). Previous studies showed that the higher the concentration of heavy metals on soil, its probability in plant tissue would be greater (Mapanda et al., 2007).

Cadmium is considered as being one of the most ecotoxic metals that exhibit adverse effects on all biological processes of humans, animals, and plants. This metal reveals its great adverse potential to affect the environment and the quality of food. Although Cd is considered to be a nonessential element for metabolic processes, it is effectively absorbed by both root and leaf systems. The distribution of Cd within plant organs is quite variable and clearly illustrates its rapid transport from roots to tops (Table 3).

Table 3. Transfer factor of heavy metals (Pb, Cd, Cr, Ni, As, Zn, Cu, Fe) in the Obiliq region

Rootstock	Transfer factor (TF)	Pb	Cd	Cr	Ni	As	Zn	Cu	Fe
M106	TF=C(shoot)/C(soil)	2.55	1.21	0.21	0.22	-	4.23	1.57	3.64
	TF=C(leaf)/C(shoot)	0.42	0.05	2.07	0.28	5	0.88	1.59	2.63
	TF=C(fruit)/C(leaf)	0.63	23.1	1.81	2.76	-	0.03	0.21	0.08
M26	TF=C(shoot)/C(soil)	1.96	0.86	0.19	0.14	-	3.51	1.94	6.25
	TF=C(leaf)/C(shoot)	0.62	0.07	1.94	0.25	2	0.56	1.66	1.89
	TF=C(fruit)/C(leaf)	0.63	29.2	1.51	3.09	-	0.05	0.15	0.01
M9	TF=C(shoot)/C(soil)	1.71	0.61	0.13	0.18	-	3.18	1.88	5.28
	TF=C(leaf)/C(shoot)	0.51	0.03	1.69	0.26	-	0.39	1.18	1.62
	TF=C(fruit)/C(leaf)	0.56	46.6	2.66	1.44	-	0.05	0.11	0.07

The interaction of Cd and Zn has received much study, and all findings may be summed up by stating that, in most cases, Zn reduces the uptake of Cd by both root and foliar systems. Chaney & Hornick (1977) suggested that when the Cd:Zn ratio in plant tissues is limited to 1%, the Cd content is restricted to below 5 mg kg^{-1}, thus below its phytotoxic level. Cd–Cu interactions are also complex. The inhibitory effect of Cu on Cd absorption is reported most often. Cd–Ca relationship seems to be highly cross-linked with variation in the soil pH. It cannot be precluded, however, that Ca^{2+} ions are able to replace Cd^{2+} in carrier mechanisms and thus Cd absorption by plants may be inhibited by an excess of Ca cations. In our study, fruit analysis from all sampling points was contaminated by an excessive amount of Cd compared to the permitted limit (0.05 mg kg^{-1}) proposed by FAO/WHO (1995). Cadmium can accumulate in the human body and can cause kidney dysfunction, skeletal damage and reproductive deficiency. The cadmium content in the literature was reported in the range of and 0.0002 0.527 mg kg^{-1} in fruit foods from the Greek market (Karavoltsos et al., 2002).

Toxic effects of Cr on plant growth and development include alterations in the germination process as well as in the growth of roots, stems and leaves. Hence, exposure to high level of Cr affected total dry matter production and yield of plants (Shanker et al., 2005). The mechanism of absorption and translocation of Cr in plants is apparently similar to those of Fe. There is some evidence that easily available Cr^{6+} is transformed into Cr^{3+} form in plant cells which readily interact with DNA and protein compounds

(Zayed et al., 1998). The concentration of Cr in delicious apple cultivars in three rootstock mm106, m26 and m9 in the contaminated studied fields was greater than in the reference area. The content of chromium in the literature was reported in the range of 1.48–6.43 mg kg^{-1} in wet weight in various summer fruits from Pakistan (Zahoor et al., 2003).

High level of Pb also causes inhibition of enzyme activities, water imbalance, alterations in membrane permeability and disturbs mineral nutrition. Pb inhibits the activity of enzymes at cellular level by reacting with their sulfhydryl groups. High Pb concentration also induces oxidative stress by increasing the production of ROS in plants (Reddy et al., 2005). Our results are in line with the results reported by Zhen et al. (2008), Xiao et al. (2010) in China. Zhen (2008) reported that cultivated fruits near the Shenyang-Dalian highway were polluted with Pb and Cd with average concentrations of 0.082 and 0.010 mg kg^{-1} in apple fruit.

Copper (Cu) is considered as a micronutrient for plants (Thomas et al., 1998) and plays important role inCO_2 assimilation and ATP synthesis. Exposure of plants to excess Cu generates oxidative stress and ROS. Oxidative stress causes disturbance of metabolic pathways and damage to macromolecules (Hegedus et al., 2001). Ni levels in fruit were many times higher than the maximum allowed limit 0.3 mg kg^{-1} (Liu et al., 2012). The content of nickel in literature was reported in the range of 1.0–8.9 mg kg^{-1} in some fruits from Pakistan (Zahoor et al., 2003).

Table 4. Transfer factor of heavy metals (Pb, Cd, Cr, Ni, As, Zn, Cu, Fe) to the reference site

Rootstock	Transfer factor (TF)	Pb	Cd	Cr	Ni	As	Zn	Cu	Fe
	TF=C(shoot)/C(soil)	3.13	4.61	0.66	1.88	nd	11.9	2.66	3.69
M106	TF=C(leaf)/C(shoot)	0.52	0.69	4.34	0.24	nd	0.41	1.66	2.31
	TF=C(fruit)/C(leaf)	0.31	0.05	1.12	0.31	nd	0.03	0.11	0.04
	TF=C(shoot)/C(soil)	2.57	3.41	0.46	1.22	nd	10.1	3.28	4.44
M26	TF=C(leaf)/C(shoot)	0.52	1.11	5.88	0.16	nd	0.85	1.85	2.53
	TF=C(fruit)/C(leaf)	0.27	0.42	0.93	0.43	nd	0.01	0.06	0.03
	TF=C(shoot)/C(soil)	2.05	2.63	1.07	1.67	nd	13.1	2.58	3.91
M9	TF=C(leaf)/C(shoot)	0.41	0.46	2.04	0.09	nd	0.47	1.97	2.62
	TF=C(fruit)/C(leaf)	0.36	0.05	0.56	1.18	nd	0.01	0.09	0.04

The obtained results showed that the fruits were powerful accumulators of heavy metals, considering that for some types of rootstocks the concentrations of heavy metals in the samples have exceeded the allowed values. High TF values (≥ 1) show the largest absorption of metals from soil to plants (Tables 3 and 4), while lower values indicate low levels of metal absorption from the plant that can be used for human consumption (Rangnekar et al. 2013). High values of transfer factors for Cd and Zn were reported (Lăcătuşu et al., 2012) in particular soil-plants systems with plants grown in the mud pond with sludge from municipal wastewater having high trace metal content and high moisture content. The highest TF was obtained for Cd in Obiliq areas. In fact, Cd is already known for its mobility from soil to plants (Kirkham, 2006). TF are even higher than 1, which means plants are accumulating Cd and should be recommended to not use them as food especially when the plants are grown in soil with high contamination factors. Thus, TF value of 23.1; 29.2 and 46.6 for Cd represent a concern for the health of the consumers.

CONCLUSIONS

The concentration of metals in the apple tissue increased with the increase of heavy metals in soils from polluted area. The accumulation ratios of heavy metals were calculated to assess the potential health risks. The mean concentrations of the heavy metals in the soil were in order of magnitude Ni > Zn > Cr > Cu > Fe > Pb > Cd > As while that in the fruits of apple were in order of magnitude Cr > Fe > Cu > Ni > Pb > Zn > Cd > As; in the leaves were Fe > Zn > Cu > Cr > Pb > Ni > Cd > As; in shoots were Zn > Fe > Cu > Pb > Ni > Cr > Cd > As.

The data of the present study showed that Cd, Cr and Ni had the highest transfer factor. The results of the recent study will help to assess the long-term impact on human health caused by the heavy metals released by mining and smelting activities in Obiliq region (Kosovo). However, much study is needed in this respect such as metal uptake studies at cellular level including efflux of different metal ions by different cell organelles and membranes. It is therefore, suggested that regular monitoring of heavy metals in plant tissues is essential in order to prevent excessive build up of these metals in the human food chain.

REFERENCES

Adamsa, M.L., Zhao, F.J., McGratha, S.P., Nicholsonb, F.A. & Chambersb, B.J. 2004. Predicting cadmium concentrations in wheat and barley grain using soil properties. *Journal of Environmental Quality* **33**, 532–541.

Arya, S.K. & Roy, B.K. 2011. Manganese induced changes in growth, chlorophyll content and antioxidants activity in seedlings of broad bean (Vicia faba L.). *J. Environ. Biol.* **32**, 707–711.

Blaylock, M.J. & Huang, J.W. 2000. Phytoremediation of Toxic Metals: Using Plants to Clean up the Environment. John Wiley, New York, vol. 9. pp. 53–71.

Cairns, J. 1980. Scenarios on alternative futures for biological monitoring, 1978-1985. In: D.L. Worf (Ed.), Biological Monitoring for Environmental Effects. Lexington Books, Lexington, pp. 11–21.

Chaney, R.L. & Hornick, S.B. Accumulation and effects of cadmium on crops, paper presented at *Int. Cadmium Conf.*, San Francisco, January 31, 125, 1977

FAO/WHO. 1995. Codex General Standard for Contaminants and Toxins in Food and Feed (193-1995), pp. 31–32.

Hegedus, A., Erdei, S. & Horvath, G. 2001. Comparative studies of H_2O_2 detoxifying enzymes in green and greening barley seedlings under cadmium stress. *Plant Science* **160**, 1085–1093.

Karavoltsos, S., Sakellari, A., Dimopoulos, M., Dasenakis, M. & Scoullos, M. 2002. Cadmium content in foodstuffs from the Greek market. *Food Additives Contam*. **19**:954962.

Kirkham, M.B. 2006. Cadmium in plants on polluted soils: Effects of soil factors, hyperaccumulation, and amendments. *Geoderma* **137**, 19–32.

Lăcătuşu, R., Lăcătuşu, AR., Stanciu-Burileanu, M.M., Lazăr, R., Lungu, M., Rizea, N. & Catrina, V. 2012. Phytoremediation of a sludge depozit proceeded from a city wastewater treatment plant. *Carpathian Journal of Earth and Environmental Sciences* **7**(1), 71–79.

Liu, J., Wang, J., Qi, J., Li, X., Chen, Y., Wang, C. & Wu, Y. 2012. Heavy Metal Contamination in Arable Soils and Vegetables around a Sulfuric Acid Factory, China. *Clean Soil Air Water* **40**, 766–772.

Mapanda, F., Mangwayana, E.N., Nyamangara, J. & Giller, K.E. 2007. Uptake of heavy metals by vegetables irrigated using wastewater and the subsequent risks in Harare, Zimbabwe. *Physics and Chemistry of the Earth* (Parts A/B/C) **32**, 1399–1405.

Müller, M. & Anke, M. 1994. Distribution of cadmium in the food chain (soil-plant-human) of a cadmium exposed area and the health risks of the general population. *Science of the Total Environment* **156**(2), 151–158.

Ramadan, M.A.E. & Al-Ashkar, E.A. 2007. The effect of different fertilizers on the heavy metals in soil and tomato plant. *Australian Journal of Basic and Applied* **1**, 300–306.

Rangnekar, S.S., Sahu, S.K., Pandit, G.G. & Gaikwad, V.B. 2013. Accumulation and Translocation of Nickel and Cobalt in Nutritionally important Indian vegetables grown in artificially contaminated soil of Mumbai, India. *Research Journal of Agricultural and Forest Sciences* **1**, 15–21.

Reddy, A.M., Kumar, S.G., Jyonthsnakumari, G., Thimmanaik, S. & Sudhakar, C. 2005. Lead induced changes in antioxidant metabolism of horsegram (Macrotyloma uniflorum (Lam.) Verdc.) and bengalgram (Cicer arietinum L.). *Chemosphere* **60**, 97–104.

Rizaj, M., Beqiri, E., McBow, Z. & Kongoli, F. 2008 "The mineral base and productive capacities of metals and non-metals of Kosovo". *JOM*. **60**(8), 18–22.

Roba, C., Roşu, C., Piştea, I., Ozunu, A. & Baciu, C. 2016. Heavy metal content in vegetables and fruits cultivated in Baia Mare mining area (Romania) and health risk assessment. *Environmental Science and Pollution Research* **23**, 6062–6073.

Shanker, A.K., Cervantes, C., Loza-Tavera, H. & Avudainayagam, S. 2005. Chromium toxicity in plants. *Environment International* **31**, 739–753.

Thomas, F., Malick, C., Endreszl, E.C. & Davies, K.S. 1998. Distinct responses to copper stress in the halophyte, Mesembryan-themum crystallium. *Physiologia Plantarum* **102**, 360–368.

Todeschini, V., Lingua, G., D'Agostino, G., Carniato, F., Roc-Cotiello, E. & Berta, G. 2011. Effects of high zinc concentration on poplar leaves: a morphological and biochemical study. *Environ. Exp. Bot.* **71**, 50–56.

Turkdogan, M.K., Fevzi, K., Kazim, K., Ilyas, T. & Ismail, U. 2003. Heavy metals in soil, vegetables and fruits in the endemic upper gastrointestinal cancer region of Turkey. *Environmental Toxicology and Pharmacology* 175e179.

Xiao, Z.L., Cong, Q. & Qu, J. 2010. Assessment of heavy metal pollution in orchard soil and its affections to fruit quality around molybdenum mining area. *Science Technology and Engineering* **10**, 5831–5834.

Yang, Z. & Chu, C. 2011. Towards understanding plant response to heavy metal stress. In: Shanker, Arun (Ed.), Abiotic Stress in Plants – Mechanisms and Adaptations. InTech, ISBN 978-953-307-394-1, pp. 59–78.

Zahoor, A., Jafar, M. & Saqib, M. 2003. Elemental distribution in summer fruits of Pakistan. *Nutr Food Sci*. **33**, 203–207.

Zayed, A., Lytle, C.M., Qian, J.-H. & Terry, N. 1998. Chromium accumulation, translocation and chemical speciation in vegetable crops. *Planta*, 206, 239,

Zhen, H. 2008. Pollution characteristics of heavy metal in grains and fruits at both sides of Shenyang-Dalian expressway. *Journal of Meteorology and Environment* **24**, 1–5.

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Eduard Stiefel's linear programming method as tool in agro metrics

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Abstract. In this paper, we consider the linear optimization models' application problems in the research processes and in the didactics processes. Our target is to convince the colleagues about preferences of Eduard Stiefel's method comparing with widespread George Bernard Dantzig's simplex method. Indeed, the Stiefel's method provides researchers and teachers with clear and pithy interpretations of linear model. Our pedagogical praxis during long time period conclusively confirms that Stiefel's method makes the theory of linear optimization match easier for understanding and for active employing to students especially in the specialities with limited mathematical education. We offer in this paper also some new theoretical concepts and methods adapted for the linear model information analysis (the concept of general optimal plan, the methods of the profounder sensitivity analysis), and we appeal economists to interpret simplex predicates as productions functions in a broad sense.

Key words: linear programming, simplex method, Stiefel's method.

INTRODUCTION

Linearity postulate often holds in the relations between economic, financial, technological indicators. For example, financial accounting and managerial accounting mostly use linear functions. That is the reason why in spite of relative simplicity linear models allow us to describe interdependence between indicators in the different scientific areas rather adequately and satisfactorily for practical application. As result, the linear programming or linear planning (LP) is one of the most widely applied quantitative decision making approach techniques in Management Science. Since the very beginning linear programming was successfully applied also in agro metrics. Well-known and included in the education courses are such linear programming models of agriculture as land utilizing planning problem, problem of the rational structure of the live-stock breeding, problem of the rational nutrition for domestic animals.

Let us mention only two lately examples published in the international Journal 'Agronomy Research'. Significant model 'Optimization of arable land use to guarantee food security in Estonia' was elaborated by Põldaru, R, Viira, A.H. & Roots, J. (2018). The authors point out: 'The supply side of the model is a typical agricultural production model that guarantees the consistency of crop and livestock farming. The objective of the model is to minimize the use of arable land for field crops to ensure fodder for animal feed, and food for human food consumption. The model is used to analyse various land use strategies'.

The linear programming can be applied in different modified forms. For example, Žgajnar & Kavčič (2009) offer 'Multi-goal pig ration formulation' using weighted goal programming supported by a system of penalty functions.

In current paper, we more rigorously will analyse the model 'Decision Making in Agriculture with Linear Programming Approach' presented by N. A. Sofi, Aquil Ahmed, Mudasir Ahmad & Bilal Ahmad Bhat (2015). The authors assert that 'Linear programming technique is relevant in optimization of resource allocation and achieving efficiency in production planning particularly in achieving increased agriculture production of food crops (rice, maize, wheat, pulses, and other crops)'. The authors applied linear programming technique to determine the optimum land allocation of 5 food crops by using agriculture data, with respect to various factors for the period 2004–2011. This model seems to us appropriate example in order to demonstrate shortly the comparative advantages of the Stiefel's method and some innovations of authors as well.

The fact that the linear models satisfactorily adequately reflect real links between indicators is a crucial reason for wide applications of linear optimal planning. Besides that linear programming owes its popularity due to George Bernard Dantzig's simplex method (Dantzig, 1949; Dantzig, 1951; Dantzig et al., 1955; Dantzig, 1963; Cottle & Dantzig, 1968; Cottle & Dantzig, 1970; Dantzig & Thapa, 1977; Dantzig & Thapa, 2003) extensively embodied in the efficient software. For example, handy tool 'Analytic Solver Upgrade' (formerly 'Premium Solver Pro') solves larges problems – up to 2,000 variables 100 times faster than the standard Solver. By the way the problem 'Optimization of arable land use to guarantee food security in Estonia' (contains 163 variables and 178 constraints in form of linear equations) was solved by authors with help of 'Premium Solver Pro'.

Observation. We have suspicion that paper (Põldaru et al., 2018) contains a fallacy. If linear model contains 163 variables and 178 constraints in form of linear equations than there is a big chance to have empty set of feasible solutions because rank of system's matrix is less or equal 163. If feasible solution exists than equations of system are linear dependent and it is worth to investigate connections between constraints.

Therefore, linear programming is one of the most successful tools to implement quantitative approaches to management decision making. A large number of applications has been published in various industries including agro metrics. Let us mention such models as Production Scheduling, Multiperiod Production and Inventory Planning, Work Force Assignment and Staff Scheduling, Environmental problems, Transportation, Assignment and Transshipment problems, Blending Problems what occur, for example, in the food industry. In our opinion, the very significant role linear programming plays through its connection with Input-Output analysis. For example, Data Envelopment Analysis (DEA) is used to compare the relative efficiency of operating units whose input and output vectors have identical structure. We must mention the Goal programming and Multicriteria decision problems. High actuality keeps classical problem of Tchebycheff Approximation in case when linear model has not feasible solutions.

The classic of Mathematical Economics professor of London School of Economics R. G. D. Allen already in 1956 in the world famous book (Allen, 1956) 'Mathematical Economics' wrote about linear model (chapter 'Marginal Analysis v. Linear Programming of the Firm', 620 page): 'The linear programming approach seems very well adapted for application to decision-taking at the level of the firm. It provides, trough emphasis on technology, just the link required between the problems of interest to the economist and those which engage the attention of entrepreneur and engineers'.

So, there exist a lot of conceptual models in different areas of management science. No doubt that proper academic course can be formed as extremely rich and interesting for students because students with help of Microsoft Solver study simulated virtual problems. But for all that, each researcher perfectly knows that sufficient difficulties arise in the practical implementation of mentioned conceptual models. In all scientific conferences we took part the researchers agreed that the estimation of the linear expressions' coefficients as a rule is the most difficult task in the construction of the relevant mathematical model.

Generally speaking, the relevant linear programming models are expensive. Therefore self-evident is the desire to obtain from constructed model as far knowledges as possible.

How to obtain more knowledge about problem utilizing expensive linear programming model?

A wide overview of scientific and educational literature persuasively shows that G. B. Dantzig's simplex method till the nowadays is the mainstream method for solving linear programming problems. The mentioned linear programming applications in 'Agronomy Research' also are made with help of Dantzig's method. In the same time already in the year 1960 Professor of Mathematics Eduard Stiefel (Swiss Federal Institute of Technology in Zurich) in the article (Stiefel, 1960) 'Stiefel, E., Note on Jordan Elimination, Linear Programming and Tchebycheff Approximation, *Numerische Mathematik*, Vol. 2, 1960, 1–17)' offered another approach to the investigation of linear programming problem based on pivot transformations of the system of linear equations.

The goal of this paper is to conduct the comparative analysis of two different linear programming solving and analysis methods: well-known Dantzig's simplex method and Eduard Stiefel's method. We are going to explore the comparative advantages of Stiefel's method and demonstrate that Stiefel's method furnishes more information easily obtained from linear programming model. Moreover, on the ground of Stiefel's method we offer new concepts and constructive approaches in the linear problem investigation with help of linear programming. The new approaches are illustrated through five applications.

MATERIALS AND METHODS

Pivot transformation (often called as Jordan-Gauss elimination) is algorithmized equivalent transformation of the system of linear equations and simultaneous equivalent transformation of corresponding dual system of linear equations. Both are interpreted as predicates. Idea of pivot transformation as simultaneous equivalent transformation of two pairs of predicates is absolutely simple but incredibly fruitful in linear algebra.

Theorem. Pivot transformation (Jordan-Gauss elimination) as simultaneous equivalent transformations of two pairs of predicates: direct and dual.

Let **E**, **F** vector spaces. Let $a, b, c, d \in \mathbf{R}$; $a \neq 0$; $X_1, X_2, Y_1, Y_2 \in \mathbf{E}$; $U_1, U_2, V_1, V_2 \in \mathbf{F}$. Direct system of equations $\{ Y_1 = a X_1 + b X_2; Y_2 = c X_1 + d X_2 \}$ can be transformed as system $\{ X_1 = a^{-1} Y_1 - b a^{-1} X_2; Y_2 = c a^{-1} Y_1 + (a d - b c) a^{-1} X_2 \}$.

Dual system of equations { $U_1 = a V_1 + c V_2$; $U_2 = b V_1 + d V_2$ } can be transformed as system { $-V_1 = a^{-1} (-U_1) + c a^{-1} V_2$; $U_2 = b a^{-1} (-U_1) + (a d - b c) a^{-1} V_2$ }.

Proof of this theorem is omitted because it is based only to the elementary algebraic transformations of equations' systems.

We offer to consider these four systems of equations as predicates. Condition $a \neq 0$ is sufficient and necessary for equivalency of direct system and it's transformed system with respect to variables a, b, c, d $\in$ **R**; $X_1, X_2, Y_1, Y_2 \in$ **E**, and for equivalency of dual system and it's transformed system with respect to variables a, b, c, d $\in$ **R**; $U_1, U_2, V_1, V_2 \in$ **F** as well.

It is handy to write both systems in a table form (Table 1).

Table 1. Pivot transformation as algorithmized equivalent transformation

	X_1	X_2	
Y_1	a	b	V_1
Y_2	c	d	V_2
	U_1	U_2	

	Y_1	X_2	
X_1	a^{-1}	$-b a^{-1}$	$-U_1$
Y_2	$c a^{-1}$	$(a d - b c) a^{-1}$	V_2
	$-V_1$	U_2	

Remark. We demonstrated just (2×2) – matrix in order to be simple. Of course, number of vectors $X_i \in$ **E** and number of vectors $Y_j \in$ **F** are arbitrary.

Idea of Eduard Stiefel − goal-directed equivalent transformations (so called pivot transformations) of simplex predicate. What was reaction?

Let us observe, that articles of Dantzig & Thapa (Dantzig & Thapa, 1977; Dantzig & Thapa, 2003) contain references to the Hestenes & Stiefel's paper (Hestenes & Stiefel, 1952). The method of Eduard Stiefel was positively appraised by Albert Tucker and Michel Balinski, and widely used in Princeton University (Tucker, 1962; Balinski & Tucker, 1969). The article (Balinski & Tucker, 1969) 'Balinski, M.L., Tucker, A.W., Duality Theory of Linear Programs: A Constructive Approach with Applications, *SIAM Review*, Vol. 11, No. 3 (Jul., 1969), 347–377' contains the reference to the article of Eduard Stiefel (Stiefel, 1960).

In the Baltic States method of Eduard Stiefel mostly was known during the book (Zukovitskii & Avdeeva, 1967) 'Зуховицкий С. И., Авдеева Л. И. Линейное и выпуклое программирование. (Серия 'Экономико-математическая библиотека') Москва, 1967'. In the preface of this book Зуховицкий С. И. wrote: 'Вычислитель-ным аппаратом в этой книге служит аппарат жордановых исключений, большие удобства которого убедительно продемонстрированы в статье Э. Штифеля 'Stiefel, E., Note on Jordan Elimination, Linear Programming and Tchebycheff Approximation, *Numerische Mathematik*, Vol. 2, 1960'.

Andrejs Jaunzems was active supporter of the Stiefel's method in Latvia (see, for example, the books (Jaunzems, 1981; Jaunzems, 1990; Jaunzems, 1993; Jaunzems, 2011; Jaunzems, 2013). In the book Jaunzems (1993) 'Mathematics for Economic Sciences. General course' theory of linear operator was based on the pivot transformations. Professor of Latvia University of Agriculture Alberts Krastiņš, which was good friend of Leonid Kantorovich (winner of the Stalin Prize in 1949 and the Nobel Memorial Prize in Economics in 1975), supported Stiefel's method through for a long time period widely used book (Krastiņš, 1976) 'Alberts Krastiņš. Matemātiskā programmēšana'.

In spite of the sufficient advantages of Stiefel's LP method comparing with Dantzig's method the method of Eduard Stiefel due to different reasons (probably, sometimes even due to peculiar reasons) does not belong to mainstream and is rarity in science and education.

For example, carefully elaborated teaching text (Border, 2004) ‘Border, K. M., The Gauss–Jordan and Simplex Algorithms’ published in California Institute of Technology utilizes traditional Dantzig’s method instead Stiefel’s LP method what is matched more suitable for teaching in the Division of the Humanities and Social Sciences. The characteristic example is also the book (Seo, 1991) ‘Seo, K.K. Managerial economics. Text, problems, and short cases, – Seventh edition, Irwin, 1991’ where solving of the extreme simple LP problem with help of Dantzig’s simplex method is demonstrated on the four (!) pages. In the same time, this book is presented as one of the most popular MBA textbooks of managerial economics in USA.

RESULTS AND DISCUSSION

Since a long period of time, we employ in our scientific and didactic praxis five kinds of Stiefel’s linear programming method applications what partly contain innovation elements. We would like to hope that our experience can be useful for many teachers and researchers.

Application 1. Duality.

Application 2. The concept of general optimal plan.

Application 3. Direct and dual simplex predicate as production functions.

Application 4. Sensitive analysis. Serious criticism of Microsoft Solver.

Application 5. Vectorial form.

Application 1. Duality

Let us examine LP standard model and it’s dual model.

$max\ \{C \cdot X \mid AX \le B, X \ge O\}$ or $max\ \{C \cdot X \mid U = -AX + B, X \ge O, U \ge O\}$;

$A \in \mathbf{R}^{m,\,n}$; $X, C \in \mathbf{R}^{n,\,1}$; $U, B \in \mathbf{R}^{m,\,1}$.

$min\ \{B \cdot Y \mid A^TY \ge C, Y \ge O\}$ or $min\ \{B \cdot Y \mid V = A^TY - C, Y \ge O, V \ge O\}$;

$Y \in \mathbf{R}^{m,\,1}$, $V \in \mathbf{R}^{n,\,1}$.

Let **X** and **Y** are the corresponding sets of feasible solutions for direct and dual problem. To solve those problems means to find the corresponding sets of optimal plans:

$\mathbf{X^*} := \{ X^* \mid X^* \in \mathbf{X}, C \cdot X^* \ge C \cdot X\ \forall X \in \mathbf{X} \}$.

$\mathbf{Y^*} := \{ Y^* \mid Y^* \in \mathbf{Y}, B \cdot Y^* \le B \cdot Y\ \forall Y \in \mathbf{Y} \}$.

Both systems of equation can be inscribed in the initial simplex table (Table 2).

Table 2. Direct and dual linear problems in the initial table form

	X	1	
U	–A	B	Y
z	C	0	1
	–V	W	

The direct problem is inscribed horizontally:

$U = -AX + B$, $z = C \cdot X + 0 \cdot 1$.

The dual problem is inscribed vertically:

$-V = -A^TY + C$, $w = B \cdot Y + 0 \cdot 1$.

We must take in account that

$X \ge O, U \ge O; Y \ge O, V \ge O$.

In order to investigate in a versatile manner this optimization problem we must make goal-oriented pivot transformations what mean simultaneous equivalent transformations of both systems – direct and dual. Duality means, that each pivot transformation of the system: $u_i \uparrow\downarrow x_j$, $x_k \uparrow\downarrow u_l$, $u_i \uparrow\downarrow u_j$, $x_k \uparrow\downarrow x_l$ simultaneously is the **modified** pivot transformation of the dual system: $v_j \uparrow^m\downarrow y_i$, $y_l \uparrow^m\downarrow v_k$, $y_j \uparrow^m\downarrow y_i$, $v_l \uparrow^m\downarrow v_k$.

Our target is to obtain such table, where z expression contains only non-positive coefficients but w expression contains only non-negative coefficients. Regular case is when coefficients in both expressions are not equal zero. Then both problems have unique solution because z has maximal value and w has minimal value then and only then if all variables in the final expressions of z and w equals zero.

Application 2. The concept of general optimal plan

If z expression in the final table contains some zero coefficients than z can take maximal value also by non-zero values of proper variables. In this case set of optimal solutions of the direct problem **X*** (generally speaking) is infinite. If w expression contains some zero coefficient than set of optimal solutions of the dual problem **Y*** (generally speaking) is infinite. If z expression and w expression both contains some zero coefficients than sets **X***, **Y*** are infinite.

We never meet the concept of general optimal plan in the literature available for us. Therefore, until the opposite is not proved, the concept of general optimal plan first is offered in the paper (Jaunzems, 2013): 'Jaunzems A. Singulārā lineārā programmēšana menedžmenta ekonomikā'. Let us examine application in the managerial accounting: the break even set in case of multiproduct output. The modified simple example from book (Coenenberg, 1997) 'Adolf G. Coenenberg. Kosten-rechnung und Kostenanalyse' is used.

The start of modified citation:

Example. Two products P_1, P_2, two machines with working time limits 200, 300 hours. Contribution margins are 300 DM, 280 DM. Fixed costs = 8,400 DM. Find the break even set.

Product	P_1	P_2
First machine (hours per unit of product)	2	1
Second machine (hours per unit of product)	2	3

The model is: $300\,x_1 + 280\,x_2 \rightarrow max$ with respect the constraints $2\,x_1 + 1\,x_2 \le 200$, $2\,x_1 + 3\,x_2 \le 300$, $x_1 \ge 0$, $x_2 \ge 0$. The end of modified citation.

We offer the simplex predicate method. Table 3 shows the initial system, Table 4 shows the final system of equations.

Table 3. Initial table

	x_1	x_2	1
u_1	-2	-1	200
u_2	-2	-3	300
CM–FC	300	280	-8,400
CM	300	280	0

CM − contribution margin, FC − fixed costs.

Table 4. Final table. Break even set

	CM–FC	x_1	1
u_2	-0.0107	1.2143	210
x_2	0.0036	-1.0714	30
u_1	-0.0036	-0.9286	170
CM	1	0	8,400

We get points on the break even set, if $x_1 \ge 0$, $x_2 \ge 0$; $u_1 \ge 0$, $u_2 \ge 0$, CM – FC = 0.
The break even set predicate is the system of equations:
$u_1 = -0.929\,x_1 + 170$; $u_2 = 1.214\,x_1 + 210$, $x_2 = -1.071\,x_1 + 30$, $0 \le x_1 \le 28$.
Answers to different 'what if' questions.
For example, let us assume, that CM – FC = 0, $x_1 = 10$.

Then x_2 = 19.29; u_1 = 160.71; u_2 = 222.14.
The point (10; 19.29) belongs to the break even set.

Application 3. Direct and dual simplex predicate as production functions

In order to be clear and short we utilize agro metrics example from article (Sofi et al., 2015). We are not responsible for quality of this article more or less correctly reflecting optimal land structure planning problem. We are going with help of this example to show Stiefel's method in action. The authors of this article tried to find the optimal land utilizing structure in order to get the maximum output of the major food crops under land, capital and two kinds of labour availability constraint. In this model the 5 variables (rice, maize, wheat, pulses and other crops) are included. The authors used linear programming – simplex method. We decided to utilize the following long citation from article (Sofi et al., 2015) as example to explain the essence of applications 3, 4, and 5.

The start of citation: The objective function is the output of various agriculture productions of food crops, inequalities is the Land / Capital/Labour (A) and Labour (B) and requirement is total. Now, our objective is to find the optimum land of food crops. Table 5 represents in simplified manner the basic information necessary in order to construct a linear programming model of land utilization.

Table 5. Output per acre and the requirements

Variable food crops	Output/acre Lakh	Land (acre)	Capital/acre	Labour (A) Man day	Labour (B) working hours
Rice	102.85	773.40	22.03	10.08	13.09
Maize	114.84	941.84	45.74	14.10	12.07
Wheat	263.50	823.13	63.47	17.33	18.08
Pulses	34.13	124.99	6.67	0.80	2.16
Other crops	98.26	89.20	19.91	7.32	9.07
Requirements		2,752.56	2,409.00	1,069.70	111.00

This model, which in the interests of simplicity ignores livestock, is as follows:
Maximize $102.85\ x_1 + 114.84\ x_2 + 263.50\ x_3 + 34.13\ x_4 + 98.26\ x_5$ subject to constraints
Land: $773.40\ x_1 + 941.84\ x_2 + 823.13\ x_3 + 124.99\ x_4 + 89.20\ x_5 \leq 2{,}752.56$
Capital: $22.03\ x_1 + 45.74\ x_2 + 63.47\ x_3 + 6.67\ x_4 + 19.91\ x_5 \leq 2{,}409.00$
Labour A: $10.08\ x_1 + 14.10\ x_2 + 17.33\ x_3 + 0.80\ x_4 + 7.32\ x_5 \leq 1{,}069.70$
Labour B: $13.09\ x_1 + 12.07\ x_2 + 18.08\ x_3 + 2.16\ x_4 + 9.07\ x_5 \leq 111.00$
$x_1 \geq 0,\ x_2 \geq 0,\ x_3 \geq 0,\ x_4 \geq 0,\ x_5 \geq 0.$
x_1 = Rice; x_2 = Maize; x_3 = Wheat; x_4 = Pulses; x_5 = Other Crops.
Applying the simplex procedure for obtaining the optimum land of Food Crops through LINGO computer-based software Global optimal solution found.
Objective Value: 1,375.996

Variable	Value	Reduced cost	Rows	Lack or surplus	Dual price
x_1	0.000000	106.5676	1	1375.996	1.000000
x_2	0.000000	102.2864	2	0.000000	0.1050571
x_3	2.565066	0.000000	3	2168.462	0.000000
x_4	0.000000	0.1500135	4	973.0925	0.000000
x_5	7.124985	0.000000	5	0.000000	9.791170

The solution to this model yields the following information: x_3 = 2.57 acres of wheat and x_5 = 7.11 acres of other food crops. The ultimate aim is to produce realistic agriculture planning model for the regions in order to examine in detail the effect of variations in prices and quantities. The end of citation.

Now we demonstrate Stiefel's method. **In the initial table** (Table 6) **two systems of equations are inscribed.** We offer to interpret such systems as productions functions in a broad sense because of these systems reflect interdependence between different indicators of the definite economic unit.

After two pivot transformations, we get **final table** (Table 7) **which contains two systems properly equivalent to initial systems.** Let us stress that absolutely all numbers in both of tables can be clearly and pithy interpreted as slope coefficients trough their role in the linear equations. We appeal colleagues to provide themselves the proper calculations in order to check correctness of all 11 equations inscribed in the Table 7.

Table 6. The initial table for the optimal land utilizing structure model

0	x_1	x_2	x_3	x_4	x_5	1	
u_1	-773.40	-941.84	-823.13	-124.99	-89.20	2,752.56	y_1
u_2	-22.03	-45.74	-63.47	-6.67	-10.91	2409	y_2
u_3	-10.08	-14.10	-17.33	-0.80	-7.32	1,069.70	y_3
u_4	-13.09	-12.07	-18.08	-2.16	-9.07	111	y_4
Z	102.85	114.84	263.5	34.13	98.26	0	1
	$-v_1$	$-v_2$	$-v_3$	$-v_4$	$-v_5$	w	

Table 7. The final table for the optimal land utilizing structure model

	u_1	x_1	x_2	u_4	x_4	1	
x_3	-0.00155	-0.99898	-1.27555	0.01524	-0.16077	2.573788	v_3
u_2	0.064653	35.39533	21.99719	0.567024	2.635808	2168.098	y_2
u_3	0.004244	3.220009	-0.86584	0.765323	1.383494	973.0686	y_3
x_5	0.003089	0.548141	1.211895	-0.14063	0.082326	7.107597	v_5
z	-0.1048	-106.522	-102.186	-9.80284	-0.14318	1376.586	1
	$-y_1$	$-v_1$	$-v_2$	$-y_4$	$-v_4$	w	

Both problems have a unique solution because z has maximal value and w has minimal value then and only then if all variables in the final expressions of z and w equal zero.

Therefore, $u_1^* = 0$, $u_4^* = 0$, $x_1^* = 0$, $x_4^* = 0$; $v_3^* = 0$, $v_5^* = 0$, $y_2^* = 0$, $y_3^* = 0$.
$z^* = 1,376.59 = w^*$; $X^* = (0; 0; 2.5738; 0; 7.1076)^T$, $U^* = (0; 2,168.10; 973.07; 0)^T$, $Y^* = (0.1048; 0; 0; 9.8028)^T$, $V^* = (106.522; 102.186; 0; 0.143; 0)^T$.

Application 4. Sensitivity analysis. Serious criticism of Microsoft Solver

Elementary sensitivity analysis means:

A. Constraint $u_i \geq 0$ is substituted with $u_i \geq \lambda$; $i \in \{1, 2, 3, 4\}$, $\lambda \in \mathbf{R}$.
B. Constraint $x_i \geq 0$ is substituted with $x_i \geq \lambda$; $i \in \{1, 2, 3, 4, 5\}$, $\lambda \in \mathbf{R}$.
C. Objective $C \cdot X$ is substituted with $C \cdot X + \mu\, u_i$; $i \in \{1, 2, 3, 4\}$, $\mu \in \mathbf{R}$.
D. Objective $C \cdot X$ is substituted with $C \cdot X + \mu\, x_i$; $i \in \{1, 2, 3, 4, 5\}$, $\mu \in \mathbf{R}$.
We must find $z^*(\lambda)$, $X^*(\lambda)$, $U^*(\lambda)$, $Y^*(\lambda)$, $V^*(\lambda)$ or $z^*(\mu)$, $X^*(\mu)$, $U^*(\mu)$, $Y^*(\mu)$, $V^*(\mu)$.

Example 1. The authors of the paper (Sofi et al., 2015) write: 'Applying the simplex procedure for obtaining the optimum land of Food Crops through LINGO computer-based software reported that value of the variable x_1 equals 0, reduced cost equals 106.57'.

The Microsoft Solver reports that optimal value for rice $x_1^* = 0$ but corresponding reduced cost is –106.52.

It is not our purpose to give in this paper the detail list of comparison between LINGO software and Microsoft Solver. We would like only to show the preferences of the Stiefel's interpretation of the simple table as direct and dual systems of equations.

We are proud to report that our students are able to receive from final table a lot of information about rice production in the concrete land utilizing economy not furnished by Solver.

For example, let us substitute the constraint $x_1 \geq 0$ with $x_1 \geq \lambda$. Than from final table (Table 7) we obtain $u_1^* = 0$, $u_4^* = 0$, $x_1^* = \lambda$, $x_2^* = 0$; $x_4^* = 0$.

$z^* = -106.52\,\lambda + 1{,}376.59$; $X^* = (\lambda; 0; -0.9990\,\lambda + 2.5738; 0; 0.5481\,\lambda + 7.1076)^T$,

$U^* = (0; 35.3953\,\lambda + 2{,}168.10; 3.2200\,\lambda + 973.07; 0)^T$,

Remark. We consider here only the direct problem and do not examine the changes in the dual problem.

As far as x_3, u_2, u_3, x_5 remain non-negative this table gives us optimal solution for each value of λ. Solving the system of inequalities

$x_3 = -1.00\,\lambda + 2.57 \geq 0$

$u_2 = 35.40\,\lambda + 2{,}168.10 \geq 0$

$u_3 = 3.22\,\lambda + 973.07 \geq 0$

$x_5 = 0.55\,\lambda + 7.11 \geq 0$,

we get $-12.97 \leq \lambda \leq 2.58$.

So, for each $\lambda \in]-12.97; 2.58[$ the unique optimal solution of problem is:

$X^* = (\lambda; 0; -1.00\,\lambda + 2.57; 0; 0.55\,\lambda + 7.11)^T$

$U^* = (0; 35.3953\,\lambda + 2{,}168.10; 3.2200\,\lambda + 973.07; 0)^T$

$z^* = -106.52\,\lambda + 1376.59$.

Remark. The border-values $\lambda = -12.97$ and $\lambda = 2.58$ have a special role.

Positive values of x_1 have natural interpretations. However it is possible to interpret pithy also negative values of rice acres.

Let us stress: this result is not included in Microsoft Solver's Sensitivity Report (Table 8). Moreover, there are labels of reduced cost in Solvers report 'Allowable Increase', 'Allowable Decrease', similar than labels of shadow prices, but misleading because of absolutely different sense.

Table 8. Part of the sensitivity report provided by Microsoft Solver

Variable Cells						
Cell	Name	Final Value	Reduced Cost	Objective Coefficient	Allowable Increase	Allowable Decrease
B	x1	0	-106.52	102.85	106.52	1E+30
Constraints						
Cell	Name	Final Value	Shadow Price	Constraint R.H. Side	Allowable Increase	Allowable Decrease
H	SUMPRODUCT	2,752.56	0.1048	2,752.56	2,300.95	1,660.92

Example 2. Interpreting the final simplex system as production function what describes post-optimized economics of the investigated unit, we can get answers to different ‘what if’ questions. Let, for example, $u_1 \geq -10$, $u_4 \geq 20$, $x_2 \geq 5$.
Than we obtain the optimal plan $X^* = (0; 5; 1.31; 0; 8.18)^T$; $z^* = 1264.49$.
Condition $u_1 \geq -10$ means that land is available 2752.56 + 10 = 2762.56 units; condition $u_4 \geq 20$ means that labour B is available 111 − 20 = 91 working hours; condition $x_2 \geq 5$ means that manager voluntary determines to produce 5 units of maize in spite of optimal plan not recommending to produce this product. It is also easy to interpret the values $u_2^* = 2190.73$; $u_3^* = 972.97$.
Important remark. We hope that with help of simple agro metrics example borrowed from article (Sofi et al., 2015) we clearly demonstrate sufficient difference between Dantzig’s method and Stiefel’s method. The Stiefel’s method allows us to interpret the content of Table 6 (Initial table for the optimal land utilizing structure model) and content of Table 7 (Final table for the optimal land utilizing structure model) as two pairs of production functions what characterizes concrete land utilizing economy. Of course, all other tables created during simplex process also can be interpreted as pairs of production functions. Our main assertion is that such interpretations are not possible using traditional Danzig’s method.

Our recommendation to Microsoft is to perfect the Solver in order to have available full final simplex table.

Application 5. Vectorial form

Absolutely the same calculations can be interpreted in vectorial form (Table 9 and Table 10). As result we find the useful linear connections between gradient of direct problem’s objective C, rows of matrix $A \in \mathbf{R}^{m,\,n}$, and vectors of standard basis I_k in the space $\mathbf{R}^{n,\,1}$, and connections between gradient of dual problem’s objective B, columns of matrix A, and vectors of standard basis J_k in the space $\mathbf{R}^{m,\,1}$ as well.

Table 9. The initial table in the vectorial form

	I_1	I_2	I_3	I_4	I_5	O	
$-A_1$	-773.40	-941.84	-823.13	-124.99	-89.20	2,752.56	J_1
$-A_2$	-22.03	-45.74	-63.47	-6.67	-10.91	2409	J_2
$-A_3$	-10.08	-14.10	-17.33	-0.80	-7.32	1,069.70	J_3
$-A_4$	-13.09	-12.07	-18.08	-2.16	-9.07	111	J_4
C	102.85	114.84	263.50	34.13	98.26	0	O
	$-A_1$	$-A_2$	$-A_3$	$-A_4$	$-A_5$	B	

Table 10. The final table in the vectorial form

	$-A_1$	I_1	I_2	$-A_4$	I_4	O	
I_3	-0.0016	-0.9990	-1.2756	0.0152	-0.1608	2.5738	J_3
$-A_2$	0.0647	35.3953	21.9972	0.5670	2.6358	2,168.0980	A_2
$-A_3$	0.0042	3.2200	-0.8658	0.7653	1.3835	973.0686	A_3
I_5	0.0031	0.5481	1.2119	-0.1406	0.0823	7.1076	J_5
C	-0.1048	-106.5220	-102.1860	-9.8028	-0.1432	1,376.5860	O
	$-J_1$	$-A_1$	$-A_2$	$-J_4$	$-A_4$	B	

For example, Table 10 shows that

$A_2 = 0.0647\ A_1 + 0.5670\ A_4 - 35.3953\ I_1 - 2.6358\ I_4$

$A_1 = -35.3953\ A_2 - 3.2200\ A_3 + 0.9990\ J_3 - 0.5481\ J_5$.

These are the agro metric connections between vectors of economic indicators. To interpret these equations we have to keep in mind economical sense of each vector. For example, let us remember that vector A_2 characterizes capital requirements and vector A_1 characterizes rice production in the concrete land utilizing economy. Coefficients 0.0647; 0.5670; −35.3953; − 3.2200 can be interpreted as slope coefficients in the proper linear equations.

CONCLUSIONS

1. The fact that linearity postulate holds in the interconnections between economic indicators in different areas leads to the wide applications of the linear models for quantitative approach to the decision making in management science.
2. Very important reason for linear planning popularity is also the comfortable possibility to solve big scale linear problems with help of effective software.
3. It is easy to observe that the linear programming mainstream applies Dantzig's simplex method, but Stiefel's method is in some kind of oblivion.
4. Of course, Dantzig's method and Stiefel's method operates with absolutely the same information and both methods differ only from interpretations point of view. For all that Hegel's dialectics teaches us that form has influence to the content. Indeed, Stiefel's method offers us the new interpretations and even new concepts, for example, the general optimal plan or simplex systems of equations as production functions.
5. The Stiefel's method allows us to interpret the content of each simplex table as two pairs of production functions what characterizes concrete economic unit. Especially fruitful for economic analysis is the content of final table what characterizes concrete economy close by the received proper optimum. Such interpretations of the simplex tables are not possible using the traditional Dantzig's method.
6. Absolutely no doubt that the Stiefel's method has didactical preferences in teaching process. Especially it concerns non-mathematical specialities' students, for example, the faculties of economics or agronomy.

REFERENCES

Allen, R.G.D. 1956. *Mathematical Economics*. London: Macmillan & Co. Ltd., 768 pp.

Balinski, M.L. & Tucker, A.W. 1969. Duality Theory of Linear Programs: A Constructive Approach with Applications, *SIAM Review* **11**(3), 347–377.

Border, K.M. 2004. *The Gauss–Jordan and Simplex Algorithms*. California Institute of Technology, Division of the Humanities and Social Sciences, 40 pp. Available at *http://www.its.caltech.edu/~kcborder/Notes/SimplexAlg.pdf* Accessed 09.09.2018.

Coenenberg, A.G. 1997. *Cost Accounting and Cost Analysis.* 3rd Edition. Publisher Modern Industry. (*Kostenrechnung und Kostenanalyse*. 3. Auflage. Verlag Moderne Industrie.) 524 pp. (in German).

Cottle, R.W. & Dantzig, G.B. 1968. Complementary Pivot Theory of Mathematical Programming. *Linear Algebra and Its Application* **1**, 103–125.

Cottle, R.W. & Dantzig, G.B. 1970. A Generalization of the Linear Complementarity Problem. *Journal of Combinatorial Theory* **8**, 79–90.

Dantzig, G.B. 1949. Programming of interdependent activities, mathematical model. *Econometrica* **17**, 200–211.
Dantzig, G.B. 1951. Maximization of a linear function of variables subject to linear inequalities. − Activity analysis of production and allocation, ed. T. C. Koopmans, Cowles Commission Monograph **13** Wiley, New York, pp. 339–347.
Dantzig, G.B. & Thapa, M.N. 1977. *Linear Programming 1: Introduction*, Springer Series in Operations Research, 435 pp.
Dantzig, G.B. 1963. *Linear Programming and Extensions*, Princeton University Press, 627 pp.
Dantzig, G.B., Orden, A. & Wolfe, P. 1955. The Generalized Simplex Method for Minimizing a Linear Form under Linear Inequality Restraints. *Pacific Journal of Mathematics* **5**(2), 183–195.
Dantzig, G.B. & Thapa, M.N. 2003. *Linear Programming 2: Theory and Extensions*, Springer Series in Operations Research, 448 pp.
Hestenes, M.R. & Stiefel, E. 1952. Methods of Conjugate Gradients for Solving Linear Systems. *Journal of Researh of the National Bureau of Standards* **49**(6), 409–436.
Jaunzems, A. 1981. Fundamentals of Linear Algebra and Linear Analysis. Zvaigzne, Riga. (*Lineārās algebras un lineārās analīzes pamati.* Zvaigzne, Rīga.) 365 pp. (in Latvian).
Jaunzems, A. 1990. Linear Optimal Planning. University of Latvia. (Яунземс А. Я. 1990. *Линейное оптимальное планирование*. Латвийский Университет) 102 pp. (in Russian).
Jaunzems, A. 1993. Mathematics for Economic Sciences. General course. University of Latvia. (Яунземс А. Я. 1993. *Математика для экономических наук. Общий курс.* − Латвийский Университет) 841 pp. (in Russian).
Jaunzems, A. 2011. *Operations Research. Course materials*. Third Edition. Supplemented and Revised. Ventspils University of Applied Sciences. (*Operāciju pētīšana. Kursa materiāli. Trešais izdevums, papildināts un pārstrādāts*. Ventspils Augstskola) 233 pp. (in Latvian).
Jaunzems, A. 2013. Singular Linear Programming in the Management Economics. In Medveckis A. (ed.): *Society and Culture*. Article Collection XV, Liepaja University. (Singulārā lineārā programmēšana menedžmenta ekonomikā. Grāmatā: *"Sabiedrība un kultūra".* Rakstu krājums, XV / Sastād. Arturs Medveckis. Liepājas Universitāte) pp. 658–672 (in Latvian).
Krastiņš, A. 1976. Mathematical Programming. Zvaigzne, Riga. 199 pp. (in Latvian).
Põldaru, R, Viira, A.H. & Roots, J. 2018. Optimization of arable land use to guarantee food security in Estonia. *Agronomy Research* **16**(4), 1837–1853.
Seo, K.K. 1991. Managerial economics. Text, problems, and short cases. Seventh edition. Irwin, 669 pp.
Sofi, N.A., Aquil Ahmed, Mudasir Ahmad & Bilal Ahmad Bhat. 2015. Decision Making in Agriculture: A Linear Programming Approach. *International Journal of Modern Mathematical Sciences* **13**(2), 160–169.
Stiefel, E. 1960. Note on Jordan Elimination, Linear Programming and Tchebycheff Approximation. *Numerische Mathematik* **2**, 1–17.
Tucker, A.W. 1962. Simplex Method and Theory. Notes on Linear Programming and Extensions – Part 62, MEMORANDUM RM-3199-PR, 36 pp.
Žgajnar, J. & Kavčič, S. 2009. Multi-goal pig ration formulation; mathematical optimization approach. *Agronomy Research* **7**(Special issue II), 775–782.
Zukovitskii, S.I. & Avdeeva, L.I. 1967. *Linear and Convex Programming*. Nauka. Moskva (Зуховицкий С. И. & Авдеева Л. И. 1967. *Линейное и выпуклое программирование.* Серия "Экономико-математическая библиотека". Москва.), 460 pp. (in Russian).

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Productivity of corn hybrids in relation to the seeding rate

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Abstract. Potential yield of corn hybrids with a different FAO number is limited by not only rainfall amount, average soil and air temperature throughout vegetation period, but also directly depends on plant density. The study and practical application of special agricultural techniques allows us to limit and mitigate the negative impact of these factors on the productivity of maize, depending on the indicators under study and the soil and climatic resources of the cultivation zone. Therefore, the study of the influence of the seeding rate on the growth and development of corn plants remains relevant. The results presented make it possible to choose optimal seeding rates for corn hybrids of early and middle groups of ripeness (FAO 180-280). Overcrowding from 61,000 to 93,000 seeds ha^{-1} leads to increase in interstage period 'sprouting–wax ripeness' of Rodnik 179SV hybrid for 4 days, of MAS 12R and AMELIOR hybrids–for 2 days, and of MAS 30K hybrid – for 3 days. Hybrids Rodnik 179SV and AMELIOR reached maximum height – 217 cm and 214 cm respectively – at seeding rate of 73,000 seeds ha^{-1}, while hybrids MAS 12R and MAS 30K grew up to their 213 cm and 223 cm respectively at seeding rate of 77,000 seeds ha^{-1}. Decrease in seeding rate to less than 73,000 seeds ha^{-1} and, contrary to it, overcrowding of seeds of more than 77,000 seeds ha^{-1} leads to decrease in corn hybrid plant height. Agronomically, the most efficient for maximizing early ripe Rodnik 179SV and MAS 12R hybrids yields (6.39 and 6.73 t ha^{-1}) and middle-early ripe AMELIOR hybrid yield (6.81 t ha^{-1}) was the seeding rate of 73,000 seeds ha^{-1}, while the highest yield of middle MAS 30K hybrid (7.21 t ha^{-1}) was at the seeding rate of 77,000 seeds ha^{-1}.

Key words: seeding rate, early ripeness, density, yield.

INTRODUCTION

Total corn yield over the world has risen in recent years, and it is assumed that its growth and absolute productivity level are in close relation to soil-climatic and macroeconomic conditions, as well as to intensity of plant growing and bio-technological progress (Hasenclever, 2000; Spiekers, 2000; Free, 2001; Heinrich, 2001; Zellner, 2004).

Corn hybrids productivity is conditioned by quantitative parameters determining its habitus, such as plant height,leaf coverage, area of leaf surface etc. These parameters' value determines the reaction of plants to changes in growing conditions. Growth and development of corn plants depend on a number of factors, primarily on existing meteo-conditions, biological properties of the hybrids and the agrotechnology applied (Jager, 2004; Spaar et al., 2009).

Studies of a number of Russian and foreign scientists give no unanimous opinion on optimal seeding rate for corn hybrids of different ripeness groups. Thus, Dorffi & Borzeny (1996) claim that optimal plants quantity per ha depends on humidification conditions of a certain year: 80,000 plants ha^{-1} in years enough humidified, 30,000 plants ha^{-1} – in droughts. Russian researchers Popov et al. (1991) claim that medium ripening hybrids form maximal yield at 50,000 plants ha^{-1}. Yakhtanigova & Topalova (2008) claim that optimal density for each hybrid is determined basing on individual parameters. Thus, the studies of Belozerny 1 MV and Kavkaz 307 MV hybrids have shown the optimal density of 60,000 plants ha^{-1}, while those of Nika 353 MV were 50,000 plants ha^{-1}. As Potapov et al. (2016) suggest, formation of optimal density of corn seedlings is not only conditioned by available humidity and nutrient reserves, but also by architectonics and drought resistance of a hybrid itself. For medium ripening hybrids, increase in plant density of more than 65,000 plants ha^{-1} results in the increase in yield, but not more than 85,000 plants ha^{-1}. The optimum for such hybrids is 75,000 plants ha^{-1}. Such scientists as Kravchenko (2010), Filin & Mikhin (2014), Zubkova & Sozin (2016) claim that the plant degree of density optimum depends on soil-climatic zones of cultivation.

Under conditions of Central Chernozem Region of Russia (CCR) unstable humidification, the influence of corn hybrid seeding rate and degree of density on yield of modern hybrids of various groups of ripeness has not been investigated to the full. Hence, the objective of the study presented is a scientific grounding of optimal seeding rate of various ripeness group corn hybrids grown under conditions of unstable humidification.

MATERIALS AND METHODS

The field experience was laid during 2013–2015 on the fields of SP by the head of the farm Kotov V.V. Bobrovsky district of the Voronezh region (N50.513678 E40.014524), according to a two-factor scheme:

Factor A – hybrids (MAS 12R, AMELIOR, MAS 30K and Rodnik 179SV (control));

Factor B – seeding rate (61,000; 67,000; 73,000; 77,000; 83,000; 87,000 and 93,000 seeds ha^{-1}).

4 hybrids were used in the experiment:

1. Rodnik 179SV is a short-season (FAO 180) three-linear maize hybrid, created jointly by breeders of the Voronezh Experimental Station VNIK and LLC Innovation and Production Agrofirm ‘Selection’. The plants are 240–260 cm tall, untillered, folious. The ear is 70–75 cm high, slightly cone-shaped, weighing 110–140 grams and 20–25 cm long. The grain is semi-toothed, yellow. The mass of 1,000 grains is 260–290 g. The grain row number on the cob is 14–16. The threshing yield of the grain is 80–82%. The recommended plant density per hectare per grain for Bogar is 60,000 plants ha^{-1}, for irrigation it is 75,000 plants ha^{-1}. If fertilizers are not applied, the density should be decreased by 5,000–10,000 plants ha^{-1}depending on the soil fertility and moisture.

2. MAS 12R is a short-season (FAO 180) hybrid, it has been produced by the MAS Seeds company, flint, cold-resistant, intended for early sowing. Rapid growth and early development. Plants are 250–265 cm tall, untillered, folious. The ear is 70–75 cm high, slightly cone-shaped, weighing 110–140 grams and 20–25 cm long. The grain is semi-toothed, yellow. The mass of 1,000 grains is 260–280 g The grain row number on the cob is 14–16. The recommended seeding rate is 58,000–62,000 seeds ha^{-1}.

3. AMELIOR is a middle-early hybrid (FAO 240), produced by the company MAS Seeds; it is flint and dent-like, has a high yield potential for grain, is environmentally plastic and drought-resistant. The grain is semi-toothed, yellow. The mass of 1,000 grains is 300–310 g. The grain row number on the cob is 14–16. The recommended plant density per ha^{-1} is 75,000–85,000 and (under the conditions of sufficient moistening) or 70,000–75,000 (under the conditions of unstable moistening).

4. MAS 30K – is a mid-season hybrid (FAO 280), produced by the company MAS Seeds; it is dent-like and has a good moisture-yielding ability due to its semi-toothed grains. The grain is semi-toothed, yellow. The mass of 1,000 grains is 320–340 g. The grain row number on the cob is 14–16. The recommended plant density per ha^{-1} is 70,000–80,000 (under the conditions of sufficient moistening) or 70,000–75,000 (under the conditions of unstable moistening).

Soils of the experimental plot are the ones of ordinary chernozem, medium loamy. Humus content in plowing layer is 4.3%, pH–5.6. The degree of soil saturation with bases is 88.9%. The content of mobile phosphorus is 73 and exchangeable potassium–103 mg kg^{-1} of soil (Chirikov, 1956).

The total area of the plot – 157 m^2, accounting–120 m^2.The experiment was laid by the method of split plots with their randomized placement inside repetitions. The experiment repetition is 4-fold. Corn's preceding crop had been winter wheat. The technology of corn cultivation in the experiment is as follows:

– plowing (John Deere 8310R + Lemken Euro Diamant) to a depth of 25–27 cm;

– cultivation (John Deere 8310R +Lemken Korund9) to a depth of 8–10 cm;

– fertilization (in the fall – anhydrous ammonia at the rate of 0.1 t ha^{-1} (82 kg AI ha^{-1}), in spring - ammophos of 75 kg ha^{-1} (39 kg AI ha^{-1}) in physical mass at sowing and Rexolin microfertilizer 0.15 kg ha^{-1}(0.22 kg AI ha^{-1}) – for top dressing;

– sowing (MTZ-1221 + Gaspardo (8 lines), planting dates – III third of April – I third of May;

– pesticide treatment (by Titus Plus herbicide – 0.387 kg ha^{-1} (dicamba 609 g kg^{-1} + rimsulfuron 32.5 g kg^{-1}), Rogor-S insecticide – 1 L ha^{-1} (dimetoat 400 g L^{-1});

– gathering (Acros 580) in lines, with recalculation on 14% humidity and 100% purity.

In the process of research, phenological observations were carried out on ten marked plants of each plot in four repetitions. The following phases of maize development were noted: the beginning and the full sprouting, the beginning and the full panicle emergence, the beginning and the full flowering of the cobs (filaments emergence), the milky, milky-wax state of the grain, the waxy ripeness, the complete ripeness. The onset of each phase was noted at its development stage: the beginning (in 15% of the plants), complete (in 75% of the plants). The standing density was determined by counting plants from 14.3 m.p. (10 m^2) in quadruplicate from each experimental plot; the field germination was determined in the phase of full germination. The plant height was measured in each phase of the growing season on 10 plants from each experimental plot. The mathematical processing of the research results was performed by B.A. Dospekhov's (1985) variance analysis method on a personal computer.

Weather conditions during the field studies for 2013–2015 had deviations from the average annual data on the main indicators. The best weather conditions for the growth and development of maize developed in 2013, the amount of precipitation during the growing season was 395.4 mm or 118% of the average annual rate. High temperatures and lack of moisture in the interphase period 'inflorescence emerge – flowering' in terms of 2014 and 2015 were the most critical to corn plants. In total, during the growing season in 2014, 191.4 mm of precipitation fell, which is 57% of the average annual norm, and in 2015 – 246.6 mm or 74% of the average annual norm.

RESULTS AND DISCUSSION

Duration of vegetation period is an important biological indicator that allows differentiating all hybrids by their earliness of ripening. To characterize the vegetation period duration of corn, two interstage periods are distinguished: sprouting–flowering and flowering–wax ripening. The duration of vegetation period of hybrids under study has been different in different years and mostly depended on the indicator of earliness of ripening. Thus, on average for three years of research, the following hybrids with FAO 180 were the most ripening: MAS 12R (102 days) und Rodnik 179SV (104 days). AMELIOR (FAO 240) hybrid vegetation period made up 113 days. MAS 30K (FAO 280) hybrid proved to be the most late ripening, with vegttion period of 122 days (Table 1).

It should be noted that the seeding rate had a slight effect on the vegetation period of all studied hybrids. There is a tendency to the increase of the vegetation period with increasing of seeding rate, especially in such early ripening hybrids as Rodnik 179SV and MAS 12R.

Table 1. Duration of vegetation period of corn hybrids at different seeding rates

Seeding rate, seeds ha^{-1}	Vegetation period (range/average), days			
	Rodnik 179CB FAO 180	MAS 12R FAO 180	AMELIOR FAO 240	MAS 30K FAO 280
61,000	$\frac{99-105}{102}$	$\frac{95-104}{100}$	$\frac{109-115}{112}$	$\frac{117-122}{120}$
67,000	$\frac{101-104}{102}$	$\frac{99-104}{101}$	$\frac{110-115}{112}$	$\frac{118-124}{120}$
73,000	$\frac{102-105}{104}$	$\frac{98-103}{100}$	$\frac{110-118}{114}$	$\frac{120-125}{123}$
77,000	$\frac{102-104}{103}$	$\frac{97-101}{99}$	$\frac{109-118}{113}$	$\frac{121-125}{123}$
83,000	$\frac{102-106}{104}$	$\frac{101-103}{102}$	$\frac{113-116}{114}$	$\frac{119-124}{121}$
87,000	$\frac{104-108}{106}$	$\frac{101-105}{103}$	$\frac{113-115}{114}$	$\frac{122-124}{123}$
93,000	$\frac{105-107}{106}$	$\frac{100-106}{103}$	$\frac{112-116}{114}$	$\frac{122-124}{123}$
Average by factor A	**104**	**102**	**113**	**122**

Increase in seeding rate from 61,000 to 93,000 seeds ha^{-1} had no significant influence on duration of 'sowing–sprouting' interstage period in all hybrids under study and made up 18–19 days(Table 2).

Table 2. Duration of interstage periods of corn hybrids at different seeding rates

Seeding rate, seeds ha^{-1}	Interstage periods, days											
	Rodnik 179SV FAO 180			MAS 12R FAO 180			AMELIOR FAO 240			MAS 30K FAO 280		
	sowing – sprouting	sprouting – flowering	sprouting - wax ripeness	sowing – sprouting	sprouting – flowering	sprouting - wax ripeness	sowing – sprouting	sprouting – flowering	sprouting - wax ripeness	sowing – sprouting	sprouting – flowering	sprouting - wax ripeness
61,000	18	50	94	17	51	93	18	64	104	18	65	112
67,000	18	53	94	18	50	93	18	64	104	18	64	112
73,000	18	52	96	17	53	93	18	63	106	19	65	114
77,000	18	53	95	17	52	92	18	64	105	19	65	114
83,000	18	53	96	18	53	94	18	65	106	18	66	113
87,000	18	53	98	18	53	95	18	64	106	19	66	114
93,000	18	54	98	18	53	95	18	65	106	19	65	114
r (yield correlation)			-0.51			-0.79			0.03			-0.18

Differencies in development of corn hybrid plants in relation to a seeding rate started to show by the stage of flowering. By the beginning of flowering of corn plants the overcrowding of seeds of such early ripening hydrids as Rodnik 179SV and MAS 12R lead to the increase in 'sprouting–flowering' interstage period up to 2–4 days, which was not observed in medium ripening (AMELIOR) and late ripening (MAS 30K) hybrids.

To the phase of wax ripeness with an increase in the seeding rate per 1 ha for all hybrids, one can note the tendency of increase in the 'sprouting–wax ripeness' interstage period. Thus, Rodnik 179SV hybrid's interstage period increased by 4 days, that of MAS 12R and AMELIOR hybrids did by 2 days,while medium ripening MAS 30K hybrid's interstage period increased up to 3 days.

At the initial stages of development, corn plants grow slowly. Within the first half of vegetation, in 12–15 days after sprouting, the highest average daily plant growth gain under favourable conditions can only reach 1.0–2.5 cm. 10 days before ear formation corn plant growth gain reaches its maximum values, that is up to 5–10 cm a day. After the flowering stage, corn plant linear growth stops.

For three years of study, the height of corn plants in the beginning of ear formation stage, in relation to seeding rate, made up 81–104 cm. MAS 12R hybrid plants grew higher – 104 cm at the seeding rate of 73,000 seeds ha^{-1}, AMELIOR hybrids were somewhat lower–95 cm at the seeding rate of 83,000 seeds ha^{-1}. Medium ripening MAS 30K hybrid grew up to its maximum of 93 cm at the seeding rate of 87,000 seeds ha^{-1} (Table 3).

Table 3. Corn hybrid plants' height by vegetation stages at different seeding rates

Seeding rate, seeds ha^{-1}	Corn hybrid plants' height by vegetation stages, cm											
	Rodnik 179SV FAO 180			MAS 12R FAO 180			AMELIOR FAO 240			MAS 30K FAO 280		
	ear formation	flowering	ripening	ear formation	flowering	ripening	ear formation	flowering	ripening	ear formation	flowering	ripening
61,000	84	196	208	101	188	194	92	202	205	91	202	210
67,000	85	188	203	98	196	203	92	200	203	87	206	209
73,000	86	202	217	104	198	205	94	210	214	88	207	216
77,000	86	198	210	97	206	213	92	206	210	92	215	223
83,000	85	201	215	99	204	210	95	207	210	86	212	221
87,000	81	197	202	98	201	203	91	199	202	93	207	211
93,000	81	196	201	94	193	199	89	198	201	91	199	209
r (yield correlation)			0.36			0.54			0.95			0.65

MAS 30K hybrid plant had the biggest height (215 cm) by the beginning of flowering stage in a variant with the seeding rate of 77,000 seeds ha^{-1}. The lowest at this stage were MAS 12R hybrid plants, that is, 193 cm at the seeding rate of 93,000 seeds ha^{-1}.

To the ripening stage, the differences in the height of all hybrid plants in relation to seeding rate became smaller. Thus, maximal difference in heights of MAS 30K and AMELIOR plant hybrids in relation to seeding rate made up 13 cm, or 6.1%.

In our experiments, the tendency has been observed of the increase in corn plant height with the increase in FAO. Thus, plant height at the ripening stage in MAS 12R hybrid with FAO 180 amounted, in relation to seeding rate, to 193–213 cm, in AMELIOR hybrid with FAO 240–to 201 – 2014 cm, and in MAS 30K with FAO 280–to 209–223 cm. The height of AMELIOR hybrid plants with seeding density of 77,000 and 83,000 seeds ha^{-1} at the ripening stage was the same and made up 210 cm. The same insignificantly did the plants height change at seeding rates of 61,000 and 67,000 seeds ha^{-1}. MAS 30K hybrid has displayed the same tendency. It should also be noted that Rodnik 179SV and AMELIOR hybrid plants at the seeding rate of 61,000 seeds ha^{-1} grew 4–7 cm higher than at the seeding rate of 93,000 seeds ha^{-1}. In medium ripening MAS 30K hybrid with FAO 280, this tendency has had little manifestation.

MAS 12R and MAS 30K hybrid plants showed top growth results at the seeding rate of 77,000 of germinable seeds ha^{-1}. Reducing seeding rate to less than 77,000 seeds ha^{-1} and, contrary to it, overcrowding of seeds to more than 77,000 seeds ha^{-1} leads to decrease in corn hybrid plants height. Thus, Rodnik 179SV and AMELIOR hybrid plants grew the highest (217 cm and 214 cm respectively) at the seeding rate of 73,000 seeds ha^{-1}, while MAS 12R and MAS 30K hybrids reached their 213 cm and 223 cm respectively at the seeding rate of 77,000 seeds ha^{-1}.

For the three years of research, germinating ability of seeds in all corn hybrids under study has been decreasing with increase in seeding rates. Of all the hybrids, MAS 12R responded most significantly to a change in seeding rate, displaying the change in field germinating capacity from 94.3% to 87.2%. MAS 30K hybrid showed the smallest change in field germinating capacity (92.3% to 87.2%) in relation to increase in quantity of seeds sown per 1 ha (Table 4).

Table 4. Plant density and germinating ability of corn seeds at the sprouting stage at different seeding rates

Seeding rate, seeds ha^{-1}	Rodnik 179SV FAO 180		MAS 12R FAO 180		AMELIOR FAO 240		MAS 30K FAO 280	
	density, thous ha^{-1}	germinating capacity,%	density, thous ha^{-1}	germinating capacity,%	density, thous ha^{-1}	germinating capacity,%	density, thous ha^{-1}	germinating capacity,%
61,000	56.0	91.7	57.5	94.3	56.9	93.3	56.3	92.3
67,000	61.1	91.1	62.2	92.8	61.7	92.1	60.4	90.1
73,000	66.1	90.5	67.6	92.6	66.8	91.5	64.5	88.3
77,000	69.7	90.5	71.2	92.5	69.7	90.6	67.8	88.0
83,000	74.1	89.3	73.6	88.7	74.2	89.4	72.8	87.8
87,000	75.5	86.7	76.8	88.3	76.0	87.3	76.2	87.6
93,000	79.7	85.7	81.1	87.2	81.0	87.1	81.1	87.2

At the sprouting stage, the best germinating capacity (94.3%) and plant density (57,500 plants ha^{-1}) were displayed by MAS 12R hybrid at the seeding rate of 61,000 seeds ha^{-1}. The worst germinating capacity (85.7%) and plant density (79,700 plants ha^{-1}) were displayed by Rodnik 179SV hybrid at the seeding rate of 93,000 seeds ha^{-1}.

In 2013–2015, the capacity of sprouts for gathering in relation to seeding rates was from 95.0 to 84.6%. AMELIOR hybrid plants showed better capacity for gathering, while MAS 12R hybrid plants deteriorated more than others (Table 5).

Table 5. Plant density and corn plant capacity for gathering at different seeding rates

Seeding rate, seeds ha^{-1}	Rodnik 179SV FAO 180		MAS 12R FAO 180		AMELIOR FAO 240		MAS 30K FAO 280	
	density, thous ha^{-1}	capacity for gathering, %	density, thous ha^{-1}	capacity for gathering, %	density, thous ha^{-1}	capacity for gathering	density, thous ha^{-1}	capacity for gathering, %
61,000	49.6	88.6	51.3	89.3	52.7	92.5	52.0	92.2
67,000	56.8	93.1	54.0	86.8	56.7	91.9	52.9	87.6
73,000	58.8	89.0	60.0	88.9	62.1	93.0	58.6	90.8
77,000	63.1	90.5	60.3	84.6	64.7	92.7	64.4	95.0
83,000	65.0	87.6	64.2	87.2	66.8	90.1	68.2	93.6
87,000	69.6	92.2	69.8	90.8	68.3	89.9	70.8	92.9
93,000	71.9	90.2	73.6	90.7	72.9	90.0	75.1	92.7

In early ripening Rodnik 179SV hybrid, the best gathering capacity level (93.1%) was at the seeding rate of 67,000 seeds ha^{-1}. In MAS 12R hybrid plant, the capacity was higher (90.7–90.8%) at maximal seeding rates of 87,000 and 93,000 seeds ha^{-1}, medium-early AMELIOR hybrid had the highest percentage of preserved plants (93.0%) at the seeding rate of 73,000 seeds ha^{-1}, while medium ripening MAS 30K hybrid displayed maximum capacity (95.0%) at the seeding rate of 77,000 seeds ha^{-1}.

The highest productivity of Rodnik 179SV (6.39 t ha^{-1}), MAS 12R (6.73 t ha^{-1}) and AMELIOR (6.81 t ha^{-1}) hybrids was at the rate of 73,000 seeds ha^{-1}, while MAS 30K hybrid gave the highest productivity of 7.21 t ha^{-1} at the seeding rate of 77,000 seeds ha^{-1}. The lowest hybrid corn yield was at the seeding rates of 61,000 and 93,000 seeds ha^{-1}. (Table 6).

Table 6. Corn hybrids yield at different seeding rates

Seeding rate, seeds ha^{-1}	Yield, t ha^{-1}							
	Rodnik 179SV FAO 180				MAS 12R FAO 180			
	2013	2014	2015	average (factor B)	2013	2014	2015	average (factor B)
61,000	5.25	5.05	4.94	**5.08**	6.08	5.31	4.83	**5.41**
67,000	7.32	6.45	5.83	**6.53**	8.29	4.66	5.49	**6.15**
73,000	6.23	6.78	6.15	**6.39**	9.49	5.66	5.04	**6.73**
77,000	5.80	6.12	5.94	**5.95**	5.71	6.19	6.93	**6.28**
83,000	6.29	5.34	4.47	**5.37**	6.63	4.94	5.82	**5.80**
87,000	-	5.52	4.93	**5.23**	-	5.05	5.17	**5.11**
93,000	-	4.91	4.76	**4.84**	-	4.47	4.26	**4.37**
average (factor A)	**6.18**	**5.74**	**5,29**		**7.24**	**5.17**	**5.36**	
LSD_{05} (A)	0.71	0.44	0.33		0.71	0.44	0.33	
LSD_{05} (B)	0.63	0.34	0.25		0.63	0.34	0.25	
LSD_{05} (AB)	1.42	0.89	0.67		1.42	0.89	0.67	
Seeding rate, seeds ha^{-1}	AMELIOR FAO 240				MAS 30K FAO 280			
	2013	2014	2015	average (factor B)	2013	2014	2015	average (factor B)
61,000	6.17	4.85	5.19	**5.40**	8.26	6.95	5.41	**6.87**
67,000	6.85	5.34	4.73	**5.64**	7.19	5.89	5.43	**6.17**
73,000	7.87	6.15	6.41	**6.81**	7.91	6.54	6.67	**7.04**
77,000	8.44	5.61	5.95	**6.67**	8.49	7.06	6.08	**7.21**
83,000	7.63	5.15	6.07	**6.28**	8.08	6.85	4.91	**6.61**
87,000	-	4.54	5.21	**4.88**	-	5.54	5.84	**5.69**
93,000	-	4.61	4.94	**4.78**	-	5.14	5.02	**5.08**
average (factor A)	**7.39**	**5.18**	**5.50**		**7.98**	**6.28**	**5.62**	
LSD_{05} (A)	0.71	0.44	0.33		0.71	0.44	0.33	
LSD_{05} (B)	0.63	0.34	0.25		0.63	0.34	0.25	
LSD_{05} (AB)	1.42	0.89	0.67		1.42	0.89	0.67	

The favorable combination of heat and moisture supply in 2013 contributed to the fullest realization of the productive potential of all the studied hybrids. The average yield of early ripe hybrids Rodnik 179SV and MAS 12R was 6.18 and 7.24 t ha^{-1}, respectively. The maximum yield of the Rodnik 179SV hybrid (7.32 t ha^{-1}) was at a seeding rate of 67,000 seeds ha^{-1}, and MAS 12R (9.49 t ha^{-1}) - at a seeding rate of 73,000 seeds ha^{-1}. An increase in the seeding rate of more than 73,000 units per hectare or a decrease of less than 67,000 units per hectare statistically significantly reduced the yield of maize for these hybrids. In the medium-early AMELIOR hybrid, the average yield was 7.39 t ha^{-1}, the maximum value (8.44 t ha^{-1}) was 77,000 seeds ha^{-1} in the variant with a seeding rate. It should be noted that with a seeding rate of 73,000 seeds ha^{-1}, this hybrid yields did not decrease statistically significantly, within the limits of experimental error. In options with low rates, less than 73,000 seeds ha^{-1}, as well as in thickened crops, more than 77,000 seeds ha^{-1}, the grain yield of this hybrid has significantly decreased. The productivity of the mid-season hybrid MAS 30K averaged 7.98 t ha^{-1}. The maximum yield values were in thickened crops with seeding rates from 73,000 to 83,000 seeds ha^{-1}. In the variant with a seeding rate of 67,000 seeds ha^{-1}, the grain yield was statistically significantly reduced. In less favorable hydrothermal conditions in 2014 and 2015, the grain yield on average in hybrids was lower by 1.01–1.70 t ha^{-1} (16.3–21.3%). For early ripening hybrids, the Rodnik 179SV and MAS 12R, the statistically significant yield increase was on varieties with a sowing of crops from 67,000 to 77,000 seeds ha^{-1} in 2014, from 73,000 to77,000 seeds ha^{-1} in 2015. An increase in the seeding rate from 77,000 to 93,000 seeds ha^{-1} resulted in a decrease in the grain yield of maize, according to the hybrid Springnik 179CB on average by 10.1–14.5%, according to MAS 12R – by 13.5–20.5%. This trend was also on the later ripening hybrids AMELIOR and MAS 30K.

Thus, the highest yield of early ripening Rodnik 179SV and MAS 12R hybrids was obtained at the seeding rate of 73,000 seeds ha^{-1}, of medium-early ripening AMELOR hybrid–also at the seeding rate of 73,000 seeds ha^{-1}, while medium MAS 30K hybrid was the most productive at the seeding rate of 77,000 seeds ha^{-1}.

CONCLUSIONS

Thus, the results of field studies for 2013–2015, showed that in conditions of unstable moistening, the rate of sowing seeds is one of the main factors influencing the formation of optimal plant density and the realization of the potential possibilities of maize hybrids, different groups of ripeness. An increase in the seeding rate from 61,000 to 93,000 seeds ha^{-1} did not contribute to an increase in the yield of maize, on the contrary, the higher or lower this indicator was from the optimum, the lower were the yields. The highest yield of early ripening Rodnik 179SV and MAS 12R hybrids was obtained at the seeding rate of 73,000 seeds ha^{-1}, of medium-early ripening AMELOR hybrid–also at the seeding rate of 73,000 seeds ha^{-1}, while medium MAS 30K hybrid was the most productive at the seeding rate of 77,000 seeds ha^{-1}.

REFERENCES

Chirikov, F.V. 1956. *Agrochemistry of potassium and phosphorus.* State Publishing House of Agricultural Literature, 463 pp. (in Russian).

Dorffi, B. & Borzheni, Z. 1996. Effect of drought, fertilizer and plant density on the yield of corn. *Abstract Journal* **8**, pp. 32–35.

Dospekhov, B. 1979. *Technique of field experience*. Moscow, Kolos, 415 pp. (in Russian).

Filin, V. & Mikhin, B. 2014. *Seed density and soil treatment mode as the factor of increasing corn productivity on Southern Chernozem of Volgograd Region*. News of Nizhnevolzhsky Agro-University Complex: Nauka y Vyscheye Proffesionalnoye Obrazovanye **1**(33), pp. 112–117 (in Russian).

Free, O. 2001. *Why corn can do more than other crops*. New agriculture, special issue corn, pp. 14–16.

Hasenclever, K. 2000. Wildscha denim maize (k) a problem*? Innovation* **4**, 10–11.

Heinrich, J. 2001. Maize can continue to compete. *Farmers newspaper* **11**, 32–33.

Jager, F. 2004. *Maize cultivation plan central Germany*. KWS Einbeck, 170 pp.

Kadyrov, S. & Kharitonov, M. 2016. Influence of seeding rate on growth and development of corn hybrids in Forest-Steppe of Central Black Earth Region. *Nauchnaya Zhizn* **9**, 6–15 (in Russian).

Kadyrov, S. & Kharitonov, M. 2016. Corn yield and seed quality at different seeding. *Vestnik Voronezhskogo Agrarnogo Universiteta* **1**(48), 12–16 (in Russian).

Kravchenko, R. 2010. *Agro-biological grounding of consistent corn crops gain under conditions of steppe zone of Central Fore-Caucasian Region*: monograph. Stavropol, 208 pp. (in Russian).

Potapov, A., Pashinin, A. &Pivovarov, I. 2016. Influence of sowing terms and seeding rates on productivity of medium-early corn hybrids. *Simvolnauki* **11–2**(23), 25–27 (in Russian).

Spaar, D., Ginapp, K., Drager, D., Zakharenko, A. &Kalenskaya, S. 2009. *Corn (Growing, harvesting and storage)*. Moscow, ID OOO ‘DLVAGRODELO’, 390 pp. (in Russian).

Spiekers, H. 2000. *Maize trace*. KWS Einbeck, 28 pp.

Spitz, H. Gute. 2002. Experiences with maize sowing. *Inovation* **2**, 22–23.

Ustimenko, G., Popov, V. & Kharlamov, M.P. 1991. *The effect of different sowing density on the productivity of mid-season corn hybrids* // Issues of increasing agricultural production in various soil-climatic zones. University of Peoples' Friendship, Moscow, 3–7. (in Russian).

Yakhtanigova, Zh. &Topalova, Z. 2008. The productivity of different types of corn depending on the different planting density. *New technologies* **6**, 48–52 (in Russian).

Zellner, M. 2004. *New Problem for the Maize*. DLG News, **9**, pp. 44–47.

Zubkova, M. & Sozin, A. 2016. Corn hybrids green mass yield in relation to seeding rate. *Molodyozh y nauka* **4**, 59 (in Russian).

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Effect of foliar fertilization of microelements on highbush blueberry (*Vaccinium corumbosum* L.) nutrient status and yield components in cutover peatlands

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Abstract. The commercial cultivation of highbush blueberry in Latvia was successfully started during the last 20 years. In 2018, there was an estimated 280 ha of highbush blueberries planted in Latvia with increasing annual hectarage. In general, blueberry is a highly specialized crop that has definite soil agrochemical and climatic requirements: it has low nutrient needs and is sensitive to excessive nutrient levels in the soil. However, balanced and precise mineral nutrition is essential for producing high and quality yield. The study was conducted in the producing planting of highbush blueberry, cultivar 'Patriot', during the vegetation season of 2018. The research was carried out to determine the effect of foliar fertilization with micronutrients (Fe, Zn, Cu, Mo, B) on the productivity, the content of mineral elements in leaves and photosynthesis of blueberries. Field experiment design included foliar fertilizer treatments 0 to 3 times per season. In general, our results revealed that foliar sprays with micronutrients had a positive effect on the berry yield, parameters of photosynthesis and microelement content in leaves of highbush blueberry. It was determined that the application of the foliar fertilizer 4 times per season provided the highest berry yield (134% compared to control) and the highest photosynthetic activity of plants. Our study suggests that correct foliar fertilization can optimize the content of Fe, Zn and B in blueberry leaves.

Key words: field experiment, soil nutrient status, leaf nutrient status, parameters of chlorophyll fluorescence.

INTRODUCTION

Highbush blueberry (*Vaccinium corymbosum* L.) is a perennial flowering plant from the family *Ericaceae* of the genus *Vaccinium*. Commercial blueberry varieties, in general, are indigenous to eastern and central North America including the eastern territories of Canada (Trehane, 2004). Commercial production of Vaccinium species in the United States of America has existed since the latter part of the eighteenth century (Eck, 1990). Today, highbush blueberries are cultivated in many non-native regions worldwide in highly different soil and climatic conditions (Strik, 2005). Many species of *Vaccinum* have a long history of being used for medical purposes. Blueberries are also valued for their fresh taste as well as their potential for being processed. Increasing demand for healthy ingredients from the food industry and changed consumer consciousness provides a great opportunity for further progress of blueberry production. The cultivation of blueberries in Latvia is comparatively recent – while the first experimental plantations were established in the middle of 1980s, commercial cultivation of these berries started in last 20 years (Osvalde et al., 2018) reaching 280 ha in 2018 (Karlsons et al., 2018). In general, about 85% of the global production of blueberries comes from the United States of America and Canada alone (Crop statistics, 2019).

There are two different soil types used for the cultivation of highbush blueberries in Latvia: light acid mineral soils rich in organic matter and peat soils. Today, about one-third of the total hectarage of blueberry plantings in Latvia are successfully developed on cutover peatlands on bare sphagnum peat (Osvalde et al., 2018).

Although highbush blueberries have specific soil requirements and are well adapted to low pH soils with limited availability of mineral elements, balanced mineral nutrition is vitally essential in producing high and qualitative yield (Hart et al., 2006; Pormale et al., 2009). Considering that blueberry plants are shallow-rooted, fruit production can be significantly reduced even with a moderate nutrient deficiency. On the other hand, excessive or inadequate fertilization is potentially damaging to blueberry cultivation especially in plantations established in environmentally sensitive areas such as excavated peat bogs. Especially considering that blueberry fruit value is relatively high; it contributes to the tendency to apply more types or quantity of fertilizers with the hope of improving yield or quality. Results from the previous studies in 2006–2017 highlighted the incompleteness in highbush blueberry providing with nutrients that could be a significant restrictive factor for obtaining high, qualitative and sustainable berry yields in Latvia. Overall, more than 50% of blueberry soils had a low content of N, S, Mo and B (Osvalde et al., 2018). Considering that average yield in Latvia (1.49 t ha^{-1}) is significantly lower to compare with the United States of America (6.91 t ha^{-1}), Canada (3.28 t ha^{-1} and Poland (3.07 t ha^{-1}) (Crop statistics, 2019) research on mineral nutrition as one of the potential limiting factors of reduced yield of blueberry in Latvia are critically important. Plant fertilizers can be applied directly to the soil for uptake by plant roots, by foliar spraying for uptake via the leaves, or in combination. Availability of nutrients from the soil may be limited in the conditions of improper soil reaction, ionic antagonism, or unfavorable weather conditions. Under such circumstances, foliar sprays are a simple, fast and effective method for supplying nutrients to plants (Fageria et al., 2009; Wach & Błażewicz-Woźniak, 2012). Main advantages of foliar fertilization: it is a possibility to react rapidly to visual symptoms or tissue analysis, fast plant response in correcting the deficiency, avoidance of soil problems, relatively low cost, small amounts of fertilizer, and reduced risk of environmental pollution. Therefore, used wisely, foliar fertilizers may be more environmentally friendly and target oriented than soil fertilization though plant responses to foliar sprays are variable and many of the principles of foliar fertilization remain poorly understood (Fernández et al, 2013).

The research was carried out to determine the effect of foliar fertilization with micronutrients (Fe, Zn, Cu, Mo, B) on the productivity, yield quality, leaf nutrient status and photosynthesis of highbush blueberries.

MATERIALS AND METHODS

Field experiment

The field experiment was carried out in 2018 by the Laboratory of Plant Mineral Nutrition, Institute of Biology, University of Latvia, on a production farm (56°70′N, 23°60′E) established on a excavated peat bog abandoned after industrial peat production (region of Jelgava) to investigate the effect of micronutrient foliar application on blueberry yield and berry quality. Together 128 (10 years old) blueberry plants (32 for each treatment) of 'Patriot' cultivar was used in the spacing of 1.2 × 2.0 m (3,888 plants ha^{-1}). The experiment followed a randomized sub-block design. Peat chemical characteristics, determined from composite peat sample of the upper 20 cm taken in April, before the start of the experiment are given in Table 1. In general, peat from the experimental field was characterized by low levels of N, S, Mo, B and consequently low $pH/_{KCl}$ (4.31), as well as high organic matter content (< 95%). At the beginning of May (beginning of the active growing period for plants in Latvia climate conditions), the complex fertilizer suitable for blueberries: 12–8–16 + microelements (Blaukorn classic, COMPO EXPERT GmbH, Germany) was applied at a dose of (according to the producer's instructions) 400 kg ha^{-1} for all treatments. Within the growing period, the bushes were sprayed with foliar fertilizer containing: Fe, Zn, Cu, Mo, B (Yara Vita, Yara International ASA) as follows:

1. Control – 0 treatments per season.
2. Treatment 1 – 1x per season (01.06. 2018).
3. Treatment 2 – 2x per season (01.06. 2018; 05.07.2018).
4. Treatment 3 – 3x per season (01.06. 2018; 05.07.2018; 03.08.2018).

The blueberry leaves for chemical analysis were collected and fluorescence parameters measured 10 days after each foliar fertilizer treatment. Berry yield, as well as the yield components, were determined for each treatment at a harvest time. Berries were harvested twice (21.07.2018 and 10.08.2018) by hand-picking, in the first harvest, all mature fruits were harvested, the second time all mature and unripe fruits were harvested, the total yield was measured for all berries, but average fruit diameter and one berry mass only for mature berries.

Fluorescence parameters were measured using a Handy PEA chlorophyll fluorometer on field conditions. Fluorescence measurements were performed according to the manufacturer's instructions as described previously (Samsone et al., 2009). Before analysis, leaves were dark adapted for 30 min using the appropriate leaf clips. For every treatment, 15 independent measurements on individual leaves were performed. Performance Index (PI) and potential maximum quantum yield of photosystem II (FV/FM) were determined. The data were analyzed by PEA Plus software.

Laboratory analysis and measurements

Soil samples were separately taken at 0 to 20 cm depth from each treatment. Each soil sample (2 L) consisted of thoroughly mixed five subsamples. To determine nutrient (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo, B) concentration the air-dried and 2 mm-sieved soil samples were extracted using 1 M HCl (soil-extractant mixture 1:5 v/v). Oxidation of soil extract with conc. HNO_3, H_2O_2 and $HClO_4$ were performed for determination of P, S, and Mo. The levels of Ca, Mg, Fe, Cu, Zn, and Mn were estimated by atomic absorption spectrophotometer (Perkin Elmer AAnalyst 700, acetylene-air flame) (Anonymous, 2000) those of N, P, Mo, B by colorimetry, S by turbidimetry, and K by a flame photometer (Jenwey PFP7, air propane-butane flame). All spectroscopic, colorimetric or photometric determinations were performed in triplicates. For soil, the concentrations of all mineral elements were given as mg L^{-1}.

Soil electrical conductivity (EC) was measured in distilled water extraction (soil – distilled water mixture 1:5) with the conductometer Hanna EC 215, but the soil reaction was detected in 1 M KCl extraction (soil-extractant mixture 1:2.5) using the pH-meter Sartorius PB-20.

For each plant sample 100–200 blueberry leaves were collected. The leaf material was oven-dried at 60 °C to a constant weight and finely ground using a laboratory mill. Then the samples were dry-ashed in concentrated HNO_3 vapors and re-dissolved in HCl solution (HCl – distilled water mixture 3:100) (Rinkis et al., 1987). Concentrations of 5 microelements (Fe, Zn, Cu, Mo, B) were determined in all leaf samples. Nutrients were analyzed using the same procedures as in the case of soil samples. Microelement concentrations in plant tissue were expressed as mg kg^{-1}.

All chemical analyses of soil and plant samples were done in the Laboratory of plant mineral nutrition of the Institute of Biology, University of Latvia.

Statistical analysis

The levels of statistical significance were determined with MS Excel 2016. Standard errors (SE) were calculated in order to reflect the mean results of chemical analysis. The Student's t-test (Two-Sample Assuming Equal Variances) was used for testing the differences between treatments.

RESULTS AND DISCUSSION

To characterize the soil nutrient status in a planting of highbush blueberry before and during the experiment, the plant available concentration of 12 essential nutrients, pH_{KCl} and EC levels were estimated in peat samples and evaluated in relation to the guideline values (Table 1).

Chemical analyses of peat before experiment establishment confirmed significant deficiency of N (9 mg L^{-1}) and S (10 mg L^{-1}) in the experimental field, as well as low level or deficiency of K, Cu, B and Mo in comparison to optimal values reported by Nollendorfs (2004) for blueberries: N – 70–150 mg L^{-1}, S – 40–80 mg L^{-1}; K – 80–120 mg L^{-1}; Cu – 4–8 mg L^{-1}; B – 0.6–1.2; Mo – 0.10–0.25 mg L^{-1}. Previously frequent deficiency of N and S in soil tests for blueberry plantations in Latvia established in peat soil was reported by Osvalde et al. (2018). As nitrogen is one of a key element in blueberry nutrition, adequate fertilization is necessary to maintain renewal growth, crop production, and flower bud development for next year's crop. Numerous studies have been proved the importance of proper N fertilization for successful blueberry growing (Bañados et al., 2012; Bryla et al., 2012; Ehret et al., 2014). However, fertilization with higher-than-recommended N rates could lead to significant yield decreases over time and risk of environmental contamination (Messiga et al., 2018). Our results demonstrated that a granular complex fertilizer applied in spring was sufficient to maintain appropriate N content in peat up to October. Though N concentrations were slightly less than recommended, it should be taken into account that a sustainable N management strategy requires the lower amounts of nitrogen for mature blueberries (Throop & Hanson, 1997; Bryla & Machado, 2011). In general, applied complex fertilizer provided a sufficient level of all macro- and microelements during the vegetation season in the experimental field, with the exception of Mo. Peat chemical analyzes suggested that established pH values (4.31–4.64) corresponded to the guidelines recommended by several authors for blueberries (Nollendorfs, 2004; Hart et al., 2006; Paal et al., 2011).

Table 1. Nutrient concentrations in 1 M HCl extraction (mg L^{-1}) in highbush blueberry peat soil (Soil standards developed by Nollendorfs (2004))

Variable	April (30.04.2018)	July (05.07.2018)	August (03.08.2018)	October (05.10.2018)	Optimal for highbush blueberries in peat soils		
N	9 ± 0.75	64 ± 6.3	49 ± 3.0	47 ± 4.2	70	-	150
P	99 ± 13.00	212 ± 33.26	168 ± 21.89	149 ± 14.32	50	-	90
K	64 ± 5.60	130 ± 15.23	117 ± 6.36	94 ± 7.42	80	-	120
Ca	2,050 ± 298.61	3,100 ± 363.32	3,300 ± 198.36	3,350 ± 302.01	500	-	1,000
Mg	276 ± 30.31	350 ± 22.36	370 ± 18.33	500 ± 32.69	100	-	180
S	10 ± 1.19	90 ± 9.56	83 ± 5.36	75 ± 2.69	40	-	80
Fe	103 ± 7.05	170 ± 9.87	203 ± 8.69	130 ± 3.23	60	-	150
Mn	4.91 ± 0.79	9.0 ± 0.63	10.0 ± 1.10	8.0 ± 0.58	3.0	-	6.0
Zn	4.95 ± 0.58	10 ± 0.66	9.5 ± 0.45	6 ± 0.36	4.0	-	8.0
Cu	1.51 ± 1.16	8.2 ± 0.53	13.5 ± 1.32	8.5 ± 0.69	4.0	-	8.0
Mo	0.02 ± 0.003	0.03 ± 0.003	0.03 ± 0.006	0.04 ± 0.003	0.1	-	0.2
B	0.13 ± 0.02	1.00 ± 0.08	0.60 ± 0.04	0.5 ± 0.09	0.6	-	1.2
$pH_{/KCl}$	4.31 ± 0.17	4.56 ± 0.23	4.64 ± 0.17	4.54 ± 0.32	4.5	-	4.8
EC (mS cm^{-1})	0.40 ± 0.03	1.66 ± 0.23	1.21 ± 0.20	1.31 ± 0.32	0.8	-	1.2

The chemical composition of leaves

It is well known that climatic conditions and various environmental factors such as relative humidity and temperature, have an effect on the properties of cuticular membrane, on the physiological processes of plants and on the properties of the fertilizer solution, and thus play an significant role in the process of absorption of mineral components by the cells of the leaf epidermis (Wójcik, 1998; Wach & Błażewicz-Woźniak, 2012). Overall, the research results from field experiment demonstrated a significant influence of the applied foliar fertilizer on the content of Fe, Zn and B in the highbush blueberry leaves. Only small impact or trend with no significant differences among treatments was established in the case of Cu and Mo, therefore, data are not presented.

Severe Fe deficiency (40–46 mg kg^{-1}) in blueberry leaves, collected prior to the use of foliar micronutrients, were detected at all experimental plots. According to current nutrient standards (Nollendorfs, 2004; Hart et al., 2006) the lower limit of the sufficiency range is 60 mg kg^{-1} (Fig. 1, A).

After the first spray, the Fe concentration in the leaves exceeded the minimum sufficiency level, but after the second treatment with foliar fertilizer reached the optimum range. In general, foliar fertilization provided from 52% (after one spraying) to 118% (after 3 sprayings) higher Fe content in leaves compared to the control group. It should be noted that despite the Fe deficiency in the leaves in the spring, Fe concentration in soils (103–203 mg L^{-1}) was optimal throughout the season. Such phenomenon could be explained by many biotic and abiotic factors influencing the availability of nutrients for plant uptake (Marshner, 2012) and underline the potential of foliar fertilization and importance of complex diagnostics (soil + pant tissue analysis) not only for correct determination of the nutritional status of plants but also for selection the most efficient mode of fertilizer application.

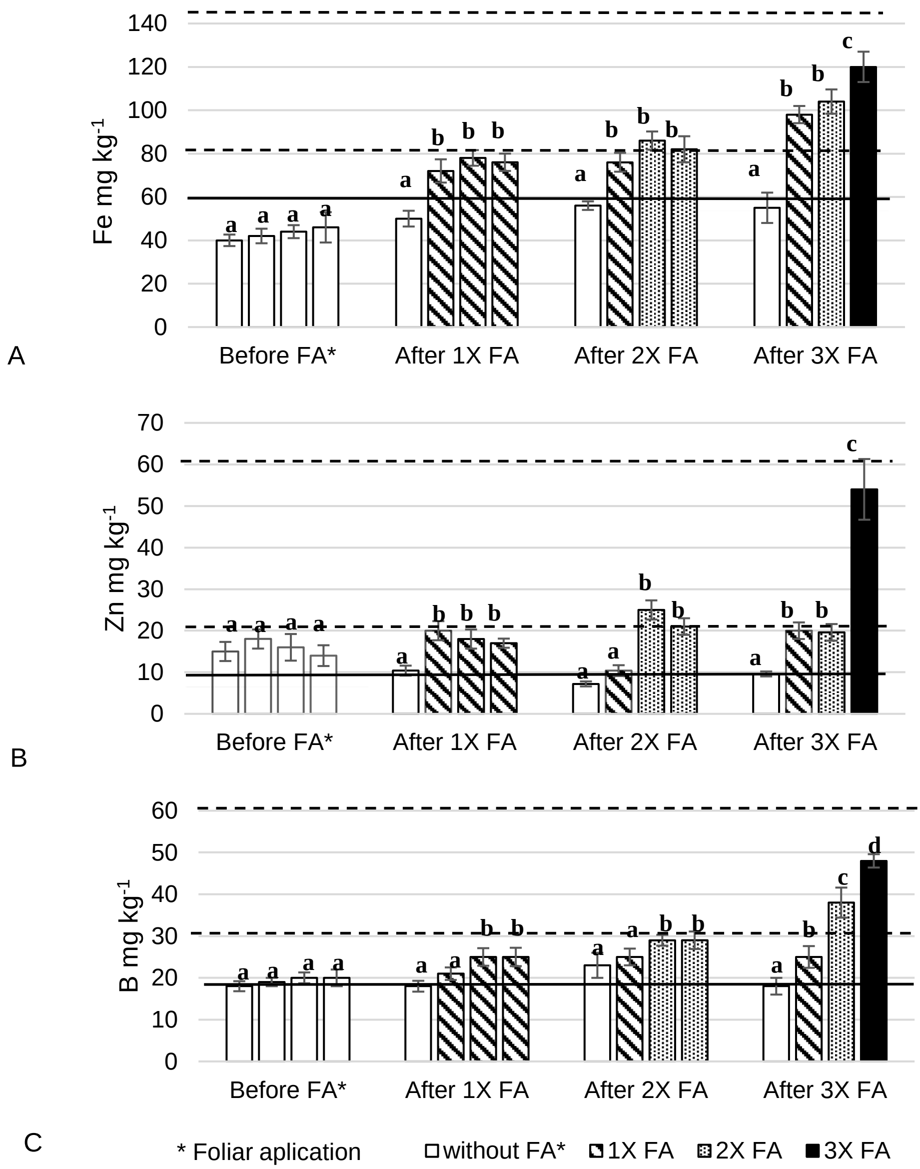

Figure 1. Concentration of Fe (A), Zn (B) and B (C) in highbush blueberry leaves.

Means with different letters for each sampling time were significantly different (t-Test, $p < 0.05$). Minimum level ———— optimum range - - - - - -.

Micronutrient Zn and B content in blueberry leaves were significantly affected by micronutrient foliar application (Fig. 1, B & C). It should be noted that sufficient Zn concentration level in soil (Table 1.) did not provide optimal content of this element in blueberry leaves at the beginning of the vegetation season As mentioned before such phenomenon is caused by limited availability of particular mineral element for uptake in plants. As the B concentration in the soil was low at the beginning of the experiment, it was not surprising that the content of B in the leaves also did not reach the optimal level in spring. In general, results indicated that micronutrient foliar application provided/ensured Zn and B absorption in plants and increased the concentrations of these nutrients in blueberry leaves. Similarly, as in the case with Fe, the highest Zn and B concentration in leaves were recorded when plants were treated 3 times per season with foliar fertilizer. Researches made by Wójcik (2005) and Arrington & De Vetter (2017) claims that the application of foliar B fertilization resulted in a significant increase of B in highbush blueberry leaves, but not resulted in higher yield of berries. Similarly, Chen et al. (1998) detected no significant differences in fruit set and yield between B treatments in 12 lowbush blueberry (*Vaccinium angustifolium* Ait.) clones. In contrast, Meriño-Gergichevich et al., (2016) found that foliar B application not only elevated B concentration in plant leaves but significantly enhanced highbush blueberry yield and quality. Considering that boron is easily leached from soils, several studies (Gupta et al., 1985; Eaton & Sanderson, 2007) suggests that foliar applications of B as a supplement for soil fertilization method are a valuable tool to enhance boron supply in blueberry leaves.

Our research clearly indicates that foliar fertilization may be effective to ensure optimal crop performance in cases when a particular mineral element is not available to plants. Since small quantities of fertilizers are used by spraying directly to the bushes, this method could contribute also to environmental protection.

Chlorophyll fluorescence

Chlorophyll a fluorescence measurements were used as an indicator to analyze the effect of foliar fertilization on the physiological state of blueberries. Within the last decades, chlorophyll a fluorescence technique has been employed in a wide range of studies in plant biology. It has been shown that the decline of the ratio of variable to maximum fluorescence or potential maximum quantum yield of the photosystem II (FV/FM) below 0.8 reflects inhibition of photosynthesis and, therefore, indicates episodes of previously suboptimal conditions (Öquist et al. 1992; Andersone et al., 2011). On the other hand, Performance Index (PI) is a more complex parameter reflecting overall efficiency of light absorption, as well as both light and dark redox reactions (Strauss et al., 2006). Although the overall positive linear correlation between PI and FV/ FM for analyzed blueberry plants was apparent, dynamics of changes in the individual parameters were not identical, suggesting the different effect of environmental factors on various aspects of the photochemistry of photosynthesis. Such difference partly can be explained by different time scales of impact by changing environmental conditions on the photochemistry, where PI shows instant general responses of the photosynthetic system while FV/FM reflects longer-lasting response. It is well known that Fe has an essential function in the whole photosynthetic apparatus and iron deficiency significantly influence parameters characterizing leaf functions, including gas exchange, water status and chlorophyll fluorescence (Abadia, 1992; Fernandez et al., 2008; Fageria, 2009). Our research demonstrates that PI and partly FV/FM value reflected the direct effect of increased microelement supply on blueberries. The lowest PI values (Fig. 2) were established in spring when Fe deficiency and suboptimal concentrations of Zn and B was evident for all treatments. In turn, the highest PI and FV/FM, above recommended 0.8, were detected after the third spraying of fertilizers. The potential maximum quantum yield of photosystem II (FV/FM) showed that it tends to be equal from May to June, with a significant decrease in July (Fig. 2, B). Such decrease of FV/FM below 0.77 in July indicated possible photoinhibition of photosynthesis in highbush blueberry. As pointed out by Baker (2008), FV/FM represents the maximum quantum yield of photosystem II, while under ambient light conditions effective quantum yield may be significantly lower. The present data show that photochemistry of photosynthesis of blueberry is strongly affected by local changes of environmental conditions. However, it is evident that mineral nutrition conditions are among the most important factors affecting the physiological status of highbush blueberry.

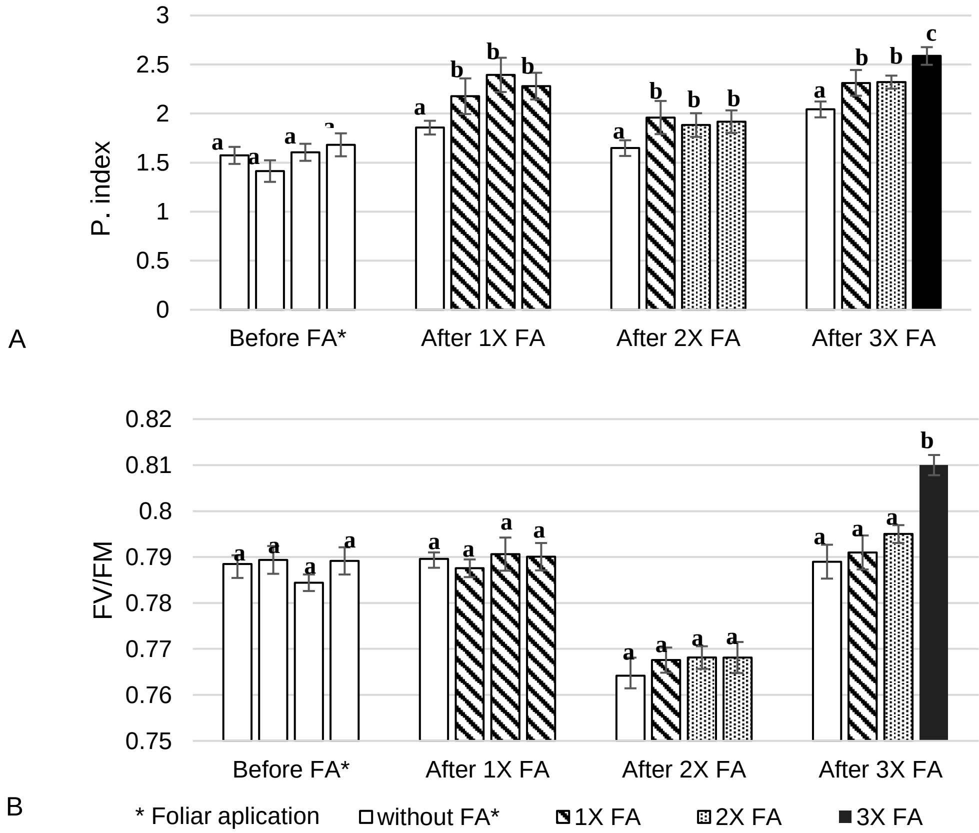

Figure 2. Changes of chlorophyll a fluorescence parameters PI (A) and FV/FM (B) in highbush blueberry leaves: PI – Performance Index; FV/FM – potential maximum quantum yield of photosystem II.

Berry yield and quality

The aim of the experiment was also to examine the blueberry yield and quality. In general, berry yield was significantly influenced by foliar fertilizer applications ($p < 0.05$). Our results showed that total blueberry yield (Table 2) achieved in the experiment was equivalent to 5.02–7.14 t ha^{-1} (3,800 bushes per hectare) which are considerably higher compare to average blueberry yield in Latvia – 1.49 t ha^{-1} (Crop statistics, 2019) and are close to average yields reached in USA (6.91 t ha^{-1} in 2017) confirming that correct mineral nutrition is one of the most significant limiting factors for high berry fields in Latvia. The highest yield was obtained from the bushes sprayed 3 times per season. Well-known that fruit size is of decisive importance for the crop quality. In our experiment, the largest berries and the highest mass of one berry (23.0 mm and 3.36 g accordingly) were also achieved from the bushes sprayed 3 times per season. On the other hand, the smallest berries and the lowest one fruit mass were detected in control treatment. Our results are consistent with Starast et al. (2002) who obtained a higher yield and higher weight of fruit of lowbush blueberry under the effect of foliar fertilization in Estonia.

Table 2. Characteristics of the highbush blueberry 'Patriot' fruits in dependence on fertilizing

Treatment	Average yield from bush (kg)	Average one fruit mass (g)	Average fruit diameter (mm)
Control	1.32 ± 0.031a*	2.90 ± 0.322a	19.5 ± 0.27a
Treatment 1	1.48 ± 0.028a	2.86 ± 0.431a	19.6 ± 0.21a
Treatment 2	1.50 ± 0.033b	2.99 ± 0.809a	22.3 ± 0.33b
Treatment 3	1.88 ± 0.042b	3.36 ± 0.286b	23.0 ± 0.56b

*Means with different letters in a column were significantly different (t-Test, $p < 0.05$).

CONCLUSION

As a conclusion, our study reveals, that foliar fertilization can become a very practical and effective method for increasing blueberry yields and berry quality. In general, foliar application of micronutrients resulted in the better provision of these elements in leaves, the better photosynthetic performance of plants as well as higher and more qualitative yield of blueberries. Based on the present study, it can be suggested that chlorophyll a fluorescence is a useful tool not only for non-destructive measurement of stress impact on the physiological status of highbush blueberry but also to search for the effect of any suboptimal conditions through the analysis of various aspects of the photochemistry of photosynthesis.

REFERENCES

Abadia, J. 1992. Leaf responses to iron deficiency - a review. *Journal of Plant Nutrition* **15**, 1699–1713.

Andersone, U., Druva-Lūsīte, I., Ieviņa, B., Karlsons, A., Ņečajeva, J., Samsone, I. & Ievinsh, G. 2011. The use of nondestructive methods to assess a physiological status and conservation perspectives of *Eryngium maritimum* L. *Journal of Coastal Conservation*, **15**, 509–522.

Anonymous. 2000. Analytical methods for atomic absorbtion spectrometry. PerkinElmer Instruments LLC.

Arrington, M. & DeVetter, L.W. 2017. Foliar Applications of Calcium and Boron Do Not Increase Fruit Set or Yield in Northern Highbush Blueberry (*Vaccinium corymbosum*). *HortScience* **52**(9), 1259–1264.

Baker, N.R. 2008. Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Annual Review Of Plant Biology* **59**, 89–113.

Bañados, M.P., Strik, B.C., Bryla, D.R. & Righetti, T.L. 2012. Response of highbush blueberry to nitrogen fertilizer during field establishment, I: Accumulation and allocation of fertilizer nitrogen and biomass. *HortScience* **47**, 648–655.

Bryla, D.R. & Machado, R.M.A. 2011. Comparative effects of nitrogen fertigation and granular fertilizer application on growth and availability of soil nitrogen during establishment of highbush blueberry. *Frontiers in Plant Science* **2**, 1–8.

Bryla, D.R., Strik, B.C., Bañados, M.P. & Righetti, T.L.2012. Response of highbush blueberry to nitrogen fertilizer during field establishment, II: plant nutrient requirements in relation to nitrogen fertilizer supply. *HortScience* **47**, 917–926.

Chen, Y., Smagula, J.M., Litten, W. & Dunham, S. 1998. Effect of boron and calcium foliar sprays on pollen germination and development, fruit set, seed development, and berry yield and quality in lowbush blueberry (*Vaccinium angustifolium* Ait.). *The Journal of the American Society for Horticultural Science* **123**, 524–531.

Crop statistics. 2019. The Food and Agriculture Organization. http://www.fao.org/faostat/en/#data/QC. Accessed 02.01.2019.

Eaton, L.J. & Sanderson, K.R. 2007. Effects of summer and fall applications of foliar boron on fruit bud winter injury in wild blueberry (*Vaccinium angustifolium* Ait.). *Canadian Journal of Plant Science* **87**(4), 923–925.

Eck, P. 1990. *The American Cranberry*. Rutgers University Press, New Brunswick, NJ, 420 pp.

Ehret, D.L., Frey, B., Forge, T., Helmer, T., Bryla, D.R. & Zebarth, B.J. 2014. Effects of nitrogen rate and application method on early production and fruit quality in highbush blueberry. *Canadian Journal of Plant Science* **94**, 1165–1179.

Fageria, N.K., Barbosa Filho, M.P., Moreira, A. & Guimarães, C.M. 2009. Foliar fertilization of crop plants. *Journal of Plant Nutrition* **32**(6), 1044–1064.

Fernandez, V., Eichert, T., Del Rio, V., Lopez-Casado, G., Heredia-Guerrero, J.A., Abadia, A., Heredia, A. & Abadia, J. 2008. Leaf structural changes associated with iron deficiency chlorosis in field-grown pear and peach: Physiological implications. *Plant and Soil* **31**, 161–172.

Fernández, V., Sotiropoulos, V.T. & Brown, P. 2013. *Foliar fertilization – scientific principles and field practices*. 1st Edn. Paris: International Fertilizer Industry Association (IFA), 140 pp.

Gupta, U.C., Jame, Y.W., Campbell, A.J., Leyshon, A.J. & Nicholiachuk, W. 1985. Boron toxicity and deficiency: A review. *Canadian Journal of Plant Science* **65**, 381–409.

Hart, J., Strik, B., White, L. & Yang, W. 2006. Nutrient management for blueberries in Oregon, Oregon State University Extension service EM 8918, 14 pp.

Karlsons, A., Osvalde, A., Čekstere, G. & Pormale, J. 2018. Research on the mineral composition of cultivated and wild blueberries and cranberries. *Agronomy Research*, **16**(2), 454–464.

Marschner, P. 2012. Mineral Nutrition of Higher Plants. Academic Press, San Diego. 651 pp.

Meriño-Gergichevich, C., Pacheco, E. & Díaz, M.R. 2016. The effect of foliar boron spraying on the fruit features of Brigitta and Legacy highbush blueberry (*Vaccinium corymbosum*) cultivars. *Ciencia e Investigación Agraria* **43**(3), 452–463.

Messiga, A.J., Haak, D. & Dorais, M. 2018. Blueberry yield and soil properties response to long-term fertigation and broadcast nitrogen. *Scientia Horticulturae* **230**(7), 92–101.

Nollendorfs, V. 2004. Highbush blueberry soil and fertilization demands. *Agropols* **12**, 8–10 (in Latvian).

Osvalde, A., Karlsons, A., Cekstere, G., Pormale, J. & Apse, J. 2018. Influence of soil type on nutrients status of highbush blueberry leaves in Latvia. *Acta Horticulturae* **1217**, 399–404.
Paal, T., Starast, M., Noormets-Sanski, M., Vool, E., Tasa, T. & Karp, K. 2011. Influence of liming and fertilization on lowbush blueberry in harvested peat field condition. *Scientia Horticulturae* **130**, 157–163.
Pormale, J., Osvalde, A. & Nollendorfs, V. 2009. Comparison study of cultivated highbush and wild blueberry nutrient status in producing plantings and woodlands. *Latvian Journal of Agronomy* **12**, 80–87.
Rinkis, G.J., Ramane, H.K. & Kunickaya, T.A. 1987. *Methods of soil and plant analysis*. Zinatne, Riga, 174 pp. (in Russian).
Samsone, I., Druva-Lūsīte, I., Andersone, U., Ņečajeva, J., Karlsons, A. & Ievinsh, G. 2009. Plasticity of a dune plant *Alyssum gmelinii* in response to sand burial in natural conditions. *Acta Universitatis Latviensis* **753**, 125–136.
Starast, M., Karp, K. & Noormets, M., 2002. The effect of foliar fertilisation on the growth and yield of lowbush blueberry in Estonia. *Acta Horticulturae* **574**, 679–684.
Strauss, A.J., Krüger, G.H.J., Strasser, R.J. & Van Heerden, P.D.R. 2006. Ranking of dark chilling tolerance in soybean genotypes probed by the chlorophyll a fluorescence transient O-J-I-P. *Environmental and Experimental Botany* **56**, 147–157.
Strik, B. 2005. Blueberry – an expanding world berry crop. *Chronica Horticulturae* **45**, 7–12.
Throop, P.A. & Hanson, E.J. 1997. Effect of application date on absorption of 15nitrogen by highbush blueberry. *Journal of the American Society for Horticultural Science* **122**, 422–426.
Trehane, J. 2004. Blueberries, Cranberries and other Vaccines. Portland, London, pp. 173–175.
Wach, D. & Błażewicz-Woźniak, M. 2012. Effect of foliar fertilization on yielding and leaf mineral composition of highbush blueberry (*Vaccinium corumbosu*m L.). *Acta Scientiarum Polonorum, Hortorum Cultus* **11**(1), 205–214.
Wójcik, P. 2005. Response of 'Bluecrop' highbush blueberry to boron fertilization. *Journal of Plant Nutrition* **28**, 1897–1906.
Wójcik, P. 1998. The uptake of mineral nutrients by above-ground parts of plants from foliar fertilization. *Advances of Agricultural Sciences: Problem Issues* **1**, 49–64 (in Polish).
Öquist, G., Chow, W.S. & Anderson, J.M. 1992. Photoinhibition of photosynthesis represents a mechanism for the long-term regulation of photosystem II. *Planta* **186**, 450–460.

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Content of malondialdehyde and activity of enzyme glutathione-S-transferase in the leaves of emmer wheat under the action of herbicide and plant growth regulator

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Abstract. The article presents the results of vegetation experiment on the influence of different rates of herbicide Prima Forte 195 – 2-ethylhexyl ether 2.4-D + aminopyralid + florasulam (0.5; 0.6 and 0.7 L ha^{-1}) under different application methods of plant growth regulator of a natural origin Wuxal BIO Vita (*Ascophyllum nodosum* extract + microelements) on the content of malondialdehyde (MDA) as an important indicator of the intensity of peroxide oxidation of lipids in plants and on the activity of enzyme glutathione-S-transferase (GST) in the leaves of emmer wheat.

Experimental scheme included 16 experimental variants: 1 – without application of preparations and pre-sowing treatment of seeds (control); 2, 3, 4 – Prima Forte 195, applied to vegetative plants at the rates 0.5, 0.6 and 0.7 L ha^{-1}; 5 – Wuxal BIO Vita at the rate of 1.0 L ha^{-1}, applied to vegetative plants; 6, 7, 8 – Prima Forte 195 at the rates of 0.5, 0.6 and 0.7 L ha^{-1} in tank mixtures with Wuxal BIO Vita at the rate of 1.0 L ha^{-1}, sprayed on vegetative plants; 9 – pre-sowing treatment of seeds with Wuxal BIO Vita at the rate of 1.0 L t^{-1} (background); 10, 11, 12 – Prima forte 195, applied to vegetative plants at the rates of 0.5, 0.6 and 0.7 L ha^{-1} at the background of pre-sowing treatment of seeds with Wuxal BIO Vita at the rate of 1.0 L t^{-1}; 13 – Wuxal BIO Vita at the rate of 1.0 L ha^{-1} (applied to vegetative plants, treated before sowing with Wuxal BIO Vita at the rate of 1.0 L t^{-1}); 14, 15, 16 – Prima Forte 195 at the rates of 0.5, 0.6 and 0.7 L ha^{-1} respectively, in tank mixture with Wuxal BIO Vita at the rate of 1.0 L ha $^{-1}$ – spraying of plants and pre-sowing treatment of seeds with Wuxal BIO Vita 1.0 L t^{-1}.

It has been found that under the application of Prima Forte 195 the redox state in the leaves of emmer wheat increased considerably in the direction of increasing the content of MDA. It has also been proved that herbicide Prima Forte 195 and its mixtures with plant growth regulator (PGR) Wuxal BIO Vita caused the changes in the activity of enzyme glutathione-S-transferase, which slightly increased in the initial period in the variants of integrated application of herbicide and plant growth regulator. However, later the GST activity in the variants of integrated application of herbicide and PGR decreased, which indicates stabilization and a positive influence of integrated application of preparations on the detoxification processes in the crops of emmer wheat.

Key words: MDA content, glutathione-S-transferase, oxidative stress, herbicide, plant growth regulator, emmer wheat (*Triticum dicoccum* (Schrank) Schuebl.)

INTRODUCTION

Weeds remain the main restricting factor of growing such an important crop as wheat. Therefore, over the last years in the technologies of growing wheat the volumes of using chemical plant protection products have increased, including herbicides, which has aggravated the problem of accumulating toxins in food chains (Hesammi, 2011). In this respect, the technological developments that provide transition from chemicals-dependent to biologically oriented arable farming are becoming more urgent. However, a drastic transition to biological arable farming leads to the increase in weed infestation of crops and a decrease of wheat yielding capacity. Therefore, nowadays when there is a shortage of foodstuffs worldwide, it is impossible to reject the application of herbicides completely. At the same time, it is necessary to look for the ways of decreasing their negative effect on farm ecosystems. Definitely, these ways should include the elements of biologization, which in the case of herbicides can be reached due to their integrated application with the preparations of natural origin, for example, with plant growth regulators (PGR), that are characterized by anti-stress and immune-stimulating properties. For the first time this possibility was proved on the example of spring barley (Karpenko et al., 2012). Though, a number of issues dealing with a comprehensive action of herbicides and PGR remains insufficiently studied. The choice and evaluation of the optimal combination of preparations in the mixtures, especially multi-component ones, are carried out without considering the mechanisms of their action on the key physiological reactions in a plant body, because herbicides, being highly-effective compounds, are able to penetrate in plants quickly, where they are subject to metabolic transformation on the part of fermentative systems.

It was proved that herbicides could cause oxidative stress (Van Camp et al.; 1994; Mitieva et al., 2010), which is accompanied by an intensive generation of active oxygen species (AOS). The latter, in their turn, lead to the structural damage of membranes, proteins, lipids, carbohydrates, and DNA, increase the level of peroxide oxidation of lipids (POL) (Hassan & Nemat Alla, 2005; Gill & Tuteja, 2010). Hence, the rate of detoxification of herbicides in cultivated plants and weeds determines their selectivity (Owen, 2000). It is in the process of destroying a toxic agent in cells when the activity of enzymes increases, as well as glutathione-S-transferase (GST) (EC 2.5.1.18) — the enzyme that metabolizes toxic products of peroxide oxidation of lipids, that damage DNA and other cell components (Liu et al., 2013). GST also catalyzes the creation of glutathione conjugates, that way decreasing the toxic level of alien compounds that are taken away from cells with the help of special ATF-dependent transport systems (Lamoureux & Rusness, 1989). Activation of glutathione-S-transferase enhances the ability of cells to detoxify toxic agents, which is explained by the participation of enzyme in the neutralization of AOS (Zama & Hatzios, 1986). In particular, GST catalyzes the conjugation of electrophilic and often hydrophobic toxic compounds with glutathione with the formation of non-toxic peptide compounds (Neuefeind et al., 1997; Dixon et al., 1998; Edwards & Dixon, 2000; DeRidder & Goldsbrough, 2006; Rochalska & Grabowska, 2007; Baimuhametova et al., 2016).

To eliminate AOS in plants there are other specific enzymes: peroxidase, catalase, and superoxide dismutase. According to our previous research (Karpenko & Pavlyshyn, 2018), under the application of herbicide Prima Forte 195 at the rates of 0.5–0.7 L ha^{-1} the activity of peroxidase and catalase in the plants of emmer wheat can increase by

18–33 and 19–32% respectively, compared to the variant without the application of herbicide, which is the result of a considerable increase of H_2O_2 in the leaves of a plant as one of the forms of AOS. It is obvious, that GST also reacts in a proper way to the increased concentration of H_2O_2 in cells. However, there is no information available in scientific literature as to the influence of herbicide Prima Forte 195, that has been used in Ukraine only for the recent two years, on the activity of GST and development of oxidative stress in the plants of emmer wheat, which makes our experiment relevant.

The aim of our experiment was to study the influence of herbicide Prima Forte 195 (0.5; 0.6; 0.7 L ha^{-1}) in vegetative plants separately and in the mixtures with PGR Wuxal BIO Vita at the rate of 1.0 L ha^{-1}, and also application of the same compositions of herbicide and PGR at the background of pre-sowing treatment of seeds with PGR Wuxal BIO Vita at the rate of 1.0 L t^{-1} on the accumulation of malondialdehyde (MDA), the product of peroxide oxidation of lipids and activity of glutathione-S-transferase. Taking into account oxidative and enzyme changes in emmer wheat we tried to determine the optimal combination of preparations, under which application the plants are subjected to the minimal stressful influence from the part of herbicide agent.

MATERIALS AND METHODS

The objects of the research were emmer wheat plants (*Triticum dicoccum* (Schrank) Schuebl.) of the cultivar Holikovska (originator – the Plant Production Institute named after V.Ya. Yuryev, Ukraine), herbicide Prima Forte 195, c.e. (Syngenta) (active substances – florasulam 5 g L^{-1}, aminopyralid 10 g L^{-1}, 2-ethylhexyl alcohol 2.4-D 180 g L^{-1}), plant growth regulator Wuxal BIO Vita (Unifer) (active substance – extract from seaweed *Ascophyllum nodosum*, nitrogen (N) – 52 g L^{-1}, manganese (Mn) – 38 g L^{-1}, sulphur (S) – 29 g L^{-1}, iron (Fe) – 6.4 g L^{-1}, zinc (Zn) – 6.4 g L^{-1}).

Plants were grown in the laboratory in plastic containers with the capacity of 12 kg filled with absolutely dry soil, typical for a field experiment. The soil was a podzolized, heavy loamy black soil. The humus content in plowing horizon made up 3.4%; nitrogen content of alkaline hydrolysis compounds was low (103 mg kg^{-1}); the content of phosphorus movable compounds (96 mg kg^{-1}) was medium; the content of potassium movable compounds was higher; reaction of soil solution was weak acid (pH of salt solution is 5.9). (above mentioned indicators were determined according to the methods, described in (Hrycajenko et al., 2003). Soil moisture was maintained by the gravimetric method at the level of 60% soil moisture. Additional illumination with fluorescent lamps 800 lux (14–16 hours) was used under controlled conditions of plants growth and development. The temperature was maintained at the level of 25 °C. Relative humidity was 60%. Treatment of seeds with PGR was carried out according to the rates, calculated per seed weight, and spraying of vegetative plants was completed per area according to concentration in relation to the rates of application under field conditions. Treatment of seeds with PGR was carried out on the day of sowing (BBCH 00). Vegetative plants were treated with herbicide and PGR at the phase BBCH 29 with hand-operated sprayer. In order to provide an even illumination and temperature regime, the location of containers with plants was changed every two days. Vegetation experiment was carried out in a 3-time repetition, observing the requirements of vegetative method (Zhurbickij, 1968). The scheme of the experiment included 16 experimental variants: 1. C – without application of preparations and pre-sowing treatment of seeds (control); 2, 3, 4. H0.5, H0.6, H0.7 – herbicide Prima Forte 195 at the rates of 0.5, 0.6 and 0.7 L ha^{-1}; 5. PV – PGR Wuxal BIO Vita at the rate of 1.0 L ha^{-1}, treatment of vegetative plants; 6, 7, 8. H0.5, H0.6, H0.7 + PV – tank mixtures of Prima Forte 195 0.5, 0.6 and 0.7 L ha^{-1} + Wuxal BIO Vita 1.0 L ha^{-1}, treatment of vegetative plants; 9. PS – PGR Wuxal BIO Vita 1.0 L t^{-1} (pre-sowing treatment of seeds, background); 10, 11, 12. H0.5, H0.6, H0.7 + PS – Prima Forte 195 0.5, 0.6, 0.7 L ha^{-1}, treatment of vegetative plants at the background of pre-sowing seed treatment with PGR; 13. PS + PV –Wuxal BIO Vita 1.0 L ha^{-1}, treatment of vegetative plants with PGR at the background of pre-sowing seeds treatment with PGR; 14, 15, 16. H0.5, H0.6, H0.7 + PS + PV – Prima Forte 195 0.5, 0.6, 0.7 L ha^{-1} + Wuxal BIO Vita 1.0 L ha^{-1}, treatment of vegetative plants with tank mixture of herbicide and PGR at the background of pre-sowing seed treatment with PGR.

The leaves of emmer wheat were selected for the analyses on the 3rd and on the 10th day after a post-germination application of preparations. Intensity of oxidative stress was evaluated by the reaction of POL – by the accumulation of a final product of peroxide oxidation of lipids – malondialdehyde (MDA), by the reaction with thiobarbituric acid (TBA) at 532 nm on spectrophotometer LEKI SS1104 according to the technique (Rogozhin, 2006). The method is based on determining the amount of a coloured product at the wave length 532 nm, obtained as the result of interaction of 2 molecules of TBA with one molecule of MDA as one of the by-products of POL. For this purpose, 1 g of leaves tissue was homogenized with 3 mL of 50% ethanol and centrifuged 10 min at 7,000 rpm. 0.5 mL of 1% triton X-100 solution, 0.2 mL of 0.6 M HCl and 0.8 mL 0.06 M of TBA were added to the obtained 0.5 mL of supernatant and heated in the boiling water bath (100 °C) for 10 min and then cooled to 15 °C for 30 min and added 0.2 mL 5 mM solution of Trilon B and 5–10 mL of 96% ethanol. As a control served test-tube in which all chemical reagents except TBA were added. MDA content was calculated, taking into account optical density of the sample and its corresponding dilutions under the coefficient of micro molar absorption TBA $\mathcal{E} = 155\ \mu M^{-1}\ cm^{-1}$ at the wave length 532 nm and was expressed in μmol g^{-1} of raw substance.

The activity of GST was determined by the method of Habig et al. (1974) in the modification of Grishko & Syschikov (1999): for that purpose 1 g of raw tissue of leaves was crashed in mortar in 3 mL water cooled to 0 °C, homogenate was centrifuged 10 min at 7,000 rpm. 0.2 mL 0.015 M solution of reduced glutathione and 0.1 mL of supernatant were added in the cuvette (optical path length l = 1 cm) that contained 2.5 mL 0.1 M potassium phosphate buffer (pH = 6.5). Reaction was initiated by adding 0.2 mL 0.015 M 1-chloro-2.4-dinitrobenzene (DNCB) in the cuvette. Simultaneously, blank sample was prepared, in which distilled water was added instead of supernatant. The change in optical density was recorded in the third minute at wave length 340 nm on spectrophotometer LEKI SS1104.

Calculations of enzyme activity were carried out considering optical density of the sample, corresponding dilutions, the time of the reaction under the coefficient of micro molar absorbtion of DNCB at wave length of 340 nm $\mathcal{E} = 9.6\ \mu M^{-1}\ cm^{-1}$. Catalytic activity of GST was expressed in μmol g^{-1} of raw substance per 1 minute.

Reliability of the experiment and significance of the difference between indexes (LSD) in the experimental research were assessed according to the results of the analysis of variance (Ehrmantraut et al., 2000) with the application of Microsoft Excel. The graphs show arithmetic means of the analyses, that were carried out three times. Sampling error doesn't exceed 5% from mean values.

RESULTS AND DISCUSSION

Herbicides, violating the key physiological reactions in plants, including enzymological, cause the activation of free-radical oxidizing processes (Enan, 2009). The most representative among them is peroxide oxidation of lipids (POL), key index that shows the intensity of stressful influence of the factors of different nature, including herbicides. Therefore the accumulation of POL products in plants, in particular MDA, can serve as an indicator of the signal system of the adaptive protection of a plant against toxic action of xenobiotic (Agostinetto et al., 2016).

According to the results of the research into the MDA content in emmer wheat leaves it has been established, that herbicide treatment of plants led to the metabolic disturbance in plants, the level of which depended on a separate or integrated application of herbicide with PGR and correspondingly affected the level of MDA accumulation (Fig. 1).

In our experiment the highest MDA content in the leaves of emmer wheat was recorded in the variants with the application of herbicide without PGR, when under the rates of Prima Forte 195 0.5; 0.6 and 0.7 L ha^{-1} its content exceeded control by 162, 190 and 226% on the 3rd day after application and by 111, 124 and 143% on the 10th day respectively.

It has been noted that along with the increase of the application rate of herbicide, there was the increase in the accumulation of MDA in the leaves of emmer wheat, which could serve as an indicator of the development of oxidative stress in the plants. Obtained results conform with the results of the experiments performed by other scientists (Rossikhina & Vinnichenko, 2004; Agostinetto et al., 2016), who established that the action of herbicides based on 2.4-Dichlorophenoxyacetic acid (which is one of the components of herbicide Prima Forte 195) in the crops of corn and wheat cause the activation of POL, which is manifested in the increased MDA accumulation.

Under the integrated application of Prima Forte 195 at the rates 0.5; 0.6 and 0.7 L ha^{-1} with Wuxal BIO Vita 1.0 L ha^{-1} the indexes of MDA content in the leaves of emmer wheat exceeded control by 78, 95 and 111% on the 3rd and by 79, 94 and 118% on the 10th day respectively.

When emmer wheat was sprayed with Prima Forte 195 at the rates of 0.5; 0.6 and 0.7 L ha^{-1} at the background of pre-sowing treatment of seeds with Wuxal BIO Vita PGR 1.0 L t^{-1}, the indexes of MDA content compared to control increased by 141,161 and 185% on the 3rd day and by 91, 107 and 135% on the10th day respectively.

Integrated application of herbicide Prima Forte 195 (0.5–0.7 L ha^{-1}) and PGR at the background of pre-sowing treatment of seeds with PGR increased MDA content compared to control in the plants of emmer wheat by 67, 91 and 103% on the 3rd and by 63, 73 and 89% on the 10th day respectively. It should be noted that MDA content in these variants was lower compared to the variants with the application on herbicide alone, which means the initial increased level of detoxification processes in plants, aimed at destroying the toxicant. It is obvious that complex application of PGR (treatment of seeds and plants) in this case was the factor of decreasing or stabilizing POL processes in the plants (Karpenko et al., 2012), which can be the result of the changes in the enzymatic system of plants in general.

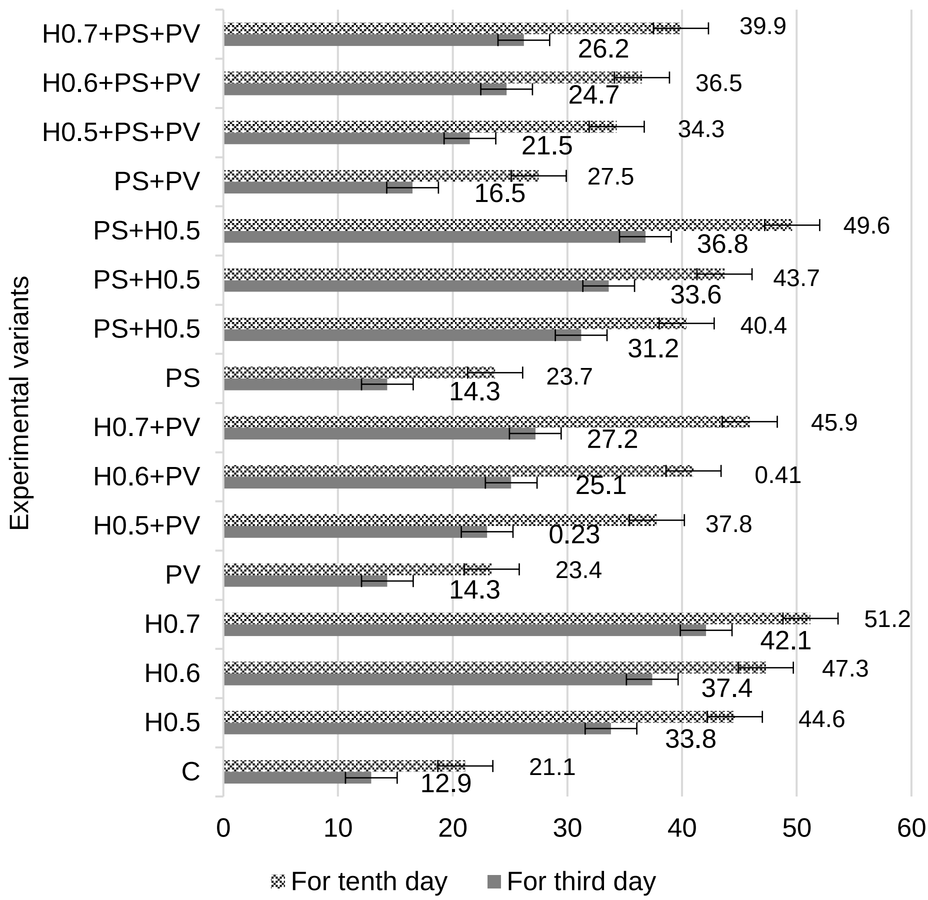

Figure 1. MDA content (μmol g^{-1} of raw substance) in the leaves of emmer wheat under the application of herbicide Prima Forte 195 and Wuxal BIO Vita PGR (LSD_{05}: for third day – 1.42; for tenth day – 2.08).
C – without application of preparations (control); H0.5, H0.6, H0.7 – Prima Forte 195 0.5, 0.6, 0.7 L ha^{-1}; PV – Wuxal BIO Vita – 1.0 L ha-^{1}; H0.5, H0.6, H0.7 + PV – Prima Forte 195 0.5, 0.6, 0.7 L ha-^{1} + Wuxal BIO Vita 1.0 L ha^{-1}; PS – Wuxal BIO Vita 1.0 L t^{-1} (pre-sowing treatment of seeds, background); H0.5, H0.6, H0.7 + PS – Background + Prima Forte 195 0.5, 0.6, 0.7 L ha^{-1}; PS + PV – Background + Wuxal BIO Vita 1.0 L ha^{-1}; H0.5, H0.6, H0.7 + PS + PV – Background + Prima Forte 195 0.5, 0.6, 0.7 L ha^{-1} + Wuxal BIO Vita 1.0 L $ha^{-1.}$

Enzymatic reactions in the mechanism of protective processes are leading and the most powerful, because they prevent not only the development of free-radical reactions but also support high intensity of oxidant-renewing processes, provide elimination of final oxygen metabolites with their involvement into the energetic exchange and activation of synthesis processes (Kobylinska & Tymochko, 2000). Metabolic response of a cell to the action of the irritant depends on its oxidizing-renovating state, that is able to influence the formation of adaptive reactions through the variability of the level of renovation of low-molecular compounds and proteins and the efficiency of the system of antioxidant protection (Kulinskii & Kolesnichenko, 1993). According to the data, obtained by the scholars (Kurganova et al., 1997; Kalashnikov et al., 1999), POL products – 'initial mediators' of stress as a special state of an organism, can trigger the corresponding mechanisms of protection in the plants, including enzymatic ones. For the reasons, that haven't been studied up to now, main agricultural crops such as wheat, sorghum, corn, soybean et al. manifest a considerably higher level of GST in the process of detoxification of herbicides than weeds, which provides the possibility to manage their selective action in relation to weeds (Dixon & Edwards, 2010).

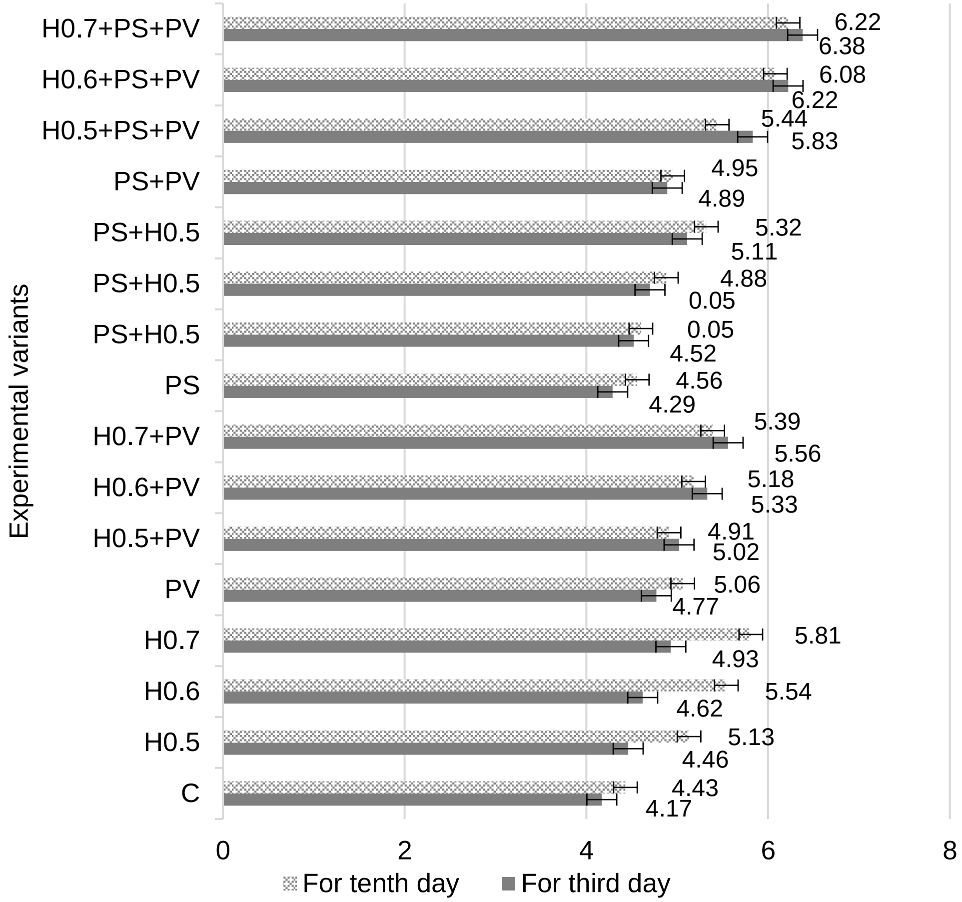

Figure 2. Activity of GST (μmol g^{-1} of raw substance per 1 min) in the leaves of emmer wheat under the action of the herbicide Prima Forte 195 and Wuxal BIO Vita PGR (LSD_{05}: for third day – 0.54; for tenth day – 0.48).
C – without application of preparations (control); H0.5, H0.6, H0.7 – Prima Forte 195 0.5, 0.6, 0.7 L ha^{-1}; PV – Wuxal BIO Vita 1.0 L ha^{-1}; H0.5, H0.6, H0.7 + PV – Prima Forte 195 0.5, 0.6, 0.7 L ha^{-1} + Wuxal BIO Vita 1.0 L ha^{-1}; PS – Wuxal BIO Vita 1.0 L t^{-1} (pre-sowing treatment of seeds, background); H0.5, H0.6, H0.7 + PS – Background + Prima Forte 195 0.5, 0.6, 0.7 L ha^{-1}; PS + PV – Background + Wuxal BIO Vita 1.0 L ha^{-1}; H0.5, H0.6, H0.7 + PS + PV – Background + Prima Forte 195 0.5, 0.6, 0.7 L ha^{-1} + Wuxal BIO Vita 1.0 L ha^{-1}.

Determining the GST activity in the leaves of emmer wheat under the application of Prima Forte 195 at the rates of 0.5; 0.6 and 0.7 L ha^{-1} showed that the index exceeded control by 7; 10 and 18 % on the 3rd day and by 16; 25 and 31% on the 10th day (Fig. 2).

Such increase in the GST activity and other enzymes of antioxidant protection of emmer wheat plants under the action of herbicides were recorded by other researchers (Yin et al., 2008; Jiang & Yang, 2009; Song et al., 2010).

When plants of emmer wheat were sprayed with Prima Forte 195 at the rates of 0.5; 0.6 and 0.7 L ha^{-1} with Wuxal BIO Vita 1.0 L ha^{-1}, the indexes of GST activity exceeded control by 20; 28 and 33% on the 3rd day and by 11, 17 and 22% compared to control on the 10th day correspondingly.

According to the data of Shorning et al. (2000), the plants constantly need AOS for the regulation of growth and development, therefore the dynamics of GST activity in the control variant and some variants testifies this process on the tenth day. However, in the variants with combined application of herbicide and PGR, compared to the indexes on the 3rd day, GST activity decreased on the 10th day, which indicates the stabilization of detoxification processes in emmer wheat plants and conforms with the data of other scientists (Bilonozhko et al., 2012).

When the plants of emmer wheat were sprayed with Prima Forte 195 at the rates of 0.5; 0.6 and 0.7 L ha^{-1} at the background of pre-sowing treatment of seeds with Wuxal BIO Vita at the rate of 1.0 L t^{-1} the GST activity increased in relation to the control by 8; 13 and 23 % (3rd day) and by 4, 10 and 20% (10th day).

Under spraying the plants with a tank mixture of Prima Forte 195 at the rates of 0.5; 0.6 and 0.7 L ha^{-1} with Wuxal BIO Vita 1.0 L ha^{-1} at the background of pre-sowing treatment of seeds with Wuxal BIO Vita at the rate 1.0 L t^{-1} the indexes of GST activity increased on the 3rd day compared to the control by 40; 49 and 53%, and on the 10th day by 23; 37 and 40% respectively. Obtained results of GST activity in the variants of a combined application of herbicide and PGR at the background of pre-sowing treatment of seeds with PGR show the balancing of pro-antioxidant status of the plants of emmer wheat plants.

The experimental data, presented in this article, give us grounds to state that under separate application of herbicide Prima Forte 195 (without PGR) MDA content in the leaves of emmer wheat is higher than in the case of a combined application of herbicide with PGR in the seeds both treated and untreated before sowing with the same PGR. The same factual findings were demonstrated on the example of pea with the application of 2.4-Dichlorophenoxyacetic acid (which is one of the components of herbicide Prima Forte 195), where MDA level increased considerably and which served as a destroying factor of haloid phenoxyacetic acids (Shevchenko et al., 1980). At the same time, combined application of herbicide and PGR at the background of pre-sowing treatment of seeds with PGR fostered the decrease of MDA content in plants. Thus, we can summarize that under complex application of herbicide and PGR the level of oxidative stress in plants slightly decreases. As to the GST activity in the leaves of emmer wheat the experiment showed its increase under lower MDA content, in particular, in the variant with the complex application of herbicide and PGR, especially at the background of pre-sowing treatment of seeds with PGR. We can, therefore, state that there is activation of metabolic processes in plants, including detoxification processes. As our previous research showed, herbicides and PGR, having different mechanisms and course of action, don't vie for the common sites (biological targets). At the same time, their complex action shows a specific form of interaction, that can be characterized as antidotal, which implements through the activation of detoxification systems of plants (Karpenko et al., 2016).

CONCLUSIONS

1. Herbicide action of Prima Forte 195, especially under the higher norms of its application, causes oxidative stress in the plants of emmer wheat that manifests in the accumulation of MDA, the product of peroxide oxidation of lipids. MDA content in the leaves of emmer wheat as a component of a signal system of the adaptive protection of a plant body considerably increases under separate action of herbicide, while it decreases under complex action of herbicide Prima Forte and PGR Wuxal BIO Vita.

2. GST activity in the leaves of emmer wheat is aimed at the decrease of oxidative stress and the formation of plant resistance to the action of herbicide agent, as evidenced by the decrease of MDA content in the corresponding variants of the experiment, where GST activity was the highest.

3. Complex application of herbicide Prima Forte 195 at the rates of 0.5; 0.6 and 0.7 L ha^{-1} with the plant growth regulator Wuxal BIO Vita at the rate of 1.0 L ha^{-1} at the background of pre-sowing treatment of seeds with the same PGR at the rate of 1.0 L t^{-1}, obviously causes faster pace of detoxification of xenobiotic in plants, because in the present variants of the experiment there is increased GST activity under decreased MDA content.

REFERENCES

Agostinetto, D., Perboni, L.T., Langaro, A.C., Gomes, J., Fraga, D.S. & Franco, J.J. 2016. Changes in Photosynthesis and Oxidative Stress in Wheat Plants Submmited to Herbicides Application. *Planta Daninha*. **34**(1), 1–9.

Baimuhametova, E.A., Taipova, R.M. & Kuluev, B.R. 2016. Glutathione and glutathione s-transferases: key components of the antioxidant protection system of plants. *Biomics*. **8**(4), 311–322 (in Russian).

Bilonozhko, V.Ya., Karpenko, V.P., Poltoretskyi, S.P. & Prytuliak, R.M. 2012. Physiological and biochemical processes in the plants of spring barley under separate and integrated application of herbicides and plant growth regulators. *Bulletin of Poltava state agrarian academy* **2**, 7–13 (in Ukrainian).

DeRidder, B.P. & Goldsbrough, P.B. 2006. Organ-Specific Expression of Glutathione S-Transferases and the Efficacy of Herbicide Safeners. *Arabidopsis*. *Plant Physiology* **140**(1), 167–175.

Dixon, D.P. & Edwards, R. 2010. Glutathione transferases. *The arabidopsis book* 8:e0131. doi:10.1199/tab.0131.

Dixon, D.P., Cummins, I., Cole, D.J. & Edwards, R. 1998. Plant glutathione S-transferases and herbicide detoxification. *Current Opinion in Plant Biology* **1**(3), 258–266.

Edwards, R. & Dixon, D.P. 2000. The role of glutathione transferases in herbicide metabolism. In Cobb, A.H. and Kirkwood, R.C. (eds.): *Herbicides and Their Mechanisms of Action*. Sheffield Academic Press, Sheffield, pp. 38–71.

Ehrmantraut, E., Shevchenko, I. & Nenyn, P. 2000. Mathematical analysis and interpretation of research. *Coll. Science. works Institute for Sugar Beet UAAS.* **2**, 189–205 (in Ukraine).

Enan, M.R. 2009. Genotoxicity of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D): Higher plants as monitoring systems. *Am.–Eurasian J. Sustain. Agric*. **3**(3), 452–459.

Gill, S.S. & Tuteja, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant. Physiol. Biochem.* **48**(12), 909–930.

Grishko, V.N. & Syshchikov, D.V. 1999. Peroxide oxidation of lipids and functioning of some antioxidant enzyme systems in maize and oat under severe affection by hydrogen fluoride. *Ukrainian biochemical journal* **71**(3), 51–57 (in Russian).

Habig, W.H., Pabst, M.J. & Jakoby, W.B. 1974. Glutathione S-transferase. The first step in mercapturic acid formation. *Journal Biol. Chem.* **249**(22), 7130–7139.

Hassan, N.M. & Nemat Alla, M.M. 2005. Oxidative stress in herbicide-treated broad bean and maize plants. *Acta Physiol. Plant*. **27**(4), 429–438.

Hesammi, E. 2011. Evaluation of herbicides Nicosulphron and Glayphusit On some weeds. *Advances in Environmental Biology* **5**, 2680–2682.

Hrycajenko, Z.M., Hrycajenko, A.O., Karpenko, V.P. 2003. Soil Nutrient Analysis. In Hrycajenko, Z.M (ed.): *Methods of biological and agrochemical investigations of plants and soil.* Nichlava, Kyiv, pp. 197–201 (in Ukrainian).

Jiang, L. & Yang, H. 2009. Prometryne-induced oxidative stress and impact on antioxidant enzymes in wheat. *Ecotoxicol. Environ. Saf*. **72**(6), 1687–1693.

Kalashnikov, Yu.Ye., Balakhnina, N.I. & Bennichelli, R.P. 1999. Activity of antioxidant system and intensity of peroxide oxidation of lipids in the plants of wheat in relation to resistance to water-logged soil. *Plant physiology* **46**(2), 268–275 (in Russian).

Karpenko, V.P. & Pavlyshyn, S.V. 2018. Activity of antioxidant enzymes in plants of amelcorn under the influence of Prima Forte 195 herbicide and Wuxal BIO Vita plant growth regulator. *Bulletin of agrarian science of Black Sea region* **99** (3), 61–65 (in Ukrainian).

Karpenko, V.P., Hrytsayenko, Z.M., Prytuliak, R.M., Poltoretskyi, S.P., Mostoviak, I.I. & Fomenko, O.O. 2012. *Biological principles of integrated action of herbicides and plant growth regulators.* Sochinskyi, Uman, 357 pp. (in Ukrainian).

Karpenko, V.P., Prytuliak, R.M., Datsenko, A.A. & Ivasiuk, I.I. 2016. Physiological and biochemical mechanisms integrated herbicide and plant growth regulator. *Bulletin of Uman National University of Horticulture*. **1**. 72–75 (in Ukrainian).

Kobylinska, L.I. & Tymochko, M.F. 2000. The role of peroxide-antioxidant balance in the adaptational processes of an organism. *Experimental and clinical phisiol. and biochem.* **12**(4), 52–57 (in Ukrainian).

Kulinskii, V.I. & Kolesnichenko, L.S. 1993. Structure, properties, biological role and regulation of glutathione peroxidase. *Achievements of modern biology* **113**(1), 107–122 (in Russian).

Kurganova, L.N., Veselov, A.P., Goncharova, T.A. & Sinitsyna, Yu.V. 1997. Peroxide oxidation of lipids and antioxidant protection system in chloroplast of peas under heat shock. *Plant physiology* **44**(5), 725–730 (in Russian).

Lamoureux, G.L. & Rusness, D.G. 1989. The role of glutathione and glutathione S-transferases in pesticide metabolism, selectivity, and mode of action in plants and insects. In Dolphin, D., Poulson R., Avramovic, O. (eds): *Glutathione: chemical biochemical and medical aspects.* John Wiley & Sons, New York, pp. 153–196.

Liu, L., Liu, Y., Rao, J., Wang, G., Li, H., Ge, F. & Chen, C. 2013 Overexpression of glutathione S-transferase gene from the fruits of *Pyrus pyrifolia* raises resistance of transgenic tobacco plants to abiotic stress. *Molecular biology*. **47**(4), 591–601 (in Russian).

Mitieva, L.P., Ivanov, S.V. & Aleksiyeva, V.S. 2010. Change of pool of glutathione and some enzymes of its metabolism in the leaves and roots of pea, treated with herbicide glyphosate. *Plant physiology* **57**(1), 139–145 (in Russian).

Neuefeind, T., Reinemer, P. & Bieseler, B. 1997. Plant glutathione S-transferases and herbicide detoxification. *Biol. Chem*. **378**(3–4), 199–205.

Owen, W.J. 2000. Herbicide metabolism as a basis for selectivity. In Roberts, T. (ed.): *Metabolism of Agrochemicals in Plants*. John Wiley and sons Ltd., London, pp. 211–258

Rochalska, M. & Grabowska, K. 2007. Influence of magnetic fields on the activity of enzymes: α- and β- amylase and glutathione S-transferase (GST) in wheat plants. *International Agrophysics*. **21**, 185–188.

Rogozhin, V.V. 2006. *Practical guide on biological chemistry*. Lan. St. Petersburg, 256 pp. (in Russian).
Rossikhina, A. & Vinnichenko, O. 2004. Herbicide treatment influence on lipoperoxidation and its regulation system in maize grain. *Visnyk of L'viv Univ. Biology series.* **37**, 227–231. (in Ukraine).
Shevchenko, N.V., Pohosian, S.I. & Merzliak, M.N. 1980. Peroxide oxidation of membrane lipid under the action of haloid phenoxyacetic acids. *Plant physiology*. **27**(2), 363–369 (in Russian).
Shorning, B.Yu., Smirnova, Ye.G., Yaguzhynskii, L.S. & Vaniushyn, B.F. 2000. Necessity of superoxide formation for the development of etiolated of wheat sprouts. *Biochemistry*. **65**(12), 1612–1618 (in Russian).
Song, N.H., Zhang, S., Hong, M. & Yang, H. 2010. Impact of dissolved organic matter on bioavailability of chlorotoluron to wheat. *Environ. Pollut*. **158**(3), 906–912
Van Camp, W., Van Montagu, M. & Inze, D. 1994. Superoxide dismutase. In Foyer, C.H. and Mullineaux, P.M. (eds.): *Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants.* Boca Raton, FL: CRC Press, Florida, pp. 317–341.
Yin, X.L., Jiang, L., Song, N.H. & Yang, H. 2008. Toxic reactivity of wheat (*Triticum aestivum*) plants to herbicide isoproturon. *J. Agric. Food. Chem.* **56**(12), 4825–4831.
Zama, P. & Hatzios, K.K. 1986. Effects of CGA-92194 on the chemical reactivity of metolachlor with glutathione and metabolism of metolachlor in grain sorghum (*Sorghum bicolor*). *Weed Science*. **34**(6), 834–841.
Zhurbickij, Z.I. 1968. Theory and practice of vegetation method. Nauka, Moskva. 268 pp. (in Russian).

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Using of high-speed mills for biomass disintegration

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Abstract. The need for mechanical disintegration of biomass is very current topic with regard to the requirements of an agrarian sector, beside the importance of a material's moisture content reduction to be used in further applications. The drawbacks of commonly applied devices are the limited use of moist biomass and high energy consumption for disintegration. In collaboration with LAVARIS company, there were tested two high-speed mills LAV 400/1R with single rotor (used for a first milling) and LAV 300/2R with double rotors (used for a second milling), which were primarily designed for crushing of concrete, rubber and construction waste. The goal of the new technical solution was a disintegration of biomass on example of pine sawdust and miscanthus together with examination of simultaneous drying in order to achieve a desired fraction (particle size) and moisture content of biomass material. Experimental tests on high-speed mills have shown the following results: in case of pine sawdust about 98% of output particle size after passing through the first and second milling was smaller than 1.5 mm, and smaller than 1 mm for miscanthus (sieve analysis method was used for determination), i.e. significant reduction was achieved comparing to initial particle size. Moisture content of the materials after disintegration (first and second) decreased from 37.08% to 8.55% for pine sawdust and from 24.43% to 7.19% for miscanthus. Based on the results, it can be concluded that the mechanical disintegration of biomass by high-speed mills has a great potential to become an effective part of raw materials' pre-treatment technology, not only in agriculture, but also in production of different types of biofuels.

Key words: pine sawdust, miscanthus, particle size, sieve analyses, moisture content.

INTRODUCTION

Size reduction of agricultural products and by-products is a key issue for the further utilization of the materials (Chitoiu et al., 2016) together with a moisture content. Particle size is an important parameter having an impact on the characteristics of loose particulate biomass (e.g. flow ability, compaction, compressibility) as well as mechanical properties of densified biofuels (bulk density, strength, mechanical durability), thus largely affecting their quality and performance (Pietsch, 2008; Tumuluru et al., 2011; Guo et al., 2012; Chaloupková et al, 2016; Muntean et al., 2017; Chaloupková et al., 2018). Mechanical disintegration significantly decreases the size of materials' particles and biomass volume resulting in the lower costs for storage, transport and use (Guo et al., 2016; Muntean, 2017). Grinding, cutting or crushing is carried out by different types of mills, using various energy sources, where the most widely used is a hammer mill (Chitoiu et al., 2016). For the purpose of biomass disintegration for biofuel production, the devices that operate on the principle of shear action (colloid mills, extruders) or shear forces (blade, striking mills) are commonly applied to disintegrated the materials with a moisture content over 20 wt% (Kratky & Jirout, 2015). However, their disadvantages are in the limited use of moist biomass and high energy demands (Tumuluru, 2018). According to Manlu et al. (2003) besides the grinding degree beeing a very important indicator, the size reduction has a direct relationship to the energy consumption per unit. Drying of biomass is another crucial operation from technological point of view, where the speed of drying is also affected by size, shape and type of the used material. Thanks to drying the material which would destruct in its natural state very quickly can be stored for a long time, however, drying often involves high energy consumption as well, and that is why it is essential to find/select an optimal drying equipment (Ivanova et al., 2012).

Generally, the importance of developing the sustainable and renewable energy sources, mainly improving the efficiency of such systems for a proper energy saving, is rapidly expanding (Shih et al., 2016). For example, thermo-chemical treatment like combustion or gasification is already known way of biomass utilization for heat and power generation (Kirsanovs et al., 2014), and since the last years a pyrolysis technology attracts an increasing scientific attention, too. However, the process of pyrolysis is energy-intensive (especially rapid pyrolysis), mainly the pre-treatment of a material before the process like pre-drying of biomass (Rogers & Brammer, 2012) as well as size reduction (Choi et al., 2012). Therefore, it is advisable to include disintegration, press or drying unit prior to the pyrolysis reactor (De Jong & Van Ommen, 2014).

High-speed grinding is one of the most efficient and promising solutions for modifying materials in which a large amount of energy is mechanically transferred to the mass of a treated material, thereby changing its reactivity and subsequent properties in addition to the reduction of a material size (Kratky & Jirout, 2015). High-speed grinding performs effective and continuous processing of a very wide range of materials. The treatment of materials by means of high-speed grinding allows to combine the milling effect (surface refinement) with a pronounced mechanochemical activation, i.e. an increase in the internal energy of the treated substances. Mechanochemistry refers to reactions, normally of solids induced by mechanical energy (Gomes et al., 2014), and mechanochemical reactions of organic compounds take place at the low milling energy (Takacs, 2014).

The intention of this paper was to introduce the results of using high-speed mills developed and manufactured by LAVARIS Ltd. for grinding of different biomass and minimising energy consumption. The present study has tested the possibility of using high-speed mills for disintegration of plant material in order to improve the pre-treatment and to expand the utilization of vast residual biomass produced in the agricultural sector, wood processing, forestry activities and other esially available biomass. The main research aim was to investigate an impact of using high-speed mills on a material moisture content and reduction of its particle size.

MATERIALS AND METHODS

For experimental purposes the following materials were used: herbaceous biomass of miscanthus (*Miscanthus sinensis)* – a promising perennial energy crop with the high yield and low input requirements (Clifton-Brown et al., 2004; Davis et al., 2010) and pine sawdust (*Pinus* L.) – a waste-wood biomass representing traditional feedstock for solid biofuels production (McKendry, 2002; Deac et al., 2015; Chaloupková et al., 2018). Both materials were initially grinded by the hammer mill (model 9FQ-40C, Pest Control Corporation, Vlčnov, Czech Republic) with the screen holes' diameter of 12 mm (i.e. initial fraction was 12 mm).

Further, two types of high-speed mills, which are an intellectual property of LAVARIS Company, were tested for the biomass disintegration within this study:

- High-speed multilevel single rotor mill (LAV 400/1R, Lavaris, Libčice nad Vltavou, Czech Republic) with the technical specifications: rotor diameter of 400 mm and rotation speed of 7,800 min^{-1} was used for the first (primary) milling (see Fig. 1);

- High-speed multilevel double rotors mill (LAV 300/2R, Lavaris, Libčice nad Vltavou, Czech Republic) with the specification: rotor diameter of 300 mm and rotation speed of each rotor of 6,200 min^{-1} was used for the second (secondary) fine milling (Fig. 2).

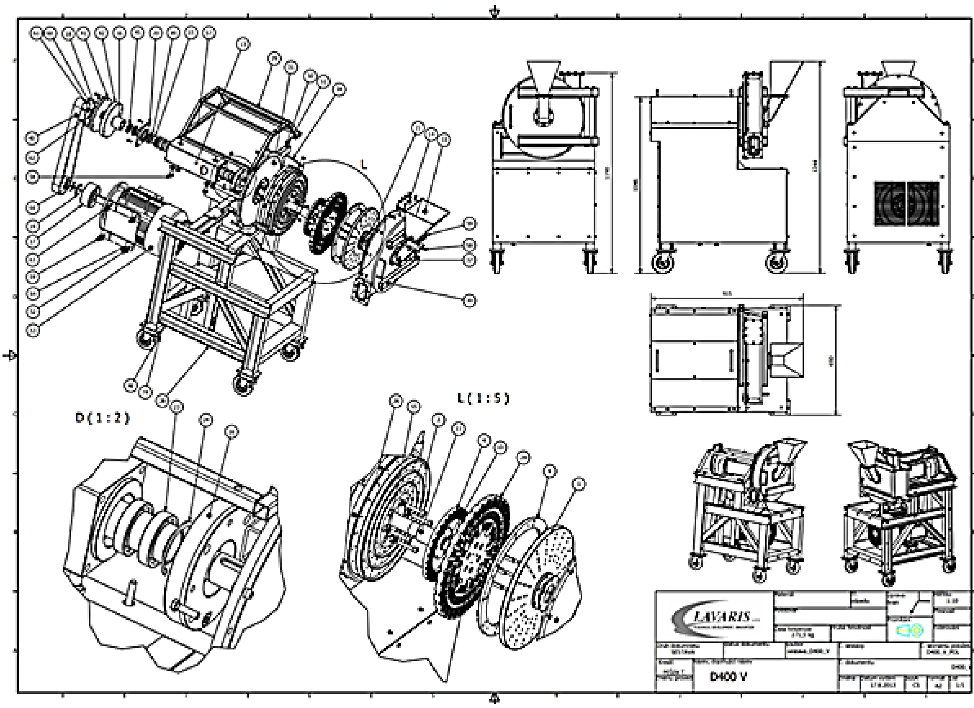

Figure 1. High-speed multilevel single rotor mill LAV 400/1R.

Basically, high-speed mills can be characterized as disintegrators with relatively simple design, low weight, which act on the materials by pressure, shear, free-hit and pulling/bending.

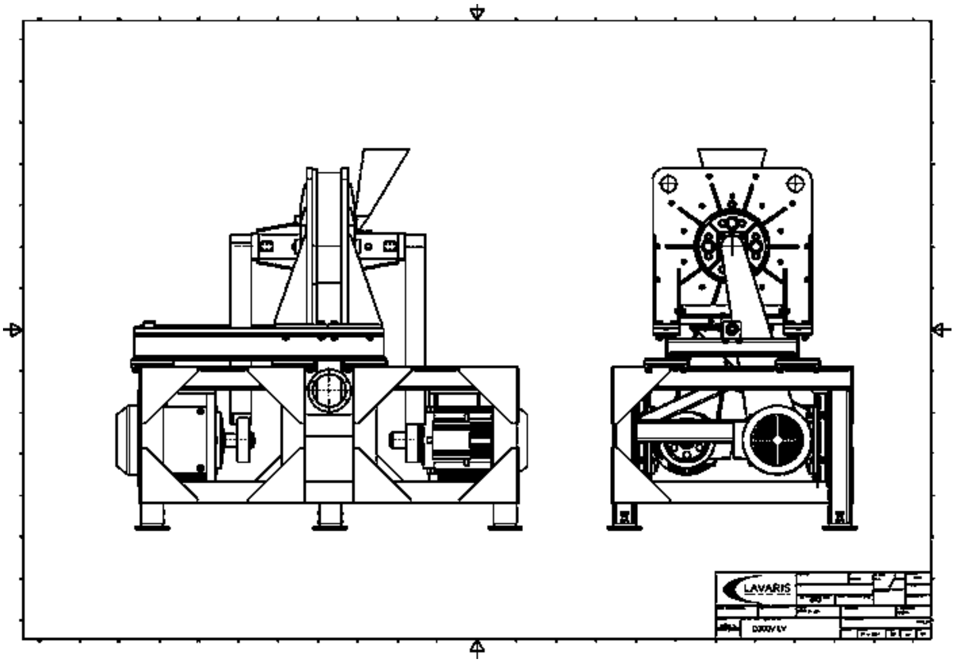

Figure 2. High-speed multilevel double rotors mill LAV 300/2R.

By Hajratwala (1982) the size reduction and heat amount produced in the high-speed milling process depend on the mill speed. The peripheral speed (v) is the speed that a point in the circumference moves per second and it was calculated for both machines by using the following equation:

$$v = \frac{\pi \times d \times n}{60}, \text{m s}^{-1} \qquad (1)$$

where π – 3.14; d – rotor disc diameter, m; n – revolutions per minute, min^{-1}.

In addition to this fact, the special and advanced construction of the tested high-speed mills, invented by LAVARIS Ltd., allowed to adjust the placements of the milling elements. Due to the flexibility in adjustable gaps between the milling elements it was possible to regulate a size of the space for the material flow, the intense of friction and the speed of the material's feeding. Thanks to the mentioned advantages, it was feasible to control the final size of output material and a temperature of the milling process.

One of the main parameters that characterize an efficient operation of a disintegrator is a particle size distribution (PSD) of the product obtained after the grinding process (Tumuluru et al., 2011; Chaloupková et al., 2016). The PSD of the input biomass, biomass after the first as well as second milling was determined by the vibrating screen method (EN ISO 17827-2, 2016) using a sieve shaker (AS 200, Retsch, Haan, Germany) with 30-min sieve shaking time for each repeated measurement and amplitude 3.0 mm g^{-1}. Therefore, for determination of particles size of the initial materials a set of calibrated sieves with the following mesh sizes was applied: 3.15 mm; 1.5 mm; 0.63 mm and the bottom pan. While determining the PSD of the materials obtained by the primary milling the sieves with the mesh sizes of 3.15 mm; 2.5 mm; 1.5 mm; 0.63 mm, including the bottom pan were selected. And, for the materials subjected to the secondary milling the sieves with apertures of 2.5 mm; 1.5 mm; 1 mm; 0.63 mm; 0.50 mm; 0.25 mm and the bottom were used. Three tests (repetitions) were performed for each material and measurement; a captured sample weight on each sieve was calculated as a percentage of total weight expressed as arithmetic mean of three repetitions. Laboratory precision balance (KERN 572-35, Kern & Sohn GmbH, Balingen, Germany) with accuracy 0.01 g was used for weighting.

Moisture content of the input biomass, biomass after the first and second milling was determined in accordance with EN ISO 18134-3 (2015) by controlled drying of the sample in the laboratory oven (MEMMERT 100-800, Memmert GmbH + Co. KG, Schwabach, Germany) at 105 °C for several hours until the weight was constant in mass. The resulting moisture content on wet basis was calculated as the mean of duplicate determinations with respect to repeatability precision (i.e. difference between two individual results of each material sample was not more than 0.2% absolute) and using the following equation:

$$w = \frac{m_2 - m_3}{m_2 - m_1} \times 100, \text{wt\%} \qquad (2)$$

where m_1 – mass of empty crucible, g; m_2 – mass of crucible with sample before drying, g; m_3 – mass of crucible with sample after drying, g.

RESULTS AND DISCUSSION

Based on the long-term experience of LAVARIS Ltd. in developing and operating high-speed mills, both machines were adjusted to the recommended position. The distance between the rotor and stator discs at the high-speed multilevel single rotor mill LAV 400/1R was 3 mm, thus the peripheral speed was found to be 163 m s^{-1}. For the high-speed multilevel double rotors mill LAV 300/2R the distance between both rotors was about 1.5 mm and the peripheral speed then 194 m s^{-1}.

Particle size distribution of tested materials including determination of PSD of input materials with initial fraction as well as PSD after first and second milling are presented at the Tables 1–2. It was obvious that the space between the rotor discs effects the final size of the treated material.

In case of herbaceous miscanthus more than 94% of initial particles were smaller than 3.15 mm, moreover more than half of the material was captured on the sieve with aperture of 1.5 mm, followed by the sieve of 0.63 mm and the bottom pan (Table 1). Almost identical results of PSD were obtained by Chaloupková et al. (2016) in case of *Miscanthus x giganteus*, which confirms similar structure of miscanthus species. Concerning PSD of pine sawdust initial fraction, the sieve analysis showed that over 81% of material's particles were smaller than 3.15 mm, where 65.74% of the material almost equally distributed between the sieves with apertures 1.5 and 0.63 mm (Table 2).

Tables 1 and 2 illustrate that the particle size was visibly reduced after the first milling. Almost 99% of miscanthus particles passed through the sieve with apertures of 2.5 mm, and moreover more than 87% of material was captured and equally divided between the sieve of 0.63 mm and the bottom pan. In case of pine sawdust more than 99% of particles were smaller than 3.15 mm and almost 60% of particles was captured on the sieve 0.63 mm, followed by the bottom pan.

Table 1. Particle size distribution of miscanthus biomass

Miscanthus sinensis								
Initial size			First milling			Second milling		
Sieve, mm	g	%	Sieve, mm	g	%	Sieve, mm	g	%
3.15	3.27	5.86	3.15	0.5	0.62	2.5	0.20	0.27
1.5	30.85	**55.32**	2.5	0.61	0.73	1.5	0.19	0.26
0.63	15.47	**27.74**	1.5	9.25	11.24	1.0	0.97	1.32
< 0.63	6.18	11.08	0.63	37.09	**45.09**	0.63	9.43	12.79
			< 0.63	34.81	**42.32**	0.50	3.04	4.12
						0.25	19.31	**26.20**
						<0.25	40.57	**55.04**
Total	55.77	100	Total	82.26	100	Total	73.71	100

Table 2. Particle size distribution of pine sawdust

Pine sawdust								
Initial size			First crushing			Second crushing		
Sieve, mm	g	%	Sieve, mm	g	%	Sieve, mm	g	%
3.15	15.97	18.60	3.15	0.51	0.59	2.5	1.22	1.72
1.5	25.78	**30.05**	2.5	2.49	2.87	1.5	0.32	0.45
0.63	30.63	**35.69**	1.5	7.44	8.57	1.0	4.77	6.74
<0.63	13.44	15.66	0.63	51.03	**58.78**	0.63	18.61	26.29
			<0.63	25.34	**29.19**	0.50	4.16	5.88
						0.25	19.83	**28.01**
						<0.25	21.88	**30.91**
Total	85.82	100		86.81	100		70.79	100

Second milling resulted in very fine particles. About 98% of miscanthus particles was smaller than 1 mm, and in case of pine sawdust smaller than 1.5 mm. Majority of miscanthus biomass (more than half) was captured on the bottom pan (< 0.25 mm), followed by the smallest sieve of 0.25 mm containing ¼ of the particles. About 60% of wood biomass was divided between the smallest sieve and the bottom pan. Moreover, in case of sawdust only minimal amount of particles (less than 2%) was found on the largest sieve of 2.5 mm, for miscanthus number of bigger particles than 1.5 mm was just negligible. Interesting phenomenon was monitored in case of the sieve with apertures 0.63 mm, where almost 13% of miscanthus biomass and twice higher amount of wood material was captured, but probably clogged. Sieve clogging during an application of the mechanical screening was priviosly observed by Igathinathane et al. (2009) and Glé et al. (2013).

As for example, modern and promising pyrolysis technology has high requirements on the size of an input material due to the extremely rapid heat transfer to a feedstock (Choi et al, 2012), and thus the size is listed among the main limitations for the majority of reactors (Trávníček et al., 2015). By Jouiad et al. (2015), Mohammed et al. (2015) and Wannapeera & Worasuwannarak (2015) the maximum size of a material's particles should be 2 mm or optimal size is 0.2–1.8 mm (Yorgun & Yildiz, 2015), even 0.5–1 mm (Henkel et al., 2016). The experimental results showed that pre-treatment of the materials by high-speed mills can satisfy these requirements.

Obtained results of moisture content are presented in the Table 3. As it can be seen, the initial moisture content of both materials was relatively high and that biomass is not suitable for efficient direct combustion or production, utilization and storage of densified biofuels like briquettes, pellets as well as use for pyrolysis process, etc. The abovementioned is based on the facts that: high moisture content has negative impact on solid biofuels properties, especially calorific value reached during the burning process (Huhtinen, 2005; Havrland et al., 2011; Ivanova et al., 2012) as well as reduction of the combustion device efficiency and a fuel efficiency (Černý et al., 2016); wet material is not bound well during densification (Muntean et al., 2017); decomposing microbial processes stop while moisture content is under 20% (Sladký & Hutla, 2000). According to Havrland et al. (2011) and Muntean et al. (2017) the proper moisture for biomass briquetting must not exceed 12–14%, depending on the technology. For the pyrolysis process it is important to pre-dry biomass to a lower moisture than 10% (Rogers & Brammer, 2012; Joubert et al., 2015), thus it will lead to a water reduction in pyrolysis products, mainly liquid one (Akhtar & Amin, 2012).

The results of the first milling showed the reduction of moisture content by 4.65% in case of miscanthus and 7.67% in case of pine sawdust (see Table 3), however the obtained moistures were still high for utilization. During the second milling the moisture content of both materials decreased significantly and resulted into the optimal values. Comparing with the first milling, the moisture of miscanthus dropped by 12.59% and by 20.86% in case of wood, which is equal to 17.24% and 28.53% moisture reduction in contrast with the initial moistures of miscanthus and pine sawdust, respectively. Biomass drying is usually a time consuming process, thus utilization of high-speed mills for coincident biomass drying provides a good solution to the time issue as well.

Table 3. Comparison of moisture content of initial materials and materials after first and second milling

Material	Moisture content, wt%
Initial material	
Miscanthus sinensis	24.43
Pine sawdust	37.08
First crushing	
Miscanthus sinensis	19.78
Pine sawdust	29.41
Second crushing	
Miscanthus sinensis	7.19
Pine sawdust	8.55

Finally, it is feasible to do a simple comparison between the tested high-speed milling technologies and the conventional systems of milling and drying in relation to the energy consumption. The engine power of commonly applied biomass crushers is about 22 kW and the production output varies between 400 to 1,000 (Deines & Pei, 2010). The engine power of typical drum dryer is 12–14 kW and hot air boiler 300–400 kW (Gigler et al, 2000; Li et al, 2012). So, both systems will need approximately 34–36 kW of electric engine power plus the energy for hot air boiler. Tested high-speed mill LAV 400/1R has maximum electric engine consumption of 10 kW and the second high-speed mill LAV 300/2R has maximum electric engine power of 32 kW. This comparison also shows the advantages of tested systems above the conventional ones.

CONCLUSIONS

The results unequivocally showed that during the milling of biomass by high-speed mills a significant reduction of moisture takes place. In case of miscanthus the moisture content changed from 24.43% (input material) to 19.78% (after the first milling) and then to 7.19% (after the second milling). In case of pine sawdust the moisture reduction was even more obvious: from 37.08% to 29.41% and finally to 8.55%, respectively. Thus, required moisture content for biofuels' production and other utilizations was achieved.

The milling resulted in obtaining of very fine particles of tested biomass materials. The granulometry/sieve analysis show that:

• For herbaceous biomass of miscanthus, 61% of the initial material particle size was above 1.5 mm, after the first disintegration 87.4% particle size was smaller than 1.5 mm and after the second disintegration 81.2% particles size was below 0.5 mm.

• For pine wood sawdust, 49% of the initial material had particle size larger than 1.5 mm, after the first disintegration almost 88% particle size was below 1.5 mm and after the second disintegration 91% particle size was smaller than 1 mm, where 58.9% was below 0.5 mm.

Hence, the experimental tests showed that application of the manufactured high-speed mills with a special construction is a promising technological solution for improved biomass pre-treatment combining both effects, drying and grinding processes, which are typically very expensive and energy demanding operations.

As it was seen during the experiments, there are three factors which affect the treated material. The gap between milling discs has impact on the final size of the output material; beside the course of milling temperature as a result of a friction force inside the machine affects the material moisture. The rotors speed is another important factor, and with increasing rotor speed the output material size was smaller and the temperature was higher. These factors related to the operating conditions of the high-speed mills will be a subject of the next paper.

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REFERENCES

Akhtar, J. & Amin, N.S. 2012. A review on operating parameters for optimum liquid oil yield in biomass pyrolysis. *Renewable and Sustainable Energy Reviews* **16**(7), 5101–5109.

Clifton-Brown, J.C., Stampfl, P.F. & Jones, M.B. 2004. Miscanthus biomass production for energy in Europe and its potential contribution to decreasing fossil fuel carbon emissions. *Global Change Biology* **10**(2), 509–518.

Chaloupková, V., Ivanova, T. & Havrland, B. 2016. Sieve analysis of biomass: accurate method for determination of particle size distribution. In: *Engineering for Rural Development.* Jelgava, Latvia, pp. 1012–1017.

Chaloupková, V., Ivanova, T., Ekrt, O., Kabutey, A. & Herák, D. 2018. Determination of particle size and distribution through image-based macroscopic analysis of structure of biomass briquettes. *Energies* **11**(2), 331/1–13.

Chiţoiu, M., Voicu, Gh., Paraschiv, G., Moiceanu, G., Vladut, V., Matache, M.G., Marin, E., Bunduchi, G., Danciu, A., Voicea, I. & Gageanu, I. 2016. Energy consumption of a hammer mill when chopping miscanthus stalks. In: *44. Symposium 'Actual Tasks on Agricultural Engineering'*, Opatija, Croatia, pp. 215–223.

Choi, S.H., Choi, Y.S. & Park, H.C. 2012. Fast pyrolysis characteristics of lignocellulosic biomass with varying reaction conditions. *Renewable Energy* **42**, 131–135.

Černý, D., Malaťák, J. & Bradna, J. 2016. Influence of biofuel moisture content on combustion and emission characteristics of stove. *Agronomy Research* **14**(3), 725–732.

Davis, S.C., Parton, W.J., Dohleman, F.G., Smith, C.M., Del Grosso, S., Kent, A.D. & Delucia, E.H. 2010. Comparative biogeochemical cycles of bioenergy crops reveal nitrogen-fixation and low greenhouse gas emissions in a *Miscanthus* × *giganteus* agroecosystem. *Ecosystems* **13**, 144–156.

Deac, T., Fechete-Tutunaru, L. & Gaspar, F. 2015. Environmental impact of sawdust briquettes use – experimental approach. *Energy Procedia* **85**, 178–183.

Deines, T.W. & Pei, Z.J. 2010. Power consumption study in knife milling of wheat straw. *Transactions of Manufacturing Research* **38**, 191–196.

De Jong, W. & Van Ommen, J.R. 2014. *Biomass as a sustainable energy source for the future: fundamentals of conversion processes.* John Wiley & Sons, Hoboken, New Jersey, 582 pp.

EN ISO 18134-3. 2015. Solid biofuels – Determination in moisture content – Oven dry method: Part 3:Moisture in general analysis sample. BSI Standards Publication, 14 pp.

EN ISO 17827-2. 2016. Solid biofuels. Determination of particle size distribution for uncompressed fuels. Vibrating screen method using sieves with aperture of 3,15 mm and below. BSI Standards Publication, 20 pp.

Gigler, J.K., van Loon, W.K.P., Vissers, M.M. & Bot, G.P.A. 2000. Forced convective drying of willow chips. *Biomass and Bioenergy* **19**, 259–270.

Glé, P., Gourdon, E. & Arnaud, L. 2013. The effect of particle shape and size distribution on the acoustical properties of mixtures of hemp particles. *Acoustical Society America* **134**, 4698–4709.

Gomes, C.S.B., Gomes, P.T. & Duarte, T. 2014. α-Diimine transition-metal complexes: Mechanochemistry − A new synthetic approach. *Journal of Organometallic Chemistry* **760**, 101–107.

Guo, L., Tabil, L., Wang, D. & Wang, G. 2016. Research paper: Influence of moisture content and hammer mill screen size on the physical quality of barley, oat, canola and wheat straw briquettes. *Biomass and Bioenergy* **94,**: 201–208.

Guo, Q., Xueli, C. & Haifeng, L. 2012. Experimental research on shape and size distribution of biomass particle. *Fuel* **94**, 551–555.

Hajratwala, BR. 1982. Particle size reduction by a hammer mill I: Effect of output screen size, feed particle size, and mill speed. *Journal of Pharmaceutical Sciences* **71** (2), 188–90.

Havrland, B., Pobedinschi, V., Vrancean, V., Pecen, J., Ivanova, T., Muntean, A. & Kandakov, A. 2011. *Biomass processing to biofuel.* PowerPrint, Prague-Chisinau, 86 pp.

Henkel, Ch., Muley, P.D., Abdollahi, K.K., Marculescu, C. & Boldor, D. 2016. Pyrolysis of energy cane bagasse and invasive Chinese tallow tree (*Triadica sebifera* L.) biomass in an inductively heated reactor. *Energy Conversion and Management* **109**, 175–183.

Huhtinen, M. 2005. *Wood Biomass as a Fuel.* Material for 5EURES Training Sessions. Joensuu, North Karelia University of Applied Sciences, 8 pp.

Igathinathane, C., Melin, S., Sokhansanj, S., Bi, X., Lim, C.J., Pordesimo, L.O. & Columbus, E.P. 2009. Machine vision based particle size and size distribution determination of airborne dust particles of wood and bark pellets. *Powder Technology* **196**(2), 202–212.

Ivanova, T., Havrland, B., Hutla, P. & Muntean, A. 2012. Drying of cherry tree chips in the experimental biomass dryer with solar collector. *Reseach in Agricultural Engineering* **58**(1), 16–23.

Joubert, J.E., Carrier, M., Dahmen, N., Stahl, R. & Knoetze, J.H. 2015. Inherent process variations between fast pyrolysis technologies: A case study on Eucalyptus grandis. *Fuel Processing Technology* **131**, 389–395.

Jouiad, M., Al-Nofeli, N., Khalifa, N., Benyettou, F. & Yousef, L.F. 2015. Characteristics of slow pyrolysis biochars produced from rhodes grass and fronds of edible date palm. *Journal of Analytical and Applied Pyrolysis* **111**, 183–190.

Kirsanovs, V., Žandeckis, A., Veidenbergs, I., Blumbergs, M., Gedrovičs, M. & Blumberga, D. 2014. Experimental study on optimisation of the burning process in a small scale pellet boiler due to air supply improvement. *Agronomy Research* **12**(2), 499–510.

Kratky, L. & Jirout, T. 2015. Mechanical disintegration of wheat straw using a roller-plate grinding system with sharp-edged segments. *Acta Polytechnica* **55**(2), 113–122.

Li, H., Chen, Q., Zhang, X., Finney, K.N., Sharifi, V.N. & Jim Swithenbank, J. 2012. Evaluation of a biomass drying process using waste heat from process industries: A case study. *Applied Thermal Engineering* **35**, 71–80.

McKendry, P. 2002. Energy production from biomass (part 1). Overview of biomass. *Bioresource Technology* **83**(1), 37–46.

Manlu, Y., Womac, A.R. & Pordesimo, L.O. 2003. *Review of biomass size reduction technology*, ASAE Annual Meeting. Paper Number: 036077. Available at: https://elibrary.asabe.org/abstract.asp?aid=15454

Mohammed, I.Y., Abakr, Y.A., Kazi, F.K., Yusuf, S., Alshareef, I. & Chin, S.A. 2015. Pyrolysis of Napier Grass in a Fixed Bed Reactor: Effect of Operating Conditions on Product Yields and Characteristics. *BioResources* **10**(4), 6457–6478.

Muntean, A., Ivanova, T., Hutla, P. & Havrland, B. 2017. Influence of raw material properties on the quality of solid biofuel and energy consumption in briquetting process. *Agronomy Research* **15**(4), 1708–1715.

Pietsch, W. 2008. *Agglomeration Processes: Phenomena, Technologies, Equipment*; John Wiley & Sons: New York, NY, USA, 614 pp.

Rogers, J.G. & Brammer, J.G. 2012. Estimation of the production cost of fast pyrolysis biooil. *Biomass and Bioenergy* **36**, 208–217.

Shih, Ch.-H., Latham, W. & Sarzynski, A. 2016. A collaborative framework for U.S. state-level energy efficiency and renewable energy governance. *The Electricity Journal* **29**(9), 21–26.

Sladký, V. & Hutla, P. 2000. Increasing caloricity of energetic wooden plants by drying. In: *Technika a technologie pro nepotravinářské využití půdy a její udřování v klidu.* Research Institute of Agricultural Engineering, Prague, pp. 36–48 (in Czech).

Takacs, L. 2014. What is unique about mechanochemical reactions? *Acta Physica Polonica Series A* **126**(4), 1040–1043.

Trávníček, P., Vitázek, I., Vítěz, T., Kotek, L. & Junga, P. 2015. *Technology for biomass processing for energy use.* Mendel University in Brno, Brno, 210 pp. (in Czech).

Tumuluru, J.S. 2018. *Biomass preprocessing and pretreatments for production of biofuels: mechanical, chemical and thermal methods.* CRC Press, Taylor & Francis Group, 458 pp.

Tumuluru, J.S., Wright, C.T., Hess, J.R. & Kenney, K.L. 2011. A review of biomass densification systems to develop uniform feedstock commodities for bioenergy application. *Biofuels Bioproducts and Biorefining* **5**(6), 683–707.

Wannapeera, J. & Worasuwannarak, N. 2015. Examinations of chemical properties and pyrolysis behaviors of torrefied woody biomass prepared at the same torrefaction mass yields. *Journal of Analytical and Applied Pyrolysis* **115**, 279–287.

Yorgun, S. & Yildiz, D. 2015. Slow pyrolysis of paulownia wood: Effects of pyrolysis parameters on product yields and bio-oil characterization. *Journal of Analytical and Applied Pyrolysis* **114**, 68–78.

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Combined application of mulches and organic fertilizers enhance shallot production in dryland

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Abstract. The objective of this study was to determine the type of mulch and organic fertilizer that can induce suitable changes in the microclimate and chemical properties of soil for the promotion of growth and yield of shallot on dryland. A factorial randomized block design experiment with two factors and three replications was constructed. The first factor was mulches consisting of rice straw, coconut husk, silver-black plastic mulch, and without mulch. The second factor was the organic fertilizers composed of either composted cow manure, *Gliricidia* leaf compost (each applied at 5 t ha^{-1}), and no organic fertilizer. Among all treatments tested, straw mulch with 5 t ha^{-1} cow manure (L_1P_1) decreased the soil temperature from 36 °C to 30 °C and increasing the soil moisture from 7% to 37%. This, in turn, increased the cation exchange capacity by 24.32 meq 100 g^{-1}, pH by 6.83, C organic from 0.74 to 2.72%, C/N ratio by 13.27%, total N by 0.29%, total P from 20.02 to 28.86 mg 100 g^{-1} and K2O by 39.16 mg 100 g^{-1}. In addition, the growth and yield of shallot were positively affected, as assessed by plant height, leaf number, root length, root dry weight, total leaf area, number of bulbs per hill, bulb diameter, weight of fresh bulbs, and bulb yield. The yield of bulbs increased from 4.27 to 10.22 t ha^{-1} after L_1P_1 treatment. This study demonstrates the application of straw mulch and 5 t ha^{-1} cow manure could enhance the yield of shallot cultivation on drylands.

Key words: *Allium cepa* var. *aggregatum*, rice straw, coconut husk mulches, cow manure.

INTRODUCTION

Palu valley shallot *(Allium cepa* L. var. *aggregatum* G. Don), cultivated in the Palu Valley, Central Sulawesi, Indonesia, is an economically important horticultural plant, used as a raw material in the preparation of fried shallots. Although the potential yield of Palu valley shallot can be as high as 10–12 t ha^{-1} (Maskar et al., 2001; Central Bureau of Statistics Central Sulawesi, 2016), generally the yield obtained by farmers is only about 5.3 t ha^{-1} (Central Bureau of Statistics Central Sulawesi, 2016). The low yield of Palu valley shallots is because they are cultivated on less fertile dryland, with low content of soil organic matter and limited water resources (Mulyani & Hidayat, 2009). In addition, the high intensity of sunlight and low rainfall also contribute to the reduced yield of shallots by directly affecting the microenvironment of the plants, especially the soil temperature, which influences the environment around the plants, from their roots to the aerial part.

Soil fertility is closely related to rainfall and climate (Kandiannan et al., 2011; Ann, 2012). It has been suggested that rainfall and the ability of soil to retain water are crucial for improving the quantity and quality of shallot bulbs (Yudiyanto et al., 2014; Rop et al., 2016).

The yield of shallots grown on drylands can be improved by modification of the microclimate by mulching and application of organic matter, as well as by increasing the input of biomass into the soil (Hani et al., 2016). This will help to optimize the use of drylands for shallot cultivation. Mulching helps in stabilizing the soil temperature and promotes moisture retention around roots (Ramakrishna et al., 2006; Hernández et al., 2016; Kader et al., 2017). It also protects the soil surface from the effects of rain and can improve soil physical properties by increasing the soil water infiltration rate and decreasing the erosion (Bhatt & Khera, 2006; Mulumba & Lal, 2008; Gholami et al., 2013). Moreover, the use of mulch as soil cover helps in the control of weeds, and it can improve the availability of soil water by reducing evaporation and increasing the soil organic matter content (Thankamani et al., 2016; Brown & Gallandt, 2018).

The addition of organic matter plays an important role in improving, and sustaining land productivity by improving the physical, chemical, and biological properties of soil. Although no single application can improve the plant growth, the use of organic fertilizers and microclimate management, together with drip irrigation system, can save water and enhance the utilization of organic residues (Sanchez-Martín et al., 2010; Reganold & Wachter, 2016). Previous studies have shown that shallot production in dryland increased by adding bokashi compost and NPK fertilizer (Lasmini et al., 2018). Application of cow dung can be used as organic fertilizer to increase grain yield and effectively reduce soil evaporation (Polthanee et al., 2008; Chang et al., 2016). The application of organic fertilizers to peas and oats significantly increased the dry weight of nodules, the rate of photosynthesis, N_2 fixation, N accumulation in pea plants, N concentration in the grain, carbon (C), N, and P content in the microbial biomass, and fungal ergosterol in soil and CO_2 production (Jannoura et al., 2014).

The improvement of soil biological properties as a result of organic fertilization is indicated by an increase in soil organic C, total N, and abundance of soil microarthropods, as well as by a decrease in soil pH. The abundance of soil microarthropods is positively correlated with soil C and N and negatively correlated with pH (Wang et al., 2015).

The present study aimed to determine the type of mulch and organic fertilizer that can be used to change the microclimate, soil chemical properties, and growth and yield of shallot cultivated on dryland.

MATERIALS AND METHODS

Experimental site and meteorological conditions

This research was conducted at Oloboju village, Sigi Regency, Central Sulawesi, Indonesia, located at an altitude of 120 m above sea level. Soil is of Inceptisol type, and the climate is characterized by air temperature around 34 °C, annual average air humidity of 72.5%, and annual average rainfall of 41.10 mm^3. The study was carried out from January 2017 to November 2017.

Plot preparation, seedling, and crop treatments

The experimental field was first cleaned of plant debris and garbage and then plowed with a tractor, then dried and dried, hoed and flattened. Then the soil is spilled again then flattened and made beds (plots) with a size of 300 × 120 (length × width) with a plot distance between 30 cm and a depth of 20 cm. While the 50 cm replicate plot distance with 40 cm depth also functions as a drainage channel. The beds are made in the north south direction.

The experiment was performed in a factorial randomized block design, consisting of two factors. The first factor was the type of mulch used (L_0 = no mulching, L_1 = rice straw mulch, L_2 = coco husk mulch, and L_3 = silver-on-black plastic mulch); the second factor was the type of organic fertilizer used (P_0 = no fertilizer used, P_1 = 5 t ha^{-1} cow manure, and P_2 = 5 t ha^{-1} *Gliricidia sepium* compost). In total, there were 12 treatments and each treatment was repeated three times, resulting in 36 experimental units. The number of plants per unit was 120; thus, the total number of plants used was 4,320.

Shallot seedlings used were of the local variety from Palu *(Allium cepa* var. *aggregatum*), obtained from local seed growers in Palu. The seeds were chosen uniformly, and has a shelf-life 2 months. A 5 grams seed were placed into each planting hole in the plot in 20 and 15 cm row and column distances respectively. Cow manure and *G. sepium* compost (5 t ha^{-1} of each) were applied a week before planting by mixing with the soil of the trial plots. A day before planting, 150 kg ha^{-1} NPK basic fertilizer was added in all plots as a non-treatment. It is conducted to provide a similar nutrition for the plant in all plots to help the plant growth in the dryland. Rice straw mulch and coconut husk mulch (5 t ha^{-1} of each), cut to a length of 10 cm, were applied on the surface of the plot directly after planting, whereas silver-on-black plastic mulch was installed on the plot surface one day before planting, with the left and right sides tied with bamboo. Watering was done in the morning and afternoon, or as needed depending on soil conditions. Weeding was performed manually every two weeks after planting.

Data collection

The parameters recorded were plant growth components, namely plant height, leaf number, root length, root dry weight, total leaf area, and bulb diameter; these were measured every week until the of 7 weeks after planting. The number of tillers per hill, bulb weight per clump, and bulb yield per hectare were noted at 8 weeks after planting.

The plant height and root length was measured using a ruler. The number of leaves and bulbs were counted manually per plant, total leaf area was counted as cm^2 per leaf. The bulb diameter was measured by callipers. The fresh weight of bulbs was measured using balance then air-dried per shallot clump until we had a constant dry weight. Shallot bulb harvested was weighted. The weight of fresh bulbs that had been separated from the leaves and roots, then converted to t ha^{-1}.

Chemical analysis of the soil was carried out in the Laboratory of Soil Science, Faculty of Agriculture, Tadulako University, Palu, Indonesia. Soil was sampled before and after the experiment for each experimental plot. Before the plot constructed and before cow manure and basic fertilizer applied, the soil sample was collected using soil corer (10 cm diameter and 10 cm depth). After harvested, soil sample was collected again using the same soil corer from each plot based on treatments. Chemical soil properties were determined by a combined glass calomel electrode (Ghosh, 1983); soil organic content was measured by wet oxidation method (Jackson, 1973); total

P measured by the method introduced by Olsen et al. (1954); soil total N estimated using modified after Kjeldahl method (Page et al., 1989); available K (Black, 1965); soil temperature, measured with a soil thermometer; air humidity, measured with a thermohygrometer; and light intensity, measured with a flux meter (van-Reeuwijk, 1993).

Statistical analysis

The data were analyzed by *F*-test. In case of significant differences between the treatments, the results were further tested by Tukey honestly significant difference (HSD) test at 5%.

RESULTS AND DISCUSSIONS

Effect of mulch and organic fertilizer on the microenvironment of growing crop

The application of rice straw mulch and cow manure were observed to reduce the soil temperature (Fig. 1) and increased the soil moisture levels (Fig. 2). The highest average temperatures were measured in the L_0P_0 treatment and the lowest in the L_1P_1 treatment (Fig. 1). The highest soil water content was determined in the L_1P_1 treatment and the lowest in the L_0P_0 treatment (Fig. 2). We also found that all of the uncovered plots with mulches had a similar trend to have lower humidity.

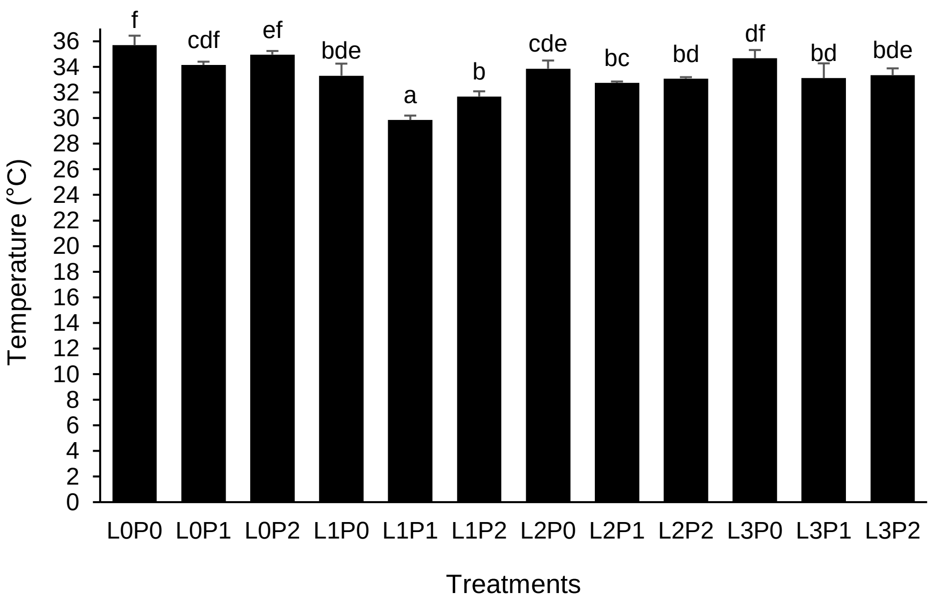

Figure 1. Effect of mulch and organic fertilizer on the average soil temperature. Data shows means (n = 3 ± SD). L_0 = no mulching; L_1 = rice straw mulch; L_2 = coco husk mulch; L_3 = silver-on-black plastic mulch; P_0 = no fertilizer used; P_1 = 5 t ha^{-1} cow manure; and P_2 = 5 t ha^{-1} *Gliricidia sepium* compost. Different letters indicate significant differences between treatments after Tukey HSD test with $P < 0.05$.

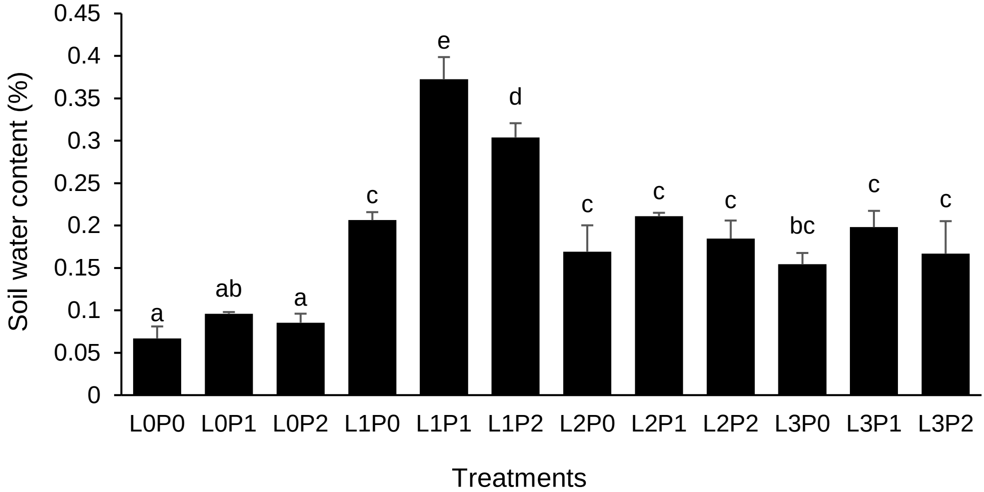

Figure 2. Effect of mulch and organic fertilizer on the soil water content. Data shows means (n = 3 ± SD). L_0 = no mulching; L_1 = rice straw mulch; L_2 = coco husk mulch; L_3 = silver-on-black plastic mulch; P_0 = no fertilizer used; P_1 = 5 t ha^{-1} cow manure; and P_2 = 5 t ha^{-1} *Gliricidia sepium* compost. Different letters indicate significant differences between treatments after Tukey HSD test with $P < 0.05$.

In this study, we found that mulches application decrease the temperature and soil water content. Decreasing soil temperature from 3 °C to 30 °C after mulches application showed a favourable growth condition for shallot production (Grubben, 1990; Sumiati, 1994; Okubo et al., 1999). Mulches application also preserved water than uncovered plant soil. Straw mulch indicate a good performance compared to the silver plastic mulch as presented by L_1P_1 treatment. Organic mulches have been reported in controlling soil temperature, increase water holding, and modified microclimate condition needed for the plant growth (Zhao et al., 2014; Abouziena & Radwan, 2015; Biswas et al., 2015; Pramanik et al., 2015). Paddy straw abundant in central Sulawesi, it also easy to applied as a mulch. Straw mulch will stick to the soil surface, reflect the sunlight (Ranjan et al., 2017), and reduce the temperature (Noorhadi & Supriyadi, 2003). The effectiveness of rice straw has been studied. It can maintain the stability of soil moisture by reducing water loss from the surface of the soil (McMillen, 2013) and reduce evaporation Dewantari et al. (2015). In addition, straw mulch able to protect the soil from erosion (Unger, 1978; Woldetsadik, 2003; Manickam et al., 2016).

Effect of Mulch and Organic Fertilizer on Soil Chemical Properties

The application of rice straw mulch and cow manure (5 t ha^{-1} each) increased the cation exchange capacity (CEC), total N content, available P, K, soil organic C, C/N ratio, and pH of the soil (Table 1). The increasing of CEC in the soil with the application of straw mulch and cow manure was caused by the increase of C-organic content in soil (Table 1).

The amendment of straw mulch and cow manure (L_1P_1) showed a significant effect ($P < 0.05$) on organic C, C/N ratio, N-total, P-total, available K and CEC, but not significantly different on soil pH (Table 1). Organic C increased by 411%, C / N ratio 85%, N total 222%, P total 44%, K_2O 42% and CEC 84% compared to L_0P_0. This finding was also reported by Meena et al. (2016), organic materials in the form of compost and straw mulch increase the soil fertility as indicated by increasing organic C, N, P, and K.

The application of straw mulch and cow manure also provided the highest levels of P and K (Table 1). Besides releasing inorganic P, decomposition and mineralization of organic matter may release molecules such as nucleic acids and organic P, which can be utilized by the plants. This was in agreement with the results of a previous study in which rice straw mulching (6 t ha^{-1}) in the red chili crop in dry season could increase soil moisture, nutrient content of N, P, and K, organic C content, and soil organic matter and reduce soil temperature (Harsono, 2012).

Table 1. Effects of application of mulch and organic fertilizer on chemical properties of soil

Treatments[a]	pH (H_2O)	Organic C (%)	C/N ratio	Total N (%)	Total P (mg 100 g^{-1})	K_2O (mg 100 g^{-1})	CEC[b] (meq 100 g^{-1})
Bare soil plots	6.17a	0.75 g	7.58 fg	0.14 ab	21.54 h	27.64 gh	14.30 h
L_0P_0	6.03 a	0.74 g	7.16 g	0.09 b	20.02 i	27.55 gh	13.21 h
L_0P_1	6.10 a	1.44 de	8.94 e	0.13 b	20.12 i	29.73 ef	17.91 e
L_0P_2	6.14 a	1.93 c	8.64 e	0.12 b	22.63 gh	28.85 fg	17.64 ef
L_1P_0	6.29 a	0.97 gf	9.16 e	0.18 ab	24.28 def	27.08 h	16.36 fg
L_1P_1	6.83 a	2.72 a	13.27 a	0.29 a	28.86 a	39.16 a	24.32 a
L_1P_2	6.10 a	2.33 b	12.69 ab	0.23 ab	26.84 b	31.01 e	20.43 b
L_2P_0	6.05 a	0.79 g	8.36 ef	0.12 b	23.19 fg	27.62 gh	16.17 g
L_2P_1	6.25 a	1.81 c	11.13 cd	0.17 ab	25.85 cd	35.53 bc	20.13 bc
L_2P_2	6.40 a	1.28 ef	10.83 d	0.21 ab	24.73 ce	36.64 b	18.49 de
L_3P_0	6.02 a	0.68 g	8.37 ef	0.13 b	23.51 ef	28.47 fh	15.73 g
L_3P_1	6.37 a	1.78 de	12.04 ef	0.22 ab	24.98 fg	33.26 e	19.38 eg
L_3P_2	6.30 a	1.31 bc	11.83 ef	0.23 ab	24.19 def	34.53 ef	18.85 def

[a]Bare soil plots (soil in the plots before planted), chemical properties were determined for soil in plots before planting of shallot plants and application of mulch and fertilizer. L_0 = no mulching; L_1 = rice straw mulch; L_2 = coco husk mulch; L_3 = silver-on-black plastic mulch; P_0 = no fertilizer used; P_1 = 5 t ha^{-1} cow manure; and P_2 = 5 t ha^{-1} *Gliricidia sepium* compost. [b]CEC: cation exchange capacity.

Values followed by the same letter in the same column are not significantly different at 5% level as assessed by Tukey honestly significant difference test.

The increasing of soil pH from 6.17 to 6.83 and C-organic from 0.75 to 2.72 (Table 1) may relate to the amendment of straw mulch with cow manure. Cow manure in its decomposition process will produce organic acids, such as humic acid and fulvic acid (Stevenson, 1994). Organic acids will react with metal cations such as Al, Fe, Mn, Zn, and Cu to form chelates so that they can become buffers which causes pH stability. In addition, organic matter is also reported to increase soil pH since organic acids produce alkaline cations (Djuniwati et al., 2007).

The role of cow manure on increasing CEC and nutrients N, P and K in the soil is related to the mineralization process which releases complete nutrient minerals (N, P, K, Ca, Mg and S, and micronutrients) that can be used by plants (Tisdale et al., 1985). The use of straw mulch will decompose to be organic matter and CEC (Chen & Weil, 2010).

Effect of Mulch and Organic Fertilizer on Shallot Growth and Yield

The application of mulch and organic fertilizer in shallot cultivation improved the plant growth (Table 2) and bulb yield of shallot (Table 3). Compared with other treatments, straw mulch and cow manure treatments increased more effectively the plant height, leaf number, total leaf area, root dry weight, root length, number of bulbs per hill, weight of fresh bulbs, and bulb yield per hectare.

Table 2. Changes in growth of shallot plants as a response to the application of mulch and organic fertilizer

Treatment	Growth				
	Plant height (cm)	Number of leaves	Leaf area (cm^2)	Root dry weight (g)	Root length (cm)
L_0P_0	20.41 c	22.41 c	208.76 g	1.01 bc	13.49 b
L_0P_1	20.43 c	23.80 bc	227.27 g	0.71 cd	13.12 b
L_0P_2	23.68 ab	23.67 bc	271.03 f	0.98 bc	13.31 b
L_1P_0	22.57 b	25.13 bc	283.01 ef	1.02 bc	13.20 b
L_1P_1	26.05 a	37.63 a	569.32 a	1.63 a	16.67 a
L_1P_2	24.91 a	30.87 b	476.89 b	1.34 ab	13.45 b
L_2P_0	22.41 b	25.73 bc	278.59 f	0.63 d	12.65 bc
L_2P_1	23.00 b	25.87 bc	338.01 d	1.27 b	12.62 bc
L_2P_2	24.96 a	24.97 bc	319.62 de	1.18 b	11.20 c
L_3P_0	22.86 b	23.60 bc	210.56 g	1.01 bc	11.89 bc
L_3P_1	22.43 b	25.13 bc	382.79 c	1.23 b	12.46 bc
L_3P_2	23.29 b	27.45b	324.95 de	0.98 bc	13.58 b

Values followed by the same letter in the same column are not significantly different at 5% level as assessed by Tukey honestly significant difference test. L_0 = no mulching; L_1 = rice straw mulch; L_2 = coco husk mulch; L_3 = silver-on-black plastic mulch; P_0 = no fertilizer used; P_1 = 5 t ha^{-1} cow manure; and P_2 = 5 t ha^{-1} *Gliricidia sepium* compost.

Table 3. Changes in yield of shallot plants as a response to the application of mulch and organic fertilizer

Treatment	Yield			
	Diameter of bulbs (cm)	Number of tillers (bulb)	Fresh weight (g $plant^{-1}$)	Yield of bulbs (t ha^{-1})
L_0P_0	1.47 d	7.33	22.48 d	4.27 d
L_0P_1	1.69 bc	7.33	28.35 d	7.56 c
L_0P_2	1.69 bc	7.00	30.00 d	8.00 c
L_1P_0	1.70 bc	7.67	34.55 bc	9.21 b
L_1P_1	2.06 a	8.00	39.27 a	10.22 a
L_1P_2	1.99 a	8.00	35.57 b	9.48c a
L_2P_0	1.77 b	7.33	29.64 d	7.90 c
L_2P_1	1,60 cd	7.33	33.75 bc	9.00 b
L_2P_2	1.50 cd	7.00	28.31 d	7.55 c
L_3P_0	1.70 bc	7.33	30.81 bc	8.02 c
L_3P_1	1.53 cb	6.33	29.70 d	7.92 c
L_3P_2	1.63 b	7.67	34.28 bc	9.14 b

Values followed by the same letter in the same column are not significantly different at 5% level as assessed by Tukey honestly significant difference test. L_0 = no mulching; L_1 = rice straw mulch; L_2 = coco husk mulch; L_3 = silver-on-black plastic mulch; P_0 = no fertilizer used; P_1 = 5 t ha^{-1} cow manure; and P_2 = 5 t ha^{-1} *Gliricidia sepium* compost.

The plant growth (plant height, leaf number and area, root length and dry weight, and bulb diameter) and yield of shallot (weight of fresh bulb) increased with the increase in soil moisture content, CEC, and N, P, K, and organic C contents in the soil in treatments after rice straw and cow manure amendments. Mulch can store and provide water for plants, especially under conditions of water scarcity, thus improving the conservation of water. The application of cow manure increased the growth (Table 2) and yield of bulbs (Table 3) because the N, P, and K contained in cow manure promoted the growth of shallot crop.

Compared to other treatments L1P1, indicate a promising number of tillers, bulb diameter, plant fresh weight, and bulb yield. The increase in of growth parameters may affect by N in cow manure combined with higher straw mulch compared to without organic fertilizer, which contributes to the increasing of vegetative growth of the plants. This is in agreement with Liu et al. (2017) that N elements can encourage the growth of plant organs to increase the number and size of plant cells. Abdissa et al. (2011) also reported that N plays role in the formation of amino acids, proteins, nucleic acids, enzymes, nucleoproteins, and alkaloids, which are needed in the process of plant growth, especially the development of leaves and plant saplings. In addition, the role of P in stimulating root growth and as a basic component in the formation of certain proteins for accelerating tuber formation. Phosphorus is a component of enzymes, proteins, ATP, RNA, and DNA, which have important functions in the photosynthesis. Adequate P can improve the development of roots and plant carbohydrate content, which ultimately increases plant growth and yield (Singh et al., 2000).

Nitrogen, phosphorus, and potassium also has been reported able to increase the size of tuber, total fresh weight of plants and number of tuber products per hectare. Potassium is important for the formation of proteins and carbohydrates, facilitating photosynthesis and increasing photosynthate translocation to plant parts, subsequently, improve the crop yields (Ali et al., 2007; Mozumder et al., 2007; Islam et al., 2008; Faten et al., 2010).

CONCLUSIONS

The application of rice straw mulch and cow manure (5 t ha^{-1} of each) to shallot crop lower the soil temperature and improve the moisture, pH, content of N, P, K, and organic C, CEC, and the C/N ratio in the soil. This study has shown that the application of mulches and organic fertilizers increase the yield of shallot bulbs significantly from 4.27 to 10.22 t ha^{-1}.

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REFERENCES

Abdissa, Y., Tekalign, T. & Pant, L.M. 2011. Growth, bulb yield and quality of onion (*Allium cepa* L.) as influenced by nitrogen and phosphorus fertilization on vertisol I. growth attributes, biomass production, and bulb yield. *African Journal of Agricultural Research* **6**(14), 3252–3258.

Abouziena, H.F. & Radwan, S.M. 2015. Allelopathic effects of sawdust, rice straw, bur-clover weed and cogongrass on weed control and development of onion. *International Journal of Chemical and Technology Research* **7**, 337–345.

Ali, M.K., Alam, M.F., Alam, M.N., Islam, M.S. & Khandaker, S.M.A.T. 2007. Effect of nitrogen and potassium levels on yield and quality seed production of onion. *Journal of Applied Sciences Research* **3**(12), 1889–1899.

Ann, Y.C. 2012. Impact of different fertilization methods on the soil, yield and growth performance of black pepper (*Piper nigrum* L.). *Malaysian Journal of Soil Science* **16**, 71–87.

Bhatt, R. & Khera, K.L. 2006. Effect of Tillage and Mode of Straw Mulch Application on Soil Erosion in the Submontaneous Tract of Punjab, India. *Soil & Tillage Research* **88**, 107–115.

Biswas, S.K., Akanda, A.R., Rahman, M.S. & Hossain, M.A. 2015. Effect of drip irrigation and mulching on yield, water-use efficiency and economics of tomato. *Plant, Soil and Environment* **61**, 97–102.

Black, C.A. 1965. Method of Soil Analysis Part I and II. Ame Soc Agron. Inc, Madison, Wiscosin, USA, p.770

Brown, B. & Gallandt, E.R. 2018. A systems comparison of contrasting organic weed management strategies. *Weed Science* **66**, 109–120.

Central Bureau of Statistics of Central Sulawesi. 2016. *Harvested Area, Production, Yield Rate of Vegetables by Regency/Municipality and Kind of Plant in Sulawesi Tengah Province, 2011–2015*. Sulawesi Tengah Province in Figures 2016. BPS statistics of Central Sulawesi province, pp. 240–249 (in Indonesia).

Chang, T., Ye, H., Shao, X., Zhang, Z. & Zhang, J. 2016. Effects of organic fertilizer on evaporation under different saline soils. In: 2016 ASABE Annual International Meeting 2016 (2–9). *American Society of Agricultural and Biological Engineers.*

Chen, G. & Weil, R.R., 2010. Penetration of cover crop roots through compacted soils. *Plant Soil* **331**, 31–43.

Dewantari, R.P., Suminarti, N.E. & Tyasmoro, S.Y. 2015. The effect of straw mulch and weeding period on growth and yield of soybean *Glycine max* (L.) Merril. *Journal Produksi Tanaman* **3**, 487–495. In Indonesia.

Djuniwati, S., Pulunggono dan Suwarno, H.B. 2007. The effect of organic matter (Centrosema pubescens) and rock phosphate application on the activity of Phosphatase and P fraction of Latosol soil in Darmaga, Bogor. Jurnal Tanah dan Lingkungan*l* **9**(1), 10–15 (in Indonesia).

Faten, S.A., El-Al, A.B.D., Shaheen, A.M., Rizk, F.A. & Hafed, M.M. 2010. Influence of irrigation intervals and potassium fertilization on productivity and quality of onion plant. *Int. J. Acad. Res.* **2**(1), 110–116.

Gholami, L., Sadeghi, S.H. & Homaee, M. 2013. Straw mulching effect on splash erosion, runoff, and sediment yield from eroded plots. *Soil Science Society of America Journal* **2013**, 268–278.

Ghosh, P. 1983. Institute of agriculture, Visva-Bharati, Srinike tan-731-236. West Bengal, India. *Indian Journal of Agriculture and Research* **32**, 75–80.

Grubben, G.J.H. 1990. Timing of vegetable production in Indonesia. *Acta Horticulturae*, 261–270.

Hani, A., Indrajaya, Y., Suryanto, P. & Budiadi. 2016. Dry land agroforestry practices in Menoreh Hills, Kulon Progo. *Agrivita, Journal of Agricultural Science* **38**, 193–203.

Harsono, P. 2012. The effects of organic mulches on microclimate, chemical soil properties and performance of red chilli in vertisol soil in Sukoharjo at dry season. *Jurnal Hortikultura Indonesia* **3**, 35–41 (in Indonesia).

Hernández, J.R.R., Pedreño, J.N. & Lucas, I.G. 2016. Evaluation of plant waste used as mulch on soil moisture retention. *Spanish Journal of Soil Science* **6**. doi: 10.3232/sjss.2016.v6.n2.05

Islam, M.A., Shamsuddoha, A.T.M., Bhuiyan, M.S.I. & Hasanuzzaman, M. 2008. Response of summer onion to potash and its application methods. *Am-Euras J. Agron.* **1**(1), 10–15.

Jackson, M.L. 1973. *Soil Chemical Analysis*. Prentice Hall of India Pvt. Ltd. New Delhi, 498 pp.

Jannoura, R., Joergensen, R.G. & Bruns, C. 2014. Organic fertilizer effects on growth, crop yields, and soil microbial biomass indices in sole and intercropped peas and oats under organic farming conditions. *European Journal of Agronomy* **52**, 259–270.

Kader, M.A., Senge, M., Mojid, M.A. & Ito, K. 2017. Recent advances in mulching materials and methods for modifying soil environment. *Soil & Tillage Research* **168**, 155–166.

Kandiannan, K., Parthasarathy, U., Krishnamurthy, K.S., Thankamani, C.K., Srinivasan, V. & Aipe, K.C. 2011. Modeling the association of weather and black pepper yield. *Indian Journal of Horticulture* **68**, 96–102.

Lasmini, S.A., Nasir, B., Hayati, N. & Edy, N. 2018. Improvement of soil quality using bokashi composting and NPK fertilizer to increase shallot yield on dry land. *Australian Journal of Crop Science* **12**, 743–749.

Li, Z., Liu, A., Wu, H., Tan, L., Long, Y., Gou, Y., Sun, S. & Sang, L. 2010. Influence of temperature, light and plant growth regulators on germination of black pepper (*Piper nigrum* L.) seeds. *African Journal of Biotechnology* **9**, 1354–1358.

Liu, X.A., Groenigena, K.J., Dijkstraa, P. & Hungatea, B.A. 2017. Increased plant nitrogen uptake of native soil fertilizer following addition – not a priming effect? *Applied Soil Ecology* **114**, 105–110.

Manickam, L., Bureau, N. & Survey, S. 2016. Effect of plastic mulch on soil properties and crop growth – A review. *Agricultura Review* **31**(2), 145–149.

Maskar, Basrum, Lasenggo, A. & Slamet, M. 2001. Multilocation assay of shallot local variety in Palu, Report of centre for application of agricultural technology. *BPTP Central Sulawesi*, p. 13 (in Indonesia).

McMillen, M. 2013. The Effect of Mulch Type and Thickness on the Soil Surface Evaporation Rate. *California Polytechnic State University*, San Luis Obispo. USA, 1–14.

Meena, M.D., Joshi, P.K., Narjary, B., Sheoran, P., Jat, H.S., Chinchmalatpure, A.R., Yadav, R.K. & Sharma, D.K. 2016. Effects of municipal solid waste compost, rice-straw compost and mineral fertilisers on biological and chemical properties of a saline soil and yields in a mustard–pearl millet cropping system. *Soil Research* **54**, 958–969. http://dx.doi.org/10.1071/SR15342.

Mozumder, S.N., Moniruzzaman, M. & Halim, G.M.A. 2007. Effect of N, K, and S on the yield and storability of transplanted onion (*Allium cepa L.)* in hilly region. *Journal of Agriculture & Rural Development* **5**(1&2), 58–63.

Mulumba, L.N. & Lal, R. 2008. Mulching effects on selected soil physical properties. *Soil &Tillage Research* **98**, 106–111.

Mulyani, A. & Hidayat, A. 2009. Increasing the production capacity of upland food crops. *Jurnal Sumberdaya Lahan.* **3**, 73–84 (in Indonesia).

Noorhadi & Supriyadi 2003. The effect application of water and mulch on the microclimate of chilli in Entizol soil. *Sains Tanah* **3**(2), 68–72 (in Indonesia).

Okubo, H., Sugiharto, A.N. & Miho, N. 1999. Bulbing response of shallot (*Allium cepa* L. var. *ascalonicum* Backer) and *Allium wakegi* Araki to daylength and temperature. *Engei Gakkai zasshi* **68**, 283–285.

Olsen, S.R., Cole, C.V., Watanabe, F.S. & Dean, L.A. 1954 Estimation of available phosphorus in soil by extraction with sodium carbonate. *USDA Circular* **939**, 1–19.

Page, A.L., Miller, R.H. & Kuny, D.R. 1989. Methods of Soil Analysis. Part 2. Chemical and microbiological properties. 2nd ed. *Madison, Wisconsin USA Publishing*. 539–641.

Polthanee, A., Tre-loges, V. & Promsena, K. 2008. Effect of rice straw management and organic fertilizer application on growth and yield of dry direct-seeded rice. *Paddy and Water Environment* **6**, 237–241.
Pramanik, P., Bandyopadhyay, K.K., Bhaduri, D., Acharyya, R.B & Aggarwal, P. 2015. Effect of mulch on soil thermal regimes–a review. *International Journal of Agriculture, Environment and Biotechnology* **8**, 645–658.
Ramakrishna, A., Tam, H.M., Wani, S.P. & Long, T.D. 2006. Effect of mulch on soil temperature, moisture, weed infestation and yield of groundnut in northern Vietnam. *Field Crop Research* **95**, 115–125.
Ranjan, P., Patle, G.T., Prem, M. & Solanke, K.R. 2017. Organic mulching – A water saving technique to increase the production of fruits and vegetables. *Current Agriculture and Research Journal* **5**, 371–380.
Reganold, J.P. & Wachter, J.M. 2016. Organic agriculture in the twenty-first century. *Nat Plants* **2**, 15221.
Rop, D.K., Kipkorir, E.C. & Taragon, J.K. 2016. Effects of deficit irrigation on yield and quality of shallot crop. *Journal of Agricultural Science* **8**, 112–126.
Sanchez-Martín, L., Meijide, A., Garcia-Torres, L. & Vallejo, A. 2010. Combination of drip irrigation and organic fertilizer for mitigating emissions of nitrogen oxides in semiarid climate. *Agriculture, Ecosystems and Environment* **137**, 99–107.
Singh, J.V., Kumar, A. & Singh, C. 2000. Influence of phosphorus on growth and yield of onion (*Allium cepa L.*). *Indian J. Agric. Res.* **34**(1), 51–54.
Stevenson, F.J. 1994. Humus chemistry, genesis-composition-reaction. *John Wiley and Sons*. New York. 512 pp.
Sumiati, E. 1994. Response of shallot and garlic to different altitudes. *Acta Horticultura,* 395–400.
Thankamani, C.K., Kandiannan, K., Hamza, S. & Saji, K.V. 2016. Effect of mulches on weed suppression and yield of ginger (Zingiber officinale Roscoe). *Sci Hortic* (Amsterdam) **207**, 125–130.
Tisdale, S.L., Nelson W.L. & Beaton, J.D. 1985. Soil Fertility and Fertilizer. *Mc Millan Publishing Co*. New York, 754 pp.
Unger, P.W.1978. Straw mulch effects on soil temperatures and sorghum germination and growth. *Agronomy Journal* **70**, 858–864.
Van-Reeuwijk, L.P. 1993. *Procedures for Soil Analysis*. International Soil Reference and Information Centre, Wageningen, The Netherlands, 101 pp.
Wang, S., Tan, Y., Fan, H., Ruan, H. & Zheng, A. 2015. Responses of soil microarthropods to inorganic and organic fertilizers in a poplar plantation in a coastal area of eastern China. *Applied Soil Ecology* **89**, 69–75.
Woldetsadik, S.K. 2003. Shallot (*Allium cepa var. Ascolonicum*) responses to plant nutrient and soil moisture in a sub-humit tropical climate. Acta Universitatis Agriculturae Sueciae. *Agraria* **367**, 1–29.
Yudiyanto, Rizali, A., Munif, A., Setiadi, D. & Qayim, I. 2014. Environmental factors affecting productivity of two Indonesian varieties of black pepper (*Piper nigrum* L.) *Agrivita* **36**(3), 278–284.
Zhao, H., Wang, R.Y., Ma, B.L., Xiong, Y.C., Qiang, S.C. & Wang, C.L. 2014. Ridge-furrow with full plastic film mulching improves water use efficiency and tuber yields of potato in a semiarid rainfed ecosystem. *Field Crops Research* **161**, 137–148.

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Effect of feed restriction on muscle fibre characteristics and meat quality traits in pigs

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Abstract. The aim of this study was to evaluate the effect of feed restriction on muscle fibre composition and meat quality traits in pigs. Forty crossbred pigs (Pietrain × Large $White_{Sire}$) × (Landrace × Large $White_{Dam}$) were divided into two feeding groups: *ad libitum* (AL) and restricted (R1). The effects of feed restriction on muscle fibre characteristics of the *musculus longissimus lumborum et thoracis* (*MLLT*) and meat quality traits were evaluated. Muscle fibres were stained and classified as fibre types I, IIA, and IIB. For each muscle fibre type, the fibre density, fibre cross-section area (CSA), and fibre proportion were determined. Fibres IIB were divided into small- (diameter < 46 µm), medium- (diameter 46–86 µm) and large-sized (diameter > 86 µm) fibres. The AL group had significantly lower ($P < 0.05$) percentage area of IIB fibres and lower ($P < 0.01$) CSA of IIB fibres than did the R1 group. The R1 group had significantly greater content of large-sized IIB fibres and smaller content of medium-sized IIB fibres than did the AL group ($P < 0.05$). The group fed *ad libitum* had greater backfat thickness and smaller lean meat content and tended to have better meat quality traits compared to the restricted group. The results of this study show that strong feed restriction had a negative effect on muscle fibre composition, especially on the amount of large-sized fibres IIB, which are associated with poor meat quality.

Key words: fibre types, meat quality, nutrition, pig.

INTRODUCTION

Muscle fibres are the basic structural unit of skeletal muscle. They make up more than 75% of muscle volume and, therefore, morphology of muscle fibre is a major determining factor of muscle mass (Lee et al., 2010). Muscle fibres are divided into individual types using various classification methods. One commonly used method is that of Brooke & Kaiser (1970), which classifies muscle fibres into three types (I, IIA, and IIB) according to their pH sensitivity in relation to myosin adenosine triphosphatase (ATPase) activity. Individual fibre types differ in their metabolic, structural, and contractile properties (Choi & Kim, 2009), and these varying characteristics are related to meat quality and carcass traits in various animal species (Lefaucheur, 2010; Kim et al., 2013a; Bogucka & Kapelanski, 2016). Qualitative and quantitative parameters of carcass traits are usually associated with muscle fibre properties, such as total number of fibres, fibre density, cross-section area of fibres (CSA), and fibre type composition in muscle (Joo et al., 2013; Kim et al., 2014). For instance, high proportion and larger area of glycolytic type IIB fibres in muscle are associated with brighter meat and lower water-holding capacity in pigs (Ryu et al., 2006; Lefaucheur, 2010; Kim et al., 2013b). Hypertrophy of fast-twitch oxido-glycolytic fibres is detrimental to meat tenderness, as well (Lefaucheur, 2010). Colour acceptability of fresh meat is positively correlated with CSA and the area and number percentages of type I muscle fibre in study of Nam et al. (2009). Number and area percentage of type I fibres positive correlate with taste of cooked pork at the same study. Kim et al. (2013a) reported that fibre size distribution of type IIB fibres also has an impact on pork meat quality. Pigs with higher percentage of large-sized IIB fibres exhibit tougher, lighter, and more exudative meat than do pigs with higher proportion of small- or normal-sized fibres.

There are many factors affecting muscle fibre characteristics, including breed, gender, age, and others (Rehfeld et al., 2004; Jeong et al., 2012; Joo et al., 2013). One of the extrinsic factors is nutrition (Bee et al., 2007; Jeong et al., 2012). The effect of feeding intensity on the proportion of different fibre types is controversial when comparing various muscles and/or species (Candek-Potokar et al., 1999). According to Harrison et al. (1996), feed restriction at an early stage (7 weeks) does not change fibre type composition in *longissimus* muscle of Large White pigs, but it does lead to a dramatic increase in the proportion of type I fibres in the red *rhomboideus* muscle and lower CSA of all fibres (Joo et al., 2013). Bee et al. (2007) reported that the fibre type composition in *longissimus* muscle is not changed due to feed restriction in post-weaning and growing-finishing barrows of Large White breed. Solomon et al. (1988) observed that feed restriction increased the proportion of red fibres in *longissimus dorsi* muscle of pigs at 55 kg of slaughter weight. Bogucka et al. (2013) observed that the decrease in the intake of protein and energy or protein alone had significant effect only on the percentage and diameter of fast-twitch glycolytic fibres in crossbred pigs (Danish Landrace × Polish Large White) at 119 days of age. They did not observed any significant differences in any of the studied microstructure traits in animals at 168 days of age.

Clarifying the inter-relationships among muscle fibre characteristics and meat quality traits as well as the factors which influence these can be helpful for improving meat quality in pigs. This study's aim is to evaluate the effect of feed restriction on meat quality and muscle fibre composition in *longissimus lumborum et thoracis* muscle in the commercial crossbred pigs used in the Czech Republic. Results of the study could help to suggest the optimal feeding strategy for the chosen hybrid combination of pigs with respect to the required slaughter weight and meat quality.

MATERIALS AND METHODS

Animals and diets

Forty crossbred pigs (20 barrows and 20 gilts) of hybrid combination (Pietrain × Large White$_{\text{Sire}}$) × (Landrace × Large White$_{\text{Dam}}$) were used in this study carried out at a swine experimental and test station belonging to Czech University of Life Sciences Prague. The animals entered the experiment at 60 days of age. Their mean body weight was 26.7 kg, and they were divided into two feeding groups. The first group was fed *ad libitum* (AL, 10 gilts and 10 barrows) and the second group (R1, 10 gilts and 10 barrows) was fed in a restricted manner. The pigs were housed with two animals per pen according to feeding group and gender.

For the R1 group, the following equation was used: MJ per day = 8.6539 + 3.9408x – $0.1435x^2$ (where x = age in days, Fig. 1). The equation for *ad libitum* fed group (MJ per day = 6.7786 + 7.4011x –$0.3534x^2$) shows the actual amount of feed received by these animals. Two experimental feed mixtures for growing and finishing pigs were used in the study.

Their amounts and choice of mixture were adjusted continuously over time depending on the actual weights of the pigs (Table 1). The compositions of the feed mixtures are presented in Table 2.

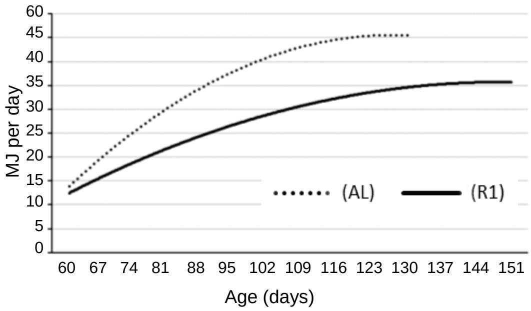

Figure 1. Feeding curves for *ad libitum* (AL) and restricted (RI) groups.

Table 1. The composition of complete feed mixture of two feeding groups during the fattening

Weight of pigs	AL			R1		
	Growing diet (%)	Finishing diet (%)	CFM per day (kg)	Growing diet (%)	Finishing diet (%)	CFM per day (kg)
27	100	0	1.80	100	0	1.25
45	85	15	2.26	85	15	1.80
72	43	57	2.92	43	57	2.57
91	10	90	3.27	10	90	2.72
112	0	100	3.44	0	100	2.81

CFM: complete feed mixture.

Table 2. Ingredient composition of the growing and finishing diets

Item	Diet	
	Growing	Finishing
Ingredient (%)		
Wheat	42.12	42.8
Barley	20.2	20.0
Soybean meal	13.8	2.9
Triticale	9.0	12.5
Rapeseed meal	4.0	7.0
Sunflower meal	3.5	6.0
Oats	2.5	2.5
Animal fat	2.0	1.15
Wheat bran	-	2.3
Calcite	1.15	1.2
Lysine-HCl	0.48	0.45
NaCl	0.45	0.45
Monocalcium phosphate	0.4	0.3
Vitamin–mineral premix	0.3	0.3
Threonine	0.2	0.15
Methionine	0.1	-

Table 2 (continued)

Nutrient composition (g per100 g of dry matter)		
Crude protein	17.48	14.95
Crude fat	3.66	2.91
Crude fibre	4.23	4.96
Lysine	1.12	0.91
Methionine	0.36	0.25
Ca	0.82	0.83
P	0.45	0.50
DE (MJ kg^{-1} of dry matter)	13.89	13.35

In order to assess the growth parameters, the individual animals were weekly weigh and daily feed intake was monitored. The average daily gain, feed conversion ratio and daily feed intake were calculated for each animal in week intervals and the final value was calculated as an average of these values. Pigs in both groups were fattened to an average slaughter weight of 112 kg. They were then slaughtered at a small commercial abattoir using electrical stunning and according to a routine procedure. The average slaughter weight, age, and selected growth parameters are presented in Table 3.

Table 3. Growth and slaughter parameters of pigs

Traits	Feeding groups		Significance[1]
	AL	R1	
Initial body weight (kg)	26.12 ± 3.02	27.27 ± 4.10	ns
Final live body weight (kg)	112.18 ± 7.15	112.41 ± 10.95	ns
Final age (days)	135.42 ± 2.63	155.65 ± 2.98	**
Average daily gain (g)	1,229.40 ± 92.44	935.60 ± 114.99	***
Feed conversion ratio (kg per kg)	2.27 ± 0.23	2.42 ± 0.25	**
Daily feed intake (kg)	2.78 ± 0.41	2.23 ± 0.10	***

[1] ns: not significant; ** $P < 0.01$; *** $P < 0.001$.

Histochemical analysis

Muscle samples for histochemical analysis were taken within 1 h after slaughter from the central part of the *musculus longissimus lumborum et thoracis* (*MLLT*). The samples were cut into 0.5 × 0.5 × 2.0 cm pieces, immediately frozen in isopentane cooled by liquid nitrogen according to the method of Dubowitz & Brooke (1973), then stored at -80 °C until analysis. Transverse serial muscle sections of 12 µm were cut from the entire blocks in a Leica CM1850 cryostat (Leica Microsystems, Nussloch, Germany) at -20 °C and mounted onto glass slides. The slides with sections were then incubated for the histochemical demonstration of myosin adenosine triphosphatase using the method of Brooke & Kaiser (1970). Stained muscle section images were obtained using an optical microscope with a Nikon Eclipse E200 camera (Nikon, Tokyo, Japan) and examined using an image analysis program (NIS - Elements AR 3.2., Nikon Instruments Europe B.V., Amsterdam, Netherlands). Approximately 300 fibres per sample were included into the analysis.

Fibres were classified into fibre types I, IIA, and IIB. For each muscle fibre type, the fibre density, fibre cross-section area (CSA), and fibre proportions (percentage by number and area) were determined. For type IIB fibres, the proportions of small (diameter < 46 μm), medium (diameter 46–86 μm) and large (diameter > 86 μm) fibres were specified.

Carcass and meat quality traits

Lean meat percentage was evaluated by ZP method ('Zwei-Punkt-Messverfahren'), which is widely used in smaller slaughterhouses in the Czech Republic (The details of the method are described in Font-i-Furnols et al., 2016). Backfat thickness was measured 45 min *post mortem* using electronic callipers at the levels of the first *thoracic vertebrae*, the first *lumbar vertebrae* and over the *gluteus medius* muscle without skin. The result value was calculated as the mean of these three measurements. Using a portable pH meter (pH 330i/set, WTW GmbH, Weilheim, Germany), pH (pH_{45}, measured together with temperature – t_{45}) was determined 45 min *p.m.* within carcasses at the 13th and 14th *thoracic vertebrae*. Electrical conductivity (EC_{50}) was evaluated 50 min *p.m.* at the same location (Conductometer EV plus, Czech Technical University in Prague, Czech Republic).

Muscle samples for evaluation of meat quality traits were taken 24 h *p.m.* from *MLLT* of right sides of carcasses. Before sampling, photos of transverse cuts of loins were obtained for evaluating loin area. The pictures were evaluated using an image analysis program (NIS - Elements AR 3.2., Nikon Instruments Europe B.V., Amsterdam, Netherlands). Meat colour (CIE L*, a* and b*) was measured 24 h *p.m.* on the muscle surface after 10 min of blooming using a Minolta CM-700d colorimeter (Konica Minolta, Osaka, Japan). Warner-Bratzler shear force (WBSF; N) values were determined by Instron Universal Texture Analyzer 3342 (Instron, Norwood, MA, USA). Muscle samples of raw and cooked meat (6 × 1 × 1 cm, cooked at 80 °C for 1 h) were cut across muscle fibres. The resulting value for each sample was calculated as an average value of at least 6 measurements. Drip loss was evaluated using a bag method (24 h at 4 °C) according to Honikel (1998). Intramuscular fat content was determined gravimetrically via Soxhlet extraction, using petroleum ether as the solvent.

Statistical analysis

The experimental data were analysed by one-way and two-way analysis of variance (ANOVA) using SAS 9.4 statistical software (SAS Institute, Cary, NC, USA). One-way ANOVA with fixed effect of feeding group was used for the data in Table 3. The effect of feed restriction was equal to the age of animals, therefore only the effect of feeding group was used. For Table 4 and Table 5, two-way ANOVA with fixed effects of feeding group and body weight was used. Results in Tables 4 and 5 are presented as least squares means (LSM ± SEM) for the main effect of feeding group and significance for the effect of feeding group and body weight. No significant interactions between feeding group and body weight were observed, therefore they were not used in the Tables 4 and 5. Differences between the LSM were determined by the Tukey's range test. Pearson correlation coefficients were calculated to evaluate the association between muscle fibre characteristics and carcass and meat quality traits.

RESULTS AND DISCUSSION

We evaluated the effect of restricted feeding at uniform slaughter weight on the characteristics of muscle fibres and qualitative parameters of meat. The average slaughter weight of all animals (n = 40) was 112.3 kg, which corresponds to the average slaughter weight of pigs slaughtered in the Czech Republic. As expected, we observed variations in growth parameters in these groups (Table 3). The AL group had significantly higher average daily gain and daily feed intake compared to the R1 group, and therefore the final age of the R1 group was almost 3 weeks greater. The feed conversion ratio was lower in the AL group, so we presume that these animals were able to utilize the maximum of their genetic potential in achieving growth performance.

The values obtained for muscle fibre parameters in relation to feed restriction are shown in Table 4. Within the evaluated groups, there were significant differences in mean CSA and CSA of IIB fibres. The higher CSA of fibres (μm^2) was found in the R1 group *(P* < 0.01). These results correspond to the values for fibre density, which values were higher in the AL group. This is probably related to the difference in lean meat content between these groups (Table 5). The group fed *ad libitum* had a significantly lower percentage of lean meat and a higher backfat thickness, which is reflected in the loin area and thus in the cross-section area of muscle fibres. Bee et al. (2007) also had evaluated the effect of feed restriction on the characteristics of muscle fibres, but they observed no differences in CSA of fibres between the groups of Swiss Large White barrows at the same body weight. Surprisingly, in their study barrows of the restricted group were not leaner than were barrows of the *ad libitum* group at 100 kg of body weight.

Table 4. Effect of feed restriction on muscle fibre characteristics of *longissimus lumborum et thoracis* muscle

Traits	Feeding groups		Significance[1]	
	AL	R1	FG	BW
Fibre density (number per mm^2)				
Type I	38.46 ± 2.75	32.27 ± 2.68	ns	*
Type IIA	32.33 ± 2.83	29.51 ± 2.76	ns	ns
Type IIB	192.25 ± 8.09	183.94 ± 8.59	ns	**
Cross-sectional area (μm^2)				
Mean	2,826.9 ± 98.3	3,063.3 ± 111.4	**	**
Type I	2,209.1 ± 89.5	2,375.2 ± 101.3	ns	*
Type IIA	1,699.1 ± 131.6	1,666.6 ± 198.8	ns	ns
Type IIB	3,174.8 ± 124.6	3,477.8 ± 161.2	**	**
Fibre number composition (%)				
Type I	14.70 ± 0.78	13.05 ± 0.76	ns	ns
Type IIA	12.00 ± 0.66	11.87 ± 0.64	ns	ns
Type IIB	73.30 ± 1.03	75.09 ± 1.00	ns	ns
Fibre area composition (%)				
Type I	11.20 ± 0.70	9.42 ± 0.69	ns	ns
Type IIA	7.43 ± 0.51	6.39 ± 0.50	ns	ns
Type IIB	81.37 ± 0.95	84.19 ± 0.92	*	ns
Proportion of fibre size IIB (%)				
Small	16.85 ± 2.03	18.04 ± 1.98	ns	ns
Medium	72.34 ± 3.24	60.70 ± 3.16	*	ns
Large	10.81 ± 3.57	21.27 ± 3.48	*	*

FG – feeding group; BW – body weight; [1] ns: not significant; * $P < 0.05$; ** $P < 0.01$.

There were no significant differences in fibre number composition between the groups. In both groups, the largest number was of IIB fibres (74.20%), followed by fibres of type I (13.89%) and of type IIA (11.94%).

The restricted group, which was significantly leaner, also had a higher percentage area of type IIB fibres (84.19%) than did the AL group, which was almost three weeks younger. The age of animals has a significant effect on the composition of muscle fibres. In general, postnatal transitions of fibres proceed from the oxidative to the glycolytic type of fibres (Lefaucheur, 2010; Joo et al., 2013), and diameters of type II fibres increase faster than diameters of type I (Oksbjerg et al., 1994). The positive relationship between high muscularity and proportional representation of type IIB fibres is reported in many studies (Wimmers et al., 2008; Lefaucheur, 2010; Joo et al., 2013; Kim et al., 2013b). Brzobohaty et al. (2015) also observed higher proportion of IIB fibres in restricted group of crossbred pigs in comparison to *ad libitum* fed group, which had lower lean meat content.

Furthermore, differences were observed in fibre size proportion of type IIB fibres. The AL group had significantly less representation of IIB fibres with a large area (> 86 μm; $P < 0.05$) in favour of medium-sized fibres IIB (46–86 μm; $P < 0.05$). Again, higher proportions of large IIB fibres in the restricted group could be associated with greater lean meat content of this group. Kim et al. (2013a) also had observed more muscularity in groups with greater content of large-sized IIB fibres. This corresponds to the statistically significant correlation coefficients that we found between lean meat content and representation of large- ($r = 0.28$) and medium-sized ($r = -0.33$) IIB fibres ($P < 0.05$). Similarly, Ryu et al. (2005) had reported that pigs with greater muscle mass had higher CSA and lower fibre density in type IIB fibres compared to pigs with lower muscle mass. Lee et al. (2016) observed that crossbred pigs with large CSA of fibres had larger loin area than pigs with middle or small CSA. They reported very high correlation coefficient between loin area and CSA of all fibres ($r = 0.92$).

Table 5 shows the effects of feed restriction on carcass and meat quality traits. Significantly greater lean meat content and lower backfat thickness ($P < 0.001$) were found in group R1. Greater backfat thickness in the AL group is probably related to the higher *post mortem* temperature (t_{45}) of meat ($P < 0.01$) measured in this group, where the thick fat layer slows the chilling of carcasses.

Electrical conductivity, pH, and water-holding capacity are parameters that can be used to classify abnormalities of meat quality. There were no differences between groups in these parameters, and we observed no meat abnormalities in the pigs used in our study.

Significant differences were observed in redness (a*) and yellowness (b*), where these values were lower in the AL group ($P < 0.01$). Variations in meat colour are related to differences in myoglobin content. Red muscle fibres (I and IIA) have greater myoglobin content and therefore darker colour (Listrat et al., 2016). During meat storage and blooming, however, myoglobin is oxidized into metmyoglobin, and that could result in a decrease in meat's redness. Faster colour change occurs during storage in muscles where red oxidative fibres predominate due to their faster oxygen consumption (Joo et al., 2013).

Table 5. Effect of feed restriction on carcass and meat quality traits of pigs

Traits	Feeding groups		Significance[1]	
	AL	R1	FG	BW
Lean meat (%)	57.27 ± 0.33	60.59 ± 0.32	***	***
Backfat thickness (mm)	23.69 ± 0.61	17.68 ± 0.59	***	***
Loin area (mm^2)	4,737 ± 112.8	5,002 ± 112.8	ns	**
Intramuscular fat content (%)	2.35 ± 0.17	2.16 ± 0.16	ns	ns
$pH_{45\ min}$	6.34 ± 0.06	6.29 ± 0.06	ns	ns
$t_{45\ min}$	39.53 ± 0.17	38.73 ± 0.17	**	**
$EC_{50\ min}$	3.42 ± 0.09	3.61 ± 0.09	ns	ns
Lightness (L*)	51.49 ± 0.66	52.91 ± 0.64	ns	ns
Redness (a*)	0.10 ± 0.25	0.94 ± 0.23	**	ns
Yellowness (b*)	9.16 ± 0.29	10.68 ± 0.28	***	ns
WBSF of raw meat (N)	38.77 ± 2.02	49.26 ± 1.96	***	ns
WBSF of cooked meat (N)	35.98 ± 1.57	38.88 ± 1.53	ns	ns
Drip loss (%)	4.27 ± 0.42	4.69 ± 0.41	ns	ns

WBSF – Warner-Bratzler shear force; EC – electrical conductivity; FG – feeding group; BW – body weight; [1] ns: not significant; ** $P < 0.01$; *** $P < 0.001$.

Although the R1 group had significantly higher WBSF of raw meat in comparison with the AL group ($P < 0.001$), no significant differences were observed in WBSF of cooked meat. The AL group had a significantly smaller CSA and lower content of IIB fibres. This corresponds to claims that higher fibre CSA, and especially of IIB fibres, increases instrumental toughness of meat (Karlsson et al., 1993; Joo et al., 2013; Kim et al., 2013a).

CONCLUSIONS

Feed restriction has a significant effect on lean meat content, which is accompanied by a different muscle fibre size. Age difference at the uniform slaughter weight, which was caused by feed restriction is reflected in the amount of fast-oxidative glycolytic fibres (IIB) in muscle and their proportion in overall fibre composition. The results of this study suggest that a greater amount in muscle of large IIB fibres, which are associated with poor meat quality, is closely related to lean meat content. Although meat quality traits tend to be better in the group fed *ad libitum*, the lean meat content is lower in this group. These results show that strongly restricting feed consumption negatively affects the composition of muscle fibres in *longissimus* muscle of pigs.

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REFERENCES

Bee, G., Calderini, M., Biolley, C., Guex, G., Herzog, W. & Lindemann, M.D. 2007. Changes in the histochemical properties and meat quality traits of porcine muscles during the growing-finishing period as affected by feed restriction, slaughter age, or slaughter weight. *J. Anim. Sci*. **85**, 1030–1045.

Bogucka, J., Polawska, E., Elminowska-Wenda, G., Raj, S., Sobolewska, A., Walasik, K., Skiba, G., Weremko, D. & Fandrejewski, H. 2013. Effect of nutritional regulation of growth on histochemical characteristics of muscles in pigs. *Acta Agriculturae Scand Section A*. **63**, 10–17.

Bogucka, J. & Kapelański, W. 2016. Microstructure of longissimus lumborum muscle and meat quality of native Polish pig breeds: Złotnicka Spotted and Puławska. *Ann. Anim. Sci*. **16**, 1199–1210.

Brooke, M.H. & Kaiser, K.K. 1970. Muscle fibre types: how many and what kind? *Arch. Neurol.* **23**, 369–379.

Brzobohaty, L., Stupka, R., Citek, J., Sprysl, M., Okrouhla, M. & Vehovsky, K. 2015. The influence of controlled nutrition intensity on the muscle fiber characteristics in fattening pigs. *J. Cent. Eur. Agric*. **16**, 92–99.

Candek-Potokar, M., Lefaucheur, L., Zlender, B. & Bonneau, M. 1999. Effect of slaughter weight and/or age on histological characteristics of pig longissimus dorsi muscle as related to meat quality. *Meat Sci*. **52**, 195–203.

Choi, Y.M. & Kim, B.C. 2009. Muscle fiber characteristics, myofibrillar protein isoforms, and meat quality. *Livest. Sci*. **122**, 105–118.

Dubowitz, V. & Brooke, M.H. 1973. Muscle biopsy: A modern approach. W.B. Saunders, London, UK, 571 pp.

Font-i-Furnols, M., Candek-Potokar, M., Daumas, G., Gispert, M., Judas, M. & Seynaeve, M. 2016. Comparison of national ZP equations for lean meat percentage assessment in SEUROP pig classification. *Meat Sci*. **113**, 1–8.

Harrison, A.P., Rowlerson, A.M. & Dauncey, M.J. 1996. Selective regulation of myofiber differentiation by energy status during postnatal development. *Am. J. Physiol*. **39**, 667–674.

Honikel, K.O. 1998. Reference methods for the assessment of physical characteristics of meat. *Meat Sci*. **49**, 447–457.

Jeong, J.Y., Kim, G.D., Ha, D.M., Park, M.J., Park, B.C., Joo, S.T. & Lee, C. 2012. Relationships of muscle fiber characteristics to dietary energy density, slaughter weight, and muscle quality traits in finishing pigs. *Journal of Animal Science and Technology* **54**, 175–183.

Joo, S.T., Kim, G.D., Hwang, Y.H. & Ryu, Y.C. 2013. Control of fresh meat quality through manipulation of muscle fiber characteristics. *Meat Sci*. **95**, 828–836.

Karlsson, A., Enfalt, A.C., Essen-Gustavsson, B., Lundstrom, K., Rydhmer, L. & Stern, S. 1993. Muscle histochemical and biochemical properties in relation to meat quality during selection for increased lean tissue growth rate in pigs. *J. Anim. Sci*. **71**, 930–938.

Kim, G.D., Jeong, J.Y., Jung, E.Y., Yang, H.S., Lim, H.T. & Joo, S.T. 2013a. The influence of fiber size distribution of type IIB on carcass traits and meat quality in pigs. *Meat Sci*. **94**, 267–273.

Kim, G.D., Kim, B.W., Jeong, J.Y., Hur, S.J., Cho, I.C., Lim, H.T. & Joo, S.T. 2013b. Relationship of carcass weight to muscle fiber characteristics and pork quality of crossbred (Korean native black pig × Landrace) F2 pigs. *Food Bioprocess Tech*. **6**, 522–529.

Kim, G.D., Ryu, Y.C., Jo, C., Lee, J.G., Yang, H.S., Jeong, J.Y. & Joo, S.T. 2014. The characteristics of myosin heavy chain-based fiber types in porcine longissimus dorsi muscle. *Meat Sci*. **96**, 712–718.

Lee, S.H., Kim, J.M., Ryu, Y.C. & Ko, K.S. 2016. Effects of Morphological Characteristics of Muscle Fibres on Porcine Growth Performance and Pork Quality. *Korean J. Food Sci. An*. **36**, 583–593.

Lee, S.H., Joo, S.T. & Ryu, Y.C. 2010. Skeletal muscle fiber type and myofibrillar proteins in relation to meat quality. *Meat Sci*. **86**, 166–170.

Lefaucheur, L. 2010. A second look into fibre typing – relation to meat quality. *Meat Sci.* **84**, 257–270.
Listrat, A., Lebret, B., Louveau, I., Astruc, T., Bonnet, M., Lefaucheur, L., Picard, B. & Bugeon, J. 2016. How muscle structure and composition influence meat and flesh quality. *The Scientific World Journal*, vol. 2016, ID 3182746, p. 14.
Nam, Y.J., Choi, Y.M., Lee, S.H., Choe, J.H., Jeong, D.W., Kim, Y.Y. & Kim, B.C. 2009. Sensory evaluations of porcine longissimus dorsi muscle: Relationships with postmortem meat quality traits and muscle fiber characteristics. *Meat Sci*. **83**, 731–736.
Oksbjerg, N., Henckel, P. & Rolph, T. 1994. Effects of salbutamol, a β2-adrenergic agonist, on muscles of growing pigs fed different levels of dietary protein: I. Muscle fibre properties and muscle protein accretion. *Acta Agr. Scand. A-An*. **44**, 12–19.
Rehfeldt, C., Fiedler, I. & Stickland, N.C. 2004. Number and Size of Muscle Fibres in Relation to Meat Production. In: Muscle development of livestock animals, te Pas, M.F.W., Everts M.E., Haagsman, H.P. Lelystad, Netherlands, 1–38.
Ryu, Y.C., Choi, Y.M. & Kim, B.C. 2005. Variations in metabolite contents and protein denaturation of the longissimus dorsi muscle in various porcine quality classifications and metabolic rates. *Meat Sci*. **71**, 522–529.
Ryu, Y.C., Lee, M.H., Lee, S.K. & Kim, B.C. 2006. Effects of muscle mass and fiber type composition of longissimus dorsi muscle on postmortem metabolic rate and meat quality in pigs. *J. Muscle Foods.* **17**, 343–353.
Solomon, M.B., Campbell, R.G., Steele, N.C., Caperna, T.J. & McMurtry, J.P. 1988. Effect of feed intake and exogenous porcine somatotropin on longissimus muscle fiber characteristics of pigs weighing 55 kilograms live weight. *J. Anim. Sci*. **66**, 3279–3284.
Wimmers, K., Ngu, N.T., Jennen, D.G.J., Tesfaye, D., Murani, E., Schellander, K. & Ponsuksili, S. 2008. Relationship between myosin heavy chain isoform expression and muscling in several diverse pig breeds. *J. Anim. Sci*. **86**, 795–803.

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The energy intensity of the briquetting process in terms of profitability of waste treatment

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Abstract. Modern agricultural industry is a source of a considerable amount of waste, which can come in various forms and states. Such waste, and not just agricultural waste in the form biomass, is highly desirable for further processing, depositing or utilising its energy potential. Briquetting technology is suitable for all these purposes. The briquetting press for industrial use is complex technical equipment. The economy of its operation has a major impact on the profitability of the produced briquettes and hence on the efficiency of waste and biomass processing as such. The paper deals with the energy demands of briquetting in terms of waste treatment and economic profitability of production in the whole context of waste processing as a whole.

Key words: briquetting, energy demands, bio-briquettes, economic profitability.

INTRODUCTION

Despite all political efforts, the share of biomass in all its forms is still relatively low and there is considerable room for increasing its production. At present, biomass accounts for about 14% of the world's annual energy consumption (Hall et al., 1992). In 2017 production of renewable energy sources (especially biofuels) generated 4 million jobs and contributed at least 2.5% to the performance of the US economy. It is clear that the small-scale production of biofuels at their very place of origin is really meaningful. It has undeniable positive environmental effects (including reduced requirements for the transport of waste and biomass). It can also have a positive impact on employment. In particular, the use of briquettes from biomass or other waste materials ensures a renewable, ecologically acceptable alternative to fossil fuels and leads to the generation of other economic income not only of farmers (Chen et al., 2009; Guo & Song, 2019). Therefore, it is very important to address the energy demands of the densification process, including the power consumption that needs to be spent on compressing the particular materials and producing the briquettes themselves.

MATERIALS AND METHODS

The energy demands of densification and production of briquettes on a briquetting press can be basically summarized into two groups:

1) Consumption of mechanical work

This is the labour consumption necessary for the compaction of the input material, it is the relationship between the density ρ (kg m^{-3}) and the desired deformation energy Ed (J). It is very important to know the necessary forces F (N) as well as the physical properties, the fraction and the moisture of the used input material. In addition to the physical properties of the materials and the required force to densification, another important factor that affects the quality of the finished briquettes is the temperature of the briquetting press and pressing chambers. The last of a group of major variables in terms of the evaluation of the consumption of mechanical work that plays important roles, is the structure, type and mode of work of the briquetting press (Repsa & Kronbergs, 2015; Muntean et al., 2017; Brunerová et al., 2018). Consumption of mechanical work is not the subject of this paper.

2) Consumption of electrical energy

The power consumption depends on the briquetting press and also on the selected input material. For this purpose, the briquetting press is understood to be a complete machine which is used to mould the input material and produce briquettes with a diameter of 50 mm.

For the experimental measurement, a typical representative of the middle classes of industrial briquetting presses used for processing biomass and other conventional waste (including paper scrap) was chosen small medium-duty single-shift operation (without an oil cooler). The machine is a product of Briklis, s.r.o. Czech Republic, specifically BrikStar 30, type 12. The basic machine parameters are summarized in Table 1.

Table 1. Selected technical parameters of the Brikstar 30, type 12 briquetting press

Parameter	Value	Unit
Performance {NL}; +-10%	20–40	kg hrs^{-1}
Installed electrical power	4.4	kW
Weight of the press with a hopper of 1 m^3 capacity	780	kg
Density of pressed briquettes	900–1,100	kg m^{-3}
Average of the briquettes	50	mm
Maximum operating pressure	180	bar
Covering of electrical elements	IP54	xxx

For the measurement itself was used Chauvin Arnoux C.A. 8334B network analyzer concatenation, which was connected in parallel to the TN-S distribution network. The block diagram is shown in Fig. 1, where the circles are the pliers for the current measurement. The device recorded the data every 1 second and stored it in its memory. The important parameters were:

- Performance (Wh)
- Time of the measurement (s)
- Voltage at each stage (V)
- Current at individual stages (A)

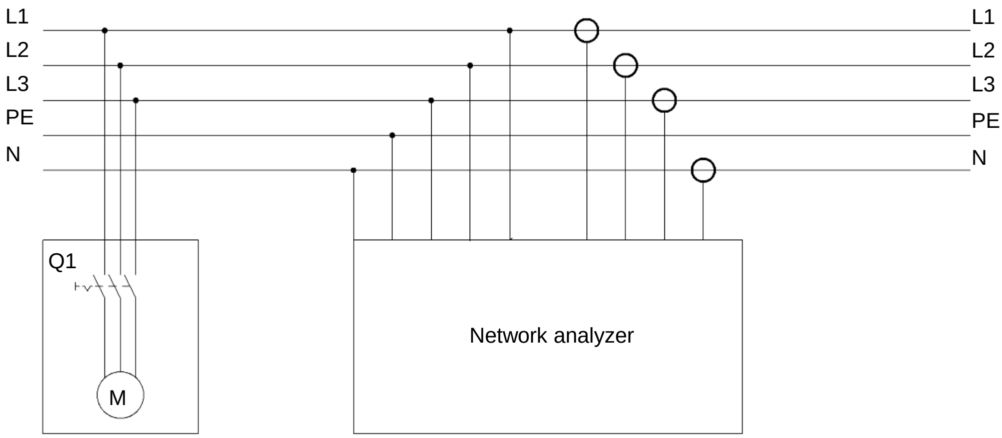

Figure 1. Block measurement scheme: L1 to L2 – phases of three-phase TN-S distribution system; PE – Protective Earthing; N – Neutral conductor; Q1 – motor terminal block; M – briquetting press three-phase asynchronous electric motor.

Theory and modelling

Based on the measured data, the energy consumption (kWh) of the briquetting press was calculated using the mathematical formula (1). The result gives the energy consumption in 1 hour of the briquetting press operation.

$$P_i = \frac{E_{ki} - E_{pi}}{k_i - p_i} \cdot 3.6 \qquad (1)$$

where P_i – power consumption of the i^{th} sample; i – data-set extent; k_i – end of measurement of the i^{th} sample; pi – beginning of measurement of the i^{th} sample; E_{ki} – the nominal end value of the reactive work of i^{th} sample; E_{pi} – the nominal initial value of the reactive work of i^{th} sample.

Depending on the briquette mass produced at the time of measurement, the actual performance of the briquetting press according to the mathematical relationship (2) was calculated (depending on the material).

$$A_i = \frac{W_{pi}}{m_i} \qquad (2)$$

where Ai – machine performance on the i^{th} same sample; W_{pi} – Consumption of the reactive work of i^{th} sample ; m – weight of the i^{th} sample.

Selected test sample

The used sampling methodology included three basic criteria:

- Material availability
- Rentability of the production
- Difficult another processing (eg, storage problem due the dustiness or bulk, other possible manufacturing processes that would be difficult or impossible in the natural form of the materials etc.)

Based on the criteria above, 8 samples were selected. These are described in Table 2. The emphasis was put on scrap paper (in several forms). There are several reasons for this. The first is its production is growing as well as the demand for its recycling. Another reason is the production of paper and scrap paper which is contaminated (mineral oils, etc.), which means it cannot be recycled in a normal way, is relatively high. The briquetting technology appears to be suitable for reducing the volume of contaminated paper and its subsequent energy utilization by incineration. This technology also appears to be useful to reduce the volume of material that is not possible to burn from environmental reasons or due to material compostion.

Table 2. Selected samples

Sample number	Material	Fraction
1	newspaper matte paper	scrap 4 x 18 mm
2	newspaper glossy paper	scrap 4 x 18 mm
3	carton	scrap 12 x 50 mm
4	old student books	scrap 4 x 18 mm
5	mix hop 50% & birch 50%	natural form/shavings
6	sugar thistle	mouldings
7	hop	natural
8	birch	shavings

First of all, it was necessary to prepare the collected material and unify its fraction. There are fractions of scrap paper in the Fig. 2 (the fraction of sample no 1, 2 and 3 are visualy the same as well as the fraction of the sample no 5 and 6). From the top left there are fractions of sugar thistle (mouldings), hop (natural form, outdoor photography) and brich (shavings). From the bottom left there are old student book (fraction 4 x 18 mm) and scrap carton (fraction 12 x 50 mm).

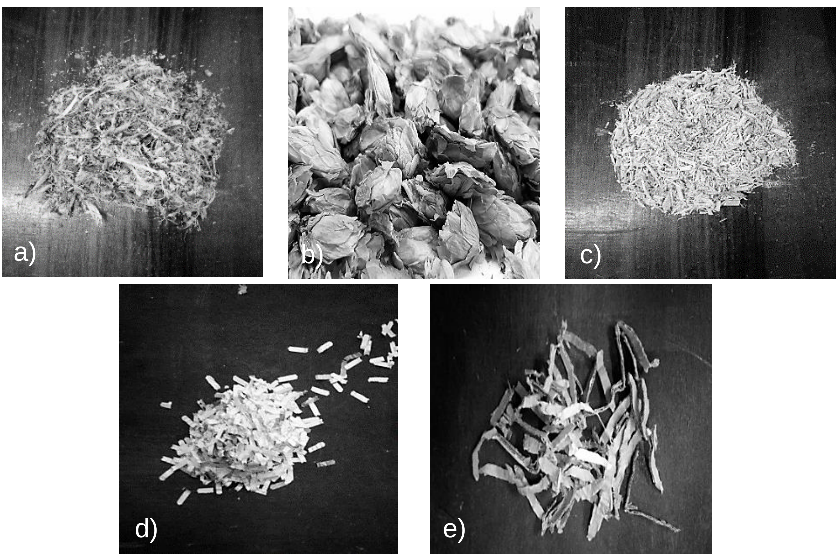

Figure 2. Photographs of the used samples. Photographs of the used samples: a) sugar thistle (mouldings); b) hop (natural form – outdoor photography); c) brich (shavings); d) old student book (scrap, fraction 4 x 18 mm); e) carton (scrap, fraction 12 x50 mm).

RESULTS AND DISCUSSION

The results of the experimental measurement carried out and evaluated by the methods described above (in the Theory and Modelling section) are clearly shown in the graph in Fig. 3. It is very clear from the graph and the displayed values that the power consumption, depending on the specifically compressed material, virtually does not change. sample no. 6 showed the largest energy demands. Scrap paper requires the most energy needed to compress scrap carton (sample no. 3). It is remarkable to note that the differences in energy demands of compressed scrap paper are virtually insignificant, namely 0.13 kWh. When we choose the other monitored materials, we find that the difference between the most energy-demanding material represented by sample no. 6 with 2.15 kWh and the least energy demanding sample no. 8 (with 1.85 kWh) is only 0.3 kWh. Due to the installed electrical power of the machine of 4.4 kW, this difference can be considered negligible. On the basis of the experiment, it can be stated that the type of compressed material does not play a fundamental role in the energy consumption demands of the industrial briquetting press. There are several factors which have impact on electricity consumption. The chemical composition of the material, the physical properties, moisture of the material and the amount of force used for pressing. Shape and particle size do not play the main role, as has also been shown in the results. Another important factor is the construction of the briquetting press itself. The principle of its operation is critical if it come to electricity consumption (hydraulics vs. mechanical transmission).

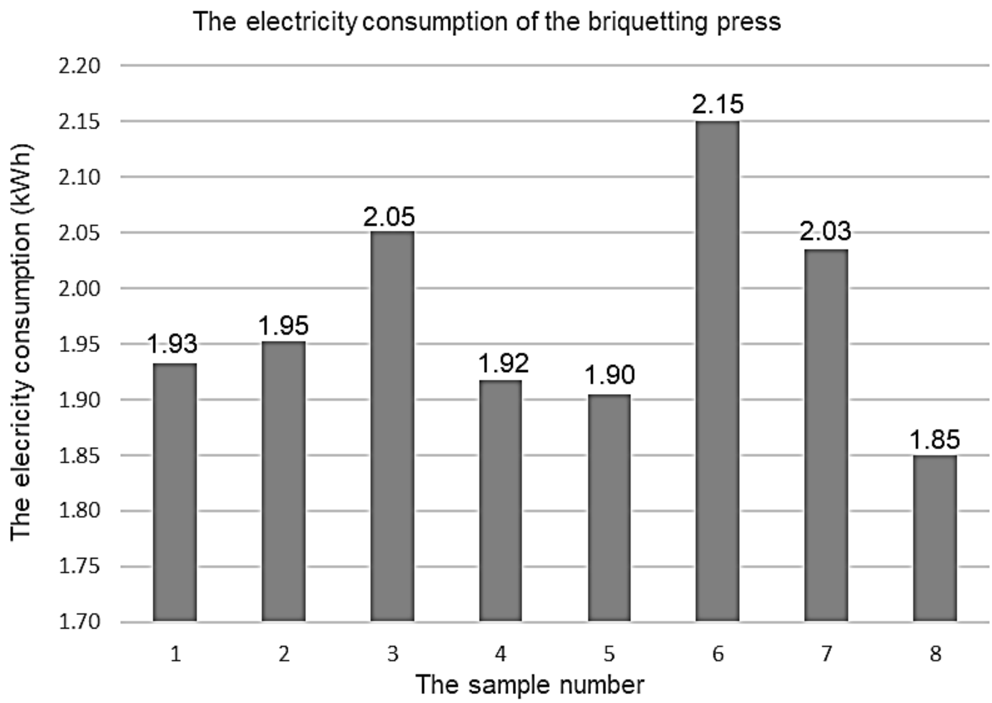

Figure 3. Electricity consumption.

For the purpose of economic evaluation, it was necessary to assess the resulting briquettes from individual tests and to determine the actual energy consumption of the machine depending on the briquetted material. The results are again clearly arranged in the table in Fig. 4. It is obvious that most of the electricity is consumed to produce the briquettes from sample no. 3. On the contrary, the least energy is needed for sample no. 2. Research presented in this paper was primary focus on the scrap paper. The other materials were (namely sample no 5, 6, 7 and 8) used to compare the energy consumption of the different scrap paper and the natural materials. It is clear from the data that the energy consumption samples no. 5, 6, 7 and 8 are practically the same. That can be caused by their very similar mechanical properties and by the size of the used fraction. From this point of view, there are more interesting the results of the scrap paper. Despite the comparable properties and similar fractions, the results differ more significantly.

In this case the results are already distinctly more pronounced, which is caused by several factors:

- The power of the briquetting press itself
  It is possible to use a machine with higher pressure. However, the purchase and operation would be more expensive.
- Compression of the compressed material due to its physical properties
- Specific weight of the particular compressed material

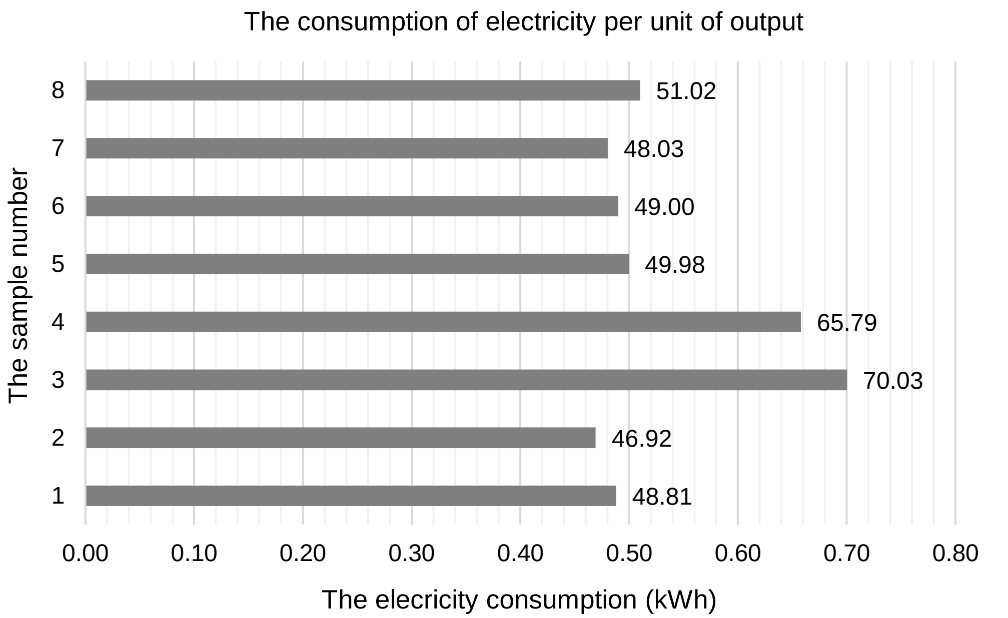

Figure 4. Specific consumption of electricity per unit of output.

In spite of the said and relatively demonstrable results, I must be critical. For the measurements a briquetting press from a leading manufacturer was chosen with paramaters which can be described as typical. However, it is likely that the values will be distinguished individually from the machine. Due to the similar design of piston hydraulic presses, it can be assumed that the resulting values will not vary significantly. Thus, it can be stated that the measurements made clearly show that most of the electricity consumption is consumed for the operation of the machine, respectively (its hydraulic systems). Moulded material does not play significant role.

Economic evaluation

Just by multiplying the cost of electricity and adding additional costs (eg, electricity transmission charges etc.) in a given country, briquette production costs of a particular briquetted material in a given region can be very accurately determined. The specific final price for 1 MWh of industrial customers can not be easily determined, depending on the size of the enterprise, its total consumption, installed power and also the conditions of specific energy providers.

From the point of view of financial fitness for electricity, the influence of briquetted material is negligible. It is clear that, from an economic point of view, it is necessary to rely more on investing in technology as a whole. In particular, it is necessary to calculate the cost of operation, maintenance and, of course, the amortization of the briquetting press, rather than considering the cost of electricity depending on the concreted briquetted material. But the performance of the machine is more interesting. Here is space for economic optimization by selecting a suitable material. In this particular case, the manufacturer reports the performance of the machine to +/- 20% accuracy, which was also measured and verified. By selecting a suitable material and its appropriate subsequent energy utilization (eg. retail sale to the end-user or industrial heating or water heating), considerable savings can be achieved with respect to the briquetting press in the order of tens of percent, and this can have the indicated multiplier effect and generate additional savings or revenue.

From the economic point of view, it is also necessary to consider the costs that need to be spent on preparing and processing the material before the briquetting process itself. There is the practical economic advantage (regardless of the electricity consumption) to use of this technology at the point of production of the material that is suitable for briquetting (eg, selected samples in this paper). From the point of view of the briquetting process itself, significant differences in processing of selected samples were not observed. Thus, it is necessary to consider all the circumstances described above and to decide what material is advantageous to briquet and which is not. Consumption of electrical dependence on material is not the only decisive factor.

CONCLUSIONS

Based on the results of the measurements and the measured values, the energy demands of the individual basic compressed materials were clearly shown. Such an evaluation can lead to the selection of material that is suitable for briquetting and which is not. In particular, economic appreciation can greatly assist in deciding and implementing technological processes in industrial and agricultural plants that produce biomass, especially in economic considerations about the profitability of its processing.

This research is basically directly preceded by the current experimental research on the briquetting press for home use of the Profilis 15 Home. The comparison of the measurements on the Briklis briquetting press with the Profilis briquetting press and subsequent economic evaluation can bring further interesting results. It will be possible to determine the point of profitability, i.e. a point in volume production from which the production of briquettes on the domestic press becomes unprofitable and it is advantageous to use an industrial press.

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REFERENCES

Brunerová, A., Brožek, M., Šleger, V. & Nováková, A. 2018. Energy Balance of Briquette Production from Various Waste Biomass. *Scientia Agriculturae Bohemica* **49**(3), 236–243.

Chen, L., Xing, L. & Han, L. 2009. Renewable energy from agro-residues in China: Solid biofuels and biomass briquetting technology. *Renewable and Sustainable Energy Reviews* **13**, 2689–2695.

Guo, M. & Song, W. 2019. The growing U.S. bioeconomy: Drivers, development and constraints. *New Biotechnology* **49**, 48–57.

Hall, D.O., Rosillo-Calle, F. & Woods, J. 1992. Biomass, its importance in balancing CO2 budgets. In Grassi, G., Collina, A. & Zibetta, H. (eds): *Biomass for energy, industry and environment. The 6th E.C. conference*. Elsevier Science, London, UK, pp. 89–96.

Muntean, A., Tatiana, I., Hutla, P. & Havrland, B. 2017. Influence of raw material properties on the quality of solid biofuel and energy consumption in briquetting process. *Agronomy Research* **15**(4), 1708–1715.

Repsa, E. & Kronbergs, E. 2015. Effect of rhomboid pressing mechanism on briquetting energy. In Malinovska, L. & Osadcuks, V. (eds): *Engineering for Rural Development. The 14th international scientific conference.* Latvia University of Agriculture, Jelgava, Latvia, pp. 278–282.

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The efficiency of humic growth stimulators in pre-sowing seed treatment and foliar additional fertilizing of sown areas of grain and industrial crops

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Abstract. The aim of the research was to establish the effectiveness of preparations, made on the basis of humic and fulvic acids on the yields of crops in case of different methods and amounts used. The experiments were held with varieties and hybrids of winter wheat, soya, corn, and sunflower. Based on the obtained results of investigation during the period of 2015–2017, the positive impact of foliar additional fertilizing with 4R Foliar Concentrate growth stimulator on the basis of humic and fulvic acids on the formation of productivity of the main crops sown areas was established.
Proceeding from the results of the research, the using of growth stimulators based on humic and fulvic acids, which contain high concentrations of these substances, can be recommended as an expedient and efficient measure of raising the productivity and improving qualitative indicators of corn, sunflower, soya, and winter wheat yields.

Key words: fertilizing, humic substances, plant development, seed treatment.

INTRODUCTION

Modern development of plant production output requires solution of the problem of stable increase of agricultural crops' yield with simultaneous preservation of soil resources. Application of growth stimulators has become very active today. They give an opportunity to open genetic potential of plants in a better way, to use fertilizers and means of plants protection more efficiently, that results in improving stability of agricultural crops' yield. Growth stimulators are highly important for increase of plant resistance to the stress situations such as moisture deficit, unfavourable temperature conditions, aftereffect of pesticides etc.

Preparations made on the basis of humic substances occupy the original place among a great number of plant growth stimulators. Favourable action of humic substances has been known for a long time. They activate nutrient uptake by plants, raise the coefficient of useful elements from mineral fertilizers, intensify soil micro-flora activity, activate synthesis of proteins, carbohydrates and vitamins in plants, increase plant resistance to radiation as well as to low and high temperatures. Humic substances decrease penetration of heavy metals and pesticides into plants, activate their growth, increase productivity, improve production quality and accelerate harvest ripening.

ANALYSIS OF THE LATEST PUBLICATIONS

Manure, composts, vermicomposts, and other organic substances are the source of humic acids for soil. They have the direct (raising the yield) and indirect impact (improving soil fertility). Thanks to organic substances the mineral composition of soil improves, its compaction decreases, and the amount of water increases. At the same time, humic substances stimulate growing root system and activate anti-stress plant systems (Garcia et al., 2014; Tahiri et al., 2014).

Considering rapid intensification and improving cultivation technologies, recently taking place in Ukraine, the reproduction of soil organic component becomes very important, because there is a real threat of losing the fertile potential of these lands. It is rather problematic to replenish the organic component in our conditions, as one of the main branches (livestock farming), which is the source of organic substances, is in a crisis condition because of considerably lower profitability of production comparing to plant growing (Minkova et al., 2016).

The preparations, received on the basis of humic and fulvic acids are one of the sources of replenishing soils with nutrients of organic origin. There are quite a lot of such substances on the market of growth stimulators in Ukraine, but their using on the farms of different size and level of production has certain specifics. First of all, they are mainly characterized as plant growth stimulators proper, which are recommended to be used for pre-sowing seed treatment and foliar fertilization.

On the other hand, the recommended amount, doses, and concentration of the substances, the compatibility and expediency of using in mixtures etc. are to be investigated. In generalized scientific reviews it is noted, that the market of growth stimulators is developing extremely dynamically, but many scholars consider these preparations not objectively and scientifically evaluated (Calvo et al., 2014).

The content of soil organic component is one of the most important indicators of its quality, potential, and suitability to growing crops, and, thus, it requires replenishment (Rosa et al., 2017). One of the solutions to this problem is using humic substances and preparations made on their basis. So, for example, humic substances, received from manure, are wonderful supplement to mineral fertilizers, which ensures effective using of nutrients and improves the development of plants (Baldotto et al., 2017). Nevertheless, the results of research presented in scientific literature, show different effectiveness of using such substances in cultivating crops depending on the origin of raw products, molecular structure of humic acids, soils or even the absence of influence or negative reaction of plants (Leventoglu & Erdal, 2014; Martinez-Balmori et al., 2014; Kalinichenko et al., 2014; Savy et al., 2016; Conselvanet al., 2017; Oliveira et al., 2017).

One of the first technological methods of growing crops is pre-sowing seed treatment and using preparations made on the basis of humic acids complex, which can be a considerable prerequisite of managing harvest from the initial stages of plant development. The treatment of corn seeds with the humic preparation Volume Humykos (18% of humic acid content) in the experiments of Brazilian scholars positively affected the germination rate, growth intensity, the length of sprouts and roots, while plant dry weight also increased considerably (Rodrigues et al., 2017). Though, as some authors note, raw products, from which humic acids were obtained, and also their concentrations and the type of soil are important for seed treatment (Melo et al., 2015).

In laboratory experiments, adding humic acids in the nutrient substrate for corn sprouts resulted in doubling the number of leaves and the length of roots (Sun et al., 2016).

In scientific publications of Russian scholars it is noted, that ultra-disperse using of humic acids in vitro resulted in better seed germination by 3.4%, and in field experiment, the difference was 10.2% as compared with the control group. Moreover, the plant weight increased by 11.5%, the leaf area increased by 5.1% and photosynthesis pure productivity – by 13.4% in comparison with the control group (Churilov et al., 2015). In other laboratory experiments, the treatment of 11-day plants with humic substances, received from different leonardites, resulted in positive effect: nitrogen metabolism accelerated, and as a result, plant growth improved (Oliveira et al., 2017).

Using humic acids favors better formation of corn yield under the conditions of water or nutrient stress or in case of soil pollution with heavy metals (Santos et al., 2014; Zhang et al., 2014; Moghadam et al., 2016).

The pre-sowing treatment of soya seeds with preparations, containing the complex of humic and fulvic acids (ligno-humate, lexin and adding them to treatment mixtures) favors not only yield increasing, but also improving the product quality, in particular, oil output (Prochazka et al., 2016). On poor sandy soils the reaction of soya on organic-mineral fertilizers, containing humic substances, was noticed concerning plant height, dry weight of root system, and, as a result, the yield. Almost the same positive effect of humic preparations was noticed under the conditions of salt and water stress (Muhammad et al., 2013; Dinler et al., 2016; Prado et al., 2016a; Prado et al., 2016b; Tuncturk et al., 2016; Matuszak-Slamani et al., 2017; Rosa et al., 2017).

Some scholars note, that although humates are distinguished by their compactness and convenience in using, their effect is inferior to the impact of organic fertilizers, in particular, manure (Daur, 2013), but combining pre-sowing seed treatment with foliar fertilizing leads to increasing soya yields by 8% (Lingaraju et al., 2016).

Using humic acids on poor soils is also effective on the sown areas of sunflower. Besides increasing biometric indices of plants, soil properties also improve and yield stability grows (Sadiq et al., 2014; Baldotto et al., 2015). In Romania using organic-mineral fertilizer, combining nitrogen, phosphorus, microelements (Fe, Cu, Zn, Mg, Mn, B), and potassium humate enabled to raise the yield by 14.4% (Parvan et al., 2013).

It is assumed, that anti-stress action of humic acids may be noticed in their participation in creating plant waxes, which explains softening effect of humic substances in stress conditions (Kulikova et al., 2014).

The experience of using humic acids on wheat in the amount of 2 kg ha^{-1} with microfertilizers containing Cu and Zn enabled to raise the yield of grain by 20.2%, and the output of general biomass grew by 17.1%. Using these substances separately increased wheat yield only by 6.52–7.52% (Manzoor et al., 2014). Under the conditions of salt stress, humic acids considerably decrease the entry of harmful amounts of elements in plants, in particular, sodium, leaving unchangeable the assimilation of other nutrients (Asik et al., 2009; Jamal et al., 2011; Jarosova et al., 2016). Such properties of humic acids make their using promising in organic cultivation of wheat (Muhammad et al., 2013).

The aim of the research was to establish the effectiveness of preparations, made on the basis of leonardite, on the yields of crops in case of different methods and amounts of using. 4R Foliar Concentrate has humic acids content of 90.06%. Nitrogen content is 1.14%; phosphorus content is 0.02%, P_2O_5 – 0.05%; potassium content is 0.02%; calcium content is 0.62%; sulphur content is 0.42%; sodium content is 0.07%; magnesium content is 0.17%. Besides, the preparation consists of nearly sixty microelements, including rare-earth ones, so the total content of microelements is about 5%.

MATERIALS AND CONDITIONS OF RESEARCH

Research was conducted during 2015–2017 on the experimental field of Poltava M.I. Vavilova State Agricultural Experimental Station of the Institute of Pig-Breeding and Agro-Industrial Production of the National Academy of Agrarian Sciences of Ukraine in the village of Stepne of Poltava district. This is the central part of the Eastern Forest-Steppe of Ukraine, almost on the conventional border with the Northern Steppe and Southern Forest-Steppe – the zone of insufficient moistening.

The soil is typical black, low-humic, heavy loamy clay (AU-BCA-Cca), the arable layer of which is characterized by the following agrochemical and agro-physical indices: humus content – 4.9–5.2%; easily hydrolyzed nitrogen (according to Turin and Kononova) – 119.1–127.1 mg; P_2O_5 in acetic acid extract (according to Chirikov) – 100.0–131.0 mg; exchangeable potassium (according to Maslova) – 171.0–200.0 mg per 1 kg of soil. Soil density is 1.05–1.17 g cm^{-3}. General layering is 55.5–59.8%. The least soil moisture is about 29.7–31.5%. The full soil moisture is about 39%. The range of active moisture is about 25 mm. The moisture of capillary connection disruption is 20–22%. According to the mechanical composition typical black soil with low humus content is heavy loam. Content of dust-like particles with size less than 0.25 mm is 9.4%, content of agronomic valuable aggregates (particle size is 0.25–10 mm) is 74.6%, content of lumpy ones (aggregates > 10 mm) is 16.0% in the soil layer of 0–30 cm. (Stolbovoy & Sheremet, 2000; World Reference base for Soil Resources, 2014)

Under such agrochemical and physical indicators, such type of soil is considered to be one of the best as to the level of fertility in Ukraine (Patyka et al., 2014).

The description of the weather conditions is given in the Table 1.

Table 1. Average weather data during the 2015–2017 crop year

Indicators	September	October	November	December	January	February	March	April	May	June	July	August	Total per year
	Air temperature, °C												
Average monthly	19.9	6.4	4.7	0.6	-7.1	0.9	4.3	13.2	16.6	21.1	23.5	22.4	10.5
Average annual	14.5	7.6	1.7	-3.4	-5.6	-4.9	0.7	9.3	15.7	19.4	21.2	20.1	8.0
± to average annual	5.4	-1.3	3.0	2.8	-1.5	4.0	3.6	3.9	0.9	1.7	2.3	2.3	2.5
	Precipitation, mm												
Monthly	3.3	1.2	54.6	40.1	85.9	31.5	74.5	45.6	115.0	26.1	30.1	200.2	708.1
Average annual	45.9	41.3	40.4	42.0	40.5	32.8	30.7	31.2	45.5	65.2	61.1	42.7	519.3
± to average annual	-42.6	-40.1	14.2	-1.9	45.4	-1.3	43.8	14.4	69.5	-39.1	-31.0	157.5	188.8

The weather conditions of vegetation period in 2015 (not uniform distribution of precipitation and active temperatures) to some extent negatively influenced plant growth, development, and grain yield formation. Arid summer and particularly July, August, and the first two autumn months were not favorable for moisture accumulation in the soil. In September and October the precipitation level was correspondingly 7.2 and 2.9% of the average index during many previous years, and the average air temperature in September was 5.4 °C higher than usually, and in October, on the contrary, it was 1.2 °C lower.

During the winter the average temperature of air was 1.9°C below zero or it was 2.7 °C higher than usually.

During the spring period of 2016, the actual average daily air temperature in March, April, and May was higher than usually on 3.6, 3.9, and 0.9 °C correspondingly. In June and July, the actual precipitation levels were 26.1 and 30.1, which are 2.5 and 2.0 times less, than the indices during many previous years.

Intensive rains at the end of summer, particularly in August and rainless, warm weather in September enabled to conduct autumn field work in optimal terms, including the sowing of winter crops, the vegetation of which continued practically to the end of October. However, at the same time, the autumn was somewhat colder, than usually for many years, especially it concerns October and November.

The precipitation during this period was also not regular. In particular, in September it was only 4.9 mm or 40.9 mm less, than usually, and in October and November – 1.4 and 55.7 mm correspondingly more.

The temperature rate during December 2016 and January 2017 was higher in comparison with average indices for the previous many years on 0.3 and 0.9 °C correspondingly, and in February it grew on 1.6 °C. During the spring period the precipitation was 21.6 mm less than usually. The total amount of precipitation during the summer months was 58.3 mm, while usually it was 169.0 mm, which is on 110.7 mm less. On the whole during the agricultural year the average temperature of air was on 1.1 °C higher, and the precipitation was on 103.0 mm less. Thus, the experiments were conducted in difficult weather conditions, which enabled to establish the effectiveness of the preparations and investigate their anti-stress action.

METHODS OF RESEARCH

The experiment was conducted during the period of 2015–2017. The experiments were held with varieties and hybrids of winter wheat, soya, corn, and sunflower. The number of experiment replication – three times. The distribution of variants was random. The results of investigating the variants of the experiment block, in which foliar application of 4R Foliar Concentrate stimulator was used, are presented in this article. Ammonium nitrate was applied by broadcasting over frozen-melted soil, and growth stimulator – by foliar additional fertilizing of the sown areas with a sprayer.

The variants of the experiment:

- for ***winter wheat:*** 1 – control group (without treatment of seeds by growth stimulators only by complex against pests and diseases); 2 – root additional fertilizing with ammonium nitrate (150 kg ha^{-1}); 3 – root additional fertilizing with ammonium nitrate (100 kg ha^{-1}); 4 – root additional fertilizing with ammonium nitrate (100 kg ha^{-1})+ foliar additional fertilizing of the sown area with 4R Foliar Concentrate in the phase of spring tillering (2 kg ha^{-1}); 5 – foliar additional fertilizing of the sown area with 4R Foliar Concentrate in the phase of spring tillering (1 kg ha^{-1}); 6 – foliar additional fertilizing of the sown area with 4R Foliar Concentrate in the phase of spring tillering (2 kg ha^{-1}); 7 – foliar additional fertilizing of the sown area with 4R Foliar Concentrate (1 kg ha^{-1}) in the phase of spring tillering and repeated foliar additional fertilizing of the sown areas with 4R Foliar Concentrate in the phase of spike formation beginning (1 kg ha^{-1}) (the variety of winter wheat – Vatazhok; the proceeding crop – soya; the sown area of the experimental plot – 0.15 ha, the record plot – 100 m^2);
- for ***corn: the main fertilization*** $\mathbf{N_{40}P_{40}K_{40}}$ 1 – control group (without using the preparation); 2 – foliar additional fertilizing of the sown area in the phase of 3–5 leaves with 4R Foliar Concentrate (1 kg ha^{-1}); 3 – foliar additional fertilizing of the sown area in the phase of 3–5 leaves with 4R Foliar Concentrate (2 kg ha^{-1}); 4 – foliar additional fertilizing of the sown area in the phase of 3–5 leaves with 4R Foliar Concentrate (1 kg ha^{-1}) and repeated foliar additional fertilizing of the sown area in the phase of 10 leaves with 4R Foliar Concentrate (1 kg ha^{-1})(corn hybrid Marsel; the proceeding crop – soya; the sown area of the experimental plot – 0.18 ha, the record plot – 35 m^2);
- for ***soya: the main fertilization*** $\mathbf{N_{15}P_{15}K_{15}}$ 1 – control group (without using the preparation); 2 – foliar additional fertilizing of the sown area with 4R Foliar Concentrate (1 kg ha^{-1}) in the phase of 2–3 ternate leaves; 3 – foliar additional fertilizing of the sown area with 4R Foliar Concentrate (2 kg ha^{-1}) in the phase of 2–3 ternate leaves; 4 – foliar additional fertilizing of the sown area with 4R Foliar Concentrate (1 kg ha^{-1}) in the phase of 2–3 ternate leaves and repeated foliar additional fertilizing of the sown area with 4R Foliar Concentrate (1 kg ha^{-1}) in the phase of budding – beginning of blossoming(soya variety –Bilosnizhka; the proceeding crop – winter wheat; the sown area of the experimental plot – 0.17 ha, the record plot – 100 m^2);

- for ***sunflower: the main fertilization*** $\mathbf{N_{30}P_{30}K_{30}}$ 1 – control (without using the preparation); 2 – foliar additional fertilizing of the sown area in the phase of 2–3 pairs of leaves with 4R Foliar Concentrate (1 kg ha^{-1}); 3 – foliar additional fertilizing of the sown area in the phase of 2–3 pairs of leaves with 4R Foliar Concentrate (2 kg ha^{-1}); 4 – foliar additional fertilizing of the sown area in the phase of 2–3 pairs of leaves with 4R Foliar Concentrate (1 kg ha^{-1}) and repeated foliar additional fertilizing of the sown area in the phase of budding with 4R Foliar Concentrate (1 kg ha^{-1}) (sunflower hybrid KC-108; the proceeding crop – winter wheat; the sown area of the experimental plot – 0.18 ha, the record plot – 35 m^2).

Variant placing in the experiments was random; repetition was three times. The farming system is conventional. The results of the research were processed using the method of multivariate disperse analysis.

RESULTS AND DISCUSSION

The analysis of the above presented theoretical material testifies about certain debatable problems. The distributors of the preparations categorically support the expediency of using humates, but lately, this method has been criticized in publications of practical character, as stated in the Ukrainian production journals. The main argument of the criticism is the unnatural method of using humic preparations for vegetative surfaces, as they must be in contact with the underground part of the plant.

Indeed, from the view point of the formation and functioning of humic acids this approach would be undisputable, but in such case physical-chemical properties of humates are ignored, in particular, considerable decreasing the solution surface tension force. As foliar additional fertilizing of plants has already been scientifically substantiated and confirmed in practice, it would be logical to foresee better availability of nutritious elements in case of using this group of preparations in mixtures with fertilizers. It should also be added, that the property of humic acids to transform nutritious macro- and microelements in the forms accessible to plants, is the peculiarity of these substances.

Humates are also surface active substances, which decrease the surface tension force, creating uniform distribution of solutions on the leaf surface and, thus, assisting in better assimilation of other substances. If humic preparations contain micro-elements, in such case there is no need to use micro-fertilizers, which is one of the sources of economizing.

As to the influence on the yield of winter wheat, foliar additional fertilizing of the sown area with 4R Foliar Concentrate in the concentration of 1 kg ha^{-1}in the phase of spring tillering was at the same level comparing to root additional fertilizing with ammonium nitrate in the amount of 100 kg ha^{-1}. Increasing the dose of 4R Foliar Concentrate preparation from 1 to 2 kg ha^{-1} ensured increasing grain yield on 0.13 t ha^{-1} or by 3.4% comparing to the option №5 and by 15.7% comparing to the control variant. The maximal crop grain yield was obtained in case of double foliar additional fertilizing of the sown area with 4R Foliar Concentrate in the phase of spring tillering (2 kg ha^{-1}) and in the phase of beginning spike formation (2 kg ha^{-1}) (Table 2).

The economic calculations confirmed a high effectiveness of additional fertilizing the sown area of winter wheat both with ammonium nitrate and 4R Foliar Concentrate growth regulator on the basis of humic and fulvic acids. Thus, the highest profitability of this element of the technology – 310% and 282% was in case of foliar additional fertilizing of the sown areas with 4R Foliar Concentrate (1.0 kg ha^{-1}) in the phase of spring tillering and 2 kg ha^{-1} of 4R Foliar Concentrate in the phase of spring tillering and 2 kg ha^{-1} in the phase of spike formation beginning. The profitability of root additional fertilizing with ammonium nitrate in the amount of 100 and 150 kg ha^{-1} was equal to 111% and 155% correspondingly. Changing a part of nitrogen in mineral fertilizers (33%) by foliar additional fertilizing with 4R Foliar Concentrate in the phase of spring tillering (2 kg ha^{-1}) was also economically effective, the profitability was 135%.

Table 2. Grain yield of winter wheat with different technologies of additional fertilizing of the sown areas

Variant	Coefficient of tillering, stems per one plant, piece	Number of grains per one plant, piece	Weight of grain per one plant, g	Plant height, cm	Yield, tha^{-1}	± comparing with the control	
						t ha^{-1}	%
1.	1.7	56.3	2.6	82.4	3.38	–	–
2.	2.3	75.2	3.5	97.3	4.05	0.67	19.8
3.	2.1	69.3	3.3	94.3	3.75	0.37	10.9
4.	2.3	75.9	3.6	97.5	4.25	0.87	25.7
5.	2.1	68.3	3.2	92.8	3.78	0.40	11.8
6.	2.1	69.5	3.3	93.9	3.91	0.53	15.7
7.	2.3	77.2	3.7	97.1	4.87	1.49	44.1
$LSD_{0.95}$					0.34	–	–

The difference between the experimental variants of foliar additional fertilizing the sown areas with 4R Foliar Concentrate as to the influence on the main structural elements forming the yield of soft winter wheat is noticeable. For example, plants were 5.5 cm higher as compared to the control, the number of grains was 10.1 pieces or by 15.5% more, grain weight was 0.5 g by 16.7% more. On the whole, spraying the sown areas positively affected the crop productivity.

Using 4R Foliar Concentrate stimulator for foliar additional fertilizing of the sownareas of corn positively influenced the realization of productivity genetic potential by the plants of this crop (Table 3). Thus, on the whole, the increase of corn grain yield was 0.87–1.25 t ha^{-1} or 12.1–17.3% as compared with the control group. Nevertheless, the most effective was foliar additional fertilizing of the sown areas with 4R Foliar Concentrate (1.0 kg ha^{-1}) in the phase of 3–5 leaves and repeated additional fertilizing with the same dose of the preparation in the phase of 10 leaves. Corn grain yield at such additional fertilizing was 8.46 t ha^{-1} or was 1.25 t ha^{-1}higher in comparison with the control.

Table 3. The crop yields in case of foliar additional fertilizing of the sown areas with 4R Foliar Concentrate preparation, t ha^{-1} (the average during 2015–2017)

Variant	Corn	Soya	Sunflower
1.	7.21	2.07	2.84
2.	8.08	2.45	3.22
3.	8.43	2.57	3.40
4.	8.46	2.74	3.50
$LSD_{0.95}$	0.51	0.18	0.23

The preparations also positively influenced the number of grains in corn ear and the weight of 1,000 grains. On the average, there were on 64 grains or by 13.1% more and the weight of 1,000 thousand of seeds – on 8.2 g or by 3.3% more. The economic analysis of the experiment results shows a high effectiveness of foliar additional fertilizing of corn sown areas with 4R Foliar Concentrate growth regulator on the basis of humic and fulvic acids. So, the highest profitability of this element of corn cultivation – 804% and 549% was in case of foliar additional fertilizing of the sown areas with 4R Foliar Concentrate (1.0 kg ha^{-1}) in the phase of 3–5 leaves and repeatedly in the phase of 10 leaves. According to economic indices, the variant of foliar additional fertilizing of the sown areas with 4R Foliar Concentrate (2 kg ha^{-1}) in the phase of 3–5 leaves was intermediate.

Foliar additional fertilizing of the sown areas under soya with 4R Foliar Concentrate growth regulator on the basis of humic acids positively influenced the crop productivity. In the variant, where the growth stimulator was used for seed treatment and foliar additional fertilizing, the height of soya plants increased on 24.0 cm, the number of beans on one plant – on 3 pieces, the number of grains on one plant – on 7.3 pieces or by 29.9%, the weight of 1,000 grains – on 7.2 g or 5.3% as compared with the control variant.

The increase of soya grain yield, as compared with the control, was 0.38–0.64 t ha^{-1} by 18.4–32.4% (Table 3). From agronomic viewpoint, foliar additional fertilizing of the sown areas with 4R Foliar Concentrate (1.0 kg ha^{-1}) in the phase of 2–3 ternate leaves and repeated foliar additional fertilizing of the sown area with the same amount of the preparation in the phase of budding-beginning of blossoming was the most effective. The soya grain yield in this case was 2.74 t ha^{-1} or was 0.64 t ha^{-1} higher as compared with the control, and also 0.14 t ha^{-1} more than when 2 kg ha^{-1} of 4R Foliar Concentrate was applied in the phase of 2–3 ternate leaves.

The increase of sunflower yield at foliar additional fertilizing of the sown areas with 4R Foliar Concentrate stimulator was 0.38–0.66 t ha^{-1} or 13.4–23.2%. The preparation also increased the area of leaf surface by 10.5%, the diameter of the head – by 7.1%, the weight of 1,000 seeds – on 0.8 g or by 1.6%. As in the previous experiments, foliar additional fertilizing of the sown area with 4R Foliar Concentrate in the phase of budding with the same amount of the preparation was the most effective. The yield of sunflower seeds in this case was 3.50 t ha^{-1}, or it was 0.66 t ha^{-1} higher as compared with the control and 0.1 t ha^{-1} higher in comparison with applying 2 kg ha^{-1} of 4R Foliar Concentrate in the phase of 2–3 pairs of leaves.

Foliar additional fertilizing in the experiment of growth regulator based on humic and fulvic acids did not affect the content and dynamics of humic and fulvic acids in the soil during the time of conducting experiments on black soils.

CONCLUSIONS

Based on the obtained test results during the period of 2015–2017, the positive impact of foliar additional fertilizing with 4R Foliar Concentrate growth stimulator on the basis of humic and fulvic acids on the formation of productivity of the sown areas of the main crops was established.

This agro-technical measure ensured the increase of winter wheat grain yield on 0.4–1.49 t ha^{-1} or by 11.8–44.1%, corn – on 0.87–1.25 t ha^{-1} or by 12.1–17.3%, soya – on 0.38–0.67 t ha^{-1}or by 18.4–32.4%, sunflower seeds – on 0.38–0.66 t ha^{-1} or 13.4–23.2%. It was found, that the best economic effect is achieved in case of double foliar additional fertilizing of the sown areas of the main field crops with 4R Foliar Concentrate preparation in phases of growth and development (1 kg ha^{-1} in both cases).

The results of the experiments show, that the conducted foliar additional fertilizing of the sown areas of the crops with the growth stimulator leads to improving the conditions of plant nutrition and raising the efficiency of applying mineral fertilizers.

REFERENCES

Asik, B.B., Turan, M.A., Celik, H. & Katkat, A.V. 2009. Effects of humic substances on plant growth and mineral nutrients uptake of wheat (*Triticum durum cv. Salihli*) under conditions of salinity. *Asian Journal of Crop Science* **1**(2), 87–95.

Baldotto, L.E.B. & Baldotto, M.A. 2015. Growth and production of ornamental sunflower grown in the field in response to application of humic acids. *Cienc. Rural* **45**(6), 1000–1005.

Baldotto, M.A., Rocha, J.E., Andrade, F.D.P., Giúdice, M.P.D. & Baldotto, L.E.B. 2016. The plant stimulant humic acid extracted from organic waste recycled by composting combined with liming and fertilization. *Semina-Ciencias Agrarias* **37**(6), 3955–3963.

Calvo, P., Nelson, L. & Kloepper, J.W. 2014. Agricultural uses of plant biostimulants. *Plant and Soil* **383**(1–2), 3–41.

Churilov, G., Polishuk, S., Kutskir, M., Churilov, D. & Borychev, S. 2015. Activators of biochemical and physiological processes in plants based on fine humic acids. In: *3rdIOP Conf. Nanobiotech, Series: Materials Science and Engineering* **98**, 012040, Tambov, Russia.

Conselvan, G.B., Pizzeghello, D., Francioso, O., Foggia, M.D., Nardi, S. & Carletti, P. 2017. Biostimulant activity of humic substances extracted from leonardites. *Plant and Soil* **420**(1–2), 119–134.

Daur, I. 2013. Comparative Study of Farm Yard Manure and Humic Acid in Integration with Inorganic-N on Wheat (*Triticumaestivum* L.) Growth and Yield. *Tarim Bilimleri Dergisi-Journal of Agricultural Sciences* **19**(3), 170–177.

Dinler, B.S., Gunduzer, E. & Tekinay, T. 2016. Pre-treatment of fulvic acid plays a stimulant role in protection of soybean (*Glycine Max* L.) Leaves against heat and salt stress. *Acta Biologica Cracoviensia Series Botanica* **58**(1), 29–41.

Garcia, A.C., Izquierdo, F.G. & Berbara, R.L.L. 2014. Effects of humic materials on plant metabolism and agricultural productivity. *Emerging Technologies and Management of Crop Stress Tolerance: Biological Techniques* **1**, 449–466.

Jamal, Y., Shafi, M. & Bakht, J. 2011. Effect of seed priming on growth and biochemical traits of wheat under saline conditions. *African Journal of Biotechnology* **10**(75), 17127–17133.

Jarošová, M., Klejdus, B., Kováčik, J., Babula, P. & Hedbavny, J. 2016. Humic acid protects barley against salinity. *Acta Physiologiae Plantarum* **38**(6), 161, 1–9.

Kalinichenko, A.V., Vakulenko, Y.V. & Galych, O.A. 2014. Ecological and economic aspects of feasibility of using crop products in alternative energy. *Actual Problems of Economics* **161**(11), 202–208 (in Ukrainian).

Kulikova, N.A., Badun, G.A., Korobkov, V.I., Chernysheva, M.G., Tsvetkova, E.A., Abroskin, D.P., Konstantinov, A.I., Zaitchik, B.T., Ruzhitsky, A.O., Perminova, I.V. 2014. Accumulation of coal humic acids by wheat seedlings: Direct evidence using tritium autoradiography and occurrence in lipid fraction. *Journal of Plant Nutrition and Soil Science* **177**(6), 875–883.

Leventoglu, H. & Erdal, I. 2014. Effect of high humic substance levels on growth and nutrient concentration of corn under calcareous conditions. *Journal of Plant Nutrition* **37**(12), 2074–2084.

Lingaraju, N.N., Hunshal, C.S. & Salakinkop, S.R. 2016. Effect of biofertilizers and foliar application of organic acids on yield, nutrient uptake and soil microbial activity in soybean. *Legume Research* **39**(2), 256–261.
Manzoor, A., Khattak, R.A. & Dost, M. 2014. Humic acid and micronutrient effects on wheat yield and nutrients uptake in salt affected soils. *International Journal of Agriculture and Biology* **16**(5), 991–995.
Martinez-Balmori, D., Spaccini, R., Aguiar, N.O., Novotny, E.H., Olivares, F.L. & Canellas, L.P. 2014. Molecular characteristics of humic acids isolated from vermicomposts and their relationship to bioactivity. *Journal of Agricultural and Food Chemistry* **62**(47), 11412–11419.
Matuszak-Slamani, R., Bejger, R., Ciesla, J., Koczańska, M., Gawlik, A., Kulpa, D., Sienkiewicz, M., Włodarczyk, M. & Gołębiowska, D. 2017. Influence of humic acid molecular fractions on growth and development of soybean seedlings under salt stress. *Plant Growth Regulation* **83**(3), 465–477.
Melo, R.O., Baldotto, M.A. & Baldotto, L.E.B. 2015. Corn initial vigor in response to humic acids from bovine manure and poultry litter. *Semina-Ciencias Agrarias* **36**(3), 1863–1874.
Minkova, O.G., Kalinichenko, A.V. & Galych, O.A. 2016. Organic farming development trends in Ukraine. *Actual Problems of Economics* **175**(1), 76–82 (in Ukrainian).
Moghadam, H.R.T., Khamene, M.K. & Zahedi, H. 2016. Effect of humic acid foliar application on growth and quantity of corn in irrigation withholding at different growth stages. *Maydica* **59**(1–4), 125–129.
Muhammad, S., Anjum, A.S., Kasana, M.I. & Randhawa, M.A. 2013. Impact of organic fertilizer, humic acid and sea weed extract on wheat production in Pothowar region of Pakistan. *Pakistan Journal of Agricultural Sciences* **50**(4), 677–681.
Oliveira, S.M., Umburanas, R.C., Pereira, R.G., de Souza, L.T. & Favarin, J.L. 2017. Biostimulants via seed treatment in the promotion of common bean (*Phaseolus vulgaris*) root growth. *Applied Research & Agrotechnology* **10**(3), 109–114.
Parvan, L., Dumitru, M., Sirbu, C. & Cioroianu, T. 2013. Fertilizer with humic substances. *Romanian Agricultural Research* **30**, 205–212.
Patyka, V.P., Taranenko, S.V., Taranenko, A.O. & Kalinichenko, A.V. 2014. Microbial biom of different soils and soil-climatic zones of Poltava region. *Mikrobiolohichnyĭ zhurnal* **76**(5), 20–25 (in Ukrainian).
Prado, M.R.V., Weber, O.L.S., Moraes, M.F., Santos, C.L.R. & Tunes, M.S. 2016a. Liquid organomineral fertilizer containing humic substances on soybean grown under water stress. *Revista Brasileira de Engenharia Agricola Ambiental* **20**(5), 408–414.
Prado, M.R.V., Weber, O.L.S., Moraes, M.F., Santos, C.L.R., Tunes, M.S. & Ramos, F.T. 2016b. Humic substances on soybeans grown under water stress. *Communications in Soil Science and Plant Analysis* **47**(21), 2405–2413.
Prochazka, P., Stranc, P. & Stranc, J. 2016. Influence of seed treatment by biologically active substances to soya seed oil content. In: *Conference on Prosperous Oil*. Crpsиe, Czech Republic, pp.153–157.
Rodrigues, L.A., Alves, C.Z., Rego, C.H.Q., Silva, T.R.B. & Silva, J.B. 2017. Humic acid on germination and vigor of corn seeds. *Revista Caatinga* **30**(1), 149–154.
Rosa, D.M., Pereira, N.L.H., Mauli, M.M., Lima, G.P. & Pacheco, F.P. 2017. Humic substances in soil cultivated with cover crops rotated with maize and soybean. *Revista Ciencia Agronomica* **48**(2), 221–230.
Sadiq, S.A., Baloch, D.M., Ahmed, N. & Kakar, H. 2014. Role of coal-derived humic acid in the availability of nutrients and growth of sunflower under calcareous soil. *Journal of Animal and Plant Sciences* **24**(6), 1737–1742.

Santos, N.M., Accioly, A.M.A, Nascimento, C.W.A., Santos, J.A.G. & Silva, I.R. 2014. Humic acids and activated charcoal as soil amendments to reduce toxicity in soil contaminated by lead. *Revista Brasileira de Ciencia do Solo* **38**(1), 345–351.

Savy, D., Cozzolino, V., Nebbioso, A., Drosos, M., Nuzzo, A., Mazzei, P. & Piccolo, A. 2016. Humic-like bioactivity on emergence and early growth of maize (*Zea mays* L.) of water-soluble lignins isolated from biomass for energy. *Plant and Soil* **402**(1–2), 221–233.

Stolbovoy, V.S. & Sheremet, B.M. 2000. Correlation of the legends of soil map of USSR with scale of 1:2.5 million and the FAO soil map of the world. *Soil Science* **3**, 277–287.

Sun, Q., Ding, W., Yang, Y., Sun, J. & Ding, Q. 2016. Humic acids derived from leonardite-affected growth and nutrient uptake of corn seedlings. *Communications in Soil Science and Plant Analysis* **47**(10), 1275–1282.

Tahiri, A., Destain, J., Druart, P. & Thonart, P. 2014. Physical-chemical and biological properties of humic substances in relation to plant growth. A review. *Biotechnologie Agronomie Societeet Environnement* **18**(3), 436–445.

Tuncturk, R., Kulaz, H. & Tuncturk, M. 2016. Effect of humic acid applications on some nutrient contents of soybean (*Glycine max* L.) cultivars. *Oxidation Communications* **39**(1)Sp.Is.SI, 503–510.

World reference base for soil resources 2014. Access mode: http://www.fao.org/3/i3794en/I3794en.pdf

Zhang, L.X., Lai, J.H., Gao, M. & Ashraf, M. 2014. Exogenous glycinebetaine and humic acid improve growth, nitrogen status, photosynthesis, and antioxidant defense system and confer tolerance to nitrogen stress in maize seedlings. *Journal of Plant Interactions* **9**(1), 159–166.

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Influence of the assimilation apparatus and productivity of white lupine plants

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Abstract. Artificial regulation of the growth and development of cultivated plants aimed to increase biological productivity and improve the quality of eco-frienfly products is an important goal of modern agricultural production. Application of the natural growth stimulators and bacterial agents is quite relevant and effective. The field research was conducted on the basis of the research farm 'Agronomichne' of Vinnytsia National Agrarian University, village Agronomichne, Vinnytsa district, Vinnytsia region, Ukraine. Features of the growth and development of white lupine *(Lupinus albus L.)* plants are examined. There has been established a positive effect of the combination of inoculation with the bacterial agent and growth stimulator on the productivity of white lupine, which is important for the formation of high and stable yields. The papers presents the results of studies on the effect of pre-sowing seed treatment and foliar nutrion under conditions of the right-bank Forest-Steppe of Ukraine on the assimilation apparatus of white lupine plants. It has been established that bacterial agents and growth stimulators increase white lupine productivity due to optimization of the studied technological methods of cultivation. The optimal leaf surface area that provided maximum grain yield has been determined. The research has established a positive effect of pre-sowing seed treatment with the bacterial agent Rhizohumin and the growth stimulator Emistym C and foliar nutrition with Emistym C on the chlorophyll content in the white lupine leaves. The influence of the investigated technological methods on the formation of the assimilation surface area and chlorophyll synthesis in the leaves of white lupine has been proved. The preparations studied induce intensive development of the photosynthetic apparatus, yield increase, improvement of the yield structure and they improve grain quality under conditions of right-bank Forest-Steppe of Ukraine. The issue of seed bacterization and application of growth stimulators requires a more detailed study. Theefore, such researches are relevant and significance in terms of both practical and scientific value.

Key words: white lupine, assimilation apparatus, chlorophyll, variety, productivity, growth stimulator, seed bacterization.

INTRODUCTION

The most important goals of modern agrarian science include the search for new ways and techniques aimed to increase crop productivity as well as to improve the product quality (Rogach, 2009; Mazur & Pantsyreva, 2017). Significant achievements in this area can be achieved through optimization of the level of fulfilment of the genetic potential of plants and simultaneous minimization of the effect of negative environmental factors in the process of their ontogenesis (Muhammad & Muhammad, 2013; Rai et al., 2017).

Climate resources are important for maximizing the biological potential of agricultural crops. The vegetative period in agricultural crops is related to the amount of precipitation and the presence of heat. Among allocated in Ukraine natural agricultural zones include: Woodland, Forest Steppe and Steppe zones. The Forest Steppe zone occupies 34.9% of the territory of Ukraine (20,291,1 thousand hectares). Right Bank Forest Steppe is characterized by moderatelycontinental climate and belongs to the zone of sufficient moisture. The absence of high altitude increases the free movement of air of various origins, which causes a significant variability of weather processes in separate seasons (Furseth, 2012; Madzikane-Mlungwana et al., 2017; Mazur & Pantsyreva, 2017).

Physiologically active substances cause restructuring of the assimilation apparatus of plants, changes in morphometric parameters, ratio of the masses of its organs, emergence of additional attractive centers, and the strengthening or weakening of the functioning of existing ones that indicates changes in the nature of donor-acceptor relationships in the plant (Kuryata et al., 2017). The effect of the growth stimulantors is associated with the acceleration of the processes of division, stretching and differentiation with the simultaneous increase in plant habitus (Madzikane-Mlungwana et al., 2017; Rai et al., 2017), the area of assimilation surface (Polyvanyj & Kuryata, 2015; Ren et al., 2017), an increase in the chlorophyll concentration (Luo et al., 2017; Ren et al., 2017) and, as a consequence, the activation of photosynthetic processes (Mohammad & Mohammad, 2013; Rai et al., 2017; Ren et al., 2017), and the growth of crop productivity (Polyvanyj & Kuryata, 2015; Gonzatto et al., 2016; Khalid et al., 2016; Alexopoulos et al., 2017; Pantsyreva, 2017; Rai et al., 2017).

It is known that the hormonal system plays an extremely important role in the regulation of the processes of plant morphogenesis, and the physiological effect depends both on the features of varieties and technological methods of cultivation. Application of the preparations based on the strains of nodule bacteria and extracts from epiphyte fungi affect the yield and quality of agricultural products. The use of growth regulators and bacterial agents provides the prospects of artificial redistribution of the flows of assimilants from vegetative growth processes to the formation and growth of grain, and, as a result, it can become an effective factor for increasing crop yields (Davis, Tim, 2017; Rai et al., 2017; Bollman &, Vessey, 2006, Merkushyna, AS, 2013; Xing et al., 2016).

In the research papers, there is enough information available on the use of natural growth stimulators and bacterial agents aimed to activate the production process through morphometric changes in the legumes (Xing et al., 2016; Pantsyreva, 2016), cereals (Muhammad & Muhammad, 2013; Luo et al., 2017; Zhao et al., 2017), oilseeds (Khodanitska & Kuryata, 2011; Fu et al., 2014; Froschle et al., 2017), vegetables (Tubiis et al., 2016; Palamarchuk, 2017, Alexopoulos et al., 2017), industrial crops (Khhodanitska & Kuryata, 2011; Mohammad & Mohammad, 2013; Rai et al., 2017), fruit crops (Ahmed et al., 2012; Cru-Castilloa et al., 2014), medicinal and decorative crops (Gouveia et al., 2012; Aremu et al., 2017; Madzikane-Mlungwana et al., 2017). Bacterial agents and growth stimulators also increase crop resistance to adverse environmental and biotic factors due to the changes in hormonal status and the activation of antioxidant plant sustems (Javid et al., 2011; Muhammad & Muhammad, 2013; Piotrowska-Niczyporuk et al., 2014; Tubi's et al., 2016; Xing et al., 2016).

Domestic and foreign authors indicate that the biological yield depends on the content of pigments, primarily chlorophylls in the assimilating organs of plants, time and intensity of their work. Chlorophyll content in the leaves affects the intensity of photosynthesis, dry matter accumulation, and, finally, their productivity. The need for research in this area is caused by the fact that the total mass of green pigment and its concentration in the leaf mesophyll as well as the assimilation surface area are considered to be the basis of the potential of photosynthetic activity of the plant organism as a whole (Pantsyreva, 2017; Rai et al., 2017).

The difference in the chlorophyll content, as a rule, is an indicator of the level of compliance with the growing conditions and it varies depending on the variety genotype. The increase in crop yield depends on both the factors affecting photosynthesis and the complex of physiological processes associated with it (water exchange, nutrition, growth). The formation of a well-developed photosynthetic apparatus that is optimal in volume, dynamics and intensity of functioning is the key to the production of organic matter, biological and commodity yield (Pantsyreva, 2017).

Scientifically substantiated foundations of the technologies for growing legume crops, including white lupine, determination of the chlorophyll accumulation in plant leaves are important as their content affects the intensity of photosynthesis and other physiological processes. The researches aimed at establishing the features of the photosynthetic apparatus, peculiarities of formation of the assimilation apparatus during plant growth and development are of primary importance for assessing the influence of the technological methods on the productivity and quality of the plant grain. Therefore, such researches are of great importance for modern agricultural production (Rogach, 2009; Mazur & Pantsyreva, 2017). Thus, the purpose of this research is to establish the specifics of the assimilation apparatus formation by white lupine crops depending on the technological methods under conditions of the right-bank Forest-Steppe.

MATERIALS AND METHODS

The field research (for 2013–2018 years) was conducted on the experimental field ‘Agronomichne’ of Vinnytsia National Agrarian University that was sown with white lupine, village Agronomichne, Vinnitsa district, Vinnytsia region. White lupine variety Veresnevyi was selected as the material for the study.

In the experiment, the effect and interaction of three factors were studied: A – variety, B – pre - sowing seed treatment, C - foliar fertilization. On the day of sowing, white lupine seeds were treated with bacterial Risogumin (600 g per hectare seed) and growth promoter Emistim C (10 mL per 1 t seed) using PKC-20 Super. Growth stimulator Emistim C with a rate of use of 15 mL ha^{-1} was used in the non-root nutrition. The first foliar nutrition of Emistim C was carried out in the budding phase, and the second in the phase of seeding. For control, an option is adopted without pre-planting and without extra-root crops. On the day of sowing, white lupine seeds were treated with water in a control.

Risogumin is used to bacterialize lupine seeds in order to improve nitrogen nutrition of plants, increase productivity of culture. Stimulator of growth of plants Emistim C a wide spectrum of action - a product of biotechnological cultivation of mushroom-epiphytes from the root system of medicinal plants. Transparent, colorless, water-alcohol solution. Contains a balanced set of phytohormones of auxin, cytokinin nature, amino acids, carbohydrates, fatty acids, trace elements.

There was applied the technology of growing white lupine varieties that was conventional for the Forest-Steppe zone of Ukraine and involved pre-sowing seed treatment with the bacterial agent Rhizohumin combined with the growth stimulator Emistym C and foliar nutrition with Emistym C. The registered area was 25 m^2. Replication was five-time. The variants were located systematically in two layers.

Evaluation of photosynthetic activity of plants was carried out in accordance with the following techniques: leaf area was measured by the method of ‘carving’, photosynthetic potential was determined by the method of A.A. Nichiporovich (1996); chlorophyll content was determined by the method of alcohol batch using a certified electrophotocololymer (KFK-2).

Statistical analysis of the experimental data was carried out using the computer program STATICA - 6. Validity of the difference of the experimental data regarding the control was determined using Student's t-criterion. The tables and figures show the average data over the years of research.

The revealed dependencies (Fig. 4) between the formation of indicators of individual productivity and grain yield of white lupine varieties can be expressed by the following regression equations:

$$У = 7.881678 + 0.066816x_1 + 0.196308x_2 – 0.026010x_3 \quad (1)$$

where У – grain yield, t ha^{-1}; x_1 – number of beans per plant, pcs/plant; x_2 – number of grains per plant, pcs; x_3 – mass of 1,000 seeds, g.

In this case, the coefficients of the multiple linear correlation in white lupine were $R = 0.904254$ and $R = 0.896057$, respectively. The pair correlation coefficients (r) between the grain yield and the number of beans, the number of seeds per plant, and the mass of 1,000 seeds were 0.145801, 0.045127, 0.09706. These indicators show a close relationship between the basic indicators of individual plant productivity and the level of grain yield of white lupine.

RESULTS AND DISCUSSION

It is known that white lupine is characterized by slow and uneven growth in the initial phase of development; however its growth rate increases in the future. White lupine grows especially intensely after the beginning of bloom. The growth rate during this period depends mainly on the environment and characteristics of the variety.

It is noted that depending on the factors studied the height of white lupine plants before the phase of budding did not change significantly, but since the beginning of the stage of full bloom the difference in height between the variants considerably increased.

The researches (Rogach, 2009; Mazur & Pantsyreva, 2017) have revealed the dependence of white lupine plant height on the effect and interaction of bacterial agent and growth stimulators that were studied (Fig. 1).

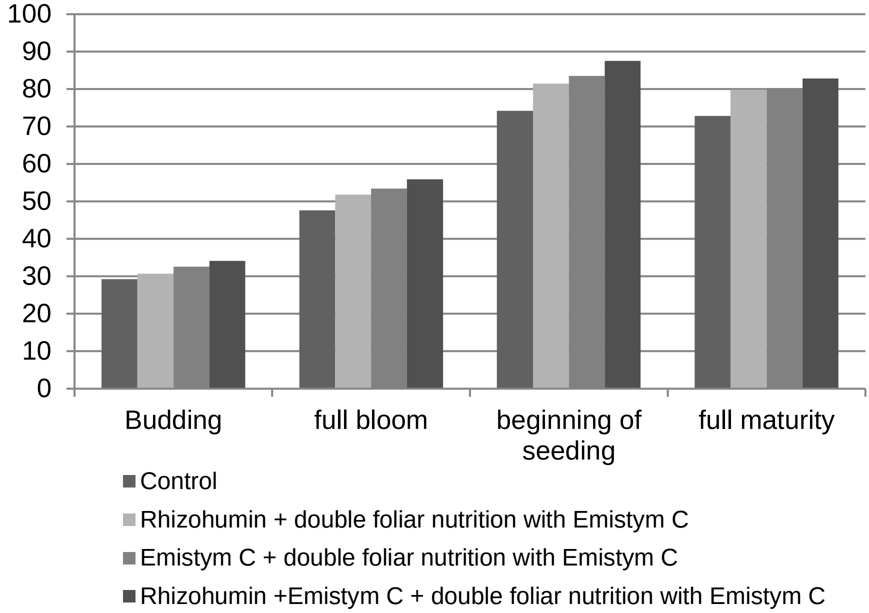

Figure 1. Dynamics of while lupine plant height in Veresnevyi variety depending on pre-sowing seed treatment and foliar nutrition, cm (average of 2013–2017).

The highest height of this variety was recorded at the beginning of the grain pouring and was 87.5 cm in the version where pre-sowing seed treatment with an inoculum Risogumin with an Emistim C growth stimulator was carried out in conjunction with two non-root nutrients. This indicator exceeded the control version without the use of pre-seed treatment on average 13.3 cm.

In the case of seedless pre-seed treatment, plant height was the lowest in all phases of growth and development of white lupine plants. The greatest value of this indicator on the control version without foliar feeding at the beginning of the pouring of grain was 74.2 cm.

In the version with the use of bacterial preparation Rizogumin without endocrine infusions in the phase of the onset of grain, the height of the plant reached 78.9 cm, which is 4.7 cm less for this variant. In the variant with pre-sowing treatment with growth stimulator Emistim C with two extra-root nutrients, the height of 83.5 cm was fixed, which is 9.3 cm less than the control variant.

The process of formation of fruit elements in white lupine plants depending on pre-sowing seed treatment and foliar nutrition is of great scientific and practical value for maximum fulfilment of the genetic potential of the variety under conditions of the right-bank Forest-Steppe. Observations of the nature of formation of fruit elements in white lupine showed that their number depends on the effect of the biological agents that were studied (Table 1).

Table 1. Formation of fruit elements in white lupine depending on pre-sowing seed treatment and foliar nutrition (average of 2013–2015)

Factors		Average number per plant, pcs.			% of mature beans	
Pre-sowing seed treatment	Foliar nutrition with Emistym C	Flowers	Beans after setting	Beans at the period of ripening	from the number of flowers	From the formed beans
Without pre-sowing seed treatment	without nutrition	25.1	7.7	5.0	19.9	64.9
	single nutrition	25.4	7.8	5.1	20.1	65.4
	double nutrition	25.8	7.9	5.2	20.2	65.8
Rhizohumin	without nutrition	25.5	8.3	5.6	22.5	67.5
	single nutrition	25.7	8.7	5.9	23.0	67.8
	double nutrition	25.9	8.9	6.0	23.2	67.4
Emistym C	without nutrition	26.3	8.8	6.1	23.2	69.3
	single nutrition	27.1	9.0	6.4	23.6	71.1
	double nutrition	27.9	9.2	6.6	23.7	71.7
Rhizohumin + Emistym C	without nutrition	28.4	9.3	6.7	23.6	72.0
	single nutrition	29.4	9.5	7.0	23.8	73.7
	double nutrition	29.7	9.6	7.1	23.9	74.0

$LSD_{0.5}$: A-0.04; B-0.8; C-0.07; AB-0.10; AC-0.11; BC-0.15; ABC-0.20
2013 $LSD_{0.5}$ t ha^{-1}: A-0.03; B-0.04; C-0.03; AB-0.07; AC-0.06; BC-0.7; ABC-0.10
2014 $LSD_{0.5}$ t ha^{-1}: A-0.04; B-0.05; C-0.04; AB-0.09; AC-0.08; BC-0.10; ABC-0.14
2015 $LSD_{0.5}$ t ha^{-1}: A-0.05; B-0.05; C-0.05; AB-0.08; AC-0.07; BC-0.10; ABC-0.13.

It was established that the largest number of flowers per plant in white lupine was formed in the variant where pre-sowing seed treatment involved the bacterial agent Rhizohumin and the growth stimulator Emistym C in combination with double foliar nutrition with Emistym C in the budding phase. Thus, the number of flowers per plant in Veresnevyi variety was 29.7 flowers per plant, which exceeded the control variant by 4.6 flowers per plant. The variants without pre-sowing seed treatment and foliar nutrition had the lowest figures, which were 25.1 flowers per plant in Veresnevyi variety.

Thus, the effect of the studied preparations on the figures of the leaf surface area was insignificant in the phases of branching and budding. On the site where Veresnevyi variety was grown, the leaf area index, depending on the pre-sowing seed treatment and foliar nutrition, was within 14.2–15.7 thousand m^2 pe ha in the budding phase, and it ranged within 22.3–27.6 thousand m^2 per ha in the phase of full bloom.

It was established that the highest index of the area of the leaf surface per hectare of lupine of the white variety of the Veresnevyi – 43.7 thousand m^2 ha^{-1} was formed in the phase of the beginning of the filling of grain in the variant with the use of the bacterial preparation Risogumin in combination with growth stimulator Emistim C with two extracorporeal feedings Emistim C. This indicator was greater than control at 8.1 thousand m^2 ha^{-1} (Fig. 2).

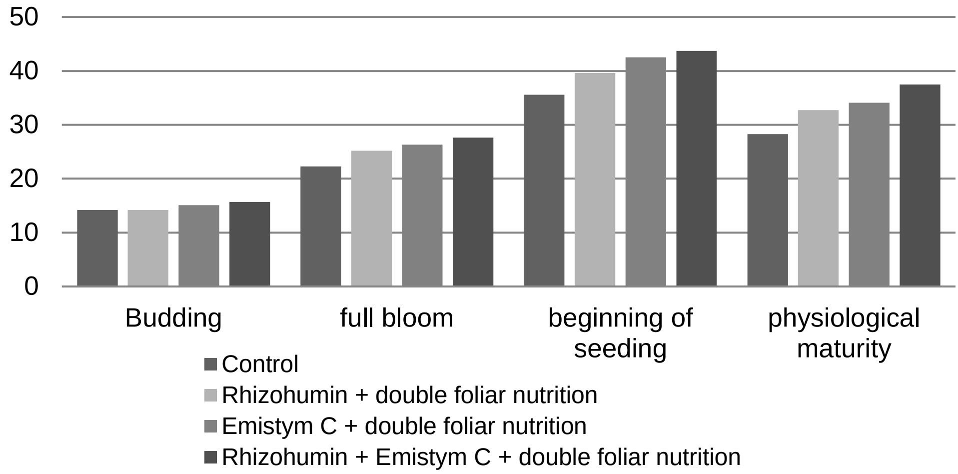

Figure 2. Dynamics of the leaf surface area of white lupine plants of Veresnevyi variety depending on technological methods, thousand m^2 per ha (2013–2015).

The records taken during the trial have showed that pre-sowing seed treatment of white lupine with the bacterial agent Rhizohumin in combination with the growth stimulator Emistym C with double foliar nutrition with Emistym C have a positive effect on the formation of photosynthetic apparatus of plants and on chlorophyll content in the leaves of white lupine (Table 2).

Table 2. Formation of the photosynthetic potential of white lupine of Veresnevyi variety depending on pre-sowing seed treatment and foliar nutrition, million m^2 per ha (average for 2013–2017)

Factors		Periods of vegetation of plants			
Pre-sowing seed treatment	Foliar nutrition with Emistym C	Full germination - budding	Full germination - full bloom	Full germination Beginning of seeding	Full germination - physiological maturity
Without pre-sowing seed treatment	without nutrition	0.321	0.601	0.989	1.505
	single nutrition	0.321	0.606	1.005	1.529
	double nutrition	0.321	0.606	1.006	1.559
Rhizohumin	without nutrition	0.326	0.616	1.050	1.588
	single nutrition	0.326	0.622	1.075	1.638
	double nutrition	0.336	0.622	1.076	1.689
Emistym C	without nutrition	0.337	0.637	1.125	1.766
	single nutrition	0.337	0.648	1.150	1.819
	double nutrition	0.337	0.648	1.151	1.860
Rhizohumin + Emistym C	without nutrition	0.354	0.675	1.125	1.941
	single nutrition	0.354	0.689	1.260	1.982
	double nutrition	0,354	0.689	1.262	2.061

$LSD_{0.5}$ million m^2 per ha: A-0.05; B-0.6; C-0.07; AB-0.12; AC-0.11; BC-0.14; ABC-0.09
2013 $LSD_{0.5}$ t ha^{-1}: A-0.04; B-0.05; C-0.04; AB-0.06; AC-0.06; BC-0.07; ABC-0.1
2014 $LSD_{0.5}$ t ha^{-1}: A-0.05; B-0.06; C-0.05; AB-0.08; AC-0.07; BC-0.08; ABC-0.1
2015 $LSD_{0.5}$ t ha^{-1}: A-0.06; B-0.07; C-0.05; AB-0.08; AC-0.07; BC-0.10; ABC-0.13.

It is proved that the effectiveness of application during pre-sowing seed treatment of the bacterial agent Rhizohumin and the growth stimulator Emistym C and foliar nutrition with the growth stimulator Emistym C is marked in the phase of physiological maturation. Thus, the highest figures of formation of photosynthetic potential of white lupine plants were observed in the period of full germination – physiological maturity in the variants where pre-sowing seed treatment involved a bacterial agent and a growth stimulator in combination with with double foliar nutrition and amounted to 2.061, which exceeded the control variant by 27.0%.

The increase in crop yield depends on both the factors affecting photosynthesis and the complex of physiological processes associated with it (water exchange, nutrition, growth). The formation of a well-developed photosynthetic apparatus that is optimal in volume, dynamics and intensity of functioning is the key to formation of organic matter, biological and commodity yields.

Many authors indicate that the biological yield depends on the content of pigments, primarily chlorophylls in the assimilating organs of plants, the time and intensity of their work. The content of chlorophyll in the leaves affects the intensity of photosynthesis, accumulation of dry matter, and, finally, their productivity. The need for research in this area is caused by the fact that the total mass of the green pigment and its concentration in leaf mesophillous and the size of the assimilation surface are considered as a basis for the potential of photosynthetic activity of the plant organism as a whole.

Availability of the positive effect of pre-sowing seed treatment and foliar nutrition on the chlorophyll content in white lupine leaves was established (Table 3).

Table 3. Chlorophyll content in while lupine depending on pre-sowing seed treatment and foliar nutrition (average of 2013–2017)

Factors		Chlorophyll content in the leaves,	
Pre-sowing seed treatment	Foliar nutrition with Emistym C	mg g^{-1} of crude mass	mg m^{-2}
without pre-sowing seed treatment	without nutrition	2.03	2,101.28
	single nutrition	2.03	2,101.28
	double nutrition	2.07	2,560.29
Rhizohumin	without nutrition	2.16	2,699.44
	single nutrition	2.23	2,707.68
	double nutrition	2.33	3,246.51
Emistym C	without nutrition	2.16	2,699.44
	single nutrition	2.23	2,707.68
	double nutrition	2.48	3,679.94
Rhizohumin Emistym C	without nutrition	2.48	3,679.94
	single nutrition	2.69	4,083.31
	double nutrition	2.87	4,802.12

Pre-sowing seed treatment of white lupine plants both with Rhizohumin and Emistym C with subsequent foliar nutrition of plants with Emistym C provided the highest pigment content in the experiment, both in terms of crude mass and per unit of area. Thus, this indicator in the plants of Varesvenyi variety was 2.87 mg g^{-1} and 4,802.12 mg m^{-2}, respectively.

Observation of the dynamics of dry matter accumulation in white lupine plants showed that the maximum output was formed in the phase of physiological maturity (Fig. 3).

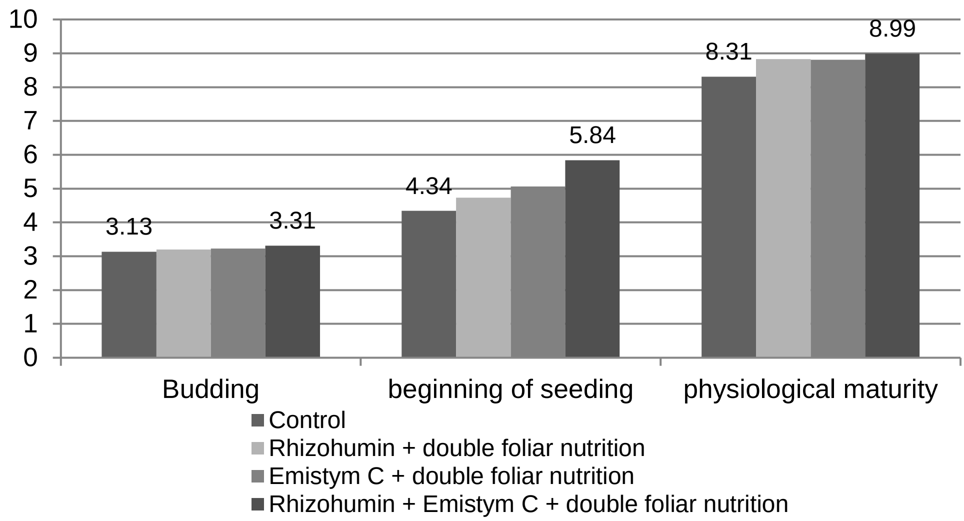

Figure 3. Dynamics of dry matter accumulation in while lupine of Veresnevyi variety depending on pre-sowing seed treatment and foliar nutrition, t ha^{-1} (average of 2013–2017).

It was noted that the intensity of dry matter accumulation during the growing season of white lupine varieties depended on the factors studied, namely, pre-sowing seed treatment and foliar nutrition. Application of the bacterial agent in combination with double foliar nutrition contributed to obtaining the largest output of dry matter of white lupine.

According to our research, individual productivity of while lupine plants depended on the features of the variety and the factors studied (Table 4).

Thus, the maximum individual productivity of white lupine plants of Veresnevyi variety was observed in the variant with pre-sowing seed treatment with a bacterial agent and a growth stimulator combined with the double foliar nutrition. In this case, the indicators of individual productivity were as follows: the number of beans per plant was 6.5 pcs, the number of grains per plant was 20.3 pcs, the mass of 1,000 grains was 335.1 g, the mass of grains per plant was 6.8 g. On the control trial sites, where no pre-sowing seed treatment and no foliar nutrition was applied, indicators of the individual productivity were the lowest, and they were as follows: the number of beans per plant was 4.9 pcs, the number of seeds per plant was 15.5 pcs, the mass of 1,000 grains was 317.2 g, the mass of grains per plant was 4.9 g.

We have found that there is a close relationship between individual productivity of plants and the level of crop yield, including white lupine.

The maximum value of the white lupine grain yield of Veresvenyi variety was obtained in the trial variants with pre-sowing seed treatment with the inoculant Rhizohumin and growth stimulator Emistym C in combination with double foliar nutrition with Emistym C (Table 5). At the same time, the grain yield was 3.61 t ha^{-1}, and it exceeded the control variant by 0.65 t ha^{-1} or 18%, respectively.

Table 4. Individual productivity of white lupine plants depending on pre-sowing seed treatment and foliar nutrition (average of 2013–2015)

Factors Pre-sowing seed treatment	Foliar nutrition	Number of beans per plant, pcs.	Number of grains per plant, pcs.	Mass of 1,000 seeds, pcs.	Mass of grains per plant, g
Without pre-sowing seed treatment	without nutrition	4.9	15.5	317.2	4.9
	single nutrition with Emistym C	5.0	16.0	318.1	5.1
	double nutrition with Emistym C	5.0	16.3	319.4	5.2
Rhizohumin	without nutrition	5.1	16.2	314.9	5.1
	single nutrition with Emistym C	5.2	17.3	317.0	5.5
	double nutrition with Emistym C	5.5	17.5	319.4	5.6
Emistym C	without nutrition	5.2	16.3	317.6	5.2
	single nutrition with Emistym C	5.4	17.6	320.1	5.6
	double nutrition with Emistym C	5.8	17.9	323.7	5.8
Rhizohumin + Emistym C	without nutrition	5.4	16.6	321.6	5.3
	single nutrition with Emistym C	6.1	18.1	325.9	5.9
	double nutrition with Emistym C	6.5	20.3	335.1	6.8

Table 5. Grain yield of while lupine depending on pre-sowing seed treatment and foliar nutrition, t ha^{-1} (average of 2013–2015)

Factors		Years			
Pre-sowing seed treatment	Foliar nutrition with Emistym C	2013	2014	2015	Average
Without pre-sowing seed treatment	without nutrition	3.08	3.24	2.55	2.96
	single nutrition	3.13	3.35	2.59	3.02
	double nutrition	3.18	3.42	2.62	3.17
Rhizohumin	without nutrition	3.15	3.71	2.90	3.25
	single nutrition	3.31	3.88	2.94	3.38
	double nutrition	3.40	3.90	3.05	3.45
Emistym C	without nutrition	3.10	3.68	2.82	3.20
	single nutrition	3.20	3.74	2.86	3.27
	double nutrition	3.31	3.81	2.93	3.35
Rhizohumin + Emistym C	without nutrition	3.08	3.62	2.88	3.19
	single nutrition	3.12	3.85	3.01	3.32
	double nutrition	3.58	4.10	3.15	3.61

$LSD_{0.5}$ t ha^{-1}: A-0.07; B-0.10; C-0.08; AB-0.14; AC-0.12; BC-0.17; ABC-0.24
2013 $LSD_{0.5}$ t ha^{-1}: A-0.04; B-0.05; C-0.04; AB-0.07; AC-0.06; BC-008; ABC-0.12
2014 $LSD_{0.5}$ t ha^{-1}: A-0.05; B-0.06; C-0.06; AB-0.09; AC-0.08; BC-0.11; ABC-0.16
2015 $LSD_{0.5}$ t ha^{-1}: A-0.04; B-0.06; C-0.05; AB-0.08; AC-0.07; BC-0.10; ABC-0.14.

It has been established that foliar nutrition with Emistym C provided an increase in the grain yield of white lupine. However, the increase in grain yield depended on the pre-sowing seed treatment, which involved foliar nutrition. Double foliar nutrition on the trial sites without pre-sowing seed treatment resulted in the yield increase of 0.21 t ha^{-1}.

While the application of double foilar nutrition with the growth stimulator Emistym C combined with pre-sowing seed treatment with the inoculant Rhizohumin and growth stimulator Emistim C provided a maximum grain yield increase of 0.65 t ha^{-1}. In the variants where pre-sowing seed treatment was conducted by the bactericidal agent Rhizohumin separately from the growth stimulator Emistym C, the application of double foliar nutrition resulted in somewhat lower yield increase of 0.49 t ha^{-1} and 0.39 t ha^{-1} or 14.2% and 12.0%, respectively. Consequently, there was revealed a significant effect of foliar nutrition with Emistym C combined with pre-sowing seed treatment with the bacterial agent Rhizohumin and growth stimulator Emistym C.

Plant functioning depends on a significant number of exogenous and endogenous factors, among which the regulation of productivity by the growth stimulator and bacterial agent is quite significant, since the changes in growth, physiological and biochemical processes cause restructuring of the entire plant organism in this way (Kuryata et al., 2017; Poprotska & Kuryata, 2017; Mazur et al., 2018). Stimulation of growth and development processes is associated with the mobilization of the genetic potential of plants and targeting assimilation resources at the enhancement of biological productivity, in contrast to the effects of inhibitors, although the effect of the latter, as it is known, may also be accompanied by the increased yields due to the redistribution of plastic substances between plant organs.

Application of pre-sowing seed treatment and foliar nutrition with the bacterial agent and growth stimulator induces changes in the processes of morphogenesis and intensification of metabolism in white lupine plants.

It is known that the leaf is the main source of assimilates in the plant. Changes in the structure and functioning of the leaf apparatus as a donor of plastic substances are the key ones in the production process. Enhancement of the activity of all types of meristem tissues under the effect of bacterial agent and growth stimulator has contributed to the formation of plants, which are bigger in their size (Mesejo et al., 2012; Aremu et al., 2017; Madzikane-Mlungwana et al., 2017) and form a more powerful leaf apparatus (Rogach, 2009; Polyvanyj & Kuryata, 2015). Setting of a bigger number of leaves, increase in the area and mass of crude matter in the leaves has resulted in the activation of photosynthetic processes and enhanced the donor function of the leaf.

Another kind of effect of the studied preparations is the mesostructure organization of the leaf. Strengthening of mitotic activity under the effect of preparations contributed to the thickening of the leaf blade due to assimilation tissue, which was manifested in increasing the number, size and volume of cells. The investigated influence of growth stimulators on the mesostructure of leaf blade in the white lupine can provide preconditions for increasing the crop photosynthetic productivity.

Assimilates, which were intensively synthesized under the effect of the bacterial preparation and growth stimulator, especially in the initial stages of ontogenesis, actively influenced the growth processes and accelerated the development of white lupine plants. Thus, the analysis of the ratio of masses of vegetative and generative organs shows that at the beginning of formation of grains, i.e. the main acceptors of plastic substances in the plant, their share increased under the effect of preparation studied. At the same time, the mass proportion of donor assimilates, i.e. the leaves in the indicated phase of ontogenesis, practically did not change, while the share of another powerful acceptor of plastic substances, i.e. the stem, significantly decreased. Their application caused changes in the activity and direction of growth processes. The treated sites showed a stronger branching of the stem compared with the control sites. An increase in the habitus of plants was due to effect of preparations.

The use of pre-sowing seed treatment and foliar nutrition contributed to the formation of a powerful photosynthetic apparatus. Under the effect of the growth stimulator, a large number of leaves were formed on the stem, and the duration of their active functioning was prolonged. At the same time, leaf surface area of white lupine plants increased. Under the effect of the preperations studied, the number and size of chloroplasts in the leaves increased, which resulted in the increased index of pure productivity of photosynthesis, increased photosynthetic performance, more intensive growth of the dry matter mass of white lupine plants. Consequently, pre-sowing seed treatment with the bacterial agent Rhizohumin and the growth stimulator Emistym C in combination with double foliar nutrition with the growth stimulator Emistym C contributed to the increase of the gross photosynthetic productivity of white lupine plants, which is an important condition for increasing the grain and fodder productivity of the crop.

Consequently, as the result of such application, enhancement of the growth processes under the effect of bacterial agents and growth stimulators and changes in plant morphometry, including the structure of the leaf apparatus, caused formation of a greater number of plastic substances with their subsequent transfer to the economically valuable organs in white lupine plants, i.e. grains, the number of which is greater under effect of the preparations. This resulted in the increase of biological productivity of the crop as a whole and the grain yield in particular.

CONCLUSIONS

Application of the bacterial agent Rhizohumin and the growth stimulator in combination with the double foliar nutrition with the growth stimulator Emistym C during pre-sowing treatment of white lupine seeds promoted the increase of the leaf area, formation of photosynthetic apparatus of plants and chlorophyll content in the leaves. The highest stimulatory effect was obtained in the variant of pre-sowing seed treatment with Rhizohumin + Emistym C + double foliar nutrition with Emistym C.

REFERENCES

Ahmed, W., Tahir, F., Rajwana, I., Raza, S. & Asad, H.U. 2012. Comparative evaluation of plant growth regulators for preventing premature fruit drop and improving fruit quality parameters in Dusehri Mango. *International Journal of Fruit Science* **12**, 372–389.

Alexopoulos, A.A., Karapanos, I.C., Akoumianakis, K.A. & Passam, H.C. 2017. Effect of gibberellic acid on the growth rate and physiological age of tubers cultivated from true potato seed. *Journal of Plant Growth Regulation* **36**(1), 1–10.

Mazur, V.A., Didur, I.M., Pantsyreva, H.V. & Telekalo, N.V. 2018. Energy-economic efficiency of growth of grain-crop cultures in the conditions of right-bank Forest-Steppe zone of Ukraine. *Ukrainian Journal of Ecology* **8**(4), 26–33.

Bollman, M. & Vessey, K. 2006. Differential effects of nitrate and ammonium supply on nodule initiation, development, and distribution on roots of pea (Pisum sativum L.). *Canadian Journal of Botany* **84**(6), 893–903.

Cruz-Castilloa, J.G., Baldicchib, A., Frionib, T., Marocchic, F., Moscatellod, S., Proiettid, S., Battistellid, A. & Famianib, F. 2014. Preanthesis CPPU low dosage application increases Hayward kiwifruit weight without affecting the other qualitative and nutritional characteristics. *Food Chemistry* **158**(1), 224–228.

Davis, T.D. 2017. Soybean photosynthesis and growth as influenced by flurprimidol. *Compar. Phisiol. and Ecol.* 1986. Vol. **11**(4), 166–169.

Froschle, M., Horn, H. & Spring, O. 2017. Effects of the cytokinins 6-benzyladenine and forchlorfenuron on fruit-, seed- and yield parameters according to developmental stages of flowers of the biofuel plant Jatropha curcas (Euphorbiaceae). *Plant Growth Regulation*, **81**(2), 293–303.

Fu, Q., Niu, L., Zhang, Q., Pan, B-Z., He, H. & Xu, Z-F. 2014. Benzyladenine treatment promotes floral feminization and fruiting in a promising oilseed crop Plukenetia volubilis. *Industrial Crops and Products* **59**, 295–298.

Furseth, B. 2012. Soybean Response to Soil Rhizobia and Seed-applied Rhizobia Inoculants in Wisconsin. *Crop Science* **52**(1), 339–344.

Gonzatto, M.P., Boettcher, G.N., Schneider, L.A., Lopes, A.A., Junior, J.C.S., Petry, H.B., Oliveira, R.P. & Schwarz, S.F. 2016. 3,5,6-trichloro-2-pyridinyloxyacetic acid as effective thinning agent for fruit of Montenegrina mandarin. *Ciencia Rural* **46**(12), 2078–2083.

Gouveia, E.J., Rocha, R.B., Galveas, B., Ramalho, L.A.R. Ferreira, M.G.R. & Dias, L.A.S. 2012. Grain yield increase of physic nut by fieldapplication of benzyladenine. *Pesquisa Agropecuária Brasileira* **47**(10), 1541–1545.

Javid, M.G., Sorooshzadeh, A., Sanavy, S.A.M.M., Allahdadi, I. & Moradi., F. 2011. Effects of the exogenous application of auxin and cytokinin on carbohydrate accumulation in grains of rice under salt stress. *Plant Growth Regulation* **65**(2), 305–313.

Khalid, S., Malik, A.U., Khan, A.S., Razzaq, K. & Naseer, M. 2016. Plant growth regulators application time influences fruit quality and storage potential of young kinnow mandarin trees. *International Journal of Agriculture and Biology* **18**, 623–629.

Khodanitska, O.O. & Kuryata, V.G. 2011. The effect of treptolem on seed yield and quality characteristics of flaxseed oil. *Forage and feed production* **70**, 54–59 (in Ukrainian).

Kuryata, V.G., Poprotska, I.V. & Rogach, T.I. 2017. The impact of growth stimulators and retardants on the utilization of reserve lipids by sunflower seedlings. *Regulatory Mechanicms in Biosystems* **8**(3), 317–322 (in Ukrainian).

Luo, Y., Yang, D., Yin, Y., Cui, Z., Li, Y., Chen, J., Zheng, M., Wang, Y., Pang, D., Li, Y. & Wang, Z. 2017. Effects of exogenous 6-BA and nitrogen fertilizers with varied rates on function and fluorescence characteristics of wheat leaves post anthesis. *Scientia Agriculturalura Sinica* **49**(6), 1060–1083.

Madzikane-Mlungwana, O., Moyo, M., Aremu, A.O., Plíhalova, L., Doleal, K., Staden, J.V. & Finnie, J.F. 2017. Differential responses to isoprenoid, N6-substituted aromatic cytokinins and indole-3-butyric acid in direct plant regeneration of Eriocephalus africanus. *Plant Growth Regulation* **82**(1), 103–110.

Mazur, V.A. & Pantsyreva, H.V. 2017. Influence of technological methods of cultivation on the yield and quality of white lupine grain in the conditions of the right-bank forest-steppe of Ukraine. *Agriculture and forestry*. Vinnytsia, VSAU, № **7**. T 1, 27–36 (in Ukrainian).

Mazur, V.A., Vdovenko, S.A., Pantsyreva, H.V., Palamarchuk, I.I. 2018. Effectiveness of the application of soil milling in the growing of the squash (Cucurbita pepo var. giraumontia) in the right-benk forest stepp of Ukraine. *Ukrainian Journal of Ecology* **8**(4), 1–5.

Merkushyna, A.S. 2013. Physiological and biological bases for increasing the productivity of peas. *Biological sciences and problems of plant growing* (Uman) **8**(4), 99–105.

Mesejo, C., Rosito, S., Reig, C., Martínez-Fuentes, A. & Agustí, M. 2012. Synthetic auxin 3,5,6-TPA provokes Citrus clementina (Hort. ex Tan) fruitlet abscission by reducing photosynthate availability. *Journal of Plant Growth Regulation* **31**(2), 186–194.

Mohammad, N. K. & Mohammad, F. 2013. Effect of GA3, N and P ameliorate growth, seed and fibre yield by enhancing photosynthetic capacity and carbonic anhydrase activity of linseed. *Integrative Agriculture* **12**(7), 1183–1194.

Muhammad, I. & Muhammad, A. 2013. Gibberellic acid mediated induction of salt tolerance in wheat plants: Growth, ionic partitioning, photosynthesis, yield and hormonal homeostasis. *Environmental and Experimental Botany* **86**, 76–85.

Palamarchuk, I.I. 2017. Influence of the variety and growth stimulator of plants on the dynamics of the growth of the area of the squash apparatus zucchini in the conditions of the forest-steppe of the right-bank. *Agriculture and forestry* (Vinnytsia) **6**, 32–40 (in Ukrainian).

Pantsyreva, H.V. 2017. Formation of grain productivity of white lupine depending on technological methods in the right-bank forest-steppe. Dissertation for obtaining a scientific degree of the candidate of agricultural sciences. Kam'ianets-Podilskyi, 100–101 (in Ukrainian).

Pantsyreva, H.V. 2016. The Influence of the Elements of the Technology of Growing on the Individual Productivity of Lupine Plants in the White Conditions of the Right Bank Forest Steppe. *Journal DDAEU, Agriculture ecology. Agronomy science* (Dnipro) **2**, 16 (in Ukrainian).

Piotrowska-Niczyporuk, A. & Bajguz, A. 2014. The effect of natural and synthetic auxins on the growth, metabolite content and antioxidant response of green alga Chlorella vulgaris (Trebouxiophyceae). *Plant Growth Regulation* **73**(1), 57–66.

Polyvanyj, S.V. & Kuryata, V.G., 2015. Effects of treptolem on morphogenesis, productivity and qualitative characteristics of poppy oil. *Agrobiologija* **117**(1), 65–72 (in Ukrainian).

Poprotska, I.V. & Kuryata, V.G., 2017. Features of gas exchange and use of reserve substances in pumpkin seedlings in conditions of skoto- and photomorphogenesis under the influence of gibberellin and chlormequat-chloride. *Regulatory Mechanicms in Biosystems* **8**(1), 317–322.

Rai, R.K., Tripathi, N., Gautam, D. & Singh, P. 2017. Exogenous application of ethrel and gibberellic acid stimulates physiological growth of late planted sugarcane with short growth period in subtropical India. *Journal of Plant Growth Regulation* **36**(2), 472–486.

Ren, B., Zhang, J., Dong, S., Liu, P. & Zhao, B. 2017. Regulations of 6-benzyladenine (6-BA) on leaf ultrastructure and photosynthetic characteristics of waterlogged summer maize. *Journal of Plant Growth Regulation* **36**(3), 743–754.

Rogach, T.I. 2009. Particularity of morphogenesis and productivity of sunflower plants under the influence of treptolem. Fiziologija Roslyn: *Problemy ta Perspektyvy Rozvytku* **2**, 680–686 (in Ukrainian).

Tubic, L., Savic, J., Mitic, N., Milojevic, J., Janosevi, D., Budimir, S. & Zdravkovic-Korac, S. 2016. Cytokinins differentially affect regeneration, plant growth and antioxidative enzymes activity in chive (Allium schoenoprasum). *Plant Cell, Tissue and Organ Culture January*, **124**(1), 1–14.

Xing, X., Jiang, H., Zhou, Q., Xing, H., Jiang, H. & Wang, S. 2016. Improved drought tolerance by early IAA - and ABA-dependent H2O2 accumulation induced by α-naphthaleneacetic acid in soybean plants. *Plant Growth Regulation* **80**(3), 303–314.

Zhao, H., Cao, H., Ming-Zhen, P., Sun, Y. & Liu, T., 2017. The role of plant growth regulators in a plant aphid parasitoid tritrophic system. *Journal of Plant Growth Regulation* **36**(4), 868–876.

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A systematic approach to exploring the role of primary sector in the development of Estonian bioeconomy

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Abstract. The aim of this paper is to provide a systematic overview of biomass production and the use of biomass for the production of key products, as well as to map businesses operating in the field of bioeconomy in Estonia. The importance of primary sector in Estonian economy has decreased over the last decade. At the same time, the competitiveness of primary sector has increased, which will, in the future, contribute towards a stable production of biomass. Therefore, bioeconomy and respective business models are some of the key ways of coping with climate change. Innovative ways to transform the use of natural resources in a conscious manner are being mapped in Estonia as well as in other member states of the European Union. Comprehending the current use of biomass is essential for finding new sustainable management solutions. Acknowledging these aspects, the study explores biomass production in Estonia. One of the aspects observed during the period 2014–2017 is the proportion of the primary sector in total gross value added and the use of biomass in the food and feed industry. The results of the paper are presented in the form of Sankey diagrams, which illustrate noteworthy connections.

Key words: bioeconomy, primary sector, biomass production, regional development, innovation.

INTRODUCTION

Different sectors of economy are usually highlighted whenever new developments arise. The emergence of bioeconomy is associated with sustainable management of the environment. In the next decade, the sustainability of economy will increasingly depend on bioresources and emerging technologies. Scientific research is applied at an increasing rate to explore the possibilities of bioeconomic sustainability. Numerous countries have also taken steps to map the potential of their bioeconomy. Since guidelines for mapping the prospects for developing bioeconomy have reached the level of European Union (EU) policy, most European countries are in the process of creating a strategic view of their bioeconomy. The Europe 2020 strategy defines bioeconomy as a key element of sustainable economic growth which reduces fossil fuel dependence (European Commission, 2012; European Commission, 2018). In OECD's strategic view, bioeconomy can increase the environmental sustainability of food, feed and fibre production, improve water quality, provide renewable energy and improve the health of animals (OECD, 2009).

Several studies – Vandermeulen et al., 2011; Bugge et al., 2016; Efken et al., 2016; D'Amato et al., 2017; Fuentes-Saguar et al., 2017; Ronzon, et al., 2017a; Ronzon, et al., 2017b; Dietz et al., 2018 – have compiled an overview of the use of the bioeconomy concept and provide solutions for aggregating the activities related to bioeconomy. Pfau et al (2014) have prepared a systematic review of scientific literature regarding bioeconomy and describe how authors address the concept of sustainability. The vision of sustainable economic development is of utmost importance in the concept of bioeconomy.

An increasing number of public and political debates are touching on the problem of potential competition between the use of bioresources for food or fuel production. Negative effects of the change of land use and the altering of existing consumer goods to incorporate more bioresources are among other frequently mentioned issues. Thus, one may observe that bioeconomy with its respective business models is one of the key ways to cope with climate change and ensure sustainable economic development (Scarlat et al, 2015).

The goal of this paper is to provide a systematic overview of biomass production and the use of biomass for the production of key products, as well as to map businesses operating in the field of bioeconomy in Estonia. As bioeconomy is widespread and encompasses different sectors, the primary challenge is to understand the current use and potential change of biomass use on the product level. In order to evaluate the size of Estonian bioeconomy, it is prudent to measure the volume of food and feed industry first. The novelty of the study consists in providing an overview of the relationship between biomass production and its use in the food industry while also mapping the primary products. Furthermore, we outline specific issues that have arisen in the process of mapping biomass production, as well as relationships between different sectors, providing focus points for further research and policy development.

The aim of mapping is also to identify underused resources and implement research at a more detailed level than biomass mappings have done thus far. The BERST project (BioEconomy Regional Strategy Toolkit for benchmarking and developing strategies, 2016) and DataM of the Joint Research Centre of the European Commission (Ronzon, et al., 2017a) have compiled large volumes of data on EU member states to illustrate biomass supply from the quantitative perspective. A recent systematic approach to understanding and quantifying the EU's bioeconomy was provided, for example, by Ronzon and others (Ronzon, et al. 2017b). They identified three main types of bioeconomy and categorized EU member states into three groups according to them. Estonia was placed in Group B together with Hungary, Cyprus, Malta, the Czech Republic and Slovakia. The bioeconomy in Group B states features below EU average labour productivity in bioeconomy and above EU average share of employment in sectors (partially) manufacturing biomass. In a nutshell, the following should be noted: in Estonia, 68.6 thousand employees were employed in bioeconomy and it generated 6 million euros of turnover or 2 million euros of value added in 2015 (Ronzon, et al., 2017a). Previous studies shed no light on the use of biomass at the product level, which this study will do. Sankey diagrams have rarely been used to present analytical results in earlier articles. Here the overview of biomass products has been presented using Sankey diagrams that illustrate bio-based raw material flows towards food processing and other industries. These diagrams are used as a reference for describing and characterizing bioeconomy in Estonia, thus demonstrating the usefulness of such visual aids for a better understanding of production flows.

It is important to note that biomass production cannot increase significantly and that competition for bioresources between different sectors is increasing. The sustainability of the secondary sector depends on how well biotechnology-based products and sustainable management are developed. The primary sector's gross value added (GVA) and business links could increase significantly in the future, compared to the business model that is currently mainly tied to the food industry. In this study, we use data on GVA to measure the size of the agriculture and food industry.

The research outlook is dependent on the direction of the Estonian bioeconomy development study under preparation (project "Bioeconomy value chains in Estonia", duration 2018–2021). Bugge et al. (2016) have found that the vision of bioeconomy could be based on different characteristics. Biotechnology vision, bioresource vision or bioecology vision are prospective perspectives. Based on findings on the development direction of Estonian bioeconomy, this study is primarily aimed at evaluating the value of the resources, which hints at an aim towards economic growth and sustainability. The creation of value depends on the conversion and upgrading of bio-resources.

MATERIALS AND METHODS

In order to ensure transparency and reproducibility, the methodology relies on Estonian official statistics (SE), which is part of Eurostat data. It is used as secondary data, supplemented by authors' calculations to fill data gaps. Based on the European Commission's (EC) definition, the study defines a methodology for the quantification of four primary dimensions: (1) classification of activity sectors (NACE Rev. 2); (2) gross production and total output; (3) gross value added (GVA); and (4) product.

As a first step, we use the official definition of bioeconomy provided in EC's communication COM(2012) 60 and interpretations of related activities defined in earlier studies, which are reflected in the range of selected activity sectors from NACE Rev.2 (Eurostat, 2008). According to the EC's communication COM(2012) 60, bioeconomy includes 'the production of renewable biological resources and their conversion into food, feed, bio-based products and bioenergy. It includes agriculture, forestry, fisheries, food and pulp and paper production, as well as parts of chemical, biotechnological and energy industries'.

Various understandings of biomass exist due to the different characteristics of such resources. As explained by Zörb & Lewandowski (2017), biomass resources can be classified according to their (1) origin (plants, animals, microorganisms), (2) sector (agriculture, fishing, forestry, waste), (3) physical conditions (solid, liquid), and (4) major component (starch, sugar, lignocellulose, oil, protein). A broad definition of biomass would include 'all resources containing non-fossil, organic carbon, recently (< 100 years) derived from living plants, animals, algae, microorganisms or organic waste streams' (Zörb & Lewandowski, 2017). This paper considers as biomass such organic resources that originate from plants or animals and are produced in agricultural, fishing and forestry sectors. Processed food is also included in our definition of biomass.

Taking into account previous studies in the selection of activities (Rönnlund et al., 2014; Haarich et al., 2017; Mainar-Causapè et al., 2017; Ronzon et al., 2017a; Vitunskienè, et al., 2017), primary sector and its industry chain include 16 main fields of activities that form the entirety of a bio-based economy. Several authors (Efken et al., 2016; Heijman, 2016) assume that primary sector belongs entirely under bioeconomy. This conclusion is logical because biomass production is traditionally dependent on natural resources, although some fossil inputs are used. It means that the production of biomass is covered by section A of NACE Rev. 2, which is comprised of agricultural (A01), forestry (A02) and fishing (A03) sectors. The study uses a three- or five-digit code from NACE Rev.2 through which it is possible to describe the links between companies in bioeconomy in more detail. The definition of sectors through the NACE Rev.2 codes allows to present a production volume, the value of output and GVA calculations based the National Accounts Statistics.

A definition of bioeconomy certainly includes the processing of biomass, which, in turn, requires defining the manufacturing industry and the selection of production activities. The choice of the latter is complicated by the fact that many industries use mineral and fossil components in addition to biomass. It has previously been assumed that food industry can be considered an industry falling under the definition of bioeconomy (NACE Rev. 2 section C, 12 downstream activity sectors (C10–12)). As it encompasses the production and manufacturing of biomass, following NACE Rev.2 classification, food industry can indeed be considered, fully or partially, a part of bioeconomy. The three- or five-digit code from NACE Rev.2 is important for monitoring the activities that use both bio-resources and fossil raw materials. The EU Bioeconomy Report 2016 (Ronzan et al. 2017a) identifies partially bio-resource based sectors, which we also use, and which are described in Fig. 1.

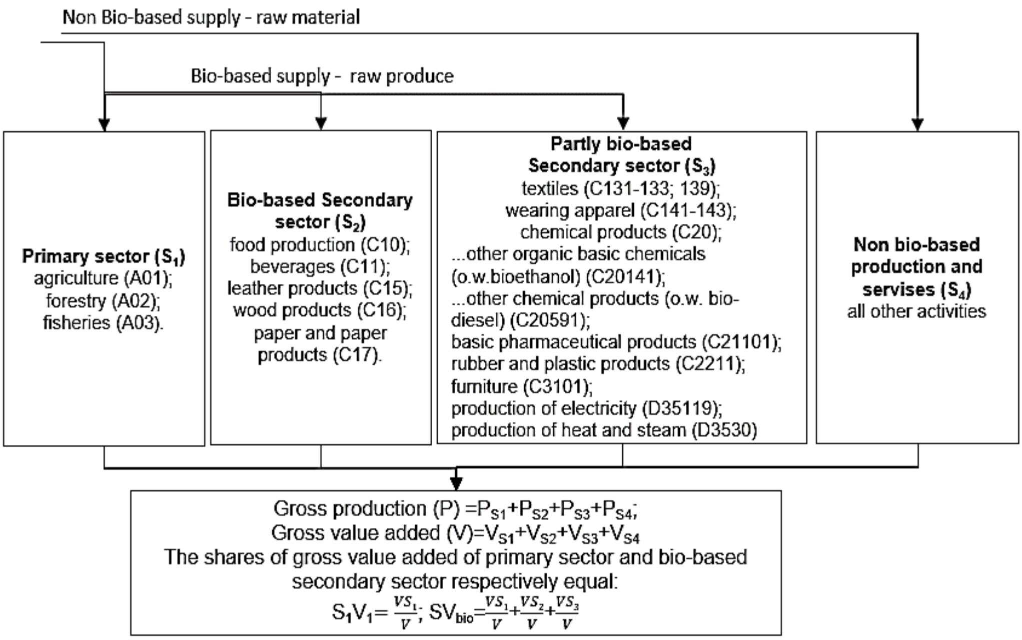

Figure 1. A simplified block scheme of selected sectors related to bioeconomy and indicators for calculations.

The indicators serving as the basis for calculations in this study are listed in Fig. 1. In order to identify the economic relevance of biomass-based products, gross production and GVA are calculated. Calculations of gross production are based on average figures from the period 2014–2016, which excludes the impact of a possible extreme year. Statistical data of GVA were recalculated according to the proportion of bioproducts fully or partly made of bio-materials. The statistics on the sale revenue (NACE C13-C14; C21 and C31) or production (NACE C20 and D35) of industrial products according to the PRODCOM list (Eurostat, 2018a) and Statistical Classification of Products by Activity was used to determine this proportion in partly bio-based manufacturing.

The main food and feed related products from the primary sector are considered when exploring the role of primary sector. The List of Products of the European Community (Eurostat, 2018a; PRODCOM list 2018) was used for product level analysis to describe biomass products.

RESULTS AND DISCUSSION

We will first take a look at the context indicators that characterize bioeconomy. The proportion of agriculture, forestry and fisheries in Estonian GVA was 2.5% in 2016, while the share of the aforementioned sectors in the GVA of other EU member states was on average 2.9% (Eurostat, 2018b), which is a markedly low level. Vásáry and Szabó (2018) discovered in the BIOEAST Initiative study that in the period between 2008 and 2015, there was a decreasing trend in the number of people employed in agriculture, forestry, fishing and aquaculture sectors, as well as in the food, beverages and tobacco sectors in the BIOEAST countries. After the global financial and economic crisis, the level of turnover and value added has remained stable since 2012. Similar changes have occurred in Estonia's labour market. The number of people employed in the above-mentioned sectors dropped from 31,361 in 2008 to 30,252 in 2015. The number of employees decreased by a further half a thousand by 2017. The fact also implies that labour productivity has increased significantly, but this is a relative presumption. It is worth pointing out that current emphasis on bioeconomy and negative changes in macro-statistics are contradictory. Conventional industry has thus far failed to develop and valorise biomaterials and it signifies the importance of innovative solutions for production in bioeconomy.

In order to provide a comprehensive overview of the main biomass production in Estonia, one must first focus on biomass production in the primary sector. The data are presented for the most important outputs of all the activities in primary sector, but it must be taken into account that part of the biomass is also used within the primary sector to produce the next level biomass (e.g. the production of milk from grass feed). The authors argue that it is necessary to know all the resources in order to find solutions for a better use of biomass. The biomass structure, production and value in Estonia are presented in Table 1 which demonstrates that the gross production of agricultural and fishery sectors is 6,348 thousand tonnes and forestry yield is 13,557 thousand m^3.

Biomass produced in agriculture is divided into two groups: the main products (cultivated crops such as cereals, potatoes or milk and meat) and the accompanying residues (e.g. straw, other plant residues or animal skins, wool, animal bristles). The analysis revealed that national statistics about accompanying residues are lacking. For a more accurate mapping of biomass and bioeconomy, it is necessary to collect more data on biological resources mentioned in Table 1. There is no data on waste management (NACE E3821) or biological waste, so discussions from the bio-waste perspective are not fact-based.

Table 1. Biomass production and value 2014–2016 average in Estonia

Sector	Commodity	Gross production, thousand tonnes; * thousand m^3	Production value, million €	Share of production value, %
Agriculture	cereal	1,244	177	8.6
	legumes	78	14	0.7
	technical crops, including oil crops	178	54	2.6
	vegetables, potatoes	185	62	3.0
	berries and fruits	6	7	0.3
	fodder roots	0.9	0.1	0.01
	grazed biomass	3,763	75	3.6
	sheep and goat meat	0.7	2	0.1
	poultry	19	30	1.5
	pork	45	79	3.8
	beef	13	46	2.2
	egg	12	14	0.7
	raw milk	723	229	11.1
Fishery	ocean fishing	13	44	2.1
	aquaculture	3	3	0.1
	Baltic Sea and inland fishing	64	10	0.5
Agriculture and fishery	total	6,348	846	-
Forestry*	fuelwood and felling waste	3,796	73	3.5
	birch and aspen pulpwood	2,034	71	3.4
	conifer pulpwood	1,762	64	3.1
	soft- and hardwood log	1,220	91	4.4
	conifer log	4,745	921	44.6
Forestry	**total**	**13,557**	**1,220**	
Total		**-**	**2,066**	**100**

Source: Statistics Estonia, 2018; State Forest Management Centre 2018 (Average roadside prices in state forest in 2018); Ministry of Rural Affairs fishing catch and prices database 2018; Authors' calculations.

Comparing two different sectors – agriculture and fisheries – with forestry, it is apparent that the calculation units of biomass are different. The quantity of wood is measured in cubic meters, which hints at a need for conversion. The conversion would scientifically be necessary, but the sector's conventions are based on cubic meters, which calls the intelligibility of the data into question. Forest felling measurement and statistics are largely model-based, where a conversion of volumes would cause an increase in the margin of error. Based on the value of production, once can surmise that since wood biomass supply amounts to 59% of total biomass, it has a significant importance in Estonia. Considering the average raw milk production share 11% and cereal production share 8.6% of the total production value, it can also be deduce that agriculture plays an important role. Thus, we conclude that the role of agriculture and forestry sectors as suppliers of biomass in Estonia is of utmost significance. Such a result confirms the results from a previous study, where Ronzon et al. (2015) exemplified that the turnover of bioeconomy is primarily generated by forestry and its downstream industries. In Finland, Sweden, Latvia and Estonia it generates more than 40% of the countries' bioeconomy. Kargytė et al. (2018) also revealed that four Norwegian regions and two Estonian regions share a high potential for developing knowledge-based circular bioeconomy. Of course, this potential can only be utilised if R&D specialisation matches the needs of identified bioeconomy sectors and if a regional business cluster takes the lead. Sweden and Finland also display an orientation towards bio-based chemical industry.

In terms of value chains, this primarily implies that the production of food and wood materials provides added value to the usage of biological resources. In order to explore the role of primary sector in ensuring food supply, product data is analysed at the level of the PRODCOM 2018 list. Due to the aforementioned results, we focus on a larger volume of resources such as the flow of cereals (1,244 thousand tonnes), raw milk (723 thousand tonnes) and oilseeds (178 thousand tonnes) (Table 1). Therefore, in order to get a realistic estimate regarding of the position of domestic production of biological resources, international trade (imports of biological raw products) as an input for downstream industries is also taken into account.

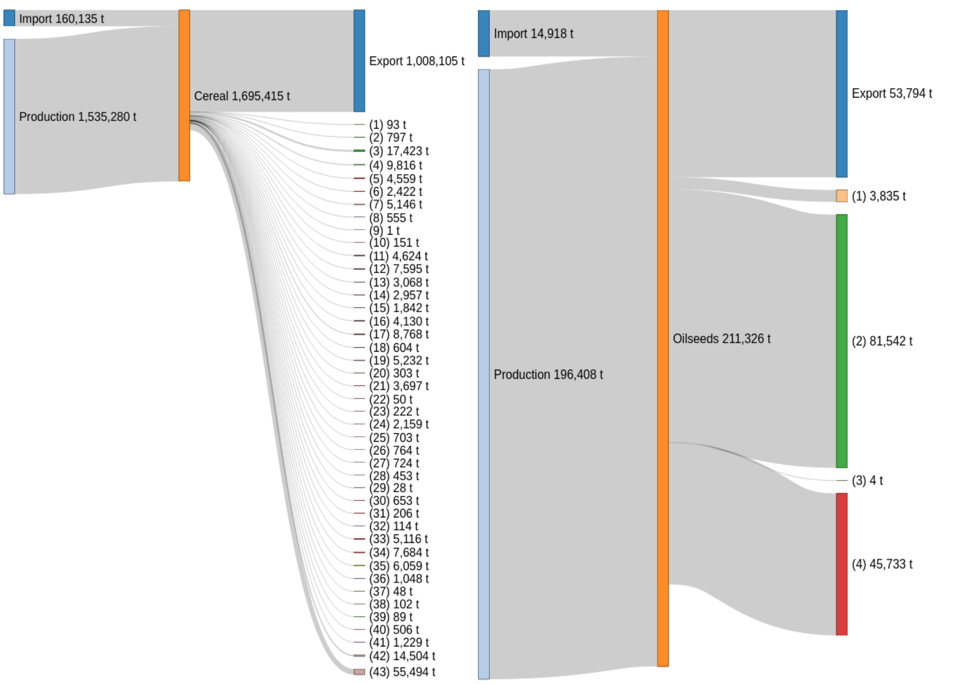

Figure 2. Cereal (on the left) and oilseeds (on the right) flows and total production in 2016. Source: Statistics Estonia, 2018 (see the meanings in Appendix A).

Compared to grain and oilseed products, the nomenclature is very different (Fig. 2; see the meanings in Appendix A) due to the characteristics of the raw material. In the case of raw materials for cereals, large quantities of cereals are imported into Estonia (160 thousand tonnes), while exports amount to more than 1 million tonnes. As the example of grain industry highlights, the importance of raw material cannot be completely attributed to domestic production. Cereal processing is diverse, but it is still characterized by traditional cereal products like wheat flour ((43) 55 thousand tonnes), residues from the sifting of wheat ((3) 17 thousand tonnes), toast ((42) 15 thousand tonnes) and fresh white bread ((17) 9 thousand tonnes). Considering the large quantities of exported cereals, further development of grain processing could be a possible area for growth. Product development and production depend on innovation and export.

When it comes to evaluating oilseed industry, its primary products are refined rape oil and its fractions ((4) 46 thousand tonnes, excluding chemically modified), and oilcake and other solid residues resulting from the extraction of rape or colza seed fats or oils ((2) 81 thousand tonnes; Fig. (2)). Other oil fractions and chemically modified oils have a very low share, reflecting the level of development of the sector and its inclination towards traditional products.

In the comparison of milk products, drinking milk has the largest volume (Fig. 3; (11) 98 thousand tonnes), but from the perspective of dry matter content, cheese is definitely noteworthy. For instance, unripened or uncured cheese (fresh cheese, (27)) production quantity per year is 18 thousand tonnes, and the annual production of (9) grated, powdered, blue-veined and other non-processed cheese (excluding fresh cheese, whey cheese and curd) is 26 thousand tonnes.

Looking at marketing figures, it is evident that processed cereal, raw milk and oilseed products are mainly geared towards the domestic Estonian market, whereas export figures show that the primary sector is a raw material provider at the global level. The results underline that food production is still dominated by traditional products and it is possible to implement new technologies to produce biomaterials.Based on the performed analysis, it is possible to observe primary connections, but local impacts of bioeconomy can also be evaluated through participatory methods.

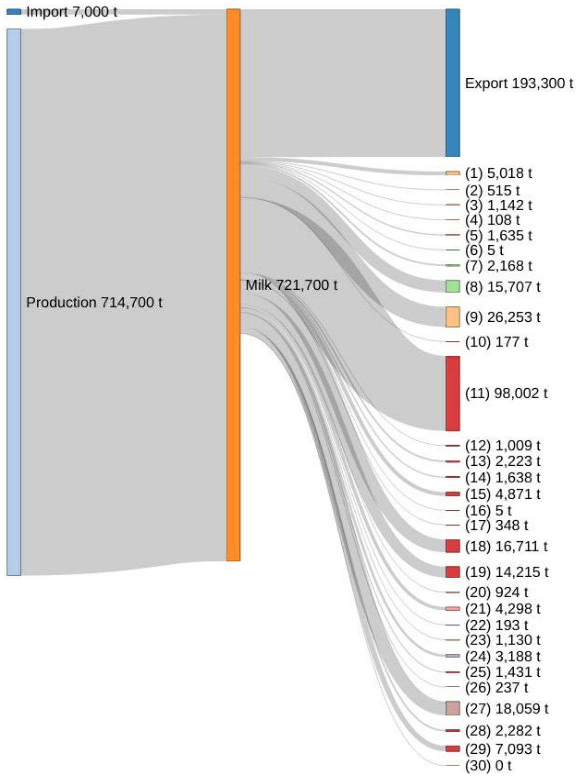

Figure 3. Milk flows and total production in 2016. Source: Statistics Estonia, 2018 (see the meanings in Appendix A).

The above shows that successful product and process development within bioeconomy sectors is demanding and multidimensional since it has to take into consideration societal concerns. Collecting data and information from local stakeholders enables recognising the value chains that complement the models (Paula & Birrer, 2006; Mattila et al., 2018).

Having identified possible product-based trends, the authors of the article with the opinion voiced in previous studies (Rönnlund et al., 2014; Ronzon et al., 2015) on Nordic countries. The largest innovation and growth potential of bioeconomy could lie in bio-based chemicals, biomaterials, biofuels, biorefineries, and certainly bioenergy.

Given the above, it can be stated that the proportion of primary sector and bio-based secondary sector in GVA is one of the basic values characterising the scale of biomass production. In Estonia, this indicator showed that the share of bioeconomy fluctuated between 12.7% to 13.7% (Fig. 4) in the period of 2014–2017. It is important to ensure the sustainable development of bioeconomy, given that the observed short-term period shows significant instability in the share of the selected sectors.

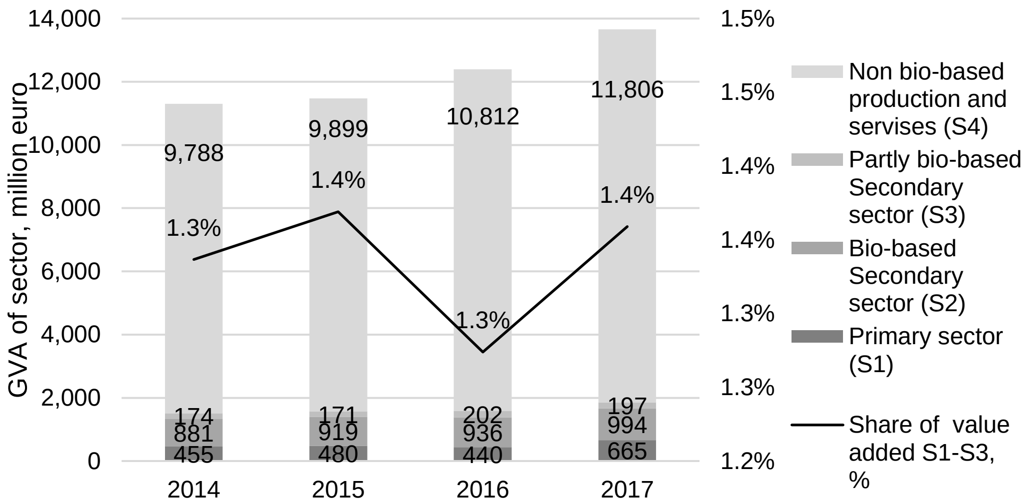

Figure 4. Development of gross value added within the selected sectors of bioeconomy in Estonia (Statistics Estonia, 2018).

It must also be stressed that despite the primary sector and food production (bio-based secondary sector) contributing only 11.8% and the primary sector separately 4.2% to the total gross value added of the entire Estonian economy, these sectors have a significant impact on the economy. At the same time, the major share of GVA of the bio-based sectors (S_1-S_3) was created in the bio-based secondary sector (S_2, 58%). The role of primary sector (S_1) is approximately 30% because one third of GVA came from all the bio-based sectors (S_1-S_3) in total.

In the period 2014–2017, gross value added increased reasonably slowly and the change has occurred in all analysed sectors. In 2014, the gross value added of bio-based and partly bio-based sectors (S_1-S_3) in total was 2,023 million euros and in 2017 the value was 2,360 million euros. This implies that the gross value added by bioeconomy is steadily improving. Non-bio-based sectors of the economy have apparently enjoyed higher growth rates than bio-economy. However, the primary aim of the study was not to monitor the change, but to illustrate biomass production and the size of bioeconomy by analysing the interconnected sectors in Estonia which make up the value chain.

Thus, industries like retail, wholesale and catering are excluded from our study of bioeconomy. We believe that further research will necessitate establishing a more precise definition of the share of bio-resource use for the aforementioned industries.

CONCLUSIONS

The scope of this study was limited to giving a systematic overview of biomass production and the use of biomass for the production of key products, and mapping businesses related to the field of bioeconomy in Estonia. However, keeping in mind the limitations of a quantitative analysis based on national statistics, the results of the analysis illustrate major aspects regarding the role of the primary sector and its possible relations to bioeconomy. The analysis of financial indicators encompassed four components: gross production, total output, gross value added, and product.

The results of the study are decisively influenced by the selection criteria of bio-based sectors and the level of detail of the data. This poses a challenge, as it means that there are inherent discrepancies in the measurement of bioeconomy. Moreover, the definition of the concept of bioeconomy as such may differ from region to region. Therefore, it is also appropriate to use databases created at the European level. The mapping of a national development strategy and development needs must take place at a level that provides an overview of possible products and value chains. Based on the value of production, one could conclude that forestry and agriculture play an important role in Estonia. In terms of value chains, this primarily indicates that the production of food and wood materials is what mainly provides added value in the use of biological resources.

In exploring the role of primary sector in ensuring the supply of food, results indicate that cereal processing is diverse on the product level, but it is still characterized by traditional products. The same applies to the milk processing sector. Looking at marketing figures, it is evident that processed cereal, raw milk and oilseed products are mainly geared towards the domestic Estonian market, whereas export figures show that the primary sector is a raw material provider at the global level. It is certainly possible to find new technologies for the production of biomaterials in the food industry.

In the Estonia, gross value added is slowly increasing in the related sectors, yet the share of bioeconomy depends on the growth of all activities and on further development of bio-based sectors. In the period 2014–2017, the share of bio-based and partly bio-based economy was around 12.7% to 13.7% of GVA. The gross value added of bio-based sectors increased at a reasonable pace throughout the period 2014–2017.

As the article pointed out, several questions arose during the analysis, the most significant one of them being related to the future potential of biomass valorisation. In order bioeconomy to develop further in the future, it is crucial to involve different business sectors and find alternatives for adding value. It should be considered that if new opportunities for refinement in bioresource utilisation are found, steps must be taken to ensure that currently operating biomass users can continue developing their businesses. There is a possibility that those companies will develop new value chains for biomass valorisation, but food production sustainability must be guaranteed. There is always a need for greater cooperation between entrepreneurs, the private sector and research institutions in analysing the potential of new value chains and developing business models. Bottlenecks in the enhancement of value chains and in bioeconomy are an outstanding target for additional research and international cooperation.

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REFERENCES

Bugge, M.M., Hansen, T. & Klitkou, A. 2016. What Is the Bioeconomy? A Review of the Literature. *Sustainability* **8**, 691.

D'Amato, D., Droste, N., Allen, B., Kettunen, M., Lähtinen, K., Korhonen, J., Leskinen, P., Matthies, B.D. & Toppinen, A. 2017. Green, circular, bio economy: A comparative analysis of sustainability avenues. *Journal of Cleaner Production* **168**, 716–734.

Dietz, T., Börner, J., Förster, J.J., Braun, J. 2018. Governance of the Bioeconomy: A Global Comparative Study of National Bioeconomy Strategies. *Sustainability* **10**, 3190.

European Commission 2012. Innovating for Sustainable Growth: A Bioeconomy for Europe. COM(2012) 60 final. Brussels, Belgium, European Commission. https://ec.europa.eu/research/bioeconomy/pdf/official-strategy_en.pdf. Accessed 7.11.2018.

European Commission 2018. A sustainable Bioeconomy for Europe: strengthening the connection between economy, society and the environment. Updated Bioeconomy Strategy. https://ec.europa.eu/research/bioeconomy/pdf/ec_bioeconomy_strategy_2018.pdf. Accessed 15.11.2018.

Fuentes-Saguar, P.D., Mainar-Causapé, A.J. & Ferrari, E. 2017. The Role of Bioeconomy Sectors and Natural Resources in EU Economies: A Social Accounting Matrix-Based Analysis Approach. *Sustainability* **9**, 2383.

Haarich, S., Kirchmayr-Novak, S., Fontenl, A., Toptsidou, M. & Hans, S. 2017. Bioeconomy development in EU regions. Mapping of EU Member States' regions' Research and Innovation plans & Strategies for Smart Specialisation (RIS3) on Bioeconomy for 2014–2020. Spatial Foresight, SWECO, ÖIR, t33, Nordregio, Berman Group, Infyde 2017. 94 pp.

Efken, J., Dirksmeyer, W., Kreins, P. & Knecht, M. 2016. Measuring the importance of the bioeconomy in Germany: Concept and illustration. *NJAS - Wageningen Journal of Life Sciences* **77**, 9–17.

Eurostat 2008. NACE Rev. 2 Statistical classification of economic activities in the European Community. RAMON Eurostat's Metadata Server Luxembourg. https://ec.europa.eu/eurostat/ramon/index.cfm?TargetUrl=DSP_PUB_WELC. Accessed 15.11.2018.

Eurostat 2018a. European System of production statistics for mining and manufacturing https://ec.europa.eu/eurostat/statistics-explained/index.php/Industrial_production_ statistics_ introduced-PRODCOM#Classifications. Accessed 7.11.2018

Eurostat 2018b. Structural business statistics https://ec.europa.eu/eurostat/web/structural-business-statistics/data/main-tables. Accessed 7.11.2018.

Heijman, W. 2016. How big is the bio-business? Notes on measuring the size of the Dutch bio-economy. *NJAS - Wageningen Journal of Life Sciences* **77**, 5–8.

Kargytė, V., Värnik, R. & Aleknevičienė, V. 2018. Assessment of bioeconomy development potential from the perspective of innovation economics in European regions. *Management Theory and Studies for Rural Business and Infrastructure Development* **40**, 492–513.

Mainar-Causapè, A., Philippidis, G. & Sanjuan, A.I. 2017. Analysis of structural patterns in highly disaggregated bioeconomy sectors by EU Membre states using SAM/IO multipliers. JRC Tehchnical reports. European Commission-Joint research Centre. http://publications.jrc.ec.europa.eu/repository/bitstream/JRC106676/kj-na-28591-en-n_.pdf. Accessed 7.11.2018.

Mattila, T.J., Jáchym Judl, J., Macombe, C. & Leskinen, P. 2018. Evaluating social sustainability of bioeconomy value chains through integrated use of local and global methods. *Biomass and Bioenergy* **109**, 276–283.

OECD 2009. The Bioeconomy to 2030: Designing a Policy Agenda. Main Findings and Policy Conclusions. http://www.oecd.org/futures/long-termtechnologicalsocietalchallenges/thebioeconomyto2030designingapolicyagenda.htm. Accessed 9.11.2018.

Paula, L. & Birrer, F. 2006. Including public perspectives in industrial biotechnology and the biobased economy. *Journal of Agricultural and Environmental Ethics* **19**, 253–267.

Pfau, S.F., Janneke E. Hagens, J.E., Dankbaar, B. & Smits, A.J.M. 2014. Visions of Sustainability in Bioeconomy Research. *Sustainability* **6**, 1222–1249.

Ronzon, T., Santini, F. & M'Barek, R. 2015. The Bioeconomy in the European Union in numbers. Facts and figures on biomass, turnover and employment. European Commission, Joint Research Centre, Institute for Prospective Technological Studies. https://ec.europa.eu/jrc/sites/jrcsh/files/JRC97789%20Factsheet_Bioeconomy_final.pdf. Accessed 10.11.2018.

Ronzon, T., Lusser, M. & Klinkenberg, M. (ed.), Landa, L., Sanchez Lopez, J. (ed.), M'Barek, R., Hadjamu, G. (ed.), Belward, A. (ed.), Camia, A. (ed.), Giuntoli, J., Cristobal, J., Parisi, C., Ferrari, E., Marelli, L., Torres de Matos, C., Gomez Barbero, M., Rodriguez Cerezo, E. 2017a. Bioeconomy Report 2016. *JRC Scientific and Policy Report.* EUR 28468 E. https://ec.europa.eu/jrc/en/publication/eur-scientific-and-technical-research-eports/bioeconomy-report-2016. Accessed 7.11.2018.

Ronzon, T., Piotrowski, S., Robert M'Barek, R. & Carus, M. 2017b. A systematic approach to understanding and quantifying the EU's bioeconomy. *Bio-based and Applied Economics* **6**, 1–17.

Rönnlund, I., Pursula, T., Bröckl, M., Hakala, L., Luoma, P., Aho, M., Alina, P., Gaia Consulting Ltd., Pallesen, B.E., Agrotech 2014. Creating value from bioresources. Innovation in Nordic Bioeconomy. Nordic Innovation Publication 2014:01. http://nordicinnovation.org/Global/_Publications/Reports/2014/2014_01%20Innovation%20in%20Nordic%20Bioeconomy_final.pdf. Accessed 7.11.2018.

Scarlat, N., Dallemand, J.-F., Monforti-Ferrario, F. & Nita, V. 2015. The role of biomass and bioenergy in a future bioeconomy: Policies and facts. *Environmental Development* **15**, 3–34.

Vandermeulen, V., Prins, W., Nolte, S. & Van Huylenbroeck, G. 2011. How to measure the size of a bio-based economy: evidence from Flanders. *Biomass and Bioenergy* **35**, 4368–4375.

Vásáry, V. & Szabó, D. 2018. Characteristics of Sustainable Bioeconomy in the CEE Macro-region. *Central European Review of Economics & Finance* **27**, 5–26.

Vitunskienė, V., Aleknevičenė, V., Miceikienė, A., Čaplikas, J., Miškinis, V., Pilvere, I., Makutėnienė, D., Dabkienė, V., Lekavičius, V., Øistad, K., Ramanauskė, N., Kargytė, V., Jazepčikas, D., Serva, E. & Markelytė, A. 2017. Lithuanian Bioeconomy Development Feasibility Study. Aleksandras Stulginskis University. pp. 179.

Zörb, C. & Lewandowski, I. 2017. Biobased Resources. *Bioeconomy: Shaping the Transition to a Sustainable, Biobased Economy*. New York, NY: Springer Berlin Heidelberg, pp. 77–86.

APPENDIX A

1. List of cereals, oilseeds and milk products based on PRODCOM 2018.

Grain products: 1 Barley meal; 2 Bran, sharps and other residues from the sifting, milling or other working of cereals (excluding maize (corn), rice, wheat); 3 Bran, sharps and other residues from the sifting, milling or other working of wheat; 4 Bread rolls (net weight less than 150 g); 5 Buns (net weight 100 g or more); 6 Cake and pastry products; other bakers' wares with added sweetening matter; 7 Cakes (in pieces, girdle-cakes); 8 Cereals in grain form, precooked or otherwise prepared (excluding maize); 9 Flour, meal, powder of edible vegetable, fruit or nuts; 10 Fresh bread containing fruit, vegetables etc.; 11 Fresh black bread (content of rye flour 50.1–89.9%); 12 Fresh black bread (content of rye flour more than 90%); 13 Fresh bread containing seeds or grain; 14 Fresh brown bread; 15 Fresh crusty bread; 16 Fresh fine rye bread (from pure white rye flour); 17 Fresh white bread (content of wheat meal at least 90%, weight more than 150 g (excluding toast); 18 Fresh white bread containing more than 2% of cereals, grain, fruit and vegetables, raisins etc.; 19 Fresh white bread from various meals (content of wheat meal more than 50%); 20 Groats of common wheat and spelt; 21 Meal of common wheat and spelt; 22 Meal of other cereals (excluding wheat, rye and barley); 23 Meslin flour; 24 Mixes and doughs for the preparation of bread, cakes, pastry, crispbread, biscuits, waffles, wafers, rusks, toasted bread and similar toasted products and other bakers' wares; 25 Muffins; 26 Oat flakes; 27 Other prepared foods obtained by the swelling or roasting of cereals; 28 Pastry products with added sweetening matter; 29 Pearl-barley; 30 Pies (baked, filled or covered, excluding strudels, pies baked in oil); 31 Pies baked in oil, doughnuts, chebureki etc.; 32 Pre-baked pastry goods; 33 Pure cream rye flour; 34 Pure white rye flour; 35 Rye whole grain meal; 36 Semolina; 37 Sponge cakes (excluding muffins); 38 Sponge, biscuit; 39 Strudels; 40 Swiss rolls; 41 Tarts; 42 Toast; 43 Wheat flour.

Oilseed products: 1 Crude rape, colza or mustard oil and their fractions (excluding chemically modified); 2 Oilcake and other solid residues resulting from the extraction of rape or colza seed fats or oils; 3 Other oils and their fractions, refined but not chemically modified, fixed vegetable fats and other vegetable oils (except maize oil) and their fractions; not elsewhere classified refined but not chemically modified; 4 Refined rape, colza or mustard oil and their fractions (excluding chemically modified).

Milk products: 1 Butter of a fat content by weight <= 85%; 2 Butter of a fat content by weight > 85% and other fats and oils derived from milk (excluding dairy spreads of a fat content by weight; < 80%); 3 Buttermilk; 4 Buttermilk powder; 5 Condensed or evaporated milk, sweetened; 6 Dairy spreads of a fat content by weight < 80%; 7 Flavoured liquid acidified milk (curdled milk, cream and other fermented products flavoured or containing added fruit, nuts or cocoa); 8 Flavoured liquid yoghurt; 9 Grated, powdered, blue-veined and other non-processed cheese (excluding fresh cheese, whey cheese and curd); 10 Milk and cream of a fat content by weight of <= 1%, not concentrated nor containing added sugar or other sweetening matter, in immediate packings of a net content <= 2 l; 11 Milk and cream of a fat content by weight of > 1% but <= 6%, not concentrated nor containing added sugar or other sweetening matter, in immediate packings of a net content <= 2 l; 12 Milk and cream of a fat content by weight of > 1% but <= 6%, not concentrated nor containing added sugar or other sweetening matter, in immediate packings of a net content > 2 l; 13 Milk and cream of a fat content by weight of > 21%, not concentrated nor containing added sugar or other sweetening matter, in immediate packings <= 2 l; 14 Milk and cream of a fat content by weight of > 21%, not concentrated nor containing added sugar or other sweetening matter, in immediate packings of > 2 l; 15 Milk and cream of a fat content by weight of > 6% <= 21%, not concentrated nor containing added sugar or other sweetening matter, in immediate packings <= 2 l; 16 Milk and cream of a fat content by weight of > 6% <= 21%, not concentrated nor containing added sugar or other sweetening matter, in immediate packings > 2 l; 17 Non-flavoured curdled milk; 18 Non-flavoured kephir; 19 Non- flavoured sour-cream; 20 Non-flavoured curdled milk drinks (excluding kephir, curdled milk and yogurt); 21 Non-flavoured yogurt; 22 Other products containing milk fats and vegetable fats, milk fat content 10–80%; 23 Processed cheese (excluding grated or powdered); 24 Skimmed milk powder of a fat content by weight of <= 1.5%, in immediate packings of > 2.5 kg; 25 Solid skim milk (excluding for drinking); 26 Substitutes of whole milk powders, of a fat content by weight of > 1.5%), in immediate packings of > 2.5 kg; 27 Unripened or uncured cheese (fresh cheese) (including whey cheese and curd); 28 Whey and modified whey in liquid or paste forms; 29 Whey and modified whey in powder, granules or other solid forms; 30 Whole milk powder, of a fat content by weight of > 1.5%, in immediate packings of > 2.5 kg.

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Assessment of wild plants for phytoremediation of heavy metals in soils surrounding the thermal power station

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Abstract. The present investigation was carried out to evaluate the phytoextraction potential of three main wild plant species: annual nettle (*Urtica urens* L.), daisy fleabane (*Stenactis annua* (L.) Ness.) and yarrow (*Achillea millefolium* L.) that grow spontaneously in heavy metal contaminated areas near the thermal power station in Kakanj, Bosnia and Herzegovina. Analyses of the heavy metal content (Ni, Fe, Cr, Cu, Zn, Cd, Pb, Mn) in soil and plant samples taken from the examined area were performed using atomic absorption spectrophotometry. The results obtained revealed that the examined soils are polluted by Ni and Pb and contain relatively high value of Cr and Fe. Annual nettle, daisy fleabane and yarrow have not shown high efficiency in the absorption and accumulation of heavy metals from polluted soils, and therefore these plants are not be considered as potential phytoremediators of soils on the examined area. Furthermore, the results of the study undoubtedly confirm the fact that the total content of heavy metals in soils is not a sufficient parameter for estimating the toxicity of heavy metals in soils and consequently for their transfer and accumulation in plants.

Key words: environment, heavy metals, pollution.

INTRODUCTION

Thermal power station in Kakanj (TPS) uses coal for electricity generation and is one of the largest environmental polluters in Bosnia and Herzegovina. Environmental deterioration is attributed to emission of large amount of carbon dioxide (CO_2), sulphur dioxide (SO_2), nitric oxide (NO), particulate matter (PM) and heavy metals as result of coal burning (Guttikunda & Jawahar, 2014). These hazardous materials are accumulated in environment and lead to severe environmental and health impacts, causing respiratory and related ailments to humans and animals and leading to disorder in physiological processes in the plant (Nagajyoti et al., 2010). Soil and water contaminated with heavy metals may pose risks and hazards to human health through: consumption of fruits and vegetables grown on these soils, drinking of contaminated ground water, reduction in land usability for agricultural production, etc. (Morais et al., 2012).

The release of heavy metals from TPS and their subsequent deposition in soil is a growing global concern that requires much greater attention of society. The necessity for harmonization of thermal engineering with environment for meeting the needs of the living and future generations has become obvious. One of the ways in which above mentioned harmonization can be achieved is restoration of heavy metal contaminated soils and investment in modern and environmentally-friendly technology, thus reducing environmental pollution due to TPS activity. Different approaches are used for the restoration of heavy metal contaminated soils: physical, biological and chemical (Sharma et al., 2018). Most physical and chemical approach are expensive and do not make soil acceptable for plant growth. In contrast, biological approach (phytoremediation) offers green technology solution for the heavy metal contamination problem without any negative impact on the environment (Marques et al., 2009).

Phytoremediation represents a group of innovative technologies that use plants to remove, detoxify, or stabilize persistent heavy metals and other contaminants in soils by inactivating contaminants in the rhizosphere or translocating them in the above-ground parts (Lone et al., 2008).

However, the ability to remediate heavy metals in soils varies significantly between plants, as different mechanisms of heavy metal absorption are operative in each plant species, based on genetic background of plants, and their morphological, anatomical, and physiological characteristics (Liu et al., 2013).

According to Yoon et al. (2006), wild plants should be preferred for phytoremediation since these plants are often better in terms of growth, development, reproduction and generally survival under stress conditions compared to plants introduced from other environments.

Some wild plants such as annual nettle (*Urtica urens* L.), daisy fleabane (*Stenactis annua* (L.) Ness.) and yarrow (*Achillea millefolium* L.) spontaneously growing on soils around TPS in high density and therefore these plants could be very interesting candidates as potential phytoaccumulator or phytostabilizer of heavy metals from contaminated soils on the examined area. These plants, as annual plants, could be used to remove heavy metals from contaminated soils by harvesting/coppicing (Jacobs et al., 2017). However, up to now, there is no systematic investigation in Bosnia and Herzegovina related to the possibility of using these plant species for that purpose, especially not in soils surrounding the thermal power stations.

The main objectives of this study were: 1) to identify the wild plants that grow in high density in soils around TPS; 2) to determine the content of heavy metals (Cu, Zn, Pb, Co, Cr, Fe, Ni and Mn) in examined soil and plant samples 3) to evaluate ability of selected wild plants to remove or stabilize heavy metals based on the determined soil-plant transfer factor (TF).

MATERIALS AND METHODS

Study Area

TPS (44°5′26″ N, 18°6′51″ E) is located on the left bank of the river Bosnia, five kilometers upstream of Kakanj town, in Central Bosnia. The TPS includes three units with a total installed capacity of 450 MW. The climate in this area is classified as Cfb by Köppen and Geiger. The average annual temperature in Kakanj is 10.7 °C, and precipitation here averages 960 mm.

Species richness estimation

Species richness (i.e., the number of species) is the simplest concept for evaluating vegetation community in some area.

Since the plants were not equally distributed around the TPS, only the areas where the individual plants grew in high density were the subject of research. Three study plots (each 100 m^2) with these characteristics were established in the immediate vicinity of TPS. A 1 m x 1 m quadrat was used to study plant population and each experimental plot had three quadrats. High density of dominant plant species and cover characteristics was criteria for quadrat selection.

Plant density was determined by counting the number of individuals of plant species in uniformly sized sample plots within a site. The dominant plant species on the experimental plot 1, 2 and 3 was annual nettle (*Urtica urens* L.), daisy fleabane (*Stenactis annua* (L.) Ness.) and yarrow (*Achillea millefolium* L.), respectively (Table 1).

Table 1. Density of dominant plant species at the experimental soil plots

Plant species	Number of individuals in each quadrat			Total number of individuals	Total number of quadrat studies	Density S/Q
	I	II	III	(S)	(Q)	
Urtica urens L. (soil plot 1)	62	56	59	177	3	59
Stenactis annua (L.) Ness. (soil plot 2)	112	96	108	316	3	105.33
Achillea millefolium L. (soil plot 3)	123	100	101	324	3	108

Since these plants have the capacity to successfully growth on the soils surrounding the TPS, they were studied as potential plant species for soil remediation.

Soil sampling and preparation

Soil sampling was carried out in March 2018 from each of the three examined soil plots in the immediate vicinity of a thermal power station. At each plot (area of 100 m^2) soil samples were collected from five spots (north, south, east, west and centre of the plot) that were thoroughly mixed to make the average sample. Samples were taken at a depth of 0–30 cm using a soil sampler probe.

Estimation of basic chemical properties of soil

The following basic chemical properties of soil were subject of analysis: soil acidity (pH), organic matter (OM), content of available forms of phosphorus (P_2O_5) and potassium (K_2O) and $CaCO_3$ content. Soil pH was determined by the potentiometric method according to ISO 10390 method (2005), OM by oxidation with potassium dichromate in the presence of sulphuric acid according to ISO 14235 method (1998), the content of available phosphorus and potassium by AL – method (Egnér et al., 1960), and $CaCO_3$ content by volumetric method (Allison & Moodie, 1965).

Extraction of heavy metals from soil samples

Extraction of heavy metals from the soil was carried out with aqua regia solution (a mixture of HCl and HNO_3 in a ratio of 3:1) as follows: 3 g of the air-dried soil was placed in 250 mL round bottom flask, and 28 mL of aqua regia (21 mL HCl and 7 mL HNO_3) was added. The flask was covered with a watch glass and allowed to stand 16 h (overnight) at room temperature. After, the mixture was heated on hotplate under reflux for two hours and cooled to room temperature. At the end, the mixture was filtered through quantitative filter paper into 100 mL Erlenmeyer flasks and diluted to the mark with deionized water according to ISO 11466 method (1995).

Plant sampling and preparation

A 1 m x 1 m quadrat was used to study vegetation cover in the examined area. Each examined soil plot had three quadrats. Quadrats were established subjectively within a study site (high density of dominant plant species and cover characteristics were criteria for quadrat selection). Plants were collected in July 2018 at the flowering stage when plants have reached their maximum height. Whole plants (with root) were sampled very carefully, then placed in clean plastic bags and transported to the laboratory. Five individual plants of dominant plant species from each quadrant were taken for analysis. The plants were carefully washed with distilled water, thereafter each plant was separately dried and grinded, and then stored in bags until extraction.

Extraction of heavy metals from plant samples

Extraction of heavy metals from the plant material was performed as follows: 1 g of air-dried and grinded plant material was placed into 100 mL round bottom flask, then 10 mL HNO_3 and 4 mL H_2SO_4 was added. The flasks were left for few hours at a room temperature and then heated gently on a hot plate for thirty minutes. After cooling to room temperature, the solution in flask was filtered through quantitative filter paper in 50 mL flask and diluted to the mark with deionized water (Lisjak et al., 2009).

Determination of heavy metals

Content of heavy metals in soil and plant samples were determined by atomic absorption spectrophotometer (AA 7000, Shimadzu, Japan), according to the instructions specified in the ISO 11047 method (1998). The standard solutions of examined heavy metals were prepared by dilution of standard stock solutions (Merck AAS solutions) with deionized water.

Determination of transfer factor soil-plant

Transfer Factor (TF) is an index used to assess the mobility of heavy metal from soil to plant. TF was calculated by dividing the content of heavy metals in the plant by the total heavy metals content in the soil (Cui et al., 2004; Osu & Ogoko, 2014).

TF = C plant / C Soil

where C plant and C soil represents the toxic metal content in the soil and plant samples on dry weight basis, respectively. If the plants have TF values higher than one, they can be potentially used for phytoextraction.

Statistical analysis

All experimental measurements with plant material were carried out in triplicate and the results were presented as mean ± standard deviation. The results were processed statistically using one-way ANOVA and differences between means were tested using the least significance difference (LSD) test at $P < 0.05$. Means that differed at $P \leq 0.05$ were considered as significantly different.

Statistical analysis was performed using Microsoft Excel software and differences between means were tested using the least significance difference (LSD) test at $P < 0.05$.

RESULTS AND DISCUSSION

Heavy metal contents (Ni, Fe, Cr, Cu, Zn, Cd, Pb, Mn) in examined soil plots are given in Table 2.

The content of toxic elements Cd, Cr, Cu, and Zn in examined soil samples did not exceed the maximum permissible value (Table 2), indicating that examined soils is not polluted by these elements. Permissible value of Fe and Mn in soils is not established by legislative, because they are not directly contaminant elements. However, the content of Mn in examined soils was lower than toxic level of Mn in soils (850 mg kg^{-1}) reported by Pais & Jones (1997), while the Fe content in analysed soils was higher than the average value of these elements in soil (0.6–1.2%) reported by Kabata-Pendias & Pendias (2001).

Table 2. Content of heavy metals in soil (mg kg^{-1} dry mass)

Soil plot	Heavy metal							
	Ni	Fe	Cr	Cu	Zn	Cd	Pb	Mn
1	106.6	19,110.6	71.9	37.1	58.8	0.3	141.3	257.9
2	111.1	1,9012.5	59.1	44.1	55.1	0.1	138.4	303.1
3	102.1	20,001.1	65.1	45.2	51.1	0.2	156.1	255.2
Permissible value[1]	50	-	100	80	200	1.5	100	-

[1]Permissible value prescribed by legislation in BIH; *Abbreviations: - = not listed in legislation.

In the present study, it was found that the content of toxic heavy metals Ni and Pb in all examined soil samples exceeded the maximum permissible value for agricultural soil prescribed by legislation in Bosnia and Herzegovina (Official Gazette of FBIH, 2009), and by legislation in some European countries (Pérez et al., 2002).

The content of heavy metals (Ni, Fe, Cr, Cu, Zn, Cd, Pb, Mn) in dominant plant species is presented in Table 3.

Table 3. Content of heavy metals in plant samples (mg kg^{-1} dry mass)

Heavy metal	Plant species			F test	$LSD_{0.05}$
	Urtica urens L.	*Stenactis annua* (L.) Ness.	*Achillea millefolium* L.		
Ni	0.71 ± 0.33	0.9 ± 0.15	0.89 ± 0.16	n. s.	-
Fe	172.55 ± 10.22^{c}	275.03 ± 16.54^{b}	295.7 ± 14.94^{a}	s.	14.33
Cr	0.87 ± 0.32	1.17 ± 0.41	1.33 ± 0.32	n. s.	-
Cu	8.53 ± 0.92^{a}	5.7 ± 1.51^{b}	7.04 ± 1.35^{a}	s.	2.02
Zn	24.65 ± 2.92^{a}	17.24 ± 5.31^{b}	19.45 ± 3.91^{b}	s.	4.19
Cd	n. d.	n. d.	n. d.	-	-
Pb	0.21 ± 0.11	0.24 ± 0.09	0.31 ± 0.14	n. s.	
Mn	18.97 ± 3.11	15.65 ± 4.14	13.34 ± 3.22	n. s.	

Means in rows followed by the same letter are not significantly different at $P = 0.05$; *Abbreviations: n. d. = not determined; n. s. = no significant; s. = significant; - = not analysed.

Annual nettle (*Urtica urens* L.), daisy fleabane (*Stenactis annua* (L.) Ness.) and yarrow (*Achillea millefolium* L.) are the spontaneous plants growing in high density on the examined area, and therefore the purpose of this study was to examine their ability to accumulate heavy metals from soils, especially Ni, Pb and Cr. If these plants have ability to absorb these elements in high amounts, then there is an objective possibility of their use to remove heavy metals from soils around TPS.

Verbruggen et al. (2009) reported that plants can be considered as a plant for soil remediation, if the content of heavy metals in their harvestable parts is approximately 1% or more of Zn, 0.1% or more of Co, Cr, Cu, Ni, Pb, Sb and 0.01% or more of Cd.

Except of ability to take up large amounts of heavy metals from soil, each plant for soil remediation must be adaptive to soil and climate characteristics on the examined area. Also, the roots of plants must fit the spatial distribution of heavy metals in soil (Keller et al., 2003). de Kroon & Hutchings (1995) noted that the root system possesses a certain level of plasticity that allows plants to cope with a wide range of soil factors, thus enhancing their ability to accumulate heavy metals from soils. The ability of plant to absorb heavy metals from soil is also dependent on a number of plant factors. Plant seasonality, the characteristics and behaviour of an individual plant species or variety are also very important in evaluating the possiblity of plants to absorb large amounts of heavy metals from soils (Mondol et al., 2011).

Unfortunately, in this study the amounts of heavy metals absorbed by above mentioned plants were significantly less than the criteria required for classifying these plants as potential hyper-accumulators. Moreover, the content of Ni, Pb, Cr and other heavy metals in tested plants; annual nettle (*Urtica urens* L.), daisy fleabane (*Stenactis annua* (L.) Ness.) and yarrow (*Achillea millefolium* L.) was lower or in the range with normal value for these elements in plants. The normal range of Ni, Fe, Cr, Cu, Zn, Cd, Pb, Mn in plants are 0.02–50 mg kg^{-1}, 30–300 mg kg^{-1}, 0.1–1 mg kg^{-1}, 5–20 mg kg^{-1}, 20–100 mg kg^{-1}, 0.1–2.4 mg kg^{-1}, 0.5–30 mg kg^{-1}, 15–150 mg kg^{-1} respectively (Chaney, 1989; Kastori et al., 1997).

The main reasons for the low level of heavy metals in plants are closely related to the chemical properties of soils, primarily to the soil reaction (Chibuike & Obiora, 2014). Wang et al. (2006) reported that the availability of most heavy metals, especially Cd and Zn, decreased with increases in soil pH. Namely, in alkaline soils, heavy metal ions bind with hydroxyl ions and carbonates to forms that have extremely low solubilities, thus becoming unavailable or low available to plants (Tack, 2010). This conclusion is consistent with some other studies that also reported the low availability of heavy metals in neutral and alkaline soils (Alkorta et al., 2004; Lenart & Wolny-Koładka, 2013).

As shown in Table 4, the all examined soil plots had an alkaline reaction, and relatively high content of $CaCO_3$ that negatively affected on the uptake of heavy metals by root of plants, so these results confirmed above mentioned hypothesis.

Table 4. Basic chemical properties of soils

Soil plot	pH (H_2O)	pH (KCl)	OM (%)	P_2O_5 (mg $100g^{-1}$)	K_2O (mg $100g^{-1}$)	$CaCO_3$ (%)
1	7.7	7.1	3.54	6.1	22.1	9.62
2	7.5	7.2	3.59	5.5	18.4	9.11
3	7.4	7.0	3.81	5.9	21.8	8.96

Except soil reaction, several other soil properties have a high influence on the availability of heavy metal in soils such as texture, content of organic matter, soil oxidation-reduction potential, activity of microorganisms, antagonistic and synergistic behaviours among heavy metals (Salgare & Acharekar, 1992; Gadd, 2004).

Also, seasonal variation of heavy metal content in soils significantly affect the ability of the plant to absorb heavy metals from the soil. Rainfall may facilitate the leaching of heavy metals from the surface layer of contaminated soils so it can be assumed that the average content of heavy metals during the dry season in the root zone is higher and this hypothesis has, in fact, been confirmed by many scientists (Rahman et al., 2012; Rouane-Hacene et al., 2018).

Generally, plants may react differently to the highly presence of heavy metals in soils, and the same plant under different conditions may exhibit a completely different efficiency in absorption of heavy metals from polluted soil. Available literature data on the ability of annual nettle (*Urtica urens* L.), daisy fleabane (*Stenactis annua* (L.) Ness.) and yarrow (*Achillea millefolium* L.) to absorb heavy metals from soils are quite limited. That was an additional reason why these plants were the subject of this research.

The hypothesis that these plants could not be considered as hyperaccumulators was also confirmed by the analysis of the results that refer to the transfer soil–plant factor (Table 5).

Table 5. Transfer soil–plant factor values of heavy metals from soil to plants

Heavy metal	Plant species		
	Urtica urens L.	*Stenactis annua* (L.) Ness.	*Achillea millefolium* L.
Ni	0.007	0.008	0.009
Fe	0.009	0.014	0.015
Cr	0.012	0.02	0.02
Cu	0.23	0.129	0.156
Zn	0.419	0.313	0.380
Pb	0.001	0.002	0.002
Mn	0.074	0.052	0.052

Transfer soil–plant factor (TF) of Zn was the highest (0.419), followed by Cu, Mn, Cr, Fe, Ni, and Pb, but TF value for all examined heavy metals were below one, regardless of which plant species were subjected to study. Moreover, the TF values of hazardous heavy metals Cd and Pb for all studied plants were below 0.01. This data indicates that tested plants: annual nettle, daisy fleabane and yarrow have a very poor ability to absorb heavy metals, especially Cd and Pb from the examined soils, so it is obvious that these plants could not be considered as hyper-accumulators of heavy metals under conditions of this study.

Although tested plants do not have a large ability to absorb heavy metals from examined soils, the results of this study have shown that there was a significantly difference in ability of plants to uptake heavy metals from examined soils. From Table 4 it is observed that among studied plants, annual nettle had a highest capability of absorbing and accumulating Zn, Cu and Mn. According to Viktorova et al. (2016) nettles belong in the group of zinc hyperaccumulating plant species, and the results of this study partially support this hypothesis.

Gounden et al. (2016) noted that Zn had an antagonistic effect on the Pb and Cd uptake by plant roots, which could be one of the reasons for the extremely low level of Pb and Cd in tested plants in our experiment. Similar results were obtained by other scientists (Pachura et al., 2016; Murtaza et al., 2017).

Interesting finding of this study was that the daisy fleabane and yarrow had a higher capability to absorb and accumulate Cr, Ni and Fe in comparison with annual nettle.

Irrespective of the fact that the examined plants have not shown high efficiency in relation to the absorption of heavy metals from polluted soils in examined area, their presence on these soils, as potentially stabilizers, is certainly desirable. Cui et al. (2007) reported that each plant with TF value lower than one can potentially be used as the stabilizer of polluted soils by heavy metals, of course, assuming that the plant can successfully develop on these soils. Furthermore, plants for successful use in soil stabilization should be able to prevent or reduce soil erosion and the distribution of the toxic heavy metals to other areas, and decrease water percolating through the soil, which may result in the formation of a hazardous leachate (Azubuike et al., 2016).

Considering that the growth and develop of annual nettle, daisy fleabane and yarrow are very successful on the soil around the thermoelectric power plant in Kakanj, and that the coverage of these soils with the examined plants is extremely large, it is assumed that these plants could have a positive effect in remediation of contaminated soils as stabilizers, but it is also obvious that their impact on the absorbing of heavy metals from examined soils is negligible.

The results of the present study also undoubtedly confirm the fact that the total content of heavy metals in soils is not suitable for estimate the solubility and mobility and consequently the availability of heavy metals in soils to plants (Pueyo et al., 2004).

CONCLUSIONS

The examined soils near the thermal power station in Kakanj are polluted with Ni and Pb and contain relatively high value of Cr and Fe. Annual nettle, daisy fleabane and yarrow have shown a very low capability to absorb and to accumulate heavy metals from examined soils, and hence phytoextraction by using these plants is not suitable for removing heavy metals from soils on the examined area. The present study also undoubtedly confirms the fact that the total content of heavy metals in soils is not a satisfactory parameter for estimating the toxicity of heavy metals in soil and consequently for their transfer and accumulation in plants. In the future, studies should focus on developing strategies to enhance phytoextraction efficiency, taking into consideration soils properties, heavy metal levels and characteristics, spatial distributions of heavy metals in soils, vegetation coverage and climatic conditions of the examined area.

REFERENCES

Alkorta, I., Hernandez-Alica, J., Becerril, J.M., Amezaga, I., Albizu, I. & Garbisu, C. 2004. Recent findings on the phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead and arsenic. *Rev. Environ. Sci. Bio*. **3**, 71–90.

Allison, L.E. & Moodie, C.D. 1965. Carbonate. *American Soc. Agron.* **9**, 1379–1396.
Azubuike, C.C., Chikere, C.B. & Okpokwasili, G.C. 2016. Bioremediation techniques-classification based on site of application: Principles, advantages, limitations and prospects. *World J. Microbiol. Biotechnol.* **32**(11), 180.
Chaney, R.L. 1989. Toxic element accumulation in soils and crops: Protecting soil fertility and agricultural foodchains. In Bar-Yosef, B., Barrow, N.J. & Goldshmid, J. (ed.): *Inorganic contaminants in the Vadose Zone*. Springer, Berlin, pp. 140–158.
Chibuike, G.U. & Obiora, S.C. 2014. Heavy Metal Polluted Soils: Effect on plants and bioremediation methods. *Appl. Environ. Soil. Sci.* **752708**, 12 pp.
Cui, S., Zhou, Q. & Chao, L. 2007. Potential hyper-accumulation of Pb, Zn, Cu and Cd in endurant plants distributed in an old smeltery, northeast China. *Environ. Geol.* **51**, 1043–1048.
Cui, Y.L., Zhi, R.H., Chen, D.Y., Huang, Y.Z. & Qui, Y. 2004. Transfer of metals from soils to vegetables in an area near a smelter in Nanning, China. *Environ. Intl.* **30**, 785–791.
de Kroon, H. & Hutchings, M.J. 1995. Morphological plasticity in clonal plants: The foraging concept reconsidered. *J. Ecol.* **83**, 143–152.
Egnér, H., Riehm, H. & Domingo, W.R. 1960. Studies on the chemical soil analysis as a basis for the assessment of the nutrient status of soils. II. Chemical extraction methods for determination of phosphorus and potassium. *K. Lantbr. Hogsk. Annlr.* **26**, 199–215 (in German).
Gadd, G.M. 2004. Microbial influence on metal mobility and application for bioremediation, *Geoderma* **122**, 109–119.
Gounden, D., Kisten, K., Moodley, R., Shaik, S. & Jonnalagadda, S.B. 2016. Impact of spiked concentrations of Cd, Pb, As and Zn in growth medium on elemental uptake of Nasturtium officinale (Watercress). *J. Environ Sci. Heal. B.* **51**(1), 1–7.
Guttikunda, S.K. & Jawahar, P. 2014. Atmospheric emissions and pollution from the coal-fired thermal power plants in India. *Atmos. Environ* **92**, 449–460.
Jacobs, A., Drouet, T., Sterckeman, T. & Noret, N. 2017. Phytoremediation of urban soils contaminated with trace metals using Noccaea caerulescens: comparing non-metallicolous populations to the metallicolous ‘Ganges' in field trials. *Environ. Sci. Pollut. Res. Int.* **24**, 8176–8188.
Kabata-Pendias, A. & Pendias, H. 2001. *Trace elements in soils and plants* (3rd edition). CRC Press, Boca Raton, 313 pp.
Kastori, R., Petrović, N. & Arsenijević-Maksimović, I. 1997. Heavy metals and plants. In: Kastori, R. (ed): *Heavy Metals in the Environment*, pp. 195–257 (in Serbian).
Keller, C., Hammer, D., Kayser, A., Richner, W., Brodbeck, M. & Sennhauser, M. 2003. Root development and heavy metal phytoextraction efficiency: comparison of different plant species in the field. *Plant Soil.* **249**(1), 67–81.
Lenart, A. & Wolny-Koładka, K. 2013. The Effect of Heavy Metal Concentration and Soil pH on the Abundance of Selected Microbial Groups Within ArcelorMittal Poland Steelworks in Cracow. *Bull. Environ. Contam. Toxicol.* **90**(1), 85–90.
Lisjak, M., Spoljarevic, M., Agic, D. & Andric, L. 2009. *Practical Plant Physiology*. Faculty of Agriculture, Osijek, 25 pp. (in Croatian).
Liu, J.G., Ma, X.M., Wang, M.X. & Sun, X.W. 2013. Genotypic differences among rice cultivars in lead accumulation and translocation and the relation with grain Pb levels. *Ecotoxicol. Environ. Saf.* **90**, 35–40.
Lone, M.I., He, Z.L., Stoffella, P.J. & Yang, X.E. 2008. Phytoremediation of heavy metal polluted soils and water: progresses and perspectives. *J. Zhejiang Univ. Sci. B*. **9**(3), 210–220.

Marques, A.P.G.C., Rangel, A.O.S.S. & Castro, P.M.L. 2009. Remediation of heavy metal contaminated soils: phytoremediation as a potentially promising clean-up technology. *Crit. Rev. Environ. Sci. Technol.* **39**(8), 622–654.

Mondol, M.N., Chamon, A.S., Faiz, B. & Elahi, S.F. 2011. Seasonal Variation of Heavy Metal Concentrations in Water and Plant Samples Around Tejgaon Industrial Area of Bangladesh. *J. Bangladesh Acad. Sci.* **35**(1), 19–41.

Morais, S., Costa, F.G. & Pereira, M.L. 2012. Heavy metals and human health. In: Oosthuizen J. (ed): *Environmental health – emerging issues and practice*, pp. 227–246.

Murtaza, G., Javed, W., Hussain, A., Qadir, M. & Aslam, M. 2017. Soil-applied zinc and copper suppress cadmium uptake and improve the performance of cereals and legumes. *Int. J. Phytoremediation* **19**(2), 199–206.

Nagajyoti, P.C., Lee, K.D. & Sreekanth, T.V.M. 2010. Heavy metals, occurrence and toxicity for plants: a review. *Environ. Chem. Lett.* **8**(3), 199–216.

Official Gazette of FBIH. 2009. *Rulebook on determination of allowable quantities of harmful and hazardous substances in soils of Federation of Bosnia and Herzegovina and methods for their testing*, No 72/09. Sarajevo, Bosnia and Herzegovina (in Bosnian).

Osu, C.I. & Ogoko, E.C. 2014. Bioconcentration and transfer of heavy metal from soil into *Verninia amydalina*, *Telfera occidendalis*, and *Amarathus spinosus*. *JAPES* **3**(4), 117–121.

Pachura, P., Ociepa-Kubicka, A. & Skowron-Grabowska, B. 2016. Assessment of the availability of heavy metals to plants based on the translocation index and the bioaccumulation factor. *Desalin. Water Treat.* **57**(3), 1469–1477.

Pais, I. & Jones, J.B. 1997. *The Handbook of Trace Elements.* Lucie Press, Boca Raton, 223 pp.

Pérez, C., Martínez, M.J., Vidal, J. & Navarro, C. 2002. Proposed reference values for heavy metals in calcaric fluvisols of the Huerta de Murcia (SE Spain). In: (Fáz, A., Ortiz, R., Mermut. A. R. (ed): *Sustainable use and management of soils in arid and semiarid regions*). Quaderna Editorial, Cartagena, Murcia, pp. 495–496.

Pueyo, M., Lopez-Sanchez, J.F. & Rauret, G. 2004. Assessment of $CaCl_2$, $NaNO_3$ and NH_4NO_3 extraction procedures for the study of Cd, Cu, Pb and Zn extractability in contaminated soils. *Anal. Chim. Acta* **504**, 217–226.

Rahman, S.H., Khanam, D., Adyel, T.M., Islam, M.S., Ahsan, M.A. & Akbor, M.A. 2012. Assessment of Heavy Metal Contamination of Agricultural Soil around Dhaka Export Processing Zone (DEPZ), Bangladesh: Implication of Seasonal Variation and Indices. *Appl. Sci.* **2**, 584–601.

Rouane-Hacene, O., Boutiba, Z., Benaissa, M., Belhaouari, B., Francour, P., Guibbolini-Sabatier, M.E. & Risso-De Faverney, C. 2018. Seasonal assessment of biological indices, bioaccumulation, and bioavailability of heavy metals in sea urchins Paracentrotus lividus from Algerian west coast, applied to environmental monitoring. *Environ. Sci. Pollut. Res. Int.* **25**(12), 11238–11251.

Salgare, S.A. & Acharekar, C. 1992. Effect of industrial pollution on growth and content of certain weeds. J. *Nature Conserve* **4**, 1–6.

Sharma, S., Tiwari, S., Hasan, A., Saxena, V. & Pandey, L.M. 2018. Recent advances in conventional and contemporary methods for remediation of heavy metal-contaminated soils. *Biotech.* **8**(4), 216.

Soil quality – Determination of cadmium, chromium, cobalt, copper, lead, manganese, nickel and zinc; Flame and electrothermal atomic absorption spectrometric methods: ISO 11047:1998 (1998) Available at: https://www.iso.org/standard/24010.html

Soil quality – Determination of organic carbon in soil by sulfochromic oxidation: ISO 14235:1998 (1998) Available at: https://www.iso.org/standard/23140.html

Soil quality – Determination of pH: ISO 10390:2005 (2005) Available at: https://www.iso.org/standard/40879.html

Soil quality – Extraction of trace elements soluble in aqua regia: ISO 11466:1995 (1995) Available at: https://www.iso.org/standard/19418.html

Tack, F.M.G. 2010. Trace Elements: General Soil Chelistry Principles and Processes. In: Hooda PS (ed): *Trace Elements in Soils*, pp. 9–38.

Verbruggen, N., Hermans, C. & Schat, H. 2009. Molecular mechanisms of metal hyperaccumulation in plants. *New Phytol.* **181**, 759–776.

Viktorova, J., Jandova, Z., Madlenakova, M., Prouzova, P., Bartunek, V., Vrchotova, B., Lovecka, P., Musilova, L. & Macek, T. 2016. Native Phytoremediation Potential of Urtica dioica for Removal of PCBs and Heavy Metals Can Be Improved by Genetic Manipulations Using Constitutive CaMV 35S Promoter. *PloS One* **11**(12), e0167927.

Wang, A.S., Angle, J.S., Chaney, R.L., Delorme, T.A., Reeves, R.D. 2006. Soil pH effects on uptake of Cd and Zn by *Thlaspi caerulescens*. *Plant and Soil* **281**(1–2), 325–337.

Yoon, J.X., Zhou, Q. & Ma, L.Q. 2006. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Sci. Total Environ* **368**, 456–464.

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Determination of heavy metals in root crops using bismuth nanoparticles modified graphene paste electrode

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Abstract. Electrochemical detection of lead (Pb^{2+}) and cadmium (Cd^{2+}) was accomplished via anodic stripping voltammetry (ASV) using bismuth nanoparticle (BiNP) modified graphene paste electrode (GPE). The electrode was fabricated by mixing bismuth nanoparticles, graphene, and mineral oil and the mixture was packed in a Teflon syringe. The best electrode was determined by varying the amount of BiNP while the amount of graphene and mineral oil were kept constant at 0.21 g and 0.80 µL, respectively. The highest peak currents were obtained using 1.5 mg BiNP modified GPE. The ASV parameters, namely accumulation time, deposition time, and accumulation potential, were optimized. The calibration curve, analytical sensitivity, limit of detection (LOD), and limit of quantitation (LOQ) of the optimized electrode were determined. The correlation values for Pb^{2+} ($R^2 = 0.9409$) and Cd^{2+} ($R^2 = 0.9086$) in the calibration curves showed a positive linear relationship between the anodic peak current and heavy metal concentration. The LOD for both Pb^{2+} and Cd^{2+} is found to be 100 ppb. The application of the modified electrode on real sample analysis was performed using root crops purchased from local supermarkets. According to ASV and AAS analyses, most of the samples contained Cd^{2+} while only a few contained Pb^{2+}. Other metals, such as Fe^{2+} and Cu^{2+}, were also detected via ASV.

Key words: bismuth nanoparticles, graphene, heavy metals, root crops, voltammetry.

INTRODUCTION

Root crops are one of the most important crops in the Philippines. These are plant roots which can be consumed for they are rich in carbohydrates and dietary fiber which make them a good alternative to rice. The demand for rice increases in the Philippines which makes its price increase as well. This makes root crops such as cassava (*Manihot esculenta*), potato (*Solanum tuberosum*), purple yam (*Dioscorea alata*), sweet potato (*Ipomoea batatas*), and taro (*Colocasia esculenta*) cheaper alternatives to rice. In addition, cassava, in particular, contributes less fat which suggests that this root crop can be used as an alternative to rice by reducing the risk of diabetes of the consumer (Nwose et al., 2017). Potatoes and purple yams are both rich in vitamins B6 and C. Sweet potatoes help in maintaining the blood sugar levels of the body. Taro has vitamins B, C and E. It also has a low content of saturated fat, cholesterol, and sodium (Pereira et al., 2015).

Commercially available root crops are those grown conventionally in farms. Toxic pesticides used to control insects and weeds on these crops contain toxic chemicals that are absorbed through the soil, making them harmful to the environment. These pesticides significantly increase the heavy metal content of plants, especially Lead (Pb^{2+}), Copper (Cu^{2+}), and Cadmium (Cd^{2+}) (Chiroma et al., 2007). Aside from this, root crops can also be contaminated through the accumulation of heavy metals in the atmosphere due to industrialization, urbanization, and deforestation. Previous researchers have investigated the presence of heavy metal concentrations in soils and vegetation within the urban areas of the Philippines (Navarrete et al., 2017).

According to the World Health Organization (WHO) and the Commission of the European Communities (CEC), the permitted threshold of Cadmium for stem and root vegetables are 0.1 ppm, but the permissible limit of Cadmium for plants, in general, is about 0.02 ppm. For Lead, the limit for metal content within plants is 2 ppm. These limitations were measured to monitor the amount of heavy metals people consume from food (Nazir et al., 2015).

Heavy metals are ingested in the body through eating and drinking contaminated foods and drinks and inhaling dust. As a result, they impose a serious health risk on the person by affecting the central nervous system, and the vital organs. In addition to this, heavy metal accumulation in the human body has long-term effects, which include degenerative diseases like cancer and Alzheimer’s disease (Chen et al., 2016). As a result, the need of detecting heavy metals in the environment increases. There are various analytical methods that can be done to detect heavy metals, namely, Atomic Absorption Spectroscopy (AAS), inductively coupled plasma-atomic emission spectroscopy (ICP-AES), Time-of-Flight Mass Spectrometry (TOFMS), and Microwave Plasma-Atomic Emission Spectrometry (MP-AES). However, these methods are complicated and require expensive machines.

The electrochemical detection of heavy metals offers several advantages such as its high sensitivity, cost-effectiveness, energy-saving, high resolution, and accuracy compared to the other techniques (Baldigowski, 2011). One of these detection techniques is anodic stripping voltammetry (ASV) which is commonly used due to its high sensitivity in detecting trace metals and its low detection limit. For many years, the most commonly used working electrode in ASV was the Mercury (Hg) electrode which possesses the excellent capability to pre-concentrate heavy metals. However, it is very toxic and requires careful handling. Hence, alternative electrodes such as chemically modified glassy carbon electrodes and carbon paste electrodes are used.

Other materials which have good electrical conductivity can be used as an alternative to Hg. In the detection of trace heavy metals, Bismuth (Bi) is usually used in modifying electrodes because of its inexpensiveness and high sensitivity. Compared to Hg electrodes, it is safer to use because it is non-toxic and it has a wider range of potential (Sadok, 2016). Aside from this, it has the ability to fuse heavy metals together (Lee et al., 2016). These properties allow Bi modified electrodes to detect various electro-active species simultaneously in a chosen sample. When used in ASV, bismuth can provide accurate, sharp, and well defined current peaks. Hence, these sharp outcomes allow the users to identify and quantify the metals present in the sample in a quick and reliable manner (Baron-Jaimez et al., 2013).

Graphene is a carbon-based conductive material that is commonly used as an electrode substrate. Its molecular structure consists of a six-membered aromatic ring with sp2 bonds on the carbons. The chemical properties of graphene include wide potential window and low background current (Li & Miao, 2013). Even though it is the lightest material known, it is very strong and dense (Ghany et al., 2017).

For decades, the most popular electrode material, carbon paste, was commonly used for the construction of different electrodes, sensors, and detectors (Vytras et al., 2009). Compared to other electrodes, carbon paste electrodes do not need any pretreatment (Olson & Adams, 1960). The universal applicabilities of the carbon paste express the specific properties and the versatility of electrode material together with advanced technology. This kind of adaptability from the electrode material is described to be the most distinguishable feature conveyed by modern electrochemistry with carbon paste-based electrodes in comparison to the past ones (Zima et al., 2009).

In this study, bismuth nanoparticle modified graphene paste electrodes (BiNP/GPE) were fabricated and were used as the working electrode in anodic stripping voltammetry (ASV) for the detection of heavy metals, mainly lead and cadmium, in commercial root crops from local supermarkets.

MATERIALS AND METHODS

Chemicals and reagents

Graphene nanopowder (multilayer graphene; average flake thickness: 60 nm) was purchased from Graphene Supermarket (Calverton, NY, USA). Bismuth nanopowder (< 100 nm particle size), sodium chloride, lead chloride, cadmium chloride, copper chloride, mineral oil, and nitric acid were purchased from Sigma, Aldrich (Sigma-Aldrich Pte Ltd, Singapore).

Glassware and equipment

The following are the equipment used in this study: BOSCH SAE200 electronic balance, BANDELIN SONOREX sonicator bath, Autolab PGSTAT128N potentiostat, Transferette® micropipette, agate mortar and pestle, AA-6300 Shimadzu atomic absorption spectrophotometer, spatula, crucible, Thermolyne 48000 furnace, La Germania general heat hot plate and glassware such as beakers, petri dish, and graduated cylinder.

Fabrication of BiNP modified graphene paste electrode

The graphene paste was prepared by mixing the graphene powder, bismuth nanopowder, and mineral oil together using agate mortar and pestle for a total of 30 minutes. The amount of graphene was 0.21 grams, which was kept constant for all of the fabricated GPE. The resulting paste was transferred to a Teflon syringe whose end was cut open to serve as its holder. All the fabricated electrodes maintained the same height (2 cm) of the graphene mixture. The diameter of the syringe opening is 3 mm. A copper wire with a diameter of 1 mm was then inserted into the GPE to obtain an ohmic contact. The electrode surface exposed to the electrolyte solution was rubbed on a qualitative filter paper to give it a smooth finish before use.

BiNP amount optimization

The amount of BiNP used was optimized in order to determine the best electrode to be used in detecting 10 ppm each of Pb^{2+}, and Cd^{2+}. The Bi was varied from 0 to 3.0 mg with 0.5 mg increments. The amounts of mineral oil and graphene powder were kept constant at 0.80 µL and 0.21 g, respectively. Thus, the corresponding BiNP:graphene:mineral oil ratios are 70.35:0.10:29.55, 82.60:0.06:17.35, 87.68:0.04:12.28, 90.47:0.03:9.50, 92.23:0.03:7.75, and 93.44:0.02:6.54.

Preparation of Stock Solutions

Stoichiometry was calculated in order to obtain the actual concentration of Cd^{2+} and Pb^{2+} ions since only $CdCl_2$ and $PbCl_2$ were available in the laboratory. First, the gravimetric factor was calculated by dividing the molar mass of the single element over the molar mass of the compound. Then, the amount of $PbCl_2$ and $CdCl_2$ was calculated to obtain a 10 ppm solution of Pb^{2+} and Cd^{2+} in a 100 mL deionized water. For the optimization of the ASV parameters, 1.6 mg of $CdCl_2$, and 1.3 mg of $PbCl_2$ with 0.5844 g of NaCl (0.1 M NaCl) was used to obtain a solution containing 10 ppm of Cd^{2+} and Pb^{2+}.

To obtain the calibration curves of each heavy metal, 10 ppm stock solution of $CdCl_2$ and $PbCl_2$ was utilized. Aliquots of the stock solution were then mixed with a 0.1 M NaCl solution to obtain a final volume of 100 mL. Six solutions were used for the calibration curve: 100 ppb, 200 ppb, 300 ppb, 400 ppb, 500 ppb, and 600 ppb. The outcome solution was utilized in retrieving the calibration curves.

Anodic Stripping Voltammetry

An AUTOLAB potentiostat/galvanostat was used for the measurements made in ASV. The fabricated BiNP/ GPE (working electrode), Ag/AgCl electrode (reference electrode), and platinum coil (counter electrode) was placed in the voltammetric cell. The solution in the voltammetric cell was made of 0.5844 NaCl and 100 mL deionized water. Lead and cadmium were detected simultaneously. In order to obtain the concentrations of the heavy metals in the real samples, the equation for the calibration curve for each heavy metal was used.

After each ASV run, the electrode underwent cleaning by applying a -0.95 V of potential for 15 minutes in order to remove the heavy metals from the surface of the electrode back to the used analyte.

Real Sample Analysis

The real samples underwent wet and dry ashing. The commercial root crop samples were cassava, potato, purple yam, sweet potato, and taro. Two sets of samples were analyzed: one from Divisoria Supermarket and the other from Balintawak Supermarket. The skin and flesh of the samples were tested separately.

One gram of each sample was placed in a crucible. Then, the crucible was transferred in a furnace, which has a temperature of 450 °C. The obtained white colored ash was mixed with 2 mL of concentrated nitric acid. It was then placed on a hot plate until it was almost dry. Finally, it was cooled to room temperature before it was added to the electrolyte solution.

Atomic Absorption Spectrometry

The results from the ASV were verified using AAS. The equipment for AAS was the AA-6300 Shimadzu Atomic Absorption Spectrophotometer apparatus. Seven stock solutions were prepared: 100 ppb, 200 ppb, 300 ppb, 400 ppb, 500 ppb, and 600 ppb. These concentrations were also tested for the calibration curve using ASV. The solutions that were used to calibrate the electrode for ASV was also used for AAS calibration. The calibration curve that was obtained was used to determine the concentrations of the heavy metals in the real samples.

RESULTS AND DISCUSSION

Determination of the Best Electrode

The optimization of the electrodes was accomplished by varying the amount of BiNP in 6 electrodes, which ranged from 0 mg to 3.0 mg of BiNP. The amount of mineral oil and graphene were held constant in all of the electrodes. Then, the fabricated electrodes were used to detect 10 ppm stock solution of Pb^{2+} and Cd^{2+} using the same ASV recipe. Consequently, 10 ppm was utilized as the standard solution for optimization to be able to discern the voltammetric peaks.

In the simultaneous detection of Pb^{2+} and Cd^{2+}, the highest peak current was obtained with the electrode modified with 1.5mg of BiNP (Fig. 1 and Fig. 2). The voltammograms (Fig. 3 and Fig. 5) and their corresponding bar graphs (Fig. 4 and Fig. 6) indicate that the highest peak for the sequential detection of Pb^{2+} and Cd^{2+} were both obtained with 1.5 mg BiNP. Since the GPE modified with 1.5 mg BiNP exhibited the highest anodic peak currents in both simultaneous and sequential detection, it was chosen as the best electrode. In addition, it was shown that as the BiNP increased, the modified electrode was able to detect more lead than cadmium compared to the lesser concentrations of BiNP. At higher Bi concentrations, bismuth will hold the target metals, and affect the voltammetric peaks of the heavy metals (Barón-Jaimez et al., 2014). It was suggested that lower concentrations of BiNP are more sensitive towards lead.

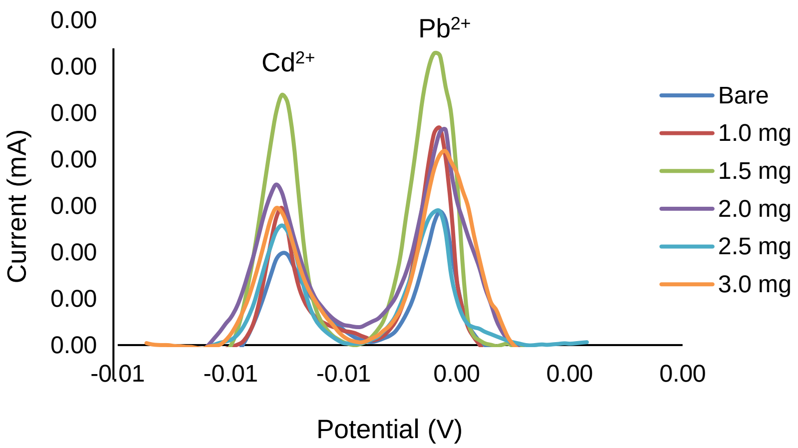

Figure 1. Anodic Stripping Voltammograms for varying BiNP amount for the simultaneous detection of Pb^{2+} and Cd^{2+}.

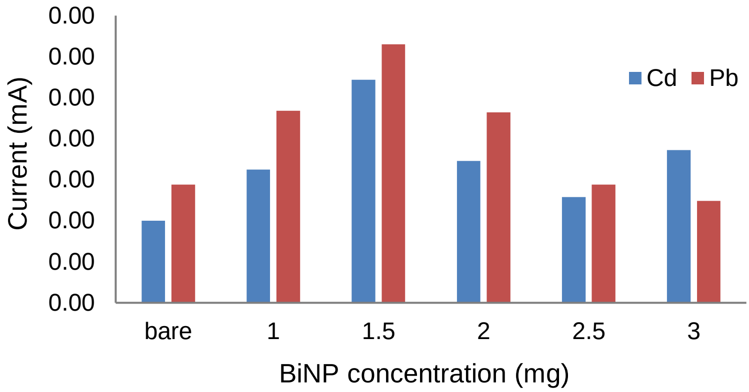

Figure 2. Anodic current peaks for varying BiNP amonut for the simultaneous detection of Pb^{2+} and Cd^{2+}.

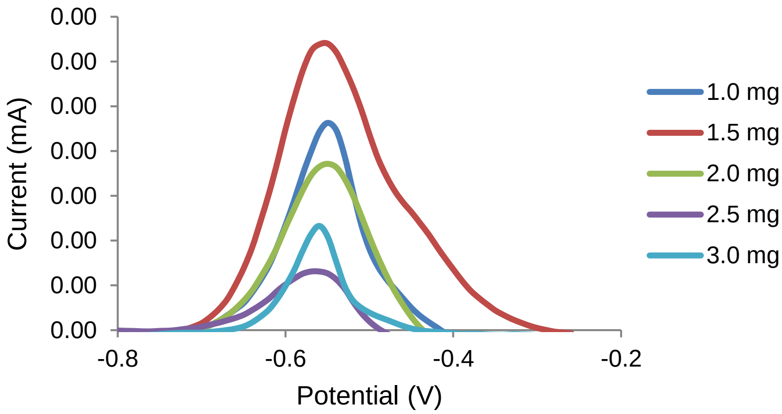

Figure 3. Anodic Stripping Voltammograms of Cd^{2+} detection using BiNP GPE modified electrodes with varying amounts of BiNP.

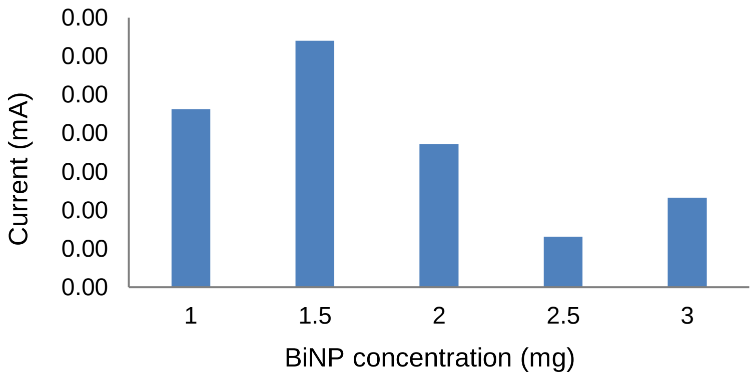

Figure 4. Anodic current peaks for varying BiNP for the sequential detection of Cd^{2+}.

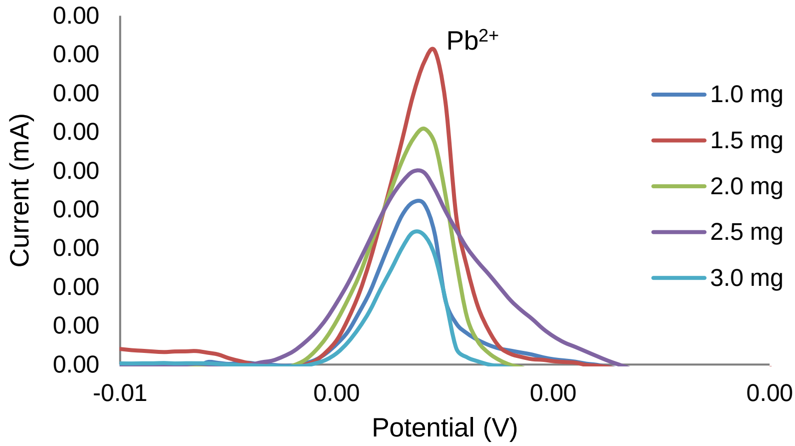

Figure 5. Anodic Stripping Voltammograms of Pb^{2+} detection using BiNP GPE modified electrodes with varying amounts of BiNP.

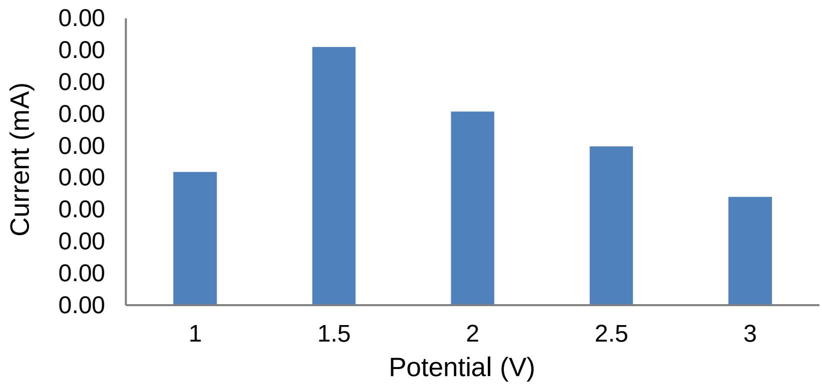

Figure 6. Anodic current peaks for varying BiNP for the sequential detection of Pb^{2+}.

Optimized Electrode

It can be deduced from the obtained results of BiNP optimization that the best electrode has 1.5 mg of BiNP. The addition of the BiNP to the electrode enhanced its sensitivity and selectivity in detecting the heavy metals in a solution due to its ability to form 'fused' alloy with Cd^{2+} and Pb^{2+}. This formation is accountable for the exceptional voltammetric performance of bismuth modified electrodes (Yang et al., 2014). Also, the large surface area of the BiNP along with the high conductivity of the graphene makes the BiNP GPE a sensitive electrode for the detection of heavy metals (Niu et al., 2015).

Characterization of the best electrode:
Scanning Electron Microscopy (SEM)

The morphological structures of the graphene nanoparticles, Bismuth Nanoparticles, and the best electrode were analyzed by SEM. The obtained images show that the graphene has a flaky appearance, while the Bismuth was globular in shape (Fig. 7).

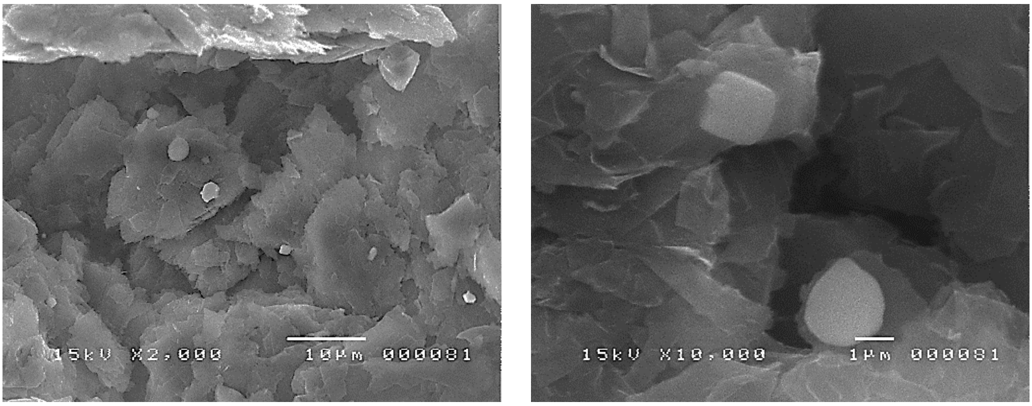

Figure 7. Scanning Electron Microscopy images of BiNP GPE.

ASV Parameters Optimization

In order to get the highest peak currents for the best electrode, the parameters in ASV were varied namely the accumulation time, deposition time, and accumulation potential. The best electrode was utilized to detect Cd^{2+} and Pb^{2+} in the stock solution. The accumulation time and deposition time were varied from 30 s to 180 s with increments of 30 s, The accumulation potential was varied from -0.8 V to -1.0 V increments of -0.05 V.

Accumulation Time

Based on the voltammograms (Fig. 8) and the corresponding histogram (Fig. 9), the peak current increased as the accumulation time increased. The peak gradually decreased after 120 s. Thus, 120 s was the optimum accumulation time.

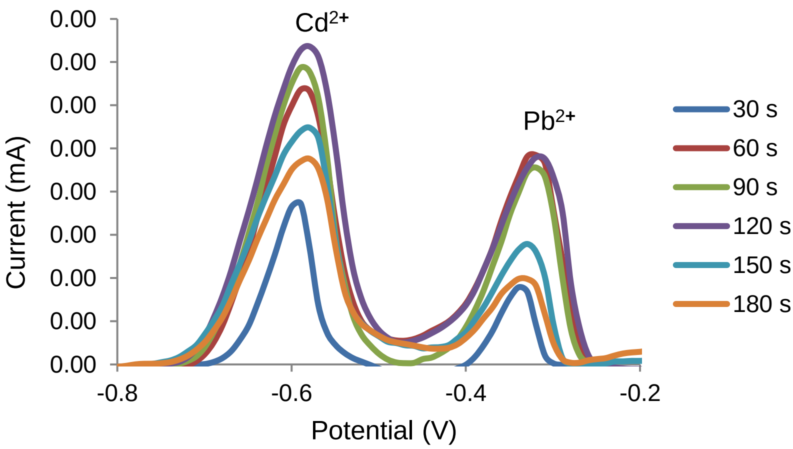

Figure 8. Anodic Stripping Voltammograms for the accumulation time of 10 ppm Cd^{2+} and Pb^{2+} from 30 s to 180 s.

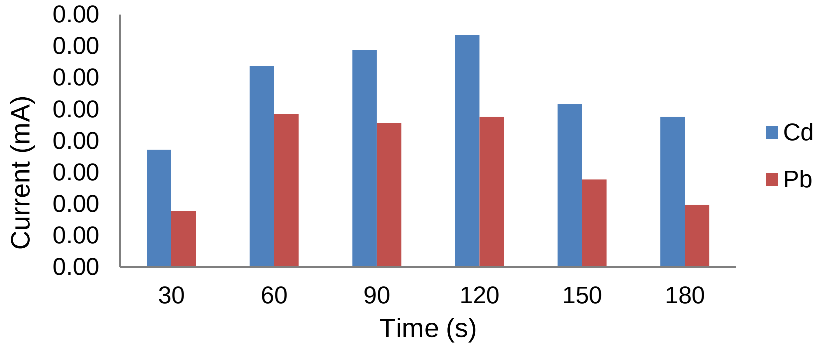

Figure 9. Accumulation time anodic current peaks for the simultaneous detection of 10 ppm Cd^{2+} and Pb^{2+} from 30 s to 180 s.

Deposition Time

The voltammograms (Fig. 10) and corresponding histogram (Fig. 11), indicate that the current peak increased as the deposition time increased. Thus, the optimum deposition time is 150 s.

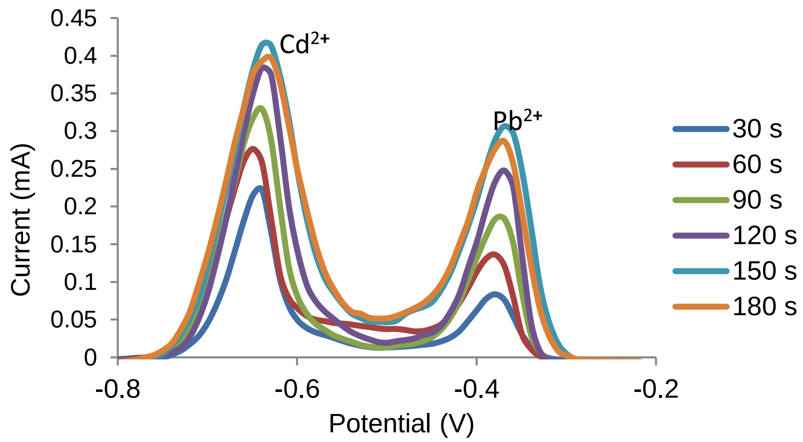

Figure 10. Anodic Stripping Voltammograms for the deposition time of 10 ppm of Cd^{2+} and Pb^{2+} from 30 s to 180 s.

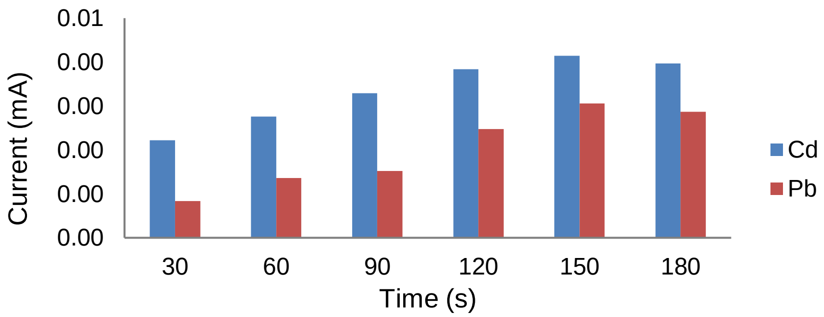

Figure 11. Deposition time anodic current peaks for the simultaneous detection of 10 ppm Cd^{2+} and Pb^{2+} from 30 s to 180 s.

Accumulation Potential

As illustrated in the voltammograms (Fig. 12) and the corresponding bar graph (Fig. 13), the highest peak was obtained at -0.95 V. Hence, the accumulation potential of -0.95 V is the optimum accumulation potential.

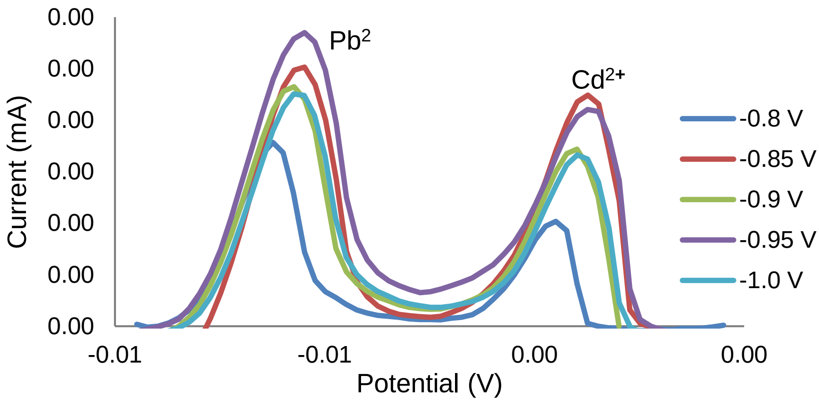

Figure 12. Anodic Stripping Voltammograms for the accumulation time of 10 ppm of Cd^{2+} and Pb^{2+} from -0.80 V to -1.0 V.

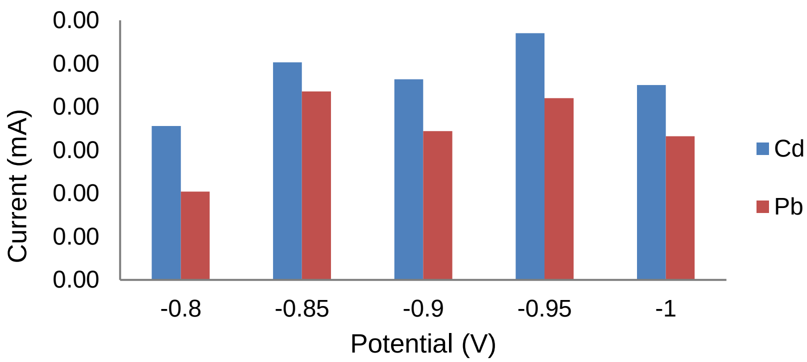

Figure 13. Accumulation potential anodic current peaks of 10 ppm of Cd^{2+} and Pb^{2+} from -0.80 V to -1.0 V.

Calibration Curve

The calibration curve of the best electrode was obtained by varying the concentrations of Cd^{2+} and Pb^{2+} from 100 ppb to 600 ppb with increments of 100 ppb. Fig. 14 shows the ASV curves for varying concentrations of Cd^{2+} and Pb^{2+}. For each heavy metal, the resulting current peaks were plotted against their respective concentrations (Fig. 15 and Fig. 16).

The R^2 value for Pb and Cd are 0.9409 and 0.9086, respectively. These values are close to 1, which indicates that there is a positive linear relationship between the peak current and the heavy metal concentration. It can also be seen from Figs 16 and 17 that the 1.5 mg BiNP modified GPE can detect concentrations as low as 100 parts per billion of both Pb^{2+} and Cd^{2+}.

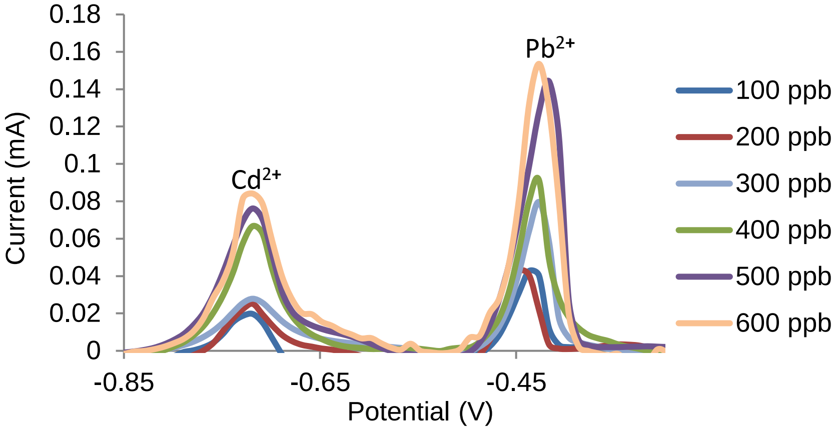

Figure 14. Anodic Stripping Voltammograms for various concentrations of Pb^{2+} and Cd^{2+}.

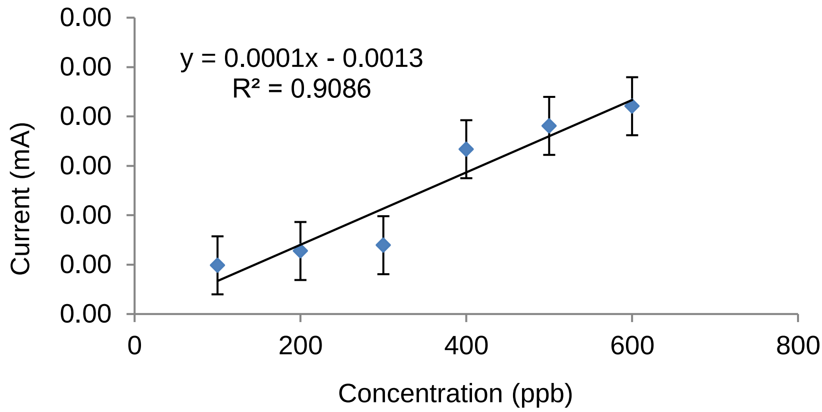

Figure 15. ASV Calibration curve for Cd^{2+} using the optimized BiNP GPE electrode.

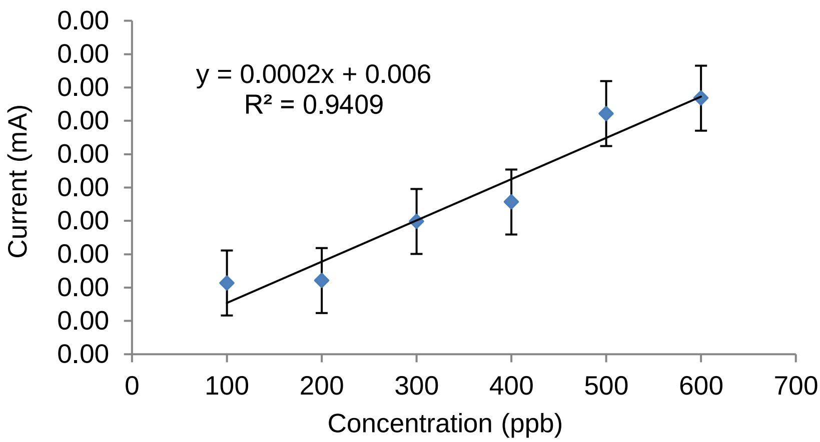

Figure 16. ASV Calibration curve for Pb^{2+} using the optimized BiNP GPE electrode.

Limit of Detection and Limit of Quantitation

The theoretical limit of detection and limit of quantitation of the best electrode were calculated to determine the minimum concentrations of Cd^{2+} and Pb^{2+} that the BiNP GPE can detect. The experimental LOD values were lower compared to the theoretical values. As seen in Table 1, the lowest concentrations that can be detected is 100 ppb for both Cd and Pb.

Table 1. Limit of Detection and Limit of Quantitation

	Experimental LOD (ppb)	Theoretical LOD (ppb)	LOQ (ppb)
Cd	100	218.93	663.42
Pb	100	172.92	523.99

Real Sample Analysis

The real sample analysis for this study was done via anodic stripping voltammetry using the 1.5 mg BiNP modified GPE, and the optimized recipe with 120 s accumulation time, 150 s deposition time, and -0.95 V initial potential.

The amount of Cd and Pb detected from ASV in the samples are shown in Table 2. Those labeled with 'SB' are the skin of the root crops from Balintawak. Those labeled with 'SD' are the skins of the root crops from Divisoria. For those labeled with 'FB' and 'FD', these are the inner part of the root crops from Balintawak and Divisoria, respectively. The modified electrode was able to detect most of the cadmium content in all the samples except for Taro- FB, and Purple Yam-SD. However, lead was detected in less than half of the samples.

Table 2. Cd and Pb concentrations detected via ASV

CADMIUM (ppb)		LEAD (ppb)	
Potato-SB	339.47	Potato-SB	N.D.
Potato-SD	68.71	Potato-SD	N.D.
Potato-FB	41.99	Potato-FB	N.D.
Potato-FD	58.93	Potato-FD	N.D.
Sweet Potato-SB	52.61	Sweet Potato-SB	N.D.
Sweet Potato-SD	18.99	Sweet Potato-SD	25.06
Sweet Potato-FB	32.68	Sweet Potato-FB	43.40
Sweet Potato-FD	116.32	Sweet Potato-FD	79.48
Taro-SB	270.55	Taro-SB	N.D.
Taro-SD	143.81	Taro-SD	N.D.
Taro-FB	N.D.	Taro-FB	47.12
Taro-FD	42.55	Taro-FD	28.21
Purple Yam-SB	112.78	Purple Yam-SB	N.D.
Purple Yam-SD	N.D.	Purple Yam-SD	N.D.
Purple Yam-FB	69.15	Purple Yam-FB	N.D.
Purple Yam-FD	197.13	Purple Yam-FD	44.67
Cassava-SB	623.70	Cassava-SB	N.D.
Cassava-SD	276.72	Cassava-SD	24.27
Cassava-FB	385.64	Cassava-FB	N.D.
Cassava-FD	104.70	Cassava-FD	53.22

Evidently, Cassava-SB has the highest cadmium concentration with the amount of 385.64 ppb, while Sweet Potato- SD has the lowest cadmium concentration of 18.99 ppb. For the lead concentration, Sweet Potato-FD has the most amount with a concentration of 79.48 ppb, while Cassava- SD had the least amount with a concentration of 24.27 ppb. For the samples that are labeled with N.D. (Not Detected), their concentrations are below the detection limit of the optimized electrode.

Atomic Absorption Spectroscopy

Atomic Absorption Spectrometry was used to verify the results obtained from the ASV. The cadmium and lead concentrations detected from AAS can be seen in Table 3. For the cadmium concentration, Purple Yam- SB with a concentration of 37.92 ppb has the highest amount, while Sweet Potato-FB with a concentration of 6.66 ppb has the lowest amount. For lead, only five samples were detected, Potato- FB, Purple Yam-SB, Purple Yam-FB, Cassava- SB, and Cassava- FB. The sample with the most amount of lead was Purple Yam- SB with the concentration of 141.58 ppb, and the least amount of lead was Potato- FB with a concentration of 2.57 ppb. For samples with a concentration that are not detected (N.D.), their concentrations are below the detection limit of AAS.

Table 3. Cd and Pb concentrations detected via AAS

CADMIUM (ppb)		LEAD (ppb)	
Potato-SB	10.71	Potato-SB	N.D.
Potato-SD	23.16	Potato-SD	N.D.
Potato-FB	17.08	Potato-FB	N.D.
Potato-FD	25.18	Potato-FD	2.57
Sweet Potato-SB	14.76	Sweet Potato-SB	N.D.
Sweet Potato-SD	20.26	Sweet Potato-SD	N.D.
Sweet Potato-FB	6.66	Sweet Potato-FB	N.D.
Sweet Potato-FD	29.24	Sweet Potato-FD	N.D.
Taro-SB	32.99	Taro-SB	N.D.
Taro-SD	18.53	Taro-SD	N.D.
Taro-FB	32.99	Taro-FB	N.D.
Taro-FD	13.32	Taro-FD	N.D.
Purple Yam-SB	37.92	Purple Yam-SB	141.58
Purple Yam-SD	27.49	Purple Yam-SD	N.D.
Purple Yam-FB	33.87	Purple Yam-FB	N.D.
Purple Yam-FD	26.92	Purple Yam-FD	10.29
Cassava-SB	34.16	Cassava-SB	46.34
Cassava-SD	20.84	Cassava-SD	N.D.
Cassava-FB	24.3153	Cassava-FB	10.297
Cassava-FD	30.39	Cassava-FD	N.D.

Comparison of ASV and AAS results

Based on the data from both ASV and AAS (Table 4), the majority of the samples contained small concentrations of cadmium in both the skin and flesh. Lead was also detected in some samples. However, the voltammograms from the ASV show that more cadmium was detected compared to AAS. According to WHO, the permissible intake limit for Cd is 100 ppb and 2 ppm for Pb^{2+}. For cadmium, nine samples exceeded the limit according to the results obtained from ASV: sweet potato-FD, taro-SB, taro-SD, purple yam-SB, purple yam-FD, cassava-SB, cassava-SD, cassava-FB, and cassava-FD. According to ASV, the lead concentrations in some of the samples were below the WHO limit. Since both the skin and flesh of the root crops contain trace heavy metals, the root crops possibly absorbed them from contaminated soil or irrigation water. Aside from Cd^{2+} and Pb^{2+}, the BiNP GPE was also able to detect iron (Fe^{3+}) and copper (Cu^{2+}). However, the concentrations of the said metals were not determined since the electrode was calibrated for Cd and Pb only. Fig. 17 and Fig. 18 are the corresponding bar graphs for Table 4.

Table 4. Comparison of ASV and AAS Results

Sample	CADMIUM		LEAD	
	ASV (ppb)	AAS (ppb)	ASV (ppb)	AAS (ppb)
Potato-SB	339.47	10.71	N.D.	N.D.
Potato-SD	68.71	23.16	N.D.	N.D.
Potato-FB	41.99	17.08	N.D.	N.D.
Potato-FD	58.93	25.18	N.D.	2.57
Sweet Potato-SB	52.61	14.76	N.D.	N.D.
Sweet Potato-SD	18.99	20.26	25.06	N.D.
Sweet Potato-FB	32.68	6.66	43.40	N.D.
Sweet Potato-FD	116.32	29.24	79.48	N.D.
Taro-SB	270.55	32.99	N.D.	N.D.
Taro-SD	143.81	18.53	N.D.	N.D.
Taro-FB	N.D.	32.99	47.12	N.D.
Taro-FD	42.55	13.32	28.21	N.D.
Purple Yam-SB	112.78	37.92	N.D.	141.58
Purple Yam-SD	N.D	27.50	N.D.	N.D.
Purple Yam-FB	69.15	33.87	N.D.	N.D.
Purple Yam-FD	197.13	26.92	44.67	10.29
Cassava-SB	623.70	34.16	N.D.	46.34
Cassava-SD	276.72	20.84	24.27	N.D.
Cassava-FB	385.64	24.31	N.D.	10.30
Cassava-FD	104.70	30.39	53.22	N.D.

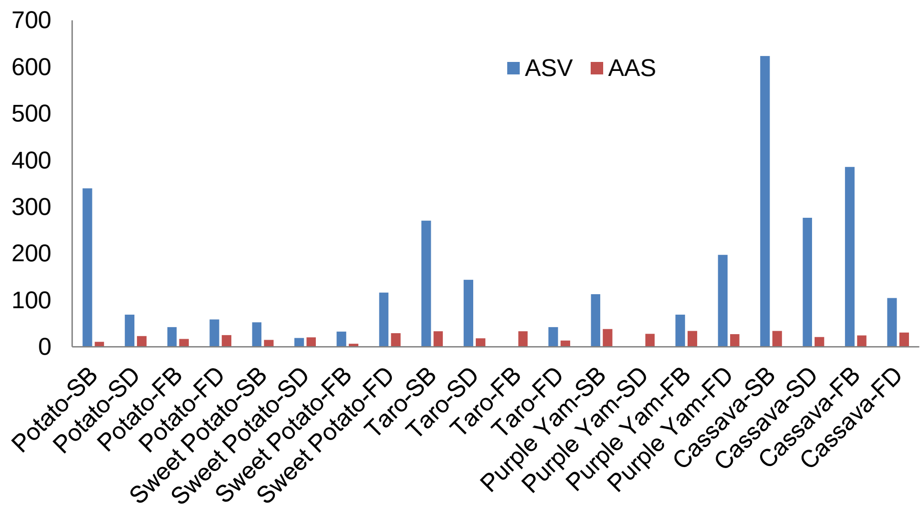

Figure 17. Bar graph of the amount of cadmium detected from the root crops via ASV and AAS.

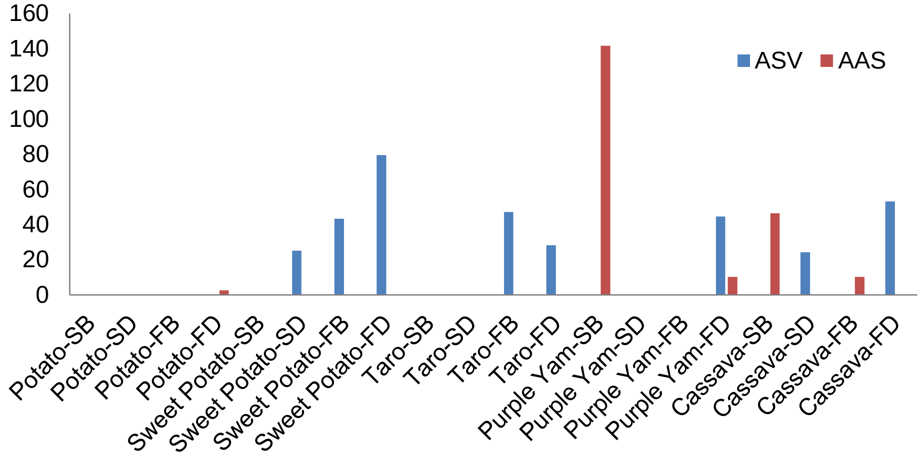

Figure 18. Bar graph of the amount of lead detected from the root crops via ASV and AAS.

CONCLUSIONS

The fabricated BiNP GPE was shown to be effective in detecting Pb^{2+} and Cd^{2+}. It is evident that the addition of BiNP improved the sensitivity of the GPE. Out of the six electrodes with different amounts of BiNP, the electrode with 1.5 mg BiNP exhibited the highest anodic current peaks for both the simultaneous and sequential detection of Cd^{2+} and Pb^{2+}. Scanning electron miscroscopy analyses confirmed the presence of BiNP and graphene in the fabricated electrode. The calibration curves obtained showed a strong linear relationship from 100 ppb to 600 ppb for both Cd^{2+} and Pb^{2+}. The LOD of the optimized modified electrode was found to be 100 ppb for both Cd^{2+} and Pb^{2+}. Real sample analysis showed that majority of the samples contained small concentrations of cadmium in both the skin and flesh. Lead was also detected in some samples.

REFERENCES

Baldigowski, M. 2011. The pros and cons of electrochemical sensors. Safety + Health. Available at: http://www.safetyandhealthmagazine.com/articles/the-pros-and-cons-of-electrochemical-sensors-2

Baron-Jaimez, J., Joya, M. & Barba-Ortega, J. 2013. Bismuth electrode: An alternative in stripping voltammetry. *Journal of Physics: Conference Series* **466**, 1–4.

Barón-Jaimez, J.A., Marulanda-Arévalo, J.L. & Barba-Ortega, J.J. 2014. Electrodes friendly with the environment for detect heavy metal. *Dyna* **81**, 122–128.

Chen, P., Miah, M.R. & Aschnerb, M. 2016. Metals and Neurodegeneration. *F1000 Research* **5**. doi:10.12688/f1000research.7431.1

Chiroma, T., Abdulkarim, B. & Kefas, H. 2007. The Impact of pesticide application on heavy metal (Cd, Pb and Cu) levels in Spinach. *Leonardo Electronic Journal of practices and Technologies* **6**, 117–122.

Ghany, N., Elsherif, S. & Handal, H. 2017. Revolution of Graphene for different applications: State-of-the-art. *Surfaces and Interfaces* **9**, 93–106.

Lee, S., Oh, J., Kim, D. & Piao, Y. 2016. A sensitive electrochemical sensor using an iron oxide/graphene composite for the simultaneous detection of heavy metal ions. *Talanta* **160**, 528–536.

Li, G. & Miao, P. 2013. Electrochemical analysis of proteins and cells. Springer-Verlag Berlin, Heidelberg, 65 pp.

Navarrete, I., Gabiana, C., Dumo, J., Salmo III, S., Guzman, M., Valera, N. & Espiritu, E. 2017. Heavy metal concentrations in soils and vegetation in urban areas of Quezon City, Philippines. *Environmental Monitoring and Assessment* **189**, 145.

Nazir, R., Khan, M., Masab, M., Rehman, H., Rauf, N., Shahab, S. & Shaheen, Z. 2015. Accumulation of heavy metals (Ni, Cu, Cd, Cr, Pb, Zn, Fe) in the soil, water and plants and analysis of physicochemical parameters of soil and water Collected from Tanda Dam kohat. 7 *Journal of Pharmaceutical Sciences & Research* **7**, 89–97.

Niu, P., Fernandez-Sanchez, C., Gich, M., Ayora, C. & Roig, A. 2015. Electroanalytical assessment of Heavy metals in water using Bismuth Nanoparticle-Porous Carbon Paste Electrodes. *Electrochimica Acta* **165**, 155–161.

Nwose, E.U., Onodu, B.C., Anyasodor, A.E., Sedowo, M.O., Okuzor, J.N. & Culas, R.J. 2017. Ethnopharmacological values of cassava and its potential for diabetes and dyslipidemia management: Knowledge survey and critical review of report. *Journal of Intercultural Ethnopharmacology* **6**, 260–266.

Olson, C. & Adams, R. 1960. Carbon paste electrodes application to anodic voltammetry. *Analytica Chimica Acta* **22**, 582–589.

Pereira, P., Silva, J., Verícimo, M., Paschoalin, V. & Teixeira, G. 2015. Crude extract from taro (Colocasia esculenta) as a natural source of bioactive proteins able to stimulate haematopoietic cells in two murine models. *Journal of Functional Foods* **18**, 333–343.

Sadok, I., Tyszczuk-Rotko, K. & Nosal-Wiercinska, A. 2016. Bismuth particles Nafion covered boron-doped diamond electrode for simultaneous and individual voltammetric assays of paracetamol and caffeine. *Sensors and Actuators B: Chemical* **235**, 263–272.

Vytras, K., Svancara, I. & Metealka, R. 2009. Carbon paste electrodes in electroanalytical chemistry. *Journal of the Serbian Chemical Society* **74**, 1021–1033.

Yang, Die, Wang, Liang, Chen, Zuliang, Mallavarapu, Megharaj & Naidu, Ravi. 2014. Anodic stripping voltammetric determination of traces of Pb(II) and Cd(II) using a glassy carbon electrode modified with bismuth nanoparticles. *Microchimica Acta* **181**, 1199–1206.

Zima, J., Svancara, I., Barek, J. & Vytras, K. 2009. Recent Advances in electroanalysis of organic compounds at carbon paste electrodes. *Analytical Chemistry* **39**, 204–227.

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https://doi.org/10.15159/AR.19.030

Digitalization as an essential growth factor contributing in SME development (experience of Latvia and Romania)

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Abstract. In today's global economy, entrepreneurship is an important economic growth engine for the European Union. Small and medium-sized enterprises (SMEs) are the backbone of Europe's economy, providing 85 % of all new jobs. The European Commission aims to promote entrepreneurship and improve the business environment for SMEs, to allow them to realise their full potential in today's global economy (EC.2015. COSME). The expansion of SMEs in today's environment is increasingly influenced by the use of digital networks. The present research focuses on the characteristics of digital environment use by SMEs in two EU Member States – Romania and Latvia. Groups of entrepreneurs that produced both goods and services and differed in number of the employed and geographical location were surveyed in each country. The information acquired revealed the technological and economic characteristics of the digitalization process in each country, highlighting both similarities and differences. An analysis of the data gives an opportunity to identify main priorities to enhance SME digitalization processes in the future. The first of them is the accessibility of competent workers in digitization and digitalization.

Key words: SMEs, digitalization, economic benefits, rural viability.
JEL codes: D2, M15, O10, R1.

INTRODUCTION

The Digital Agenda presented by the European Commission forms one of the seven pillars of the Europe 2020 Strategy which sets objectives for the growth of the European Union (EU) by 2020. The Digital Agenda proposes to better exploit the potential of Information and Communication Technologies (ICTs) in order to foster innovation, economic growth and progress (EC, 2010). It will help rebooting Europe`s economy by delivering sustainable economic and social benefits from a digital single market (EC. 2014).

Digitalization as an important phenomenon has been discussed at several forums held in Europe. At the TUAC (Trade Union Advisory Committee) Forum (February 2017), analysing the positive effect of business digitalization on economic growth, a focus was placed on the effects of this change on the labour market: employment as such and particularly training and new working conditions for employees aimed at raising their competences and opportunities in the labour market. The entry of technologies has to be aligned with building up the workforce's knowledge and skills, as new quality jobs are created. Only in this way, it is possible to maintain and increase prosperity for the population (TUAC, 2017).

At the Stakeholders Forum (March 2018), the main tracks of industry digitising have been widely discussed, stressing that the future of Europe and industry is digital, and the needs of digitalization are enormous. Therefore, the European Platform of national initiatives on the digitising industry is being established. The participants of the forum set the following key functions for the platform: coordination of national and regional activities, expansion of private-public partnerships not only in usual fields but also in the financial field, exchange of positive experience and the build-up of employee and employer competences needed because of changes in economic activity (EC, 2018b).

Since SMEs comprise a significant share of the total enterprises in the European Union, one of the key messages from the Stakeholder Forum is dedicated to SMEs. The message stresses that only one in five such enterprises is engaged in digitalization, as this process is hindered by financial, human skill and time constraints. For this reason, there is a need to provide digitalization and financial help to SMEs (EC, 2018a:59)

The nature of digitalization and the impact of it on the society are important research problems for scientists. Digitalization is characterised as the most striking feature of the Fourth Industrial Revolution (Schwab K.; 2017; Rachinger M. et al., 2018). The concepts of 'digital production' and 'digital people' emerged, referring to changes both in production processes and in people involved in the processes and stressing that the digital people represent the new technologies that entered the production of goods and services (Perkowitz S., 2005; Rhoades L, 2005). In order for digitalized activities to emerge, the territory where the activities develop has to have a digital environment, i.e. Fiber to the Premises (FTTP), meaning the Internet is available to any group of individuals interested in the use of it (Broadband...2017). Consequently, a new business model is emerging, which encompasses the customer dimension, the benefit dimension, the value added dimension, the partner dimension and the financial dimension and which has to form a system for networking of actors (Shalmo et al., 2017).

Scientists focus also on the spatial distribution of the digitalized economy in the European Union, examining the performance of the EU as a whole and each individual Member State and giving an opportunity to do a comparative analysis. In this respect, an example is the research on the digital economy that compared Poland with the other EU Member States (Moroz, 2017). A similar analysis was done on the pace of business digitalization in Sweden, assessing the results achieved (Mahring et al., 2018). A focus is placed also on an analytical combination of the spatial and content aspects. An example is the research on the digitalization of microenterprises that compared the situations in Eastern European and Western European countries and used an online survey of 36 microenterprises as an information source (Pytkovska & Korynski, 2017).

The present research continues detailing the spatial and content aspects of business digitalization. The research object is a combination of SMEs as the content aspect and two EU Member States – Latvia and Romania – as the spatial aspect. **The research aims are:** a) to assess the positions of Romanian and Latvian SMEs within the EU based on the DESI index and b) to compare and assess the technological and economic characteristics of the digitalization process in each country. **Methodology and methods:** The research used the following information sources: the Digital Economy and Society Index (DESI), which showed the situation of each country examined by the research within the EU, while a more detailed situation in both countries was identified by conducting a survey in each of the countries (n = 100 in Romania and n = 100 in Latvia operating in the manufacture of goods, in the supply of services and marketing). Since most of the respondents represented microenterprises (81.0% in Romania and 70.0% in Latvia) located outside cities, the data acquired gave an opportunity to get insight into the manifestations of digitalization contributing to maintaining the viability of rural space both in Romania and in Latvia. To maintain the viability of rural areas, the society has to move with the times and engage in modernisation processes. Nowadays, the key path of modernisation is digitalization that significantly affects the economy and the society's life as a whole.

To assess the situation, the research used the DESI index component 'Business technology integration: the digitalization of business and development of the online sales channel' (EC. 2018a).

RESULTS AND DISCUSSION

1. Positions of Rumania and Latvia within the EU with regard to SME digitalization

The DESI is a composite index (five components) that shows the overall situation in a spatial unit examined. According to the latest data, both Latvia and Romania still lagged behind the EU average. However, the data indicated the two countries progressed and continued approaching the EU average (Fig. 1).

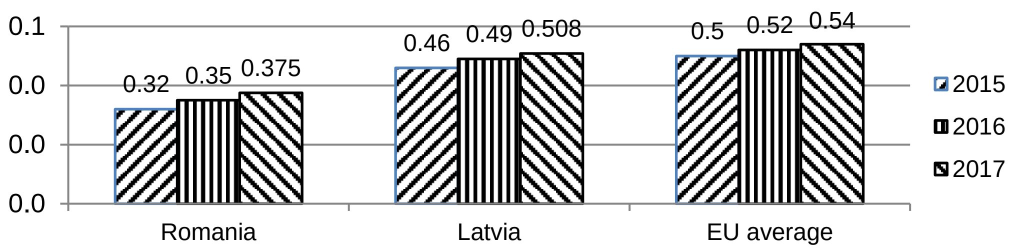

Figure 1. Digitalization progress in Rumania and Latvia in the last three years. Source: (DESI composite index, EC. 2018a).

In the last three years, the EU average rose by 8.0%, while the index for Latvia increased by 10.4% and for Romania by 17.2%. In the result, Latvia reached 94.1%, while Romania – 69.4% of the EU average. The population's interest in the use of the Internet and the build-up of digital skills to be used for mutual communication as well as communication with national regulatory authorities increased.

As regards the research problem, the authors were particularly interested, first, in the composite index components showing residents' use of the Internet for shopping (3c2) (Table 1), as the Internet serves as a source of various information and a means of communication and as a platform for shopping. Online shopping is a form of electronic commerce, which allows consumers to buy goods and services directly from a seller (Moroz, 2017).

Table 1. Increase in the use of the Internet as a tool for shopping, (%)

Online shopping	2015	2017	Growth, %-points
Romania	18%	23%	5%
Latvia	48%	74%	26%
EU average	65%	68%	3%

Source: authors' calculations based on the DESI index (3c2) (EC. 2018a).

According to the authors' calculations, online shopping as an option of consumer behaviour rose sharply in Romania and in Latvia in particular over the last years, exceeding the average growth rate for the EU. Nevertheless, a lot of efforts have to be made to increase the availability of digital services and reach the most optimum level (84% in Sweden, 82% in Denmark), in Romania in particular.

Second, the composite index components for SMEs reveal important facts regarding the research problem, as they show the SMEs engagement in this form of electronic commerce (DESI 4b1, 2, 3) (Table 2).

Table 2. Engagement of SMEs in online selling (% of total SMEs)

	2015	2017	Growth, %-points
Selling online			
Romania	7.4%	7.7%	0.3%
Latvia	8.3%	10.6%	2.3%
EU average	16.0%	17.2%	1.2%
Selling online cross-border			
Romania	1.9%	1.8%	- 0.1%
Latvia	3.9%	4.7%	0.8%
EU average	7.5%	8.4%	0.9%

Source: authors' calculations based on the DESI index (4b1, 3) (EC. 2018a).

SME electronic commerce in Romania and Latvia has begun and expands. The number of SMEs engaged in such activities rose, which was indicated by an increase in the share of those engaged in the digitalization process. Online selling in Romania approached the EU level (44.8% of the EU average), while in Latvia this level slightly exceeded the EU average (61.6% of the EU average). The levels for online cross-border selling were lower, yet this kind of activity grew. Overall, electronic commerce is an indicator of successful economic activity for SMEs, which is confirmed by an increase in e-commerce turnover in the last year (2017/2016) by 0.9% in Romania and 0.4% in Latvia.

If analysing SME e-commerce, a comparison of an indicator for an individual country with the EU average represents only an intermediary comparison. A comparison of it with the optimum level among the EU Member States has to be the next step. For example, 30.0% of the population in Ireland, 28.5% in Sweden, 27.8% in Denmark and 21.9% in Lithuania were involved in online commerce. This is an obvious target to move towards for the employees of SMEs in Romania and Latvia (The Digital... 2018).

2. Technological and economic characteristics of the digitalization process in Romania and Latvia

The DESI composite index components give a rough notion of the phenomenon researched, as they indicate the overall situation in the countries, whereas the survey of SME businesspersons conducted by the authors and an analysis of the data give some insight into the digitalization process at the enterprises engaged in e-commerce in each of the countries and allow assessing the process. Since the survey involved respondents from enterprises producing goods and services and trading enterprises with less than ten employees as well as with a larger number of employees, this gives an opportunity to identify whether the kind of economic activity and the size of enterprises can affect the SME digitalization process. Progress in the SME digitalization process both in Romania and in Latvia has been confirmed by the DESI index (4b1, 2, 3). The survey, however, gives insight into the purposes of engagement in Internet marketing or e-commerce, the results achieved and the problems related to this activity.

2.1. Purposes of engagement in Internet marketing or e-commerce

Marketing as a phenomenon involves at least three components – communication with customers, advertising goods or services and, finally, sales of the goods or services offered.

The survey results allow concluding that overall, communication with customers was almost of the same importance for SMEs in both countries. Selling goods as a purpose was more stressed by the respondents in Latvia, yet there was a considerable difference in perception of the role of advertising between both countries. It was referred to as dominant by the Romanian respondents, whereas only a tenth of the Latvian respondents mentioned it (Table 3). This could be explained by the fact that online shopping as an option of shopping was more popular in Latvia than in Romania (EC 2018, 3c2). In Latvia, there are service enterprises developing Internet tools (websites, social networks, mini blogs etc.) for other enterprises ordering the tools (Marketing Angels Ltd).

Table 3. Main purposes of using the Internet environment by enterprises, % (several answers possible)

Purpose	Average	Goods-producing enterprises	Services-producing enterprises	Trading enterprises	Others
Romania					
Advertising	73.0	64.3	67.3	90.9	88.9
Selling goods or services	67.0	60.7	86.5	36.4	44.4
Communication with customers	72.0	78.6	73.1	45.4	33.7
Latvia					
Advertising	11.0	96.0	83.8	44.4	23.5
Selling goods or services	77.o	78.3	73.0	66.7	64.7
Communication with customers	71.0	0.0	5.4	27.8	70.6

Since the SMEs surveyed were comprised of those producing goods or services and trading ones, the data acquired in the survey gave an opportunity to analyse the components of interest in e-commerce as well as the kinds of economic activity represented. In both Romania and Latvia, the interest in the use of the Internet environment differed between business entities producing goods or services and trading ones as well as those engaged in other kinds of economic activity. In Romania, the role of advertising increased for the mentioned groups of enterprises, whereas the role of sales promotion and communication with consumers decreased. In Latvia, the situation was completely opposite – the demand for advertising decreased, whereas the demand for the Internet environment increased in order to communicate with consumers of goods and services.

As regards the size of enterprises, in both Romania and Latvia the replies given by the representatives of microenterprises with up to ten employees were consistent with the average for all the SMEs (Table 3) – communication with consumers dominated, followed by opportunities for selling goods or services. However, the needs for advertising were different. In Romania, advertising as an important purpose was noted by 69.1% of the respondents, whereas in Latvia – only by 12.9%. In Romania, the larger the enterprise in terms of employment, the higher the interest in digitalized advertising. The interest of enterprises with more than 25 employees could reach even 100%. In Latvia, the situation was opposite – the larger the enterprise, the lower the interest in business digitalization.

2.2. Gains from engagement in Internet marketing or e-commerce

Net turnover includes the revenues from the sale of products, goods, and services. The ability to use electronics provides an opportunity to increase net turnover from both producing goods and producing services (21st century...). In the opinion of the Romanian and Latvian respondents, the SMEs benefited from it in both countries.

Table 4. Net turnover increase after commencing Internet marketing or e-commerce, % (enterprises operating for more than one year)

Turnover increase	Average	Goods-producing enterprises	Services-producing enterprises	Trading enterprises	Other
Romania					
No increase	8%	20.0%	4.2%	9.1%	0%
Insignificant	23%	**48.0%**	12.8%	18.2%	33.3%
Significant	52%	28.0%	**70.2%**	**72.7%**	**44.5%**
Very significant	9%	4.0%	12.8%	0%	22.2%
Latvia					
No increase	5%	0%	0%	5.6%	11.8%
Insignificant	31%	**52.2%**	32.4%	33.3%	29.4%
Significant	47%	47.8	**45.9%**	**44.4%**	**58.8%**
Very significant	12%	0%	21.6%	16.7%	0%

Even though the percentages of increase in net turnover differed between Romania and Latvia, the dominant positions were the same. An insignificant increase in net turnover was specific to goods-producing enterprises, while a significant increase in net turnover was specific to services-producing enterprises irrespective of the kind of services they provided. For such enterprises, an increase in net turnover was very significant in Romania. This indicates Romania expanded activities paving the way to approach the EU average for financial gains from Internet marketing or e-commerce activity.

Both in Romania and in Latvia, e-commerce took both forms – domestic trade as well as cross-border sales (EC. 2018a - 4b1, 3). The survey results revealed a breakdown of domestic e-commerce transactions for the SMEs that had engaged in digital marketing. In both countries, the percentages of enterprises engaged only in domestic trade were equal (71%). For this reason, the percentages of SMEs engaged in cross-border commerce were also equal (29%). Nevertheless, the degrees of engagement in the European Union or even the global markets were quite different (Fig. 2).

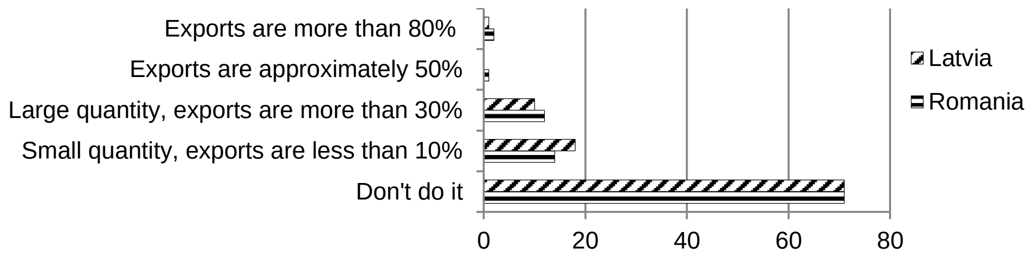

Figure 2. Exports of goods or services abroad, as a % of SMEs using e-commerce.

The data acquired in the survey were consistent with the DESI index values presented in Table 2, i.e. SMEs slowly commenced their cross-border commerce. A breakdown of the enterprises by kind of economic activity revealed that in Romania, their engagement in cross-border commerce ranged from 9.1% (trading enterprises) to 38.5% (providers of various services). Just like in Romania, in Latvia too trading enterprises were minimally (18.8%) engaged in cross-border commerce, whereas goods-producing enterprises were maximally (45.5%) engaged in cross-border commerce. This means there were both differences and similarities between the countries.

The survey data revealed the effect of enterprise size on their extent of cross-border commerce. A comparison of the enterprises by size (in terms of employment) revealed that the engagement of the enterprises employing more than 25 people in cross-border commerce was the highest both in Romania and in Latvia (80.0% in Romania, 83.3% in Latvia), whereas the engagement of the microenterprises (up to 10 employees) was the lowest: 13.5% in Romania, 14.3% in Latvia.

The slow engagement of SMEs in e-commerce and particularly in cross-border commerce was affected, in the opinion of the respondents, by two factors. The first one was high costs of maintaining the e-commerce environment. A larger problem, according to the Romanian and Latvian respondents, was a lack of competent specialists at enterprises (12% in Romania and 35% in Latvia). To expand e-commerce and particularly cross-border commerce, specialists having competences in production and particularly competences for organising sales on the Internet were needed.

A survey of Western and Eastern European microenterprises done by Polish researchers identified a lack of finances as a factor constraining the expansion of digitalization; another obstacle was the insufficient skill of consumers themselves to use the Internet for shopping (Pytkovska & Korynski, 2017). The aspects of a digital buyer have become a research problem (Managing..., 2018). The European programme 'COSME' (2014 – 2020) was established for strengthening the positions of SMEs within the business sector and getting access to financial assistance for entry into the single market and for cooperation networks, with a special focus being placed on promoting business digitalization (EC, 2015). Successfully implementing the programme could contribute to mitigating the mentioned problems. How fast this could be done largely depends on the implementation of the digitalization policy in each EU Member State.

2.3. Assessment of the results achieved of Internet marketing efforts according to the goals set

Commencing any innovative activity is associated with the goals to be achieved by implementing this activity. Commencing Internet marketing or e-commerce, as described in section 2.1 of the paper, is associated with specific goals. The survey of SMEs in Romania and Latvia gave an opportunity to identify not only the goals of beginning something new but also the extent to which the goals, in the opinion of SME representatives, had been achieved at their enterprises.

A positive fact, of course, is that engagement in business digitalization and e-commerce has yielded real results. In both countries, the SMEs achieved some results for each of the goals set: advertising goods and services, communication with buyers of their goods and services as well as selling their goods and services (Fig. 3).

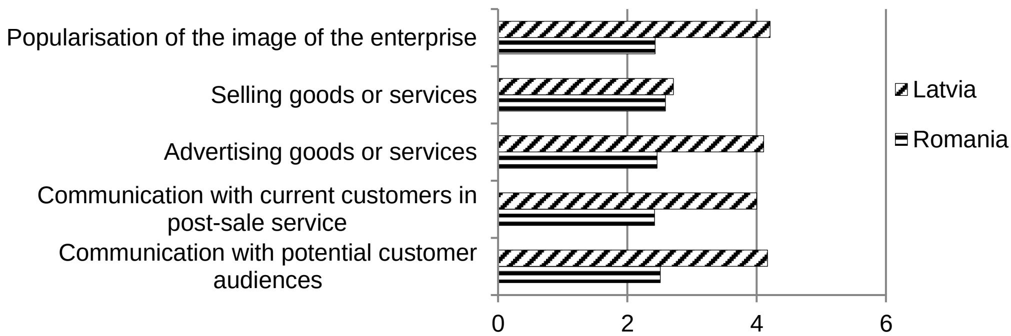

Figure 3. Respondents' ratings of e-commerce activity at their enterprises (weighted average on a five-point scale).

The survey revealed both similarities and differences between the countries. The first difference was the respondents' perspective on opportunities provided by e-commerce. In Romania, all the use options of digitalization were rated almost equally (ratings varied around 2.5 on a 5-point scale). In contrast, in Latvia the respondents' ratings of use options of digitalization were different. Communication, advertising and image popularisation were rated relatively high (ratings varied around 4.0), whereas selling goods and services was rated relatively low (only 2.7). It is interesting that in both countries particularly the ratings of selling goods and services were almost equal. This, in the opinion of the authors, confirms the opinion of the respondents of both countries once more that the relatively high development level of the Internet environment providing a possibility to advertise goods or services as well as communicate with buyers of goods and services does not ensure e-commerce activity is successful. As noted by the SME representatives, specialists competent in both digitalization and commerce organisation were needed. This was convincingly confirmed by the DESI indicator. In Romania, ICT specialists accounted for 2.0% of the total employees, in Latvia – 2.2%, while in Sweden where e-commerce activity was two-fold higher, they comprised 6.3%, i.e. three times more (EC. 2018a).

Table 5. Role of e-commerce in selling goods or services (weighted average on a five-point scale)

Country	Goods-producing enterprises	Services- producing enterprises	Trading enterprises	Other
Romania	2.33	2.54	2.90	3.0
Latvia	2.30	3.52	3.57	4.33

In both countries, in the opinion of the respondents, the largest problems were faced by goods-producing enterprises selling their goods through e-commerce, as the representatives of this group of enterprises gave the lowest ratings of e-commerce. This means that this group of enterprises in particular has to seriously and actively consider hiring ICT specialists, and – what is important – the specialists being able to also improve the performance of their e-commerce units. Swedish researchers associate it with their skill to better understand the needs and wishes of diverse consumer groups and the fact that the demand for goods is differentiated if the society is differentiated. Since the dominant part of the market is represented by buyers/consumers, this fact has to be taken into consideration in e-commerce and particularly cross-border selling management in order to promote the sales of goods and services (Monitoring ...2018).

CONCLUSIONS

The DESI index data aggregated by the authors confirm that the digitalization process progresses both in Romania and in Latvia. It is showed both by the DESI composite index and by the characteristics of SME economic activity, indicating that both countries are approaching the level of the old European Union Member States.

The data of the survey of Romanian and Latvian SME representatives gives an opportunity to express some conclusions on the pace of digitalization in two new European Union Member States that are very different in area size and population density as well as geographical location. There are similarities in SME digitalization between both countries. Communication with buyers/consumers promote sales of goods and services. In Latvia and Romania e-commerce takes the form of both domestic and cross-border selling. As result most of the SMEs financially benefit from digitalization, as their net turnovers rise. Also released problematic similarities. The Internet environment for the purpose of e-commerce is expensive. Lack of ICT specialists that can manage the e-environment and also competently organise cross-border selling, as it is not the same job. The smaller the enterprise (in terms of employment, net turnover), the lower the share of the enterprises engaged in e-commerce because of the two mentioned factors.

At the same time, one quite important difference in opinion on e-commerce between the Romanian and Latvian SME representatives has to be stressed. The Romanian respondents rated communication, advertising and sales with regard to e-commerce quite equally. The Latvian respondents' opinions on the same phenomena were different. The Latvian SME representatives rated advertising goods or services and communication with consumers higher than the Romanian counterparts did. However, the key purpose of economic activity – selling goods and services – that brings financial gains was rated considerably lower.

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REFERENCES

Broadband Coverage in Europe 2017. Final report. European Commission, Luxemburg. Access: https://ec.europa.eu/digital-single-market/en/news/study-broadband-coverage-europe-2017

European Commission (EC). 2010. Europe 2020. A European strategy for smart, sustainable and inclusive growth. Access: http://ec.europa.eu/eu2020/pdf/COMPLET%20EN%20BARROSO%20%20%20007%20-%20Europe%202020%20-%20EN%20version.pdf

European Commision (EC). 2014. Digital agenda for Europe. Rebooting Europe's economy. Access: https://eige.europa.eu/resources/digital_agenda_en.pdf

European Commission (EC). 2015. COSME. Europe's programme for small and medium-sized enterprises. Access: https://ec.europa.eu/growth/smes/cosme_en

European Commission (EC). 2018a. The Digital Economy and Society Index (DESI). Access: https://ec.europa.eu/digital-single-market/en/desi

European Commission (EC). 2018b. Digitising European Industry. Stakeholder Forum. Access: https://ec.europa.eu/digital-single-market/en/news/digitising-european-industry-stakeholder-forum-2018

Mahring, M., Wennberg K. & Demir R. 2018. Reaping Value From Digitalization in Swedish Manufactoring Firms: Untapped Opportunities? *In: Managing Digital Transformation.* pp. 41–63. Stockholm School of Economics Institute for Research.

Managing Digital Transformation. 2018. 4st Chapter – The Digital Customer, 97–154. SSE Institute for Research.

Marketing Angels. Ltd. Access: https://www.marketingangels.lv/lv/

Moroz, M. 2017. The level of development of the digital economy in Poland and selected European countries: a comparative analysis. *Foundations of management* **9**(1), 175–190.

Perkowitz, S. 2005. Digital People in Manufacturing: Making Them and Using Them. *The Bridge* **35**(1), 21–5.

Pytkovska, J. & Korynski P. 2017. Digitalizing Microfinance in Europe. Microfinance Centre. Access: https://www.european-microfinance.org/sites/default/files/document/file/Digitalization-research-paper.pdf

Rachinger, M., Reuter R.,Muller Ch., Vorraber W. & Shirgi E. 2018. Digitalization and its influence on business model innovation. Journal of Manufactoring Technology Management (Earlysite Online) https://www.emeraldinsight.com/doi/pdfplus/10.1108/JMTM-01-2018-0020

Rhoades, L.J. 2005. The Transformation of Manufacturing in the 21th Century. *The Bridge* **35**(1), 13–20.

Schallmo, D., Williams, C.A. & Boardman, L. 2017. Digital Transformation of Business Models - Best Practice, Enablers, and Roadmap. *International Journal of Innovation Management* **21**(08), 1–17.

Schwab, K. 2017. *The Fourth Industrial revolution.* UK, Penguin Random House. 184 pp.

TUAC. 2017. Digitalisation and the digital economy. Trade union key messages. Access: https://www.ituc-csi.org/digitalisation-and-the-digital

21st century manufactoring, 2013. UNIDO, Vienna. 103 pp.

Agronomy Research **17**(1), 271–280, 2019
https://doi.org/10.15159/AR.19.026

Modelling of operator's focusing scheme along working hours: harvesting operation

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Abstract. In consistent with the growing research activities regarding the Farm 4.0 concept, it is valuable to consider each possible chance of enhancement which is expected to contribute positively to the productivity and the safety of planned operations. Human centred design concept is becoming essential for the multitasking vehicles market, which promotes the research experiments aiming to understand the human behaviour inside the vehicle cabins to proceed with upgrading the design, planning and production procedures based on validated inputs leading to introducing reliable solutions for more productive and safety conduct of operations. The accurate and deep analysis of the operator behaviour inside the cabin will lead to a better understanding for the problems and issues need to be resolved in new designs in addition to providing the production planning (i.e. manpower planning and working shift period) with the necessary data to ensure achieving the maximum efficiency and effectiveness. In this research, the operator's glance behaviour inside the tractor cabin is studied during the harvesting operation to develop a model for the change of operator's focusing scheme along working hours.

Key words: off-road vehicle, operator's behaviour, eye tracking, focusing scheme, passive fatigue, precision farming.

INTRODUCTION

Herdovics (2013) mentioned a very important role of the academic sector improving the development of agricultural operations by problem resolving and developing the recommendations based on the advanced analysis of data to be used in advanced farming processes easily. To the purpose of assisting the decision-making activity during the planning phase (Man-power planning, operational procedures, …etc.) this research activity is conducted. Thereafter; the availability of such models will provide decision makers by a factual based method coming from deterministic data analysis, which is usually done based on managerial assumptions in the absence of validated models from research and development centres and/or academic sector.

In continuation of the research work done by Szabo et al. (2017) and Szabo et al. (2018) regarding Operator's behaviour measuring methodology inside off-road vehicle cabin and the developed two models for windrowing and cultivating agricultural operations, in which the passive fatigue is examined due to the nature of the said two operations requiring a continual physical movement to turn back and check for the rear attached tool. However; it is stated that the increasing mental load is inherent inside the resulted models.

This research activity depends on the validated methodology to develop a model representing the change on the resulted operator's focusing scheme along working hours in harvesting agricultural operation in which the operator doesn't need to physically turn back and check for a rear attached tool, but the operator is still requested to pay enough attention to the front mounted tool doing the necessary steering and monitoring.

Due to the operational nature of multi-tasking off-road vehicles, operators need to spend long working hours, which increases the level of mental workload leading to human error. Li & Haslegrave (1999) introduced similar conclusion of which the vehicle design should be human oriented in order to maximize comfort and ability to perform the driving task perfectly and safely by reducing the human error possibility.

In the in-road vehicles market, such a study is conducted more thoroughly to check for the distraction caused to the driver due to the increasingly added systems and technologies to the driving cabin. Indirect measures are usually applied to assess safety and attention of drivers due to complexity of definitions and criteria of safety and attention. That is why some studies such as (Alm & Nilsson, 1995; Hulst et al., 2001; Lai et al., 2001; Lee et al., 2001; Broy et al., 2006) commonly used measures of driver distraction related to secondary task interaction by primary task measures such as lateral control (e.g. measurement of lane keeping performance) and longitudinal control (e.g. speed maintenance).

Therefore, it is quite common to make inferences, such that higher driver workload when interacting with an in-vehicle system implies greater lateral movement and more frequent lane exceedances. It is interesting to note that a measure such as the number and length of lane exceedances during in-vehicle interaction is not considered primarily safety-relevant by everyone.

Cnossen et al. (2000) and Liua & Lee (2006) studies argue that if there are no other traffic users nearby, if the lane exceedance is small or of short duration, or if the lane exceedance or speed reduction reflects the driver's strategy for compensation and reducing workload during concurrent task execution, there is no safety implication at all.

It is a general aim to improve comfort and safety (Sheridan, 1992; Endsley, 1996; Fukunaga et al., 1997; Scheding et al., 1999; Shen & Neyens, 2017; Zewdie & Kic, 2017; S. Kumar et al., 2018), additionally, it is stated that, in the automated driving condition, driver responses to the safety critical events were slower, especially when engaged in a non-driving task. At the same time in their paper – dealing with driver visual attention (Louw & Merat, 2017) reached a conclusion shows that the drivers understanding of the automated system increases as time progressed, and that scenarios which encourage driver gaze towards the road centre are more likely to increase situation awareness during high levels of automation.

Operating an off-road vehicle is a complex task, requiring a concurrent execution of various cognitive, physical, sensory and psychomotor skills (Young & Regan, 2007), additionally to control attached tools to perform in-field productive tasks such as agricultural and industrial operations. Ensuring the comfortable ride is considered essential for any vehicle, as well as executing happily and safely requested operational tasks, to that end, the driver ergonomics comes to play as considered as an important parameter that can't be neglected in the design phase of the vehicle (Hsiao et al., 2005).

Driving is not only a physical task but also visual and mental tasks. The eyes of a driver are indispensable in performing visual tasks such as scanning the road and monitoring in-vehicle devices. Mental tasks are important during driving, and include such factors as understanding vehicle dynamics, making situation-dependent decisions, and judging time/space relationships (Kramer, 1990), (De Waard, 1996), (Brookhuis & De Waard, 2010) and (Marquart et al., 2015) were examined the eye-related measures of drivers' mental workload. The mental workload could be defined as the relation between demands resulted from various tasks to be performed on the operator and his ability to fulfil; with satisfactory; these demands. While (Sporrong et al., 1998) described the mentioned demands as multidimensional, as it involves tasks, operator and system demands together with other factors. Additionally; many studies showed that; the need for well fitted architectural space to the operator's dimensions is considered crucial. The mental workload level is found to be increasing with the time passing.

For the purpose of this research, the passive fatigue is investigated. This type of fatigue is characterized by being the indirect product of the human driver's exertion of a set of tasks whose demands are low, monotonous or repetitive (Saxby et al., 2013). These rules out any sort of physical fatigue or mental active fatigue.

MATERIAL AND METHODS

Tobii equipment and software packages

Tobii solutions were used to conduct the eye tracking and glance measuring of the operator inside the off-road vehicle cabin.

Tobii glasses 2 (Fig. 1) package was selected due to its mobility feature in addition to the powerful properties enable the operator to use it in the daylight and night in the field. A brief description of the package is illustrated in the figure below:

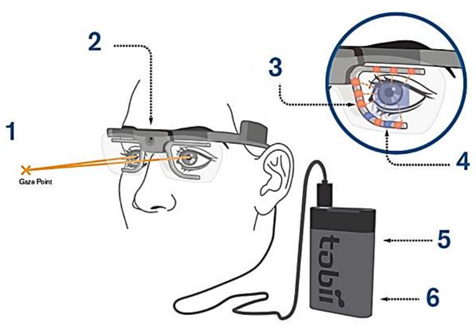


Figure 1. Tobii glasses 2 package.

1 – Eye tracker: consists of cameras, illuminators, and algorithms;
2 – Scene camera: a camera is recording what the operator is looking at;
3 – Illuminators: creates a pattern of near infrared light on the eyes;
4 – The cameras: take a high-resolution image of the user's eyes and patterns;
5 – The image processing algorithms: find specific details in the user's eyes and reflection patterns;
6 – The eye position and gaze point are calculated using a sophisticated 3D eye model algorithm based on the inputs and configurations mentioned previously.

MATLAB

The Curve Fitting Toolbox is used for modelling the resulted data of the change in operator's focusing scheme along working hours for the samples collected from harvesting agricultural operation.

Research methodology

Followed methodology is summarized in process map showed in (Fig. 2).

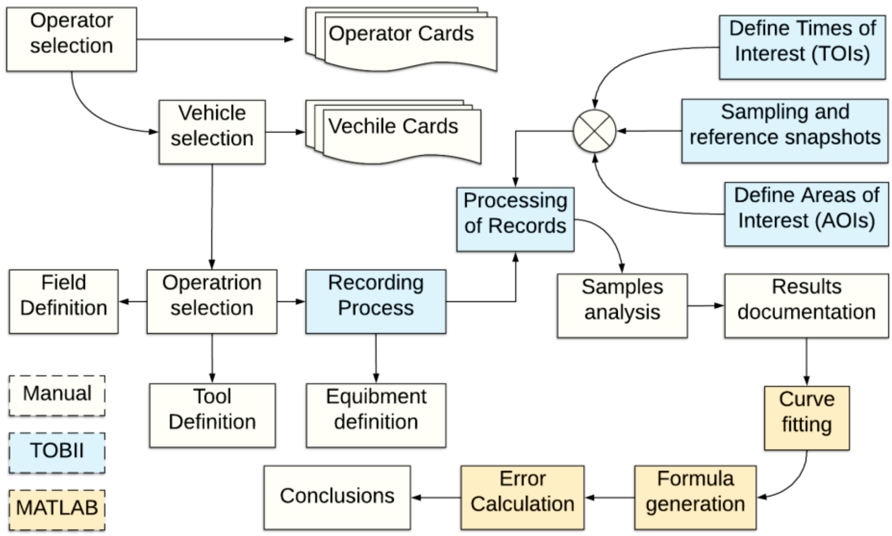


Figure 2. Methodology process map (Szabo et al., 2018).

The selected area of interest (AOI), to the purpose of this research, is the front mounted harvesting tool (Fig. 3). The operator needs to keep focusing on AOI for continuously monitoring and steering the vehicle into the right path, which requires more mental load than the additional physical passive fatigue for turning around and check a rear tool as an example.

A reference snapshot (Fig. 4) is taken for the selected AOI form videos recorded by the Tobii Glasses 2 equipment.

Figure 3. The front mounted tool for harvesting operation.

Figure 4. Reference snapshot for the harvesting tool.

The operator is requested to wear Tobii glasses and to go through the calibration process whenever a new recording is started. The glasses are connected wirelessly to the windows tablet which is running the Tobii controller software to register the recording information, monitor the real-time view of the operator, conduct the calibration process and to stop, pause and start the recording process.

Thereafter, the collected video recordings are transferred to the PC which is running the Tobii Pro Lab software to be analyzed using the real-time mapping and available filtering packages to obtain the accumulated times. Recorded videos are splitted out into number of recording samples, each one represents 600 seconds of the real-time recording of the operator's gaze during the windrowing operation.

Due to the differences between the planned and actual recoding time, each sample is normalized to represent the 600 seconds of recording with a factor (*N*). However, collected gaze time spent on the attached tool (*X*) is multiplied by the Normalization factor (*N*) according to the formula:

$$(X)_{\text{Normalized}} = (N) \cdot (X)_{\text{Actual}}$$

Cultivating operation

The experimental trials are conducted for harvesting the sunflowers field using the vehicle (CLAAS Dominator 202) (Fig. 5), in a field beside Gödöllői airport to the south west of Gödöllő city.

Figure 5. CLAAS Dominator 202.

RESULTS AND DISCUSSION

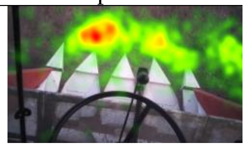
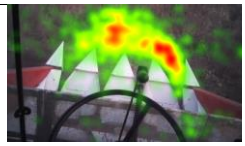
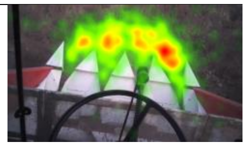
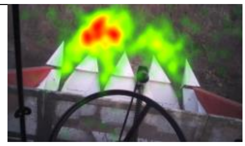
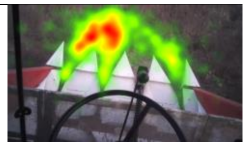
Measurements and analysis results

After recording the harvesting operation along one working day with calibration process conducted successfully whenever it was needed, the recorded samples were analyzed by Tobii Pro Lab analyzer software and exported to MS Excel sheet.

The samples were normalized in accordance to the mentioned normalization formula and the exported results (Table. 1) showed the following:

- The sample reference in the original video (column 1); which represents the reference of a certain sample inside the used analyzer software (Tobii Pro Lab).
- The sample serial number (X) (column 2); which will represent the X-Axis on the resulted curve.
- The tool gaze times in (X) sample (column 3); which will represent the accumulated time of operator's gaze inside the AOI on the Y-Axis on the resulted curve.
- The normalization factor (N) for the sample (X) (column 4).
- The Normalized tool gaze times (X*N) (column 5).
- The generated heat map for the sample (X) (column 6); which is a graphical representation for the operator's gaze distribution and accumulated time over the reference image along the sample recording time.

Table 1. Sample Experiment results of windrowing operation

Sample Reference	X value	Tool gaze time (Sec)	N Factor	Time (Normalized) (Sec)	Generated Heat map
1	1	481	0.90	245.52	
2	2	413.5	1.00	274.79	
3	3	500.05	1.00	369.99	
4	4	409.89	1.00	282.74	
5	5	440.73	1.00	362.42	

Putting the resulted data of accumulated time of each sample on the y-axis and the and the samples sequence on the x-axis, after the normalization of results based on the actual recording time to represent 600 seconds of recording for each sample, the results are shown in (Fig. 6).

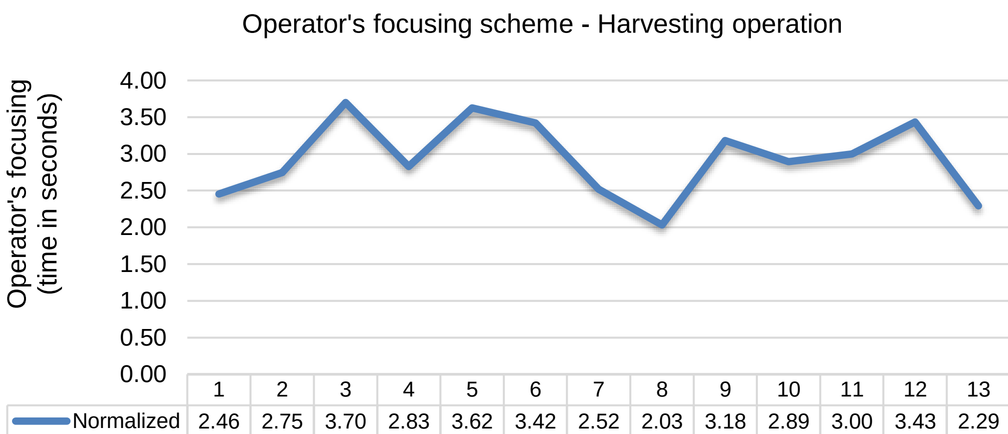


Figure 6. Accumulated time of operator's focusing scheme for each sample in harvesting agricultural operation.

Modelling results

The curve fitting operation is conducted using the MATLAB Curve Fitting Toolbox™, the resulted curve (Fig. 7) for the harvesting operation was processed selecting the Linear model (Poly 4) which generates a polynomial equation with the forth degree and using Bi-square robust method.

Where:

X-Axis represents the samples serial number (1 unit = 600 working seconds)

Y-Axis represents the OFS (Accumulated gaze time on the AOI in seconds)

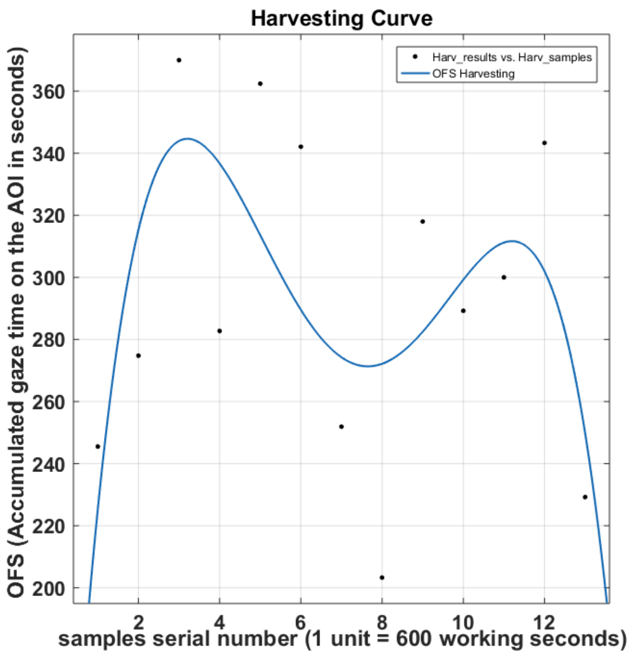


Figure 7. Curve fitting of results.

The results showed the operator's gaze on selected area of interest. The used equipment and supporting software packages easily defined the time in which the operator paid his attention to the front mounted tool during working time in the harvesting operation developing the model describing the change on the OFS along working hours $X_{harv}(t)$:

$$X_{harv}(t) = 274.3 - 35.07t + 103.3t^2 + 18.12t^3 - 50.04t^4$$

Resulted models and the goodness of fit as per exported from MatLab Curve fitting toolbox:

Linear model Poly4:

f(x) = p1*x^4 + p2*x^3 + p3*x^2 + p4*x + p5
p1 = -50.04
p2 = 18.12
p3 = 103.3
p4 = -35.07
p5 = 274.3

Goodness of fit:

R-square: 0.2816
RMSE: 54.34

DISCUSSION

The used equipment and supporting software packages easily defined the time in which the operator paid attention to the defined areas of interest during the operations. All experimental trials were conducted in similar environmental and operational conditions. The daylight recording, use of closed cabin controlling the temperature and humidity inside the cabin, protection from dust and insects… etc.; all of it; are considered to be similar along executing all experimental trials in order to keep on consistency of environmental and operational conditions trying to include the same uncertainties sources along all developed models which is reflecting the routine duties conducting by the operator in agricultural operations.

The resulted models can be used to give an indication estimating the effort required by operators to conduct different agricultural operations based on deterministic data driven models.

The impact of the learning process on the operator's focusing scheme is subjected to be under more investigation in order to assess the contribution of the experience of the operator to the production phase in a certain agricultural operation which is proposed to be conducting by developing different models for the same operation executed by different operators with differentiated levels of operating experience.

The resulted models are developed to be used as a simple tool predicting the behaviour of an operator inside the off-road vehicle cabins based on deterministic data analysis. The contribution of the implemented models is expected to assist the decision-making process regarding many aspects (i.e. scheduling of breaking times, working hours and payment estimation). Which make it necessary not to exclude any uncertainties expected to accrue during the real-time implementation of the model.

Taking into consideration keeping on the simplicity of the model and not excluding of uncertainties, the resulted models are showing low R2 coefficient of determinization. This small number is resulted from the huge variation of accumulated operator's gaze from each sample to other samples. Each sample result represents summation of operator's gaze along the 10 minutes of the sample record analysis. Repeating some routine tasks require more operator attention to the AOI might be repeated twice in the same sample while it would not happen in next or previous sample (i.e. lifting the tool to turn over at the end of the field will be reflected twice in one sample and might not happen in the next recording sample).

However; the resulted models for the tested agricultural operations are found to be the first attempt to modelling the change on operator's focusing scheme along working hours, which is subjected to be improved on a continual base.

CONCLUSIONS

This work presents a method for measuring change of operator focusing scheme over time during an agricultural operation. The time is not the only factor affecting the operator focusing scheme. Other factors such as age, gender, experience, working conditions... etc. are not within the scope of this work and are left for future works.

The resulted model showed a notable decrement behaviour in operator's focusing scheme along working hours, which is correlated to the passive fatigue and mental load accumulated along working hours. However, the resulted trend of decrement is slower than it was in the previous experiments which require the operator contribution to monitor and control the rear attached tool on a continual base.

The resulted model is subjected for further development in term of the modelling quality. However; it is still usable to provide an indication for comparing different agricultural operations.

REFERENCES

Alm, H. & Nilsson, L. 1995. The effects of a mobile telephone task on driver behaviour in a car following situation in *Accident analysis & prevention* **27**(5), 707–715.

Brookhuis, K. & De Waard, D. 2010. Monitoring drivers' mental workload in driving simulators using physiological measures. *Accident Analysis and Prevention* **42**, 898–903.

Broy, V., Althoff, F. & Klinker, G. 2006. Designing for the end user: short papers: iflip: a metaphor for in-vehicle information systems in AVI`06 Conference

Cnossen, F., Rothengatter, T. & Meijman, T. 2000. Strategic changes in task performance in simulated car driving as an adaptive response to task demands in *Transportation research part F* **3**, 123–140.

De Waard, D., Van der Hulst, M. & Brookhuis, K.A. 1999. Elderly and young drivers' reaction to an in-car enforcement and tutoring system. *Applied ergonomics* **30**(2), 147–157. doi:10.1016/s0003-6870(98)00002-7

Endsley, M.R. 1996. Automation and situation awareness. *Automation and Human Performance: Theory and Applications,* CRC Press. 163–181.

Fukunaga, A., Rabideau, G., Chien, S. & Yan, D. 1997. Towards an application framework for automated planning and scheduling. *Aerospace conference*. In Proceedings, IEEE, pp. 411–427.

Herdovics, M. 2013. *Agroinformatics*. University of Debrecen. ISBN:978-963-473-671-4

Hsiao, H., Whitestone, J., Bradtmiller, B., Whisler, R., Zwiener, J., Lafferty, C. & Gross, M. 2005. Anthropometric criteria for the design of tractor cabs and protection frames. *Ergonomics* **48**(4), 323–353. doi:10.1080/00140130512331332891

Hulst, M. van der, Meijman, T. & Rothengatter, T. 2001. Maintaining task set under fatigue: a study of time-on-task effects in simulated driving in *Transportation research part F: Traffic psychology and behaviour* **4**(2), 103–118.

Kramer, A. 1990. Physiological metrics of mental workload: A review of recent progress. In D. Damos, (Ed.). 2015. Multiple-task Performance, London: Taylor & Francis. 279–328.

Kumar, S., Cerny, J. & Kic, P., 2018. Air-conditioning in the cabins of passenger cars. *Agronomy Research* **16**(5), 2088–2096.

Lai, J., Cheng, K., Green, P. & Tsimhoni, O. 2001. *On the road and on the web: comprehension of synthetic and human* speech while driving in *CHI 2001*. IBM/UMTRI

Lee, J.D., Caven, B., Haake, S. & Brown, T.L. 2001. Speech-based interaction with invehicle computers: the effect of speech-based e-mail on drivers' attention to the roadway in *Human Factors,* 631–640.

Li, G. & Haslegrave, C.M. 1999. Seated work postures for manual, visual and combined tasks. *Ergonomics* **42**(8), 1060–1086. doi:10.1080/001401399185144

Liua, B. & Lee, Y. 2006. In-vehicle workload assessment: effects of traffic situations and cellular telephone use in *Journal of safety research* **37**, 99–105.

Louw, T. & Merat, N. 2017. Are you in the loop? Using gaze dispersion to understand driver visual attention during vehicle automation. *Transportation Research Part C* **76**, 35–50.

Marquart, G., Cabrall, C. & Winter, J. 2015. Review of eye-related measures of drivers' mental workload. *Procedia Manufacturing* **3**, 2854–2861.
Saxby, D.J., Matthews, G., Warm, J.S., Hitchcock, E.M. & Neubauer, C. 2013. Active and passive fatigue in simulated driving: discriminating styles of workload regulation and their safety impacts. *J. Exp. Psychol. Appl.* **19**(4), 287–300.
Scheding, S., Dissanayake, G., Nebot, E.M. & Durrant-Whyte, H. 1999. An experiment in autonomous navigation of an underground mining vehicle. *IEEE Transactions on Robotics and Automation* **15**(1). 85–95.
Shen S., & Neyens, D.M. 2017. Assessing drivers' response during automated driver support system failures with non-driving tasks. *Journal of Safety Research* **61**, 149–155
Sheridan, T.B. 1992. Telerobotics, automation, and human supervisory control. 1–10. MIT Press.
Sporrong, H., Palmerud, G., Ka defors, R. & Herberts, P. 1998. The effect of light manual precision work on shoulder muscles-an EMG analysis. *Journal of Electromyography and Kinesiology* **8**, 177–184.
Szabó, I., Hushki, M., Bártfai, Z. & Kátai, L. 2017. Operator's behaviour measuring methodology inside off-road vehicle cabin, operator's focusing scheme. *Agronomy Research* **15**(5), 2172–2182. doi: 10.15158/AR.17.033
Szabó, I., Hushki, M., Bártfai, Z. & Kátai, L. 2018. Modelling of operator's focusing scheme along working hours: windrowing and cultivating operations. *Agronomy Research* **16**(4), 1885–1895. doi: 10.15159/AR.18.155
Young, K. & Regan, M. 2007. Driver distraction: A review of the literature. In: I.J. Faulks, M. Regan, M. Stevenson, J. Brown, A. Porter & J.D. Irwin (Eds.). Distracted driving. Sydney, NSW: Australasian College of Road Safety, pp. 379–405.
Zewdie, R. & Kic, P., 2017. Substantial factors influencing drivers' comfort in transportation. *Agronomy Research* **15**(5), 2236–2246. doi: 10.15159/AR.17.037

Agronomy Research **17**(1), 281–294, 2019
https://doi.org/10.15159/AR.19.025

The effect of the 1,2,3-triazolo[5,1-*b*][1,3,4]thiadiazines on *Solanum lycopersicum* L. seed germination

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Abstract. In recent years chemical pesticides are being replaced by environmentally friendly and universal means of plant protection that are able to exert a complex effect on the plant (stimulate growth and development, improve metabolic processes, develop resistance to pathogens, etc.). The effect of new synthetic growth regulators of the 1,2,3-triazolo[5,1-b][1,3,4]thiadiazine class and trade phytohormones (6-BAP, GA and TDZ) on the growth processes, growth energy and vitality of tomato seeds, as well as morphological parameters of seedlings was researched in this article. It was revealed that the effect of synthesized compounds on seed vigor and viability of seedlings are superior to commercial phytohormones. In the early stages of germination the seed vigor of tomato seeds treated with the compounds TT1-TT5 were superior to one in treatments with phytohormones and in control experiment. As a result of the experiment, the most viable seedlings were formed into the treatments **TT1, TT2** and **TT3** (in all studied concentrations). Tomato seedlings treated with 6-BAP and GA at a concentration of 5 mg L^{-1} produced the worst results.

Key words: seed germination, plant grow regulators, *Solanum lycopersicum*, tomato seeds, 1,2,3-triazolo[5,1-*b*][1,3,4]thiadiazine, 6-benzylaminopyrine, gibberellic acid, thidiazurone, leaf tissue, development/growth, root, shoot.

INTRODUCTION

Both seed germination and the earliest stages of seedling development are crucial for the establishment of viable plantlets of agricultural plants and are important processes that determine the competitiveness and fitness of the plants in various environments, such as lighting, temperature, humidity, etc (Luo et al., 2019). The establishment of seed dormancy, subsequent germination and other developmental processes in plants, are heavily influenced by hormones. Abscisic acid is required for the establishment of seed dormancy and inhibits germination. Gibberellins, on the other hand, counteract abscisic acid responses and are considered a promoting factor in seed germination. Cytokinins also appear to act positively on germination, possibly by stimulating ethylene synthesis (Toh et al., 2011).

Both natural and synthetic phytohormones are used for regulation of seed germination and plant growth. In addition, regular fertilization, and/or growth stimulants are required for normal growth and development of plants as well as increasing yields and increasing resistance to pathogens of various types (Senberga et al., 2018).
After a long period of uncontrolled use of chemical fertilizers, strict measures have been taken to regulate and use the latter. Crop producers are interested in the emergence of new natural and synthetic compounds that do not harm the environment (Pal & McSpadden Gardener, 2006).

Currently, growth regulators and elicitors are a vast group of natural and synthetic organic compounds that influence the metabolism which stimulate plants' immunity to many bacterial and viral diseases, as well as adverse environmental factors (Pekarskas & Sinkevičienė, 2015; Nugmanova, 2017).

The requirements for the properties of chemically synthesized growth regulators are rather high: physiological activity, rapid disintegration in tissues after their action, and the absence of harmful effects on the environment and humans (Harman, 1991). A special property of a physiologically active compound should be its ability to act in low concentrations (a low number of milligrams per liter of solution).

Plant seeds are a convenient test system in the study of certain properties of substances or the study of completely new chemical compounds because the growth and development of the seed can be used as an integral indicator of the state of the plant, which reflects the physiological processes at the level of the whole organism (Taiz & Zeiger, 2002). The seeds of agricultural crops are the best for studying stimulation effects.

This article is a continuation of a study on the growth-regulating properties of newly synthesized chemical compounds of the 1,2,3-triazolo[5,1-b][1,3,4]thiadiazine class (Fig. 1) on plants of the Solanaceae family. Previously, some of the studied substances showed a stimulating effect on the growth and development of seeds of common pine and animal cells (Kalinina et al., 2015; Kalinina et al., 2018). In this paper, we studied the growth-regulating properties of five synthesized compounds and three commercial phytohormones (gibberellic acid, 6-benzylaminopurine, thiadiazuron) on tomato seeds. The aim of this work was to investigate the influence of tested compounds on seed germination and seedling development.

MATERIALS AND METHODS

The objects of study were 8 chemical compounds. 3 commercial phytohormone were used (gibberellic acid (GA), 6-benzylaminopurine (6-BAP), thiadiazuron (TDZ)), as were 5 synthesized substances: **TT1, TT2, TT3, TT4, TT5** (Fig. 1).

The tested compounds (TT1-TT5) were produced according to the previously described method (Kalinina et al., 2017). The spectroscopic characteristics of the compounds **T1–T4** are in accordance with the previously reported data (Kalinina et al., 2017). Compound **T5** was synthesized for the first time according to the procedure described in (Kalinina et al., 2017).

^{1}H and ^{13}C NMR spectra were acquired on a Bruker Avance II spectrometer (400 and 100 MHz, respectively) in DMSO-d_6, with TMS as the internal standard. Mass spectra were recorded on a GCMS-QP2010 Plus gas chromato-mass spectrometer (EI ionization at 70 eV). Elemental analysis was performed on a Perkin Elmer PE 2400 CHNS-analyser. Melting points were determined on a Stuart SMP3 apparatus.

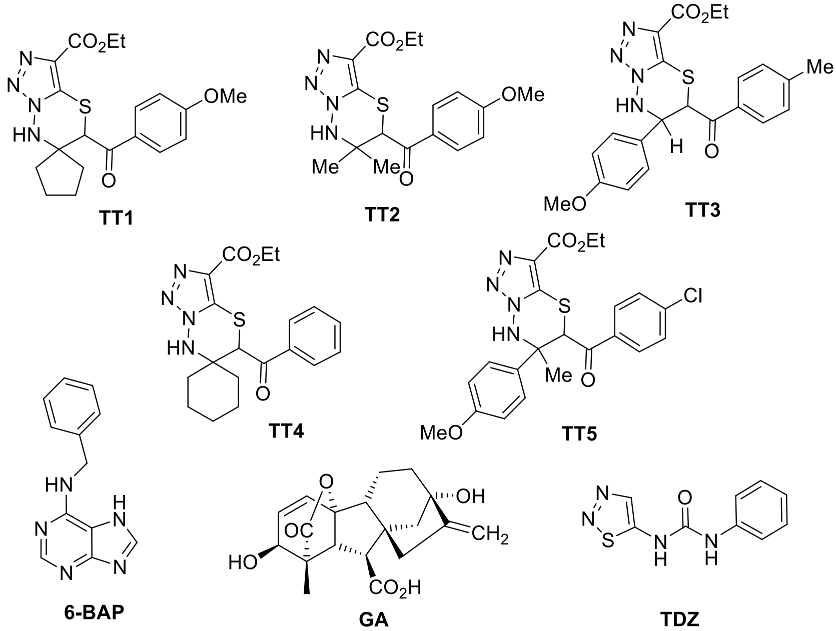


Figure 1. The structure of the studied compounds and commercial phytohormones.

Ethyl 5-(4-chlorobenzoyl)-6-methyl-6-*p*-tolyl-6,7-dihydro-5*H*-[1,2,3]triazolo[5,1-*b*][1,3,4]thiadiazine-3-carboxylate (TT5). Reaction yield is 76%, white powder, melting temperature 217–218 °C. IR υ (cm^{-1}): 3234 (NH), 2964 (CH), 2834 (CH), 1738 (CO), 1712 (CO). ^1H NMR (*J*, Hz): δ 1.29 (3H, t, *J*=6.5, Me), 1.47 (3H, s, Me), 3.73 (s, 3H, OMe), 4.22 (q, 2H, *J*=6.5, OCH_2), 5.75 (s, 1H, CH), 6.82 (d, 2H, *J*=7.9, ArH), 7.52 (d, 2H, *J*=7.9, ArH), 7.63 (d, 2H, *J*=7.8, ArH), 7.96 (s, 1H, NH), 8.31 (d, 2H, *J*=7.8, ArH). Attached proton test (APT) NMR: δ 13.9 (CH_3), 27.8 (CH_3), 40.6 (OCH_3), 54.9 (CH_{TDZ}), 59.7 (C_{TDZ}), 60.4 (CH_2), 113.8 (CHAr), 126.5 (CHAr), 127.1, 129.0 (CHAr), 130.8 (CHAr), 133.9, 134.8, 139.4, 158.4, 159.8, 195.7 (CO). MS-spectrum (m/z relative intensity): 472 $[M]^+$ (5), 416 (7), 400 (4), 285 (3), 251(2), 148 (100), 141 (11), 139 (35), 134 (59), 119 (18), 113 (5), 111 (14), 107 (13), 105 (5), 103 (4), 92 (18), 77 (32), 65 (7), 51 (3). Found, %: C 57.78; H 4.70; N 12.08. $C_{22}H_{21}ClN_4O_3S$. Calculated, %: C 57.83; H 4.63; N 12.26.

Biological study

The effect of the synthesized chemical compounds on seed germination was studied in 3 concentrations: 0.5, 1 and 5 mg L^{-1}. Negative control was administered with water. We used seeds of the 'Siberian early' tomato variety (Russia).

The experiment on the effect of the studied substances on the germination of tomato seeds was run in the Binger climate chamber for full control of the temperature and humidity conditions, as well as creating conditions close to natural ones: 20 °C, 55–60% humidity, and 16 daylight hours. In the experiment, we used mature tomato seeds with a stated commercial germination rate of 90%. The seeds were placed in Petri dishes on filter paper, 100 pcs per dish. For each concentration of the substance, the experiment was run three times. A total of 7,000 'Siberian early' tomato seeds were sown. To each

Petri dish 3 mL of solutions of the studied substances were added (distilled water for negative control). The measurement of the weight of the Petri dishes for humidity control was carry out every day, the difference from the starting weight was determined and the necessary amount of water to return to the starting weight was added.

The measurement of seed vigor parameters was carried out at 3 time control points: 5, 10 and 15 days (FAO, 2010). The experiment was over on 15th day. Germination (%) is percent of germinated seeds from total seeds. Seeds were counted as germinated when the length of the radicle/root was more than twice the length of the seed. To assess the quality of the seedlings, the following parameters were selected: the total length and thickness of the root, the length and width of the cotyledons, the thickness of the stem at the base of the cotyledons, the elongation of the cotyledons and the root, and the branching of the root system. These features give an estimate of the vitality of the seedling and the quality of the assimilation apparatus being formed. Simultaneously with the measurement of the linear parameters of the seedlings, a visual assessment of the condition of the seedlings was carried out on plant health scale of 0 to 5, where 0 is unsatisfactory (signs of wilting, drying out, lodging, underdevelopment, etc.), and 5 is excellent (all seedlings are viable, crops do not lodge, the cotyledons are open and have a rich green colour, etc.). The fungal contamination was evaluated visually. For this, the Petri dish was separated into 4 sectors and infested area determined in percent. The average for all experimental treatments are presented in Table 1. The experimental results were processed using the STATISTICA 8.0 software package (ANOVA, Kruskal-Wallis test, Tukey test; graph is based on the calculated Mahalanobis distances in the discriminant analysis module).

The variability of the linear parameters and the shape of the cells were studied on the first true leaf in triplicate for each treatment. To study the effect of the substances under investigation on the growth of individual cells, the tissue maceration method was used. Maceration was carried out in KOH solution on pre-dried and cut (FastPrep-24) leaflets, which were taken three times from the middle of the crown. After the solution with leaf tissues was evaporated, the tissues were additionally crushed on a glass slide with a dissecting needle, covered with a slide and examined under a CarlZeizz microscope (15 × 40) in five visual fields for each concentration. The photographs were measured in the program AxioVizion CarlZeizz.

RESULTS AND DISCUSSION

The effect of 1,2,3-triazolo[5,1-b][1,3,4]thiadiazine derivatives and trade phytohormones (6-BAP, GA and TDZ) on the growth processes, growth energy and vitality of tomato seeds was evaluated.

Assessment of the morphological and physiological parameters. On the 5th day of the experiment, an assessment of seed germination energy was performed. In general, the seed vigor of tomato seeds ranged from 2% (GA treatment at a concentration of 5 mg L^{-1}) to 77.6% (**TT3** treatment at a concentration of 1 mg L^{-1}). Inhibition of growth processes was observed in one Petri dish in the TDZ treatment at a concentration of 0.5 mg L^{-1}. Influence of concentration of substance on the germination process was not detected for compound TT2. Seed vigor of tomato seeds treated TT2 was more than in the control and phytohormone treatments for all concentration of compound TT2 and ranged from 41 to 51% (Table 1). By the 10th day of the experiment, the proportion of germinated seeds reached maximum values in almost all treatments and concentrations, as well as in the control (water), with the exception of GA at a concentration of 5 mg L^{-1}, which demonstrated 35.7% (Table 1). By the 15th day of the experiment, the proportion of germinated seeds in the GA treatment, regardless of the concentration, increased slightly. It was noted that with increasing GA concentration, a general inhibition of seed germination rate was observed (Gupta & Chakrabarty, 2013).

Table 1. The share of germinated seeds of the 'Siberian early' tomato variety

Concentration, mg L^{-1}	5th day		10th day			15th day		
	Germinatio, %	Fungi infected, %	Germinatio, %	Fungi infected, %	Plant health scale	Germinatio, %	Fungi infected, %	Plant health scale
TT1								
0.5	27.6 ± 4.5	0	92.7 ± 2.6	0	5.0	100 ± 0.0	9.0 ± 3.3	5.0
1	30.0 ± 9.5	10.0 ± 7.6	94.7 ± 2.0	5.0 ± 2.9	3.8	100 ± 0.0	9.0 ± 2.1	4.5
5	40.6 ± 3.5	3.3 ± 1.7	97.7 ± 0.3	10.0 ± 5.8	4.2	97.7 ± 0.3	10.7 ± 0.3	4.5
TT2								
0.5	51.3 ± 3.8*	23.3 ± 7.3	97.4 ± 0.9	8.3 ± 1.7	3.7	97.0 ± 1.5	8.3 ± 1.7	4.0
1	41.3 ± 3.8*	11.7 ± 73	94.0 ± 0.6	8.3 ± 2.9	3.7	97.4 ± 0.9	8.3 ± 2.9	4.0
5	49.0 ± 7.8*	16.7 ± 8.3	94.7 ± 0.3	9.0 ± 3.3	3.0	97.4 ± 0.3	10.0 ± 2.9	4.0
TT3								
0.5	48.0 ± 24.2*	16.7 ± 3.3	94.4 ± 2.7	3.3 ± 1.7	4.3	97.0 ± 0.6	5.0 ± 0.0	4.0
1	77.6 ± 1.9*	0	98.4 ± 1.2	5.0 ± 2.9	4.0	97.4 ± 0.9	6.7 ± 1.7	4.0
5	37.6 ± 19.6	8.3 ± 4.4	92.0 ± 3.0	5.0 ± 3.0	5.0	97.4 ± 2.3	6.7 ± 1.0	4.0
TT4								
0.5	65.6 ± 6.4*	8.3 ± 3.3	92.7 ± 0.7	3.3 ± 3.3	4.3	95.0 ± 1.2	10.0 ± 5.8	4.0
1	64.3 ± 4.8*	21.7 ± 3.3	92.7 ± 2.4	8.3 ± 1.7	4.0	95.4 ± 1.5	11.7 ± 1.6	3.8
5	47.0 ± 6.7*	10.0 ± 5.8	91.7 ± 2.4	8.3 ± 1.7	3.3	94.0 ± 2.4	21.7 ± 7.3	3.3
TT5								
0.5	59.6 ± 1.2*	36.7 ± 1.7	92.0 ± 2.1	6.7 ± 1.7	4.3	97.4 ± 0.3	15.0 ± 5.0	3.0
1	36.3 ± 1.7*	3.3 ± 3.3	95.4 ± 0.7	8.3 ± 3.3	4.7	95.4 ± 0.9	20.0 ± 1.7	3.0
5	64.0 ± 12.0*	21.7 ± 7.3	94.4 ± 1.2	18.3 ± 4.4	4.7	94.0 ± 9.2	25.0 ± 5.6	3.0
TDZ								
0.5	0	–	84.4 ± 8.4	16.7 ± 4.4	1.3	83.4 ± 14.3	18.3 ± 8.3	1.8
1	11.3 ± 9.4	26.7 ± 26.7	78.4 ± 14.5	18.3 ± 9.3	1.8	77.4 ± 3.8	21.7 ± 11.7	2.5
5	25.6 ± 2.3	53.3 ± 3.3	89.4 ± 3.3	18.3 ± 5.3	2.8	80.4 ± 3.0	21.7 ± 1.7	2.5
GA								
0.5	20.6 ± 7.5	31.7 ± 4.4	93.0 ± 0.6	18.3 ± 7.3	3.2	93.0 ± 0.3	33.3 ± 12.0	3.0
1	17.0 ± 9.5	23.3 ± 12.0	79.0 ± 9.0	20.0 ± 7.3	2.5	89.0 ± 9.6	40.0 ± 10.1	3.0
5	5.0 ± 2.0	16.7 ±	35.7 ± 23.7	30.0 ± 10.0	1.0	66.3 ± 21.0	50.0 ± 26.5	1.3
6-BAP								
0.5	19.0 ± 4.0	13.3 ± 3.3	88.4 ± 0.7	23.3 ± 3.3	3.0	95.0 ± 2.5	36.7 ± 11.7	1.0
1	29.0 ± 9.0	16.7 ± 3.3	84.0 ± 0.6	18.3 ± 8.3	2.8	93.7 ± 0.7	39.7 ± 13.0	1.0
5	15.0 ± 5.1	40.0 ± 5.8	83.4 ± 5.5	23.3 ± 9.3	2.8	97.4 ± 1.2	40.0 ± 5.0	0.8
water								
–	34.0 ± 4.0	45.0 ± 0.1	91.5 ± 2.5	20.0 ± 10.0	3.3	94.0 ± 1.8	10.0 ± 5.0	4.0

Note: * – significant differences from water (Tukey test, ANOVA, $p < 0.001$).

By day 10, the tomato seedlings treated with compounds **TT1** and **TT2** at a concentration of 1 mg L^{-1} were in the lead in terms of the parameter of growth processes (Table 2, Fig. 2). The maximum linear dimensions of the root, which were noted in the **TT2** treatment at a concentration of 0.5 mg L^{-1}, were 49.07 ± 2.55 mm. The maximum linear dimensions of the cotyledons (7.78 ± 0.55 mm) were noted in the **TT1** treatment at a concentration of 1 mg L^{-1}. The maximum thickness of the young spine was noted in the 6-BAP treatment in two concentrations – 0.5 mg L^{-}1 and 1 mg L^{-1} (1.42 ± 0.23 mm and 0.99 ± 0.12 mm), more than 2.5 times the size of the control. Earlier, it was noted that high concentrations of 6-BAP (more than 1 mg L^{-1}) are able to inhibit the growth of cells following stretching, which leads to the formation of short internodes and highly branched plant forms (Pan et al, 2013). In the **TT2** and **TT4** treatments, the formation of lateral roots was noted in all concentrations. Probably, these substances, like cytokines, are capable of removing the apical dominance, causing inhibition via the growing apical bud of lateral shoots (Engelbrecht, 1971).

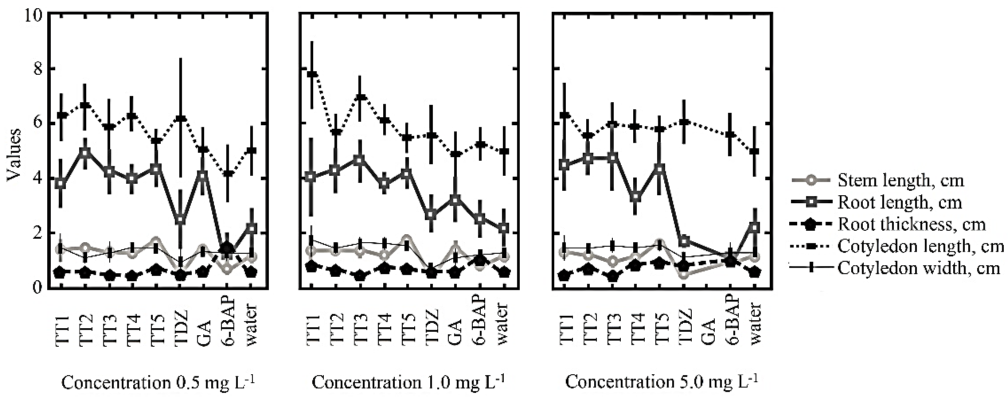
Table 2. Linear parameters of the seedling of the 'Siberian early' tomato variety on the 10th experimental day

Concentration, mg L^{-1}	Stem length, cm (M ± m)	Root length, mm (M ± m)	Root thickness, mm (M ± m)	Root elongation (M ± m)	Cotyledon length, mm (M ± m)	Cotyledon width, mm (M ± m)	Cotelydon elongation (M ± m)
TT1							
0.5	1.36 ± 0.17	38.22 ± 3.79	0.52 ± 0.01	0.014 ± 0.003	6.22 ± 0.36	1.48 ± 0.22	0.23 ± 0.04
1	1.33 ± 0.14	40.56 ± 6.21	0.79 ± 0.16	0.024 ± 0.006	7.78 ± 0.55$^{I, III}$	1.70 ± 0.23	0.23 ± 0.03
5	1.26 ± 0.11	44.83 ± 4.13	0.46 ± 0.07$^{II, III}$	0.011 ± 0.002$^{II, III}$	6.29 ± 0.53	1.48 ± 0.19	0.26 ± 0.04
TT2							
0.5	1.45 ± 0.07$^{I, II, III}$	49.07 ± 2.55^{II}	0.59 ± 0.04	0.012 ± 0.001	6.60 ± 0.40	1.13 ± 0.07	0.18 ± 0.01
1	1.37 ± 0.06^{II}	42.87 ± 3.69	0.59 ± 0.03	0.016 ± 0.002	5.73 ± 0.28	1.46 ± 0.09	0.26 ± 0.02^{II}
5	1.24 ± 0.03	47.40 ± 2.88	0.59 ± 0.02	0.013 ± 0.001	5.60 ± 0.29	1.44 ± 0.03^{II}	0.27 ± 0.02
TT3							
0.5	1.33 ± 0.08	42.18 ± 3.56	0.39 ± 0.04$^{III, V}$	0.010 ± 0.001	5.91 ± 0.46	1.33 ± 0.16	0.24 ± 0.04
1	1.39 ± 0.12^{II}	46.36 ± 3.58	0.41 ± 0.04^{III}	0.010 ± 0.001$^{I, II}$	6.93 ± 0.39	1.64 ± 0.09^{III}	0.24 ± 0.01
5	0.99 ± 0.09^{II}	47.70 ± 5.21$^{I, II}$	0.36 ± 0.04$^{II, III,V}$	0.009 ± 0.001$^{I, II,III}$	6.00 ± 0.33	1.55 ± 0.12^{II}	0.26 ± 0.02
TT4							
0.5	1.27 ± 0.07	39.67 ± 2.49	0.41 ± 0.04$^{III, V}$	0.011 ± 0.001	6.33 ± 0.30	1.49 ± 0.07	0.24 ± 0.02
1	1.23 ± 0.09	38.29 ± 1.87	0.69 ± 0.09	0.019 ± 0.003	6.14 ± 0.25	1.62 ± 0.09	0.27 ± 0.01^{II}
5	1.12 ± 0.07	33.79 ± 3.03	0.79 ± 0.10^{VI}	0.028 ± 0.006	5.93 ± 0.27	1.46 ± 0.08	0.25 ± 0.02
TT5							
0.5	1.61 ± 0.08$^{I, III}$	43.53 ± 3.01$^{I, III}$	0.60 ± 0.02	0.015 ± 0.002	5.40 ± 0.19	1.47 ± 0.04	0.28 ± 0.01
1	1.67 ± 0.09^{II}	42.07 ± 2.65$^{I, III}$	0.62 ± 0.02^{VII}	0.015 ± 0.001	5.47 ± 0.26	1.56 ± 0.05	0.30 ± 0.02
5	1.61 ± 0.10	43.71 ± 4.33	0.84 ± 0.11^{VI}	0.022 ± 0.003^{III}	5.79 ± 0.24	1.56 ± 0.09^{II}	0.28 ± 0.02

Table 2 (continued)

TDZ							
0.5	0.62 ± 0.06	25.00 ± 3.91^{V}	0.50 ± 0.10	0.021 ± 0.003	6.20 ± 0.80	0.93 ± 0.26	0.14 ± 0.02
1	0.57 ± 0.06	27.20 ± 3.03^{VI}	0.55 ± 0.06	0.024 ± 0.005	5.60 ± 0.48	0.72 ± 0.04^{V}	0.14 ± 0.01^{V}
5	0.58 ± 0.04^{I}	16.73 ± 1.06	0.81 ± 0.06	0.052 ± 0.007	6.07 ± 0.36	1.10 ± 0.07^{V,VI}	0.19 ± 0.02
GA							
0.5	1.39 ± 0.09	40.69 ± 3.19	0.53 ± 0.02	0.014 ± 0.001	5.08 ± 0.37	1.28 ± 0.08	0.27 ± 0.03
1	1.38 ± 0.14	31.89 ± 3.30	0.54 ± 0.04	0.019 ± 0.002	4.89 ± 0.35^{IV}	1.12 ± 0.14	0.23 ± 0.03
6-BAP							
0.5	0.74 ± 0.17^{IV,V,VI}	13.20 ± 2.46	1.42 ± 0.23^{IV}	0.128 ± 0.041^{IV}	4.20 ± 0.37	1.32 ± 0.13	0.32 ± 0.02
1	0.88 ± 0.09^{II}	25.17 ± 3.06	1.03 ± 0.11^{VI,I}	0.054 ± 0.014^{V,VI,VIII}	5.25 ± 0.28	1.17 ± 0.06^{VII}	0.23 ± 0.01
5	0.96 ± 0.07^{V}	11.10 ± 1.21^{I,III}	0.99 ± 0.12^{VI,VII}	0.106 ± 0.023^{V,VI}	5.60 ± 0.34	1.30 ± 0.15	0.24 ± 0.03
water							
–	1.15 ± 0.07^{II}	21.90 ± 3.03^{VI}	0.53 ± 0.03	0.033 ± 0.009	5.00 ± 0.39	1.30 ± 0.04	0.28 ± 0.03

Note: M – mean value; m – standard error of mean.
^{I} – significant differences from water (Kraskall-Wallis test, ANOVA, $p < 0.001$); ^{II} – significant differences from TDZ (Kraskall-Wallis test, ANOVA, $p < 0.001$); ^{IV} – significant differences from TT4 (Kraskall-Wallis test, ANOVA, $p < 0.001$); ^{V} – significant differences from TT5 (Kraskall-Wallis test, ANOVA, $p < 0.001$); ^{VI} – significant differences from TT3 (Kraskall-Wallis test, ANOVA, $p < 0.001$); ^{VII} – significant differences from TT1 (Kraskall-Wallis test, ANOVA, $p < 0.001$).



Note: • – mean value; | – standard error of mean.

Figure 2. The visualisation of some of the linear parameter of the seedling of the "Siberian early" tomato variety on the 10th day of the experiment.

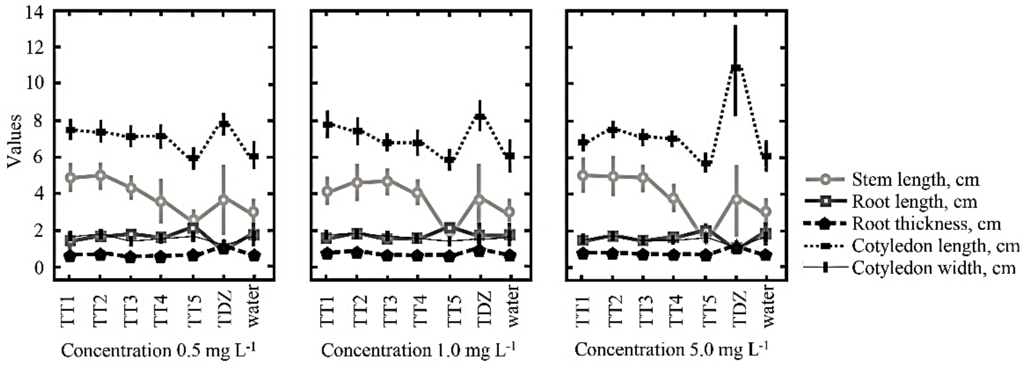
The maximum length of a young root on the 15th day is seen in seedlings treated with the **TT2** compound (5.24 ± 0.49 cm) at a concentration of 1 mg L^{-1}, which is almost 1.5 times more than in the control (3.64 ± 0.69 cm), while the minimum length was demonstrated by seedlings treated with the TDZ compound (1.92 ± 0.20 cm) in the same concentration, which is 1.9 times less than in the control. In the remaining treatments **TT2, TT3, TT4,** and **TT5**, the seedlings were characterized by some increase in the length of the linear parameters (with respect to the control) (Table 3, Fig. 3).

Table 3. Linear parameters of the seedlings of the "Siberian early" tomato variety on the 15th experimental day

Concentration, g L^{-1}	Stem length, cm (M ± m)	Root length, mm (M ± m)	Root thickness, mm (M ± m)	Root elongation (M ± m)	Cotyledon length, mm (M ± m)	Cotyledon width, mm (M ± m)	Cotelydon elongation (M ± m)	Branching of the root system
TT1								
0.5	3.85 ± 0.36 VI	1.49 ± 0.11	0.66 ± 0.03	0.19 ± 0.02	7.46 ± 0.29	1.72 ± 0.08	0.23 ± 0.01	0.31 ± 0.01
1	5.24 ± 0.49	1.52 ± 0.07	0.72 ± 0.02	0.15 ± 0.01	7.77 ± 0.34 I, V	1.85 ± 0.07 II	0.24 ± 0.01	0.85 ± 0.01
5	4.37 ± 0.40	1.45 ± 0.07	0.75 ± 0.02 II	0.20 ± 0.02	6.80 ± 0.22	1.53 ± 0.09	0.23 ± 0.02*	1.00 ± 0.01
TT2								
0.5	4.88 ± 0.36	1.68 ± 0.09	0.60 ± 0.04	0.13 ± 0.02	7.40 ± 0.31	1.78 ± 0.04	0.25 ± 0.01	1.00 ± 0.01
1	4.15 ± 0.35	1.79 ± 0.08	0.72 ± 0.03	0.19 ± 0.02	7.40 ± 0.34 I, V	1.77 ± 0.07	0.25 ± 0.02	0.80 ± 0.01
5	5.05 ± 0.45	1.65 ± 0.07	0.72 ± 0.02 II, IV, V	0.16 ± 0.02	7.53 ± 0.22 II	1.63 ± 0.05	0.22 ± 0.01 II	1.00 ± 0.01
TT3								
0.5	4.95 ± 0.35	1.77 ± 0.05	0.55 ± 0.03	0.12 ± 0.01	7.13 ± 0.26	1.48 ± 0.08	0.21 ± 0.02	0.80 ± 0.01
1	4.61 ± 0.48 I, II	1.53 ± 0.07	0.56 ± 0.03	0.15 ± 0.02	6.80 ± 0.22	1.66 ± 0.05	0.25 ± 0.01	0.93 ± 0.01
5	4.99 ± 0.52	1.49 ± 0.09	0.57 ± 0.02 II, IV,V	0.14 ± 0.02	7.07 ± 0.23	1.47 ± 0.06	0.21 ± 0.01	0.73 ± 0.01
TT4								
0.5	4.30 ± 0.30	1.65 ± 0.09	0.58 ± 0.02	0.15 ± 0.02	7.13 ± 0.31	1.60 ± 0.07	0.23 ± 0.01	1.00 ± 0.01
1	4.65 ± 0.34	1.51 ± 0.07	0.59 ± 0.03	0.13 ± 0.01	6.80 ± 0.33 II	1.52 ± 0.07	0.23 ± 0.02	0.93 ± 0.01
5	4.82 ± 0.35	1.58 ± 0.05	0.63 ± 0.02	0.14 ± 0.01	7.00 ± 0.20	1.43 ± 0.08	0.21 ± 0.01	0.86 ± 0.01
TT5								
0.5	3.57 ± 0.51 II	2.14 ± 0.09	0.64 ± 0.03	0.21 ± 0.03	5.90 ± 0.28 VII	1.70 ± 0.12	0.30 ± 0.03	0.70 ± 0.01
1	4.04 ± 0.33 II,VII	2.11 ± 0.11	0.58 ± 0.03	0.16 ± 0.02	5.87 ± 0.26 II	1.44 ± 0.09 II	0.25 ± 0.02	0.40 ± 0.01
5	3.76 ± 0.35	2.01 ± 0.18	0.57 ± 0.03 II, VII	0.18 ± 0.02	5.73 ± 0.23 V	1.52 ± 0.10	0.27 ± 0.02 II	0.53 ± 0.01
TDZ								
0.5	2.58 ± 0.25 I, V	1.03 ± 0.05 I	0.96 ± 0.09 I, IV,V	0.44 ± 0.07	7.85 ± 0.27 V	1.26 ± 0.09 I, V	0.16 ± 0.10 I, V	0.00*
1	1.92 ± 0.20 I, V, VI, VII	1.02 ± 0.05 I, VI	0.86 ± 0.05 V	0.50 ± 0.05 VI	8.23 ± 0.38 I, V	1.52 ± 0.12	0.19 ± 0.01	0.00*
5	1.53 ± 0.18 I, V, VI	1.10 ± 0.04 I, V, VI	0.99 ± 0.07 VI, VII	0.73 ± 0.10 I	10.75 ± 1.0 I, V, VII	1.23 ± 0.13	0.12 ± 0.02 V	0.00*
water								
–	3.64 ± 0.69	1.80 ± 0.22	0.62 ± 0.08	0.21 ± 0.05	6.00 ± 0.32	1.71 ± 0.15	0.29 ± 0.03	0.40 ± 0.01

Note: M – mean value; m – standard error of mean.
* – significant differences from all groups (Kraskall-Wallis test, ANOVA, $p < 0.001$); I – significant differences from water (Kraskall-Wallis test, ANOVA, $p < 0.001$); II – significant differences from TDZ (Kraskall-Wallis test, ANOVA, $p < 0.001$); IV – significant differences from TT4 (Kraskall-Wallis test, ANOVA, $p < 0.001$); V – significant differences from TT5 (Kraskall-Wallis test, ANOVA, $p < 0.001$); VI – significant differences from TT3 (Kraskall-Wallis test, ANOVA, $p < 0.001$; VII – significant differences from TT1 (Kraskall-Wallis test, ANOVA, $p < 0.001$).

All seedlings treated with GA and BAP on 15th day were contaminated by fungi and most of them were putrefied. The minimal linear parameters of seedlings were noted in the TDZ treatment (Table 3) in all concentrations. The seedlings with minimal linear parameters were observed in experimental treatment TDZ in a concentration of 5 mg L^{-1}: the length of the root was 1.53 ± 0.18 cm, which is 2.4 times lower than in the control, and 1.23 ± 0.13 mm wide, which is 1.4 times lower than in the control. However, a number of authors have noted the positive effect of low concentrations of 6-BAP and TDZ (0.5 mg L^{-1}) on plant rhizogenesis (Shein et al, 2004; Laplaze et al, 2007).



Note: • – mean value; | – standard error of mean.

Figure 3. The visualisation of some linear parameter of the seedling of the "Siberian early" tomato variety on the 15^{th} day of the experiment.

In the course of this experiment, a positive effect of TDZ at a concentration of 5 mg L^{-1} on the formation of cotyledons was revealed, the length of which was 10.75 ± 1.08 mm (maximum). This is probably due to compensatory mechanisms: blocking the development of the root and the apical meristem by a maximum concentration of TDZ provoked an increase in the linear parameters of the cotyledons, it is important for incresing of photosynthetic activity.

By the 15^{th} day, the share of fungal invasion in the studied treatments remained at an average level of 10–15%, which indicates a certain inhibition by these compounds of the development of the fungal mycelium. An increase in the pathogenic fungal mycelium in the **TT4** treatment at a concentration of 5 mg L^{-1} to 35% was noted once. In the comparison group (in the GA, 6-BAP, and TDZ treatments), only in the TDZ treatment at a concentration of 5 mg L^{-1} did the proportion of fungi by the end of the experiment range from 5 to 10%. In the remaining treatments, GA and 6-BAP, the share of fungi increased to 55% by the 15^{th} day, and once amounted to 100% (GA, 5 mg L^{-1}) (Table 3).

Tomato seeds treated with these compounds formed weakened seedlings by 15^{th} day, which later quickly died when planted into the soil substrate.

The general condition of the tomato seedlings formed was significantly different depending on the studied compounds and their concentration. By the end of the experiment, the most viable seedlings grew in treatments **TT2**, **TT1**, and **TT4** (in all concentrations), where a low percentage of cup infection with mold fungi was also noted. The seedlings treated with 6-BAP and GA in the maximum concentrations, where fungal invasion was high, produced the worst results (Table 1). In a number of works

(Yamauchi, et al, 2004; Kucera et al, 2005; de Lucas et al, 2008), growth was inhibited against a background of an increase of mitoses in the shoot meristems with high concentrations of GA, 6-BAP, and TDZ, applied both separately and in various combinations. It is known that GA plays an important role in the revival of seeds from the state of rest, has a stimulating effect during the period of cell division, and also ensures the growth of an interstice by stretching the cells. With prolonged exposure to GA, as well as high concentrations in plants, seedlings are formed with a changed ratio of cotyledons to root system: equally, the seedlings have a pale green colour and the root may lag behind or not develop at all and also effect on cell elongation and as a result in long thin hypocotyls. (Lang, 1970; Bewley, 1997; Kucera et al., 2005).

Assessment of the anatomical parameters of the leaf. Significant statistical differences in the size and shape changes of mesophyll cells of tomato leaves grown using synthesized compounds and trading phytohormones were not found. We selected three substances - gibberellic acid (GA), one synthesized compound (TT3) and water as control to illustrate cell structure of a mesophyll of treated tomato plants (Fig. 4).

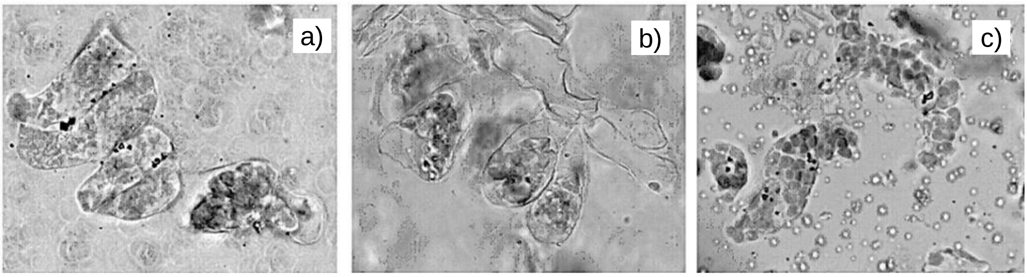


Figure 4. Cell structure of a mesophyll of tomato plants treated with: a) water, b) GA (1 mg L^{-1}), and c) TT3 (5 mg L^{-1}).

Comparison of the studied parameters of the leaf showed that the length, width, and area of cells leads in plants treated with GA at a concentration of 1 mg L^{-1} exceeds control by 56%, 43%, and 36%, respectively (Table 4). It is interesting to note that a sharp increase in the linear dimensions of cells in the GA treatment (1 mg L^{-1}) somewhat reduces their tortuosity. The treatment of GA at concentrations of 0.5 and 5 mg L^{-1} does not give such a pronounced result, although the most tortuous cells were noted at a concentration of 0.5 mg L^{-1} (Table 4). It was once again possible to confirm that one of the most important stimulation effects of GA is the acceleration of cell growth by stretching them (Gupta & Chakrabarty, 2013).

The length of the cells of plants treated with **TT3** at a concentration of 5 mg L^{-1} and GA at a concentration of 1 mg L^{-1} have a similar value. At the same time, the concentration of **TT3** 5 mg L^{-1} even exceeds that of GA (only 55.9 ± 3.22 μm). The width and area of cells in plants treated with **TT3** exceeds the control by an average of 1.4–2.1 times, but is inferior to cells of healthy plants after exposure to GA, except for the cell area at a concentration of 5 mg L^{-1} (Table 4). The mesophyll cells of plants treated with TDZ had the most minimum linear parameters in comparison with mesophyll cells of the other experiment treatments.

Table 4. Average parameters of the mesophyll cells of tomato leaves

Compound	Concentration, mg L^{-1} (M ± m)	Length, μm (M ± m)	Width, μm (M ± m)	Area, $μm^2$ (M ± m)	Perimetr, μm (M ± m)
TT1	0.5	62.01 ± 6.80	29.28 ± 1.90	1,382.97 ± 92.42	132.77 ± 11.60
	1	59.34 ± 4.90	27.80 ± 1.45	1,295.24 ± 98.33	148.39 ± 12.80
	5	64.12 ± 8.01	22.45 ± 2.09	1,404.67 ± 101.08	139.99 ± 10.42
TT2	0.5	52.13 ± 6.44	22.69 ± 1.88	1,136.23 ± 136.08	142.83 ± 12.45
	1	55.06 ± 4.81	25.07 ± 1.95	1,354.98 ± 104.73	125.70 ± 10.80
	5	59.86 ± 5.05	25.33 ± 1.64	1,476.65 ± 128.12	149.64 ± 13.17
TT3	0.5	56.18 ± 3.20	24.66 ± 1.14	1,143.53 ± 87.65	154.12 ± 9.12
	1	**69.27 ±** 6.17	25.62 ± 1.86	1,544.36 ± 160.40	180.49 ± 11.77
	5	68.01 ± 7.97	**31.81 ±** 2.36	**1,638.25 ±** 197.81	155.85 ± 28.08
TT4	0.5	54.72 ± 4.99	21.86 ± 1.90	1,451.22 ± 142.08	150.33 ± 14.98
	1	63.97 ± 4.01	27.77 ± 2.23	1,539.17 ± 127.84	171.45 ± 17.80
	5	67.90 ± 5.62	25.90 ± 2.56	1,595.38 ± 160.55	163.98 ± 11.23
TT5	0.5	53.81 ± 5.29	26.38 ± 2.43	1,206.87 ± 109.70	134.66 ± 10.65
	1	58.32 ± 6.87	27.53 ± 2.53	1,387.90 ± 129.58	139.43 ± 13.99
	5	55.43 ± 5.33	27.84 ± 1.69	1,364.73 ± 111.17	154.87 ± 14.04
TDZ	0.5	51.06 ± 4.02	19.15 ± 1.96	983.72 ± 231.56	105.44 ± 9.52
	1	59.34 ± 5.12	21.22 ± 1,55	1,108.56 ± 126.90	138.33 ± 10.20
	5	49.74 ± 5.33	20.68 ± 2.49	949.01 ± 195.40	122.84 ± 11.15
GA	0.5	65.42 ± 6.41	31.43 ± 4.31	1,501.62 ± 241.13	**421.38 ±** 254.84
	1	**70.08** ± 7.09	**41.74** ± 2.59	**2,191.54 ±** 300.30	193.2 ± 16.12
	5	55.95 ± 3.22	37.49 ± 2.02	1,622.78 ± 123.96	283.29 ± 124.99
6-BAP	0.5	55.82 ± 4.65	25.62 ± 1.96	1,254.38 ± 121.40	138.59 ± 26.97
	1	58.21 ± 6.01	24.06 ± 1.54	1,304.25 ± 98.65	129.31 ± 19.55
	5	56.93 ± 3.44	25.22 ± 2.07	1,298.03 ± 109.21	140.50 ± 10.39
water	–	39.55 ± 3.38	17.93 ± 2.54	789.19 ± 227.90	122.47 ± 11.12

Note: M – mean value; m – standard error of mean.
No significant differences (Kraskall-Wallis test, ANOVA, $p \geq 0.001$).

Fig. 5 shows the results of discriminant analysis. The scatter plot is based on the calculated values of the Mahalanobis square of the studied treatments. In terms of linear parameters, the tomato seedlings formed from seeds treated with **TT1–TT4** treatments are close. An exception is the **TT5** treatment, where the maximum dimensions of the young stalk (2.14 ± 0.09 cm) and the minimum length of the cotyledons (5.90 ± 0.27 mm) are noted. The differences of the linear dimensions of the cotyledons is well illustrated in the graph (Fig. 4). Along the Y-axis, the separation of the treatments passed the branch parameter of the root system. In the **TT1–TT4** treatments, the appearance of well-developed lateral roots was observed by the 15th day in all concentrations, with the exception of the **TT5** treatment. In the comparison group (6-BAP, TDZ, GA) branching was not observed. In the control, the beginning of lateral root growth was observed in 30% of cases.

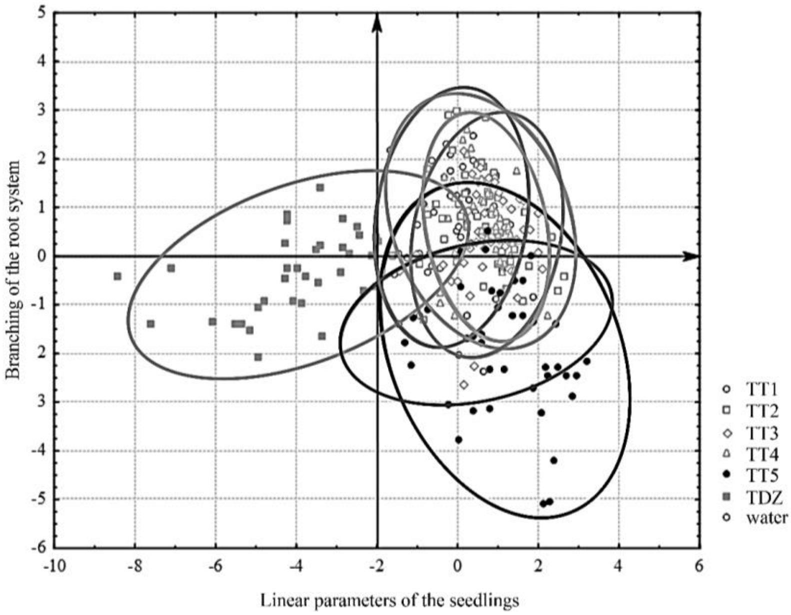


Figure 5. Visualisation of discriminant analysis results.

CONCLUSIONS

Thus, seed treatment with TT series compounds positively affected seed vigor and viability of seedlings (plant health scale on the 10th and 15th day of the experiment, Table 1) compared with treatments with commercial phytohormones (GA, 6-BAP, TDZ). Among the tested treatments of the **TT** series, the best properties for stimulating germination, subsequent removal of the apical blockade, the formation of the photosynthetic apparatus, and the suppression of the development of pathogenic fungal mycelium are possessed by compounds **TT1** and **TT4**. It should be noted that, in terms of their properties, these compounds are close to the cytokine group (triggering embryogenesis, stimulating the development of meristem, removing the apical dominant, accelerating the growth of the axial root and the development of lateral roots, etc.) (Kim et al, 2005; Osugi, A. & Sakakibara, 2015). From the compounds **TT2** and **TT3** in individual concentrations on 5th day, up to 77.6% showed good stimulation in the early stages of germination.

Despite the high concentrations (5 mg L^{-1}), there was not only inhibition of growth processes (as opposed to TDZ, GA, and 6-BAP), but also a reduction in the linear parameters of the roots and cotyledons of tomato seedlings of the 'Siberian early' variety.

Synthetic compounds **TT1–TT4** can be recommended, after checking their safety, for stimulating the germination and subsequent development of crops with a low percentage of seed germination or low viability of young seedlings, as well as for increasing the quantity and quality of planting material.

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REFERENCES

Bewley, J.D. 1997. Seed germination and dormancy. *Plant Cell* **9**, 1055–1066.

de Lucas, M., Davière, J.M., Rodríguez-Falcón, M., Pontin, M., Iglesias-Pedraz, J.M., Lorrain, S, Fankhauser, C., Blázquez, M.A., Titarenko, E. & Prat, S. 2008. A molecular framework for light and gibberellin control of cell elongation. *Nature* **451**, 480–484.

Engelbrecht, L. 1971. Cytokinins in buds and leaves during growth, maturity and aging (with a comparison of two bio-assays). *Biochemie und Physiologie der Pflanzen* **162**, 547–558.

Food & Agriculture Organization (FAO). 2011. *Seed in emergencies: a technical handbook. FAO Plant Production and Protection Papers (No 202)*. Food and agriculture organization of the United Nations, Rome, 86 pp.

Gupta, R. & Chakrabarty, S.K. 2013. Gibberellic acid in plant: Still a mystery unresolved. *Plant Signaling & Behavior* **8**, e25504.

Harman, G.E. 1991. Seed treatments for biological control of plant disease. *Crop Protection* **10**, 166–171.

Kalinina, T.A., Bystrykh, O.A., Glukhareva, T.V. & Morzherin, Y.Y. 2017. Transformation of 1,2,3-thiadiazolyl hydrazones as method for preparation of 1,2,3-triazolo[5,1-b][1,3,4]thiadiazines. *Journal of Heterocyclic Chemistry* **54**, 137–146.

Kalinina, T.A., Bystrykh, O.A., Pozdina, V.A., Glukhareva, T.V., Ulitko, M.V. & Morzherin, Y.Y. 2015. Synthesis of spiro derivatives of 1,2,3-triazolo[5,1-b][1,3,4]thiadiazines and biological activity thereof. *Chemistry of Heterocyclic Compounds* **51**, 589–592.

Kalinina, T.A., Vysokova, O.A., Khamidullina, L.A., Kochubei, A.A., Cherepanova, O.E. & Glukhareva, T.V. 2018. The effect of ethyl 5′-(4-methoxybenzoyl)-5′,7′-dihydrospiro[cyclopentane-1,6′-[1,2,3]triazolo[5,1-b][1,3,4]thiadiazin]-3′-carboxylate on Pinus sylvestris L. seed germination. *Agronomy Research* **16**, 103–112.

Kim, S.K., Kim, J.T., Jang, S.W., Lee, S.C., Lee, B.H. & Lee, I.J. 2005. Exogenous effect of gibberellins and jasmonate on tuber enlargement of *Dioscorea opposite*. *Agronomy Research* **3**, 39–44.

Kucera, B., Cohn, M.A. & Leubner-Metzger, G. 2005. Plant hormone interactions during seed dormancy release and germination. *Seed Science Research* **15**, 281–307.

Lang, A. 1970. Gibberellins: structure and metabolism. *Annual Review of Plant Physiology* **21**, 537–570.

Laplaze, L., Benkova, E., Casimiro, I., Maes, L., Vanneste, S., Swarup, R., Weijers, D., Calvo, V., Parizot, B., Herrera-Rodriguez, B., Offringa, R., Graham, N., Doumas, P., Friml, J., Bogusz, D., Beeckman, T. & Bennett, M. 2007. Cytokinins act directly on lateral root founder cells to inhibit root initiation. *The Plant Cell* **19**, 3889–3900.

Luo, T., Xian, M., Zhang, C., Zhang, C., Hu, L. & Xu, Z. 2019. Associating transcriptional regulation for rapid germination of rapeseed (*Brassica napus* L.) under low temperature stress through weighted gene co-expression network analysis. *Scientific Reports* **9:55**, 1–16.

Nugmanova, T. 2017. Biopreparations for the production of environmentally safe food – Part I. *Ecological Engineering and Environment Protection* **2**, 63–69.

Osugi, A. & Sakakibara, H. 2015. How do plants respond to cytokinins and what is their importance? *BMC Biology*. **13**, 102.

Pal, K.K. & McSpadden Gardener, B. 2006. Biological control of plant pathogens. *The Plant Health Instructor*, 1–25.

Pan, S., Rasul, F., Li, W., Tian, H., Mo, Z., Duan, M. & Tang, X. 2013. Roles of plant growth regulators on yield, grain qualities and antioxidant enzyme activities in super hybrid rice (*Oryza sativa* L.). *Rice* **6**, 9.

Pekarskas, J. & Sinkevičienė, J. 2015. Effect of biopreparations on seed germination and fungal contamination of winter wheat. *Biologija* **61,** 25–33.

Senberga, A., Dubova, L. & Alsina, I. 2018. Germination and growth of primary roots of inoculated bean (*Vicia faba*) seeds under different temperatures. *Agronomy Research* **16**, 243–253.

Shein, I.V., Polyakova, G.G., Zrazhevskaya, G.K., Pashenova, N.V., & Vetrova, V.P. 2004. Accumulation of phenolic compounds in conifer callus cultures in response to wood blue-stain fungi. *Russian Journal of Plant Physiology* **48**, 216–221.

Taiz, L. & Zeiger, E. 2002. *Plant physiology.* Sinauer Associates, Sunderland, 690 pp.

Toh, S., Kamiya, Yu., Kawakami, N., Nambara, E., McCourt, P. & Tsuchiya, Yu. 2011. Thermoinhibition Uncovers a Role for Strigolactones in Arabidopsis Seed Germination. *Plant and Cell Physiology* **53**, 107–117.

Yamauchi, Y., Ogawa, M., Kuwahara, A., Hanada, A., Kamiya, Y. & Yamaguchi, S. 2004. Activation of gibberellin biosynthesis and response pathways by low temperature during imbibition of *Arabidopsis thaliana* seeds. *Plant Cell* **16**, 367–378.

Agronomy Research **17**(1), 295–306, 2019
https://doi.org/10.15159/AR.19.007

Mathematics education for sustainable agriculture specialists

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Abstract. One of the Sustainable Development objectives is to promote life-long learning opportunities for all, but one of the Lifelong Learning competences is mathematical competence, which can be obtained studying mathematics at schools and universities. The question is how much and whether the course of mathematics should be included in the curriculum of the agronomy specialties at universities. The aim of the article is to highlight the insufficient amount of higher mathematics in the education of agriculture specialist in the context of sustainable development.
The objectives of the study: to identify the importance of mathematics for agronomy specialists by analysing the role of the mathematics education for agronomy specialists and to analyse the proportion of mathematics courses in the curriculum of the agronomy programmes in the Baltics States and the Baltic Sea region's higher education institutions. The mathematics education is important for agronomy specialists in many fields. Agronomy specialists need knowledge and skills in basic mathematics, in statistical analysis and interpretation, mathematical modelling, in scientific methods, in economic analysis. The knowledge and skills in a direct and indirect means is provided by mathematical studies at universities. In order to analyse the proportion of mathematics courses in study programmes of Agriculture, Agronomy and Horticulture, three universities of the Baltic States were compared: the Latvia University of Life Sciences and Technologies, Aleksandras Stulginskis University (Lithuania) and Estonian University of Life Sciences. For a more comprehensive analysis and comparison several universities from the Baltic Sea region were chosen that provide studies in agricultural sciences. Unfortunately, not all agronomy programmes in the Baltic Sea region contain the higher mathematics course that would help to understand the role of derivatives, integrals, and differential equations in the modelling process, as well as further developing general problem-solving skills.

Key words: agronomy specialist, mathematics competences, mathematics education, sustainable agriculture.

INTRODUCTION

We all live in any age where we need to think about the sustainable development of our planet and our people. To get rid of poverty and hunger in the world, as well as the worst of climate change effects, leaders from 193 countries created a plan called the Sustainable Development Goals (SD) in 2015 which consists of 17 goals (Sustainable

Development goals, 2015). The fourth SD objective is 'Ensure inclusive and equitable quality education and promote life-long learning opportunities for all'. It is essential for each individual in a knowledge-based society to acquire lifelong learning competences. The Recommendation of the European Parliament and of the Council sets out eight key competences for lifelong learning: 1) Communicating in a mother tongue: 2) Communicating in a foreign language; 3) Learning to learn; 4) Social and civic competences; 5) Cultural awareness and expression; 6) Mathematical, scientific and technological competence; 7) Digital competence; 8) Sense of initiative and entrepreneurship. The sustainability citizens need to have certain key competencies which would allow responsible engagement in today's world. Competences include cognitive, emotional, electoral and motivating elements. It is the interaction of knowledge, abilities and skills, motivation and affective attitudes (Education for Sustainable Development, 2017).

Sustainable agriculture is often described as a set of ideal objectives which it is supposed to achieve (Goals). Overarching goals are ethics, multi-functionality, safety, stability and resilience (Velten et al., 2015). Overarching goals can be divided into Environmental goals (Production- Specific and Non- Production- Specific), Social goals and Economic goals. In order to achieve these goals, different approaches and principles (strategies) can be used, such as adaptive management, cooperation, ecology-based strategy and economics-based strategy, holistic & complex systems thinking, knowledge & science, subsidiarity. In order to be able to acquire these strategies, agronomy specialists need to develop lifelong learning competences.

One of the Lifelong Learning competences is Mathematical, Scientific and Technological competence, which can be obtained by studying mathematics at schools and universities. Mathematical competence in general is the ability to design and apply mathematical thinking to solve a range of problems in everyday situations.

Mathematical education provides the skills to solve problems related to the acquisition of algorithms and formulas necessary for calculations. What is sustainability in mathematics? First, mathematical studies must develop mathematical competences. Secondly, studying mathematics, we can reveal three qualitatively different concepts of mathematics (Petocz & Reid, 2003):

– Components - Mathematics made up of individual components and we must concentrate our attention on different mathematical activities or aspects of mathematics, including the concept of calculation interpreted in the broadest sense, for example, the statistical components of a census of population;

– Models - Creating mathematical models we should translate some aspects of reality or specific situations into mathematical form, for example, a production line;

– Life - Mathematics is a way of thinking that helps to represent reality in mathematical terms, creating a strong personal relationship between mathematics and your life.

At the same time, learning objectives for sustainable education can overlain upon almost any mathematics course without losing the appropriate content. In the study programme we must carefully review the content of mathematics, enrich it with real examples and increase the involvement of students in the study process (Hamilton & Pfaff, 2013).

Like many other areas of life, mathematics plays an important role in agriculture, for example, in soil analysis, calculation of various fertilizer contents, use of statistics in estimation. Mathematics is also needed in estimation of requirements of fertilizers or insecticides, estimation of yield based on the sample of crop etc. (Pande, 2017).

The problem is that in recent years, the proportion of mathematics courses has decreased in the curricula of agronomy specialties of the Baltic States' universities which was highlighted in the XI Nordic-Baltic Agrometrics conference held in September 2018 (Nordic-Baltic Agrometrics conference, 2018). The question is how much mathematics should be taught in schools and whether the course of high mathematics should be included in the curriculum of agriculture specialties at higher education institutions.

The aim of the article is to highlight the insufficient amount of higher mathematics in the education of agriculture specialist in the context of sustainable development.

The objectives of the study:

1) to identify the importance of mathematics for agronomy specialists, to analyse the role of the mathematics education for specialists of agronomy, and

2) to analyse the proportion of mathematics courses in curriculum of the agronomy studies in the Baltic States universities and compare with the Baltic Sea region's higher education institutions.

MATERIALS AND METHODS

In the process of the study inductive approach, appropriate qualitative and quantitative research methods have been used: the general research, monographic, analysis and synthesis, comparative method, data grouping, statistical data processing method.

Mathematics studies have two impacts (Zeidmane & Sergejeva, 2013): i) a direct impact, which provides skills for solving and calculating various real, conjunct problems; ii) an indirect impact, which develops general problem-solving skills (Fig. 1).

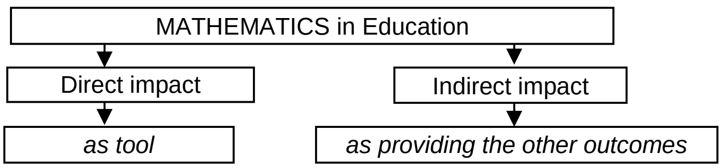

Figure 1. Mathematics impact in Education.

In order to investigate the first main role of mathematics in agronomy education, *a direct impact*, mathematics textbooks offered by the Internet resources to agronomists were examined to find out the necessary topics of mathematics. Further, agricultural areas requiring knowledge of mathematics were identified. In addition, trends in sustainable agriculture were studied in order to clarify the increasing role of mathematics in them.

To analyse the *indirect impact* of mathematics, the competences acquired with the help of mathematics were identified.

To analyse and compare the content of Bachelor's study programmes as well as to measure a proportion of mathematics courses, universities from the Baltic States which provide education related to agricultural sciences and agronomy and which are active Baltic and Nordic Agrometrics network members since 1998, i.e., Latvia University of Life Sciences and Technologies (LLU), Alexandras Stulginskis University (ASU), Estonian University of Life Sciences (EMU), were selected. The purpose of this cooperation network was the necessity to understand what kind of mathematics and what amount of it agriculture specialists need as well as to achieve common standards in higher agriculture education. To extend the scope of the research, universities from the Baltic Sea region were chosen for the comparison: University of Hohenheim (Germany), University of Helsinki (Finland), Swedish University of Agricultural Sciences (SLU) (Sweden), Aarhus University (Denmark).

Detailed descriptions of the study programmes were downloaded from the websites of each of the above mentioned universities. The courses of study programmes were divided into several groups: mathematics courses (higher mathematics, statistics, biometry with or without IT application), physics, general courses, industrial courses, the practice or project and a thesis. Based on the description of a study programme and study courses, the amount in ECTs credits and proportion of each group were determined.

To calculate the proportion of mathematics courses and other groups of courses in a study programme, the formula was used:

$$proportion\ of\ course\ group, \% = \frac{sum\ of\ courses\ credits\ in\ group\ (ECTS)}{total\ credits\ in\ study\ programm\ (ECTS)} \cdot 100\%$$

RESULTS AND DISCUSSION

There is no doubt that prospective farmers need mathematics, but the questions are:

- what kind of mathematics should be learned in the study process;
- how and what subjects related to mathematics should be included in the agronomy study course;
- whether the study process requires mathematics as a separate subject?

Importance of mathematics for agronomy specialists

The main objective in mathematics for agriculture is to provide an adequate overview of basic arithmetic, statistical interpretation and algebraic concepts for preparing students in mathematics involved in other agricultural and horticultural courses. There are textbooks for this purpose, for example, B.C Rogers 'Mathematics for Agriculture' (Rogers, 2000) which deals with parts of mathematics such as: Whole Numbers, Common Fractions, Decimals, Percent and Percentages, Interpretation and Analysis of Data, Introduction to Algebra, Linear Equations, Ratios and Proportions, Special Formulas, Measurement. The role of mathematics in agronomy courses has been studied by various authors. For example, an interview with 15 professional farmers, 13 vocational teachers in agriculture, 11 mathematics teachers who teach agricultural students and 40 secondary vocational education students was conducted (Muhman, 2015), and the results showed that percent, geometry and statistics were considered as the main areas of mathematics according to the respondents' opinion. Similar information can be found in online resources (Masula, 2015; Jenkins, 2017) etc.. These subjects in mathematics are at the secondary level and textbooks are for special vocational schools. The problem is only that graduates from the secondary schools may also join the agricultural specialties at universities and they have not mastered this specific mathematics.

Research into agricultural sectors that require mathematics has been considered to be insufficient to complete secondary school mathematics. Agronomic work is closely linked to the knowledge of mathematics in economics. G. Beer mentions areas where mathematical skills are vital (Beer, 2016):

- Soil Analysis
- Calculation of various fertilizer contents
- For foreign markets, conversions to/from metric
- Areas of various shapes
- Market Protection Strategies
- Evaluation of Retailer Performance
- Application Rates
- Finance Decisions
- Piloting of airplane for more efficient travel

This creates the need for special courses in mathematics in the curriculum of agronomy at universities.

The emergence of new trends in sustainable agriculture causes the necessity of modelling different processes. The computational methods in applied mathematics blog (The Importance of Maths, 2017) indicate seven ways how and why mathematical models are used in agriculture:

1) Mathematics allows you to design better climate models;
2) Mathematics improves the accuracy of soil analysis;
3) Mathematics makes chemical content analysis for fertilizers more accurate;
4) Conversion of units at A drop of A hat requires A strong background in maths;
5) Planning, plotting, and laying out plot sizes and dimensions;
6) Improving estimates for expenditure and yields;
7) Mathematics lays the foundation for innovation in agricultural methods.

The question can be raised for a discussion: will an agronomy specialist be a creator or a user of these models. If agronomy specialists are involved in developing these models, then they need higher mathematics knowledge (even if they work in a team with a mathematician). In addition, agronomists should understand the mathematical meaning of the operation of these models when using them. For example, a model for the a nitrogen cycle in pasture has to deal with Distributed-delay-differential equations $y'(t) = y(t-T)$. The integral calculus and differential equations must be solved in the fundamental problem of optimal control theory to determine the probable control that maximizes (or minimizes) where u (t) is the parent infusion rate, and x (t) is the current mass of the fruit (Wake, 2008).

The area of agriculture, which has benefited from mathematics, is precision agriculture which increases agricultural efficiency by helping farmers identify which plants to plant, where and how to harvest them. Nowadays, more and more farmers introduce precision agriculture. Precision agriculture or precision farming, is a modern farming management concept which uses digital techniques to monitor and optimise agricultural production processes. CEMA, the European Agricultural Machinery Industry (CEMA, 2018), has given a detailed list of the most common technologies used in precision farming practices (Fig. 2).

Precise farming production has increased the demand for high-quality training in order to accurately assess the spatial variability in the countryside.

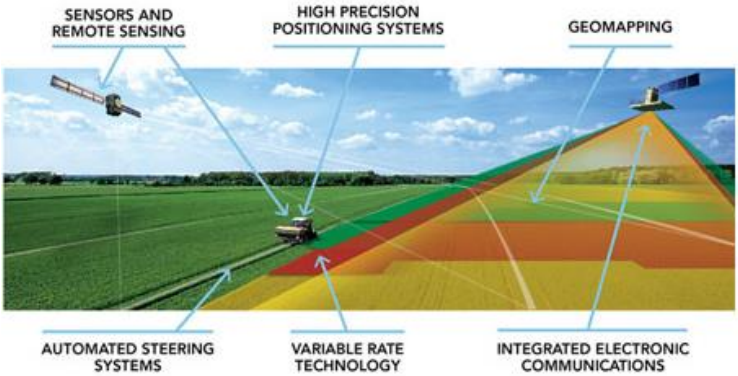

Figure 2. Precision Farming: key technologies & concepts, (adapted by CEMA).

With the increasing impact of precision farming, there has been an increased demand for quality training to accurately evaluate spatial variability within fields. The book 'Practical Mathematics for Precision Farming', which was issued in 2017, provides hand-on training and examples (Clay et al., 2017). The target audience for this book include certified crop consultants (CCAs), farmers, crop consultants, and undergraduate and graduate students. This book covers examples, how to conduct and analyse on-farm studies, write simple programmes, use precision techniques to scout for pests and collect soil samples, develop management zones, determine the cost of production, assess the environmental consequences of precision techniques, understand soil test results, and develop site-specific nutrient and plant population algorithms. Again, the questions arise: what core mathematics knowledge and skills of agronomists are necessary to be able to master the course and should this course or part of the course be included in the agronomy study curriculum in universities?

Mathematics plays an important part in everyone's life, but the increasing complexity of agricultural technology makes it mandatory that workers in agricultural occupations have skills in the analysis and solution of mathematical problems. In addition, the 13th International Conference on Precision Agriculture (USA), describing the current situation in agriculture (Ericson et al., 2016), emphasized that:

- It is often difficult to find skilled professionals to work in precision agriculture
- Ag school graduates not always 'field ready' to hit ground running
- Information-intensive future vs. current automated systems
- Lack of maths/analytic skills.

It makes us think about the competences, learning by studying mathematics. This also shows the indirect impact of mathematics in education. Mathematical competence in general is the ability to design and apply mathematical thinking to solve a range of problems in everyday situations. Mathematical studies at universities should develop the ability to ask and answer questions in and with mathematics, as well as the ability to deal with mathematical language and tools (Niss & Hojgaard, 2007). The ability to ask and answer questions cover four competences: mathematical thinking, problem tackling, modelling and reasoning competences. The ability to deal with mathematical language and tools cover other four competences: representing, symbol and formalism, communicating and aids and tools competences (Fig. 3).

Based on the above, it should be concluded that the proportion of maths courses in agronomy specialties in universities should increase. The reality is exactly the opposite: the proportion of mathematics courses has been reduced.

The analysis of the proportion of mathematics courses in curriculum of the agronomy programmes

Seven Bachelor's level study programmes were analysed in the framework of this study. These programmes have been chosen based on available information about their content on the webpages of universities. Fig. 4 contains a summary of information about volume, duration and other details of each programme. The universities provide 3 and 4 years study programmes in equal proportion. Only the Polish university provides 3.5 years study programme.

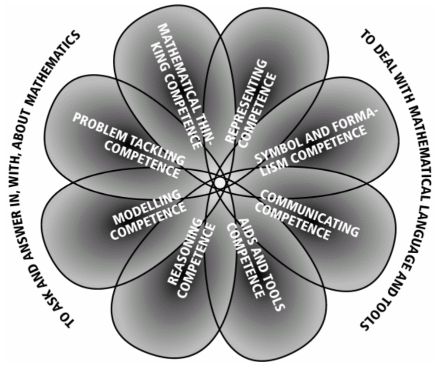

Figure 3. A visual representation of the 'KOM (Competences and the Learning of Mathematics) flower' of the eight mathematical competences presented and exemplified in the KOM report (Niss & Jensen, 2002).

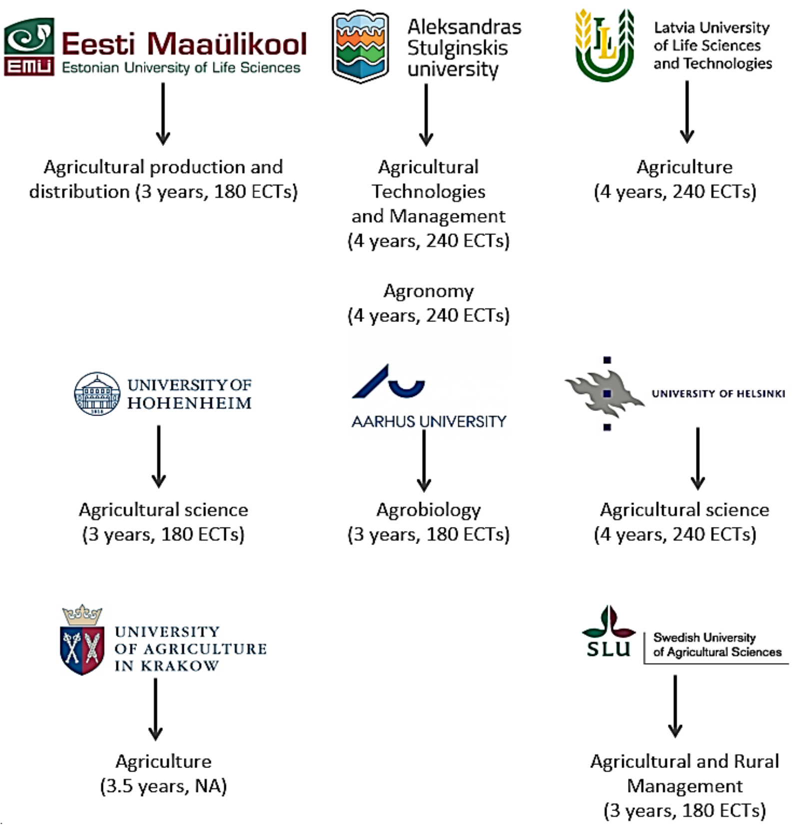

Figure 4. The basic information about programmes.

In this research programme courses are divided in six groups: mathematics, physics, other general courses, industrial courses, the practice or project and the thesis. Fig. 5 shows the proportion of different courses' groups in each particular programme of Baltic States universities: Alexandras Stulginskis University (ASU) in Lithuania, Estonian University of Life Sciences (EMU) and Latvia University of Life Sciences and Technologies (LLU).

For the comparison several programmes from the Baltic Sea Universities (Fig. 6.) have been analysed: University of Hohenheim (Germany, GE), University of Helsinki (Finland, FI), Swedish University of Agricultural Sciences (Sweden, SW), Aarhus University (Denmark, DM).

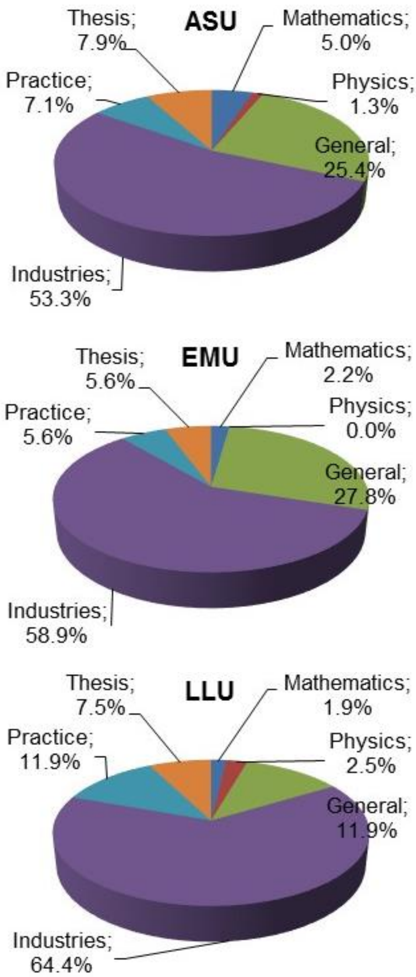

Figure 5. The proportion of courses groups in study programmes of Baltic States universities.

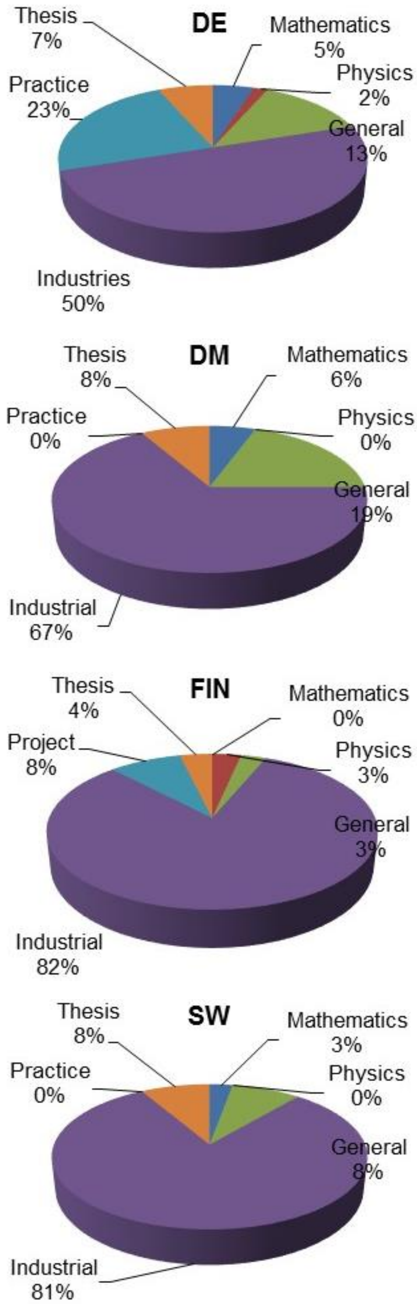

Figure 6. The proportion of courses groups in study programmes.

The largest proportion of mathematics courses is observed in programmes of Danish, German and Lithuanian universities. Physics is not always included in study programmes of the investigated universities. Physics is mostly studied in the Finnish university. The largest part of study programmes in all universities is devoted to industrial specialized courses.

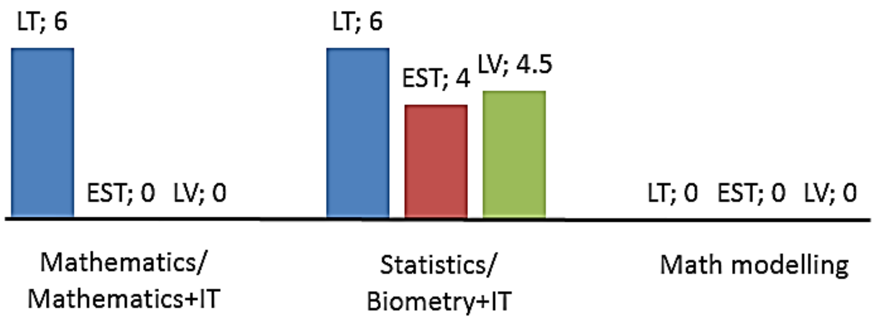

Figure 7. The distribution of credit points with mathematical content in the Baltic States' universities.

The comparison of the credit points given to courses with mathematical content in the Baltic States' universities (Fig. 7.) and other Baltic Sea region's universities (Fig. 8.) shows that Lithuanian study programme is the programme which provides students with knowledge and skills about basic concepts of higher mathematics. But the amount of mathematics in the course accounts only for 3 credits from 6 credits. The other focus in the course is on informatics, i.e., preparation of documents with spreadsheet, graphical analysis, database technology, projection of tables and their connections, completion of forms, questionnaires and reports.

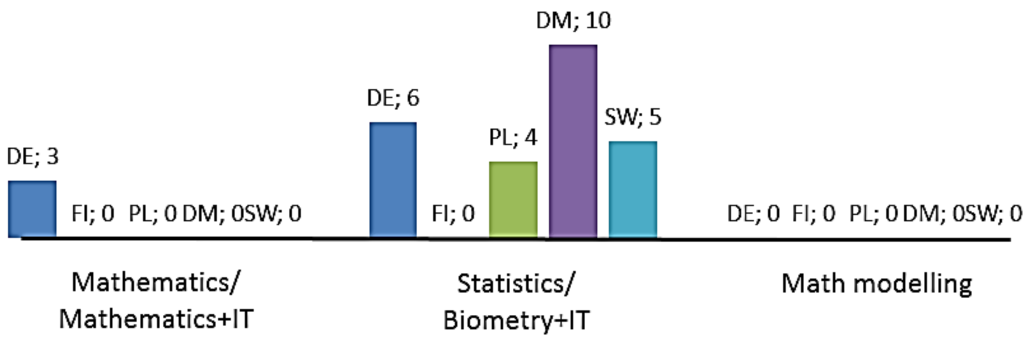

Figure 8. The distribution of credit points with mathematical content in the Baltic sea region's universities.

All three Baltic States' universities provide students with the statistical subjects. The detailed list of subjects can be seen in Table 1.

Table 1. The content comparison of Mathematics and Statistics in programmes of the Baltic States' universities

Subject	EMU	ASU	LLU
Matrices and actions with matrices. Determinants		x	
Linear equation systems and their solutions methods		x	
Elements of limit theory. Concept of a continuous function		x	
Function derivative. Derivative applications. Concept of function differential		x	
Concept and characteristics of indefinite integral. Applications of definite integrals		x	
Basic concepts of probability theory		x	
Random variables, characteristics, distributions and densities	x	x	x
Descriptive statistics	x	x	x
Testing of normal distribution	x		
Introduction to interferential statistics. Research methodology: statistics application		x	
Population. Statistical samples. Data classification		x	x
Statistical hypotheses and tests: t- test	x	x	x
F-test, chi-square test	x		x
Dispersion analysis - one and two factors			x
One and two-way ANOVA and their applications	x	x	
Correlation, regression analysis. Single and multifactor linear regression Non-linear single factor models	x	x	x
Interpretation and presentation results of statistical data analysis	x	x	x
Statistics with agronomic and economic databases	x		
Use of software packages (MS Excel)	x	x	x
Computer based application of statistics to agricultural research		x	

The content in statistical course programmes is almost the same, only the number of hours in auditoriums varies. Mathematical modelling is not included in any of the programmes. A similar situation can be observed in other universities of the Baltic Sea region. Only the university in Germany includes learning of mathematical basic concepts. The particular attention should be paid to the Finnish University which has not included mathematical courses in their programme at all. The Danish university devotes the largest amount of time to statistics. Similarly to the Baltic States' universities, the Baltic Sea region's universities have not included mathematical modelling.

The Lithuanian university is the only one that provides knowledge and skills about basic concepts of higher mathematics including an overview of main concepts of the probability theory. The set of statistical topics in study programmes of the Baltic States' universities is almost the same. The Lithuanian university devotes more attention to statistics application and research methodology including experiments in laboratories and field experiments which form 4.5 ECTs of the course volume from 6 ECTs.

CONCLUSIONS

Based on the literature studies on the need for mathematical content for agronomy specialists, it was concluded that agronomists need knowledge in mathematical areas such as algebra, geometry, percent and statistics. These subjects in mathematics are taught at the secondary level and textbooks are designed for special vocational schools.

In addition, work in agronomy is closely linked to the knowledge of mathematics in economics. This creates the need for special courses in mathematics in the curriculum of agronomy at universities.

The Lithuanian university is the only Baltic States' university that provides knowledge and skills about basic concepts of higher mathematics including an overview of the main concepts of the probability theory. All three Baltic universities provide students with the statistical subjects in their programmes, but the largest amount of credit points for statistics is devoted by Denmark among the universities of the Baltic Sea region.

Mathematical modelling is not included in the Bachelor's level studies of the Baltic Sea region's universities.

The analysis of different study programmes shows that the most proportion of mathematical courses is provided in programmes of the Danish university (6%), the German university (5%) and the Lithuanian university (5%).

The emergence of new trends in sustainable agriculture demands modelling of different processes. Using these models, agronomists should understand the mathematical meaning of the operation of these models. It requires additional knowledge in higher mathematics (differential, integrals, differential equations, etc.).

Nowadays, more and more farmers introduce precision agriculture. This requires the development of mathematical competences which, of course, are provided by mathematics studies. Based on the above, it should be concluded that the proportion of mathematics courses in agronomy specialties in universities should be increased. The reality is exactly the opposite: the proportion of mathematics courses has been reduced.

REFERENCES

Beer, G. 2016. Mathematics in Agriculture. *http://www.opsu.edu/www/education/Mathematics%20in%20Agricul ture.pdf*. Accessed 02.09.2018.

CEMA. 2018. Precision Farming: key technologies & concepts. http://cema-agri.org/page/precision-farming-key-technologies-concepts. Accessed 30.08.2018.

Clay, D., Clay, S. & Bruggeman, S. 2017. *Practical Mathematics for Precision Farming*. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Inc. *https://dl.sciencesocieties.org/publications/books/tocs/acsesspublicati/practicalmath*. Accessed 31.08.2018.

Education for Sustainable Development Goals: Learning Objectives 2017. *http://unesdoc.unesco.org/images/0024/ 002474/247444e.pdf.* Accessed 08.09.2018.

Erickson, B., Fausti, S., Clay, D. & Clau, S. 2016. Knowledge, Skills and Abilities Needed in the Precision Ag Workforce: An Industry Survey. In: *13th International Conference on Precision Agriculture.* St. Louis, Missouri, USA, pp. 1–20. *https://infoag.org/presentations/2187.pdf.* Accessed 31.08.2018.

Hamilton, J. & Pfaff, T. 2014. Sustainability Education: The What and How for Mathematics. Journal of Problems, Resources, and Issues in Mathematics Undergraduate Studies **24**, 61–80. *https://www.tandfonline.com/doi/abs/10.1080/ 10511970.2013.834526*. Accessed 09.09.2018.

Jenkins, L. 2017. Mathematical solutions to agricultural problems. Clemson scientist featured by prestigious American Mathematical Society, Clemson College of Science, Washington. *https://www.youtube.com/watch?v=uq1aUh Yucvw*. Accessed 29.08.2018.

Masula, J. 2015. How Mathematics is used by people in agriculture? *https://www.youtube.com/watch?v=Sc5l_e4JcLk.* Accessed 29.08.2018.

Muhrman, K. 2015. Mathematics in agriculture and vocational education for agricultures. In: *Proceedings of the Ninth Congress of the European Society for Research in Mathematics Education.* Prague, Czech Republic, pp. 1669–1670 *https://hal.archives-ouvertes.fr/hal-01287937.* Accessed 28.08.2018.

Niss, M. & Hojgaard, T. (Eds.). 2002. Competences and mathematics learning - Ideas and inspiration for the development of mathematics teaching in Denmark. The Danish Agency for Education's thematic series Copenhagen: The Ministry of Education (in Danish). Available at: *https://www.researchgate.net/publication/290429774_Kompetencer_og_matematiklaering_Ideer_og_inspiration_til_udvikling_af_matematikundervisning_i_Danmark*

Nordic-Baltic Agrometrics conference. 2018. The 11th Nordic-Baltic Agrometrics conference Mathematics, statistics and physics for agronomy research and teaching. September 13–14, 2018 Tartu, Estonia *https://agrometrics.emu.ee/.* Accessed 15.10.2018.

Pande, S. 2017. What is the relation between maths and agriculture? Quora Platform. *https://www.quora.com/What-is-the- relation-between-maths-and-agriculture.* Accessed 10.09.2018.

Petocz, P. & Reid, A. 2003. What on earth is sustainability in mathematics? *New Zealand Journal of Mathematics* **32**, 135–144.

Recommendation 2006/962/EC on key competences for lifelong learning. 2006. Official Journal of the European Union L 394/10, 10–18. *https://eur-lex.europa.eu/eli/reco/2006/962/oj*. Accessed 02.09.2018.

Rogers, B. 2000. Mathematics for Agriculture. Pearson, 336 pp. *https://www.pearson.com/us/higher-education/program/Rogers-Mathematics-for-Agriculture-2nd-Edition/PGM42304.html.* Accessed 28.08.2018.

The Division for Sustainable Development Goals (DSDG). Sustainable Development goals 2015. *https://sustainabledevelopment.un.org/topics/sustainabledevelopmentgoals*. Accessed 08.09.2018.

The Importance of Mathematics as Applied to Agriculture and Farming: Computational methods in applied mathematics, 2017. A blog about mathematical contributions to computational methods and numerical analysis. *http://www.cmam.info/the-importance-of-mathematics-as-applied-to-agriculture-and-farming.html.* Accessed 02.09.2018.

Velten, S., Leventon, J., Jager, N. & Newing, J. 2015. What is Sustainable Agriculture? A systematic Review. *Sustainability* **7**, 7833–7865. *https://www.mdpi.com/2071-1050/7/6/7833*. Accessed 09.09.2018.

Wake, G. 2008. Industrial Mathematics Initiatives: An (inter)national need? *Centre for Mathematics in Industry*, Massey University, Auckland, New Zealand. *http://www.mathsinindustry.co.nz g.c.wake@massey.ac.nz.* Accessed 02.09.2018.

Zeidmane, A. & Sergejeva, N. 2013. Indirect Impact of Mathematics in Engineering Education. In: *Proceedings of 12th International conference 'Engineering for Rural Development'*. Jelgava, Latvia, pp. 611–615.

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- Use font Times New Roman, point size for the title of article **14 (Bold)**, author's names 12, core text 11; Abstract, Key words, Acknowledgements, References, tables, and figure captions 10.
- Use *italics* for Latin biological names, mathematical variables and statistical terms.
- Use single ('…') instead of double quotation marks ("…").

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- All tables must be referred to in the text (Table 1; Tables 1, 3; Tables 2–3).
- Use font Times New Roman, regular, 10 pt. Insert tables by Word's 'Insert' menu.
- Do not use vertical lines as dividers; only horizontal lines (1/2 pt) are allowed. Primary column and row headings should start with an initial capital.

Figures

- All figures must be referred to in the text (Fig. 1; Fig. 1 A; Figs 1, 3; Figs 1–3). Use only black and white or greyscale for figures. Avoid 3D charts, background shading, gridlines and excessive symbols. Use font **Arial, 10 pt** within the figures. Make sure that thickness of the lines is greater than 0.3 pt.
- Do not put caption in the frame of the figure.
- The preferred graphic format is Excel object; for diagrams and charts EPS; for half-tones please use TIFF. MS Office files are also acceptable. Please include these files in your submission.
- Check and double-check spelling in figures and graphs. Proof-readers may not be able to change mistakes in a different program.

References

- **Within the text**

In case of two authors, use '&', if more than two authors, provide first author 'et al.':

Smith & Jones (1996); (Smith & Jones, 1996);

Brown et al. (1997); (Brown et al., 1997)

When referring to more than one publication, arrange them by following keys: 1. year of publication (ascending), 2. alphabetical order for the same year of publication:

(Smith & Jones, 1996; Brown et al., 1997; Adams, 1998; Smith, 1998)

- **For whole books**

Name(s) and initials of the author(s). Year of publication. *Title of the book* (*in italics*). Publisher, place of publication, number of pages.

Shiyatov, S.G. 1986. *Dendrochronology of the upper timberline in the Urals*. Nauka, Moscow, 350 pp. (in Russian).

- **For articles in a journal**

Name(s) and initials of the author(s). Year of publication. Title of the article. *Abbreviated journal title* (*in italic*) volume (in bold), page numbers.

Titles of papers published in languages other than English, should be replaced by an English translation, with an explanatory note at the end, e.g., (in Russian, English abstr.).

Karube, I. & Tamiyra, M.Y. 1987. Biosensors for environmental control. *Pure Appl. Chem.* **59**, 545–554.

Frey, R. 1958. Zur Kenntnis der Diptera brachycera p.p. der Kapverdischen Inseln. *Commentat.Biol.* **18**(4), 1–61.

Danielyan, S.G. & Nabaldiyan, K.M. 1971. The causal agents of meloids in bees. *Veterinariya* **8**, 64–65 (in Russian).

- **For articles in collections:**

Name(s) and initials of the author(s). Year of publication. Title of the article. Name(s) and initials of the editor(s) (preceded by In:) *Title of the collection (in italics)*, publisher, place of publication, page numbers.

Yurtsev, B.A., Tolmachev, A.I. & Rebristaya, O.V. 1978. The floristic delimitation and subdivisions of the Arctic. In: Yurtsev, B. A. (ed.) *The Arctic Floristic Region*. Nauka, Leningrad, pp. 9–104 (in Russian).

- **For conference proceedings:**

Name(s) and initials of the author(s). Year of publication. Name(s) and initials of the editor(s) (preceded by In:) *Proceedings name (in italics)*, publisher, place of publishing, page numbers.

Ritchie, M.E. & Olff, H. 1999. Herbivore diversity and plant dynamics: compensatory and additive effects. In: Olff, H., Brown, V.K. & Drent R.H. (eds) *Herbivores between plants and predators. Proc. Int. Conf. The 38th Symposium of the British Ecological Society*, Blackwell Science, Oxford, UK, pp. 175–204.

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Please note

- Use '.' (not ',') for decimal point: 0.6 ± 0.2; Use ',' for thousands – 1,230.4;
- Use '–' (not '-') and without space: pp. 27–36, 1998–2000, 4–6 min, 3–5 kg
- With spaces: 5 h, 5 kg, 5 m, 5 °C, C : D = 0.6 ± 0.2; p < 0.001
- Without space: 55°, 5% (not 55 °, 5 %)
- Use 'kg ha^{-1}' (not 'kg/ha');
- Use degree sign ' ° ' : 5 °C (not 5 ^{O}C).