

Influence of nitrogen fertilizer on Cd and Zn accumulation in rapeseed (*Brassica napus* L.) biomass

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Abstract. Diffuse soil contamination with heavy metals and Cd in particular is a matter of serious concern. Application of conventional remediation methods usually is not feasible due to the large territories and relatively low heavy metal content. Thus, phytoremediation is seen as an alternative. Rapeseed was grown on Cd and Zn contaminated as well as clean soil under the greenhouse conditions. Solid and liquid nitrogen fertilizers were applied during the pot experiment in order to test their influence on heavy metal accumulation in plant tissues. Vegetative parameters were measured four times during the pot experiment and it was concluded, that the elevated concentrations of Cd and Zn in the soil did not disrupt the development of rapeseed plants. Furthermore, plants from contaminated soil produced significantly bigger seeds in comparison to plants from uncontaminated soil. Calculated Bioconcentration factors for rapeseed grown on Cd and Zn contaminated soil in all cases were below unity, thus possibility to use this plant species for phytoextraction purposes is limited, but it can be successfully grown on contaminated land as an energy crop. Application of nitrogen fertilizers had a significant effect on heavy metal accumulation and decreased Cd and Zn concentrations in rapeseed roots and stems with leaves were recorded. Accumulation differences between the liquid and solid fertilizer applications were negligible.

Key words: cadmium, zinc, contaminated soil, phytoremediation, nitrogen fertilizer.

INTRODUCTION

Biomass is one of the most abundant sources for the renewable energy. However, due to the shortage of arable lands energy cropping is enforced to compete with traditional agriculture (Campbell et al., 2008). Furthermore, there is another serious concern regarding the upper soil layer pollution with trace elements as a consequence of intensive farming. This problem is faced in many regions worldwide (Nagajyoti et al., 2011; Witters et al., 2012). Soils with exceeding threshold values for heavy metals (HM) are no longer proper for food and feedstock production (Lithuanian..., 2004; Oves et al., 2012).

Many remediation techniques, such as soil flushing, vitrification, thermal destruction, etc., are not reasonable in the case of diffuse pollution due to the large areas of contaminated land, therefore phytoremediation is being proposed as an alternative. On the other hand, plants–hyperaccumulators used for this purpose usually grow slowly

and produce low-yield biomass. Thus, a numerous rotation cycles are required for tolerable soil clean up (Bhargava et al., 2012; Ali et al., 2013). Those are the main reasons why phytoremediation exhibits a scanty number of the successfully carried out field-scale projects.

Quite recently a new approach emerged (Meers et al., 2010), which suggests that combining traditional phytoextraction with energy cropping could help not only to reduce heavy metal concentrations in soil, but also to use biomass grown on contaminated land for energy recovery.

Worldwide use of mineral fertilizers is still increasing in large numbers. On one hand, it is well acknowledged, that mineral fertilizers, especially phosphoric, are responsible for carrying HM to the arable lands (Nagajyoti et al., 2011). On the other hand, information about the influence of fertilizers on HM bioavailability is still insufficient. Due to the fertilizer application, plant development is improved and consequently physiological stress, induced by contaminants in the soil, is reduced. Furthermore, fertilization increases the competition for membrane carriers between additional macronutrients and HM ions, so less contaminants gets through plant cell barriers (Sarwar et al., 2013).

As crop fertilization is such a common practise in agriculture, alleviation of HM induced toxicity to plants and reduction of accumulated concentrations in the biomass hereby could be seen as a relatively low-cost, time saving and effective aid for the phytoremediation.

The results presented in this paper are a part of a larger research study. The research is designated to investigate the possibility to grow high biomass yielding plants on heavy metal contaminated soil and to use harvest for bioenergy purposes. The aims of the research in particular are 1) to investigate vegetative parameters of rapeseed (*Brassica napus* L.) plant when cultivated on HM contaminated soil; 2) to determine rapeseed capability to extract Cd and Zn from contaminated soil and 3) to analyse the possible effects on HM accumulation in rapeseed tissues due to the application of liquid and solid nitrogen fertilisers.

MATERIALS AND METHODS

Soil sample collection

A contaminated soil used in this experiment was taken from the former septic drain fields in Molainiai. This territory covers 96.4 ha area and is located in Panevėžys city in mid-Lithuania. Septic drain fields were used for wastewater biological filtering purpose not only by households but also by several heavy industry companies which did not have their own wastewater treatment facilities. Because processes like tin dipping and galvanisation were involved, varying content of heavy metals was brought along with the wastewater and sank in the soil. Although, septic drain fields were used only for 3 years in the early sixties of the 20th century, soil pollution with heavy metals did not withdraw.

A composite contaminated soil sample was pooled from sub-samples taken at three spots. Soil was taken from 0–20 cm depth using plastic shovel, sieved to pass 20 mm mesh screen, homogenized and then brought to the lab for the pot experiment.

Soil without known contamination was taken from agricultural field of Aleksandras Stulginskis University plant-growing experimental station (mid-Lithuania).

A composite uncontaminated soil sample was pooled from three spots as well. This soil was also sieved and thoroughly homogenized.

Primary analytical characteristics including pH, electrical conductivity (EC), macronutrients (NPK) and heavy metal content were detected for both soils. Soil type was identified as well.

Pot experiment

Both soils after homogenization were separately subdivided and put into the plastic buckets of 26 L volume and placed at the greenhouse. Each bucket was seeded with 100 seeds of summer rapeseed cultivar 'Fenja' in mid-May. Temperature in the greenhouse chamber was maintained at $25^{\circ} \pm 2^{\circ} \text{C}$; plants were watered with tap water. The experiment was implemented in triplicates and lasted 13 weeks. In order to determine plant development and HM accumulation rate differences induced by the excess of HM in the soil and addition of macronutrients, plants were thinned thrice: at the initial stem growth phase (I), at the bud formation phase (II) and at the flowering phase (III). Rapeseed was harvested in late August (IV). Pods were cut with scissors and chucked, whereas roots were separated from stem. Roots were washed with distilled water and as well as stem and leaves air-dried at room temperature.

Fertilizers

To improve plant growth and biomass yield as well as to test HM interaction with fertilizers, two of them were chosen for this pot experiment: liquid fertilizer with amide nitrogen and magnesium 'Lyderis Mg' and solid nitrogen urea fertilizer 'Karbamidas'. Liquid and solid nitrogen fertilizers were applied 5 weeks after the seeding, just before flowering.

Liquid amide nitrogen fertilizer with magnesium 'Lyderis Mg' is designated to supplement the plants with nitrogen and to intensify photosynthesis. The fertilizer contained 15% of amide nitrogen, 7% of magnesium (as MgO) and 14% of sulphur (as SO_3). The recommended application is by spraying it out onto the leaves during the vegetation period. The intended dose for activation of the photosynthesis is 15 L ha^{-1} and it is recommended to dilute concentrate with water up to 200 L. Such solution would supplement the soil with 1.5 kg of MgO and $3.2 \text{ kg of N ha}^{-1}$. Therefore, for the spraying rapeseed in the buckets with the surface area of 0.0875 m^2 , only 2 ml of prepared solution (15 ml of concentrated fertilizer diluted up to 200 ml of distilled water) were used. The volume of one spraying click was measured and then the same amount of clicks were used to dispense the solution for all buckets.

Solid fertilizer containing 46.2% of urea nitrogen 'Karbamidas' was used as an alternative for liquid fertilizer 'Lyderis Mg'. Urea is considered as a main supplement of nitrogen for the spring crops, tuber-plants, various vegetables and berry-bushes. The proposed application for summer rapeseed is $160\text{--}230 \text{ kg ha}^{-1}$ before sowing and smaller dose of $60\text{--}100 \text{ kg ha}^{-1}$ during flower budding period. However, for this experiment dose of solid urea fertilizer was recalculated to match on the dose of nitrogen in liquid fertilizer 'Lyderis Mg'. Thus, to reach 3.2 kg ha^{-1} dose, 6.86 kg of urea should be applied. The surface area of buckets with rapeseed plants was 0.0875 m^2 , so approximately 0.065–0.075 g were added. The pellets were distributed evenly on the freshly watered soil surface. It is assumed, that the temperature in the greenhouse

(25° ± 2 °C) was not too high to cause evaporation losses. The pellets of the urea dissolved within 24 hours.

Analytical procedure

Rapeseed stem with leaves, roots, pods and seeds were separately grinded into a powder using lab grinder mill. Wet digestion was performed adding 2 ml of 30% H₂O₂, 5 ml of concentrated HNO₃ and 1 ml of deionized water step-wisely into the aliquots (0.500 ± 0.005 g) of plant sample. The Teflon bombs containing wet samples were heated for 10 minutes at a temperature of 195 °C in a microwave digestion oven (CEM Mars 5). After cool down, samples were diluted with deionised water up to 100 ml and filtered using PVDP syringe filters (pore size 0.45 µm). Inductively coupled plasma optical emission spectrometry (Perkin-Elmer Optima 8000 ICP–OES spectrometer) was carried out and HM concentrations were detected in the liquid plant samples. Reference material was analysed to verify the reliability of the results.

Data evaluation

Average concentrations of accumulated Cd and Zn in single rapeseed plant parts were calculated from mg L⁻¹ to mg kg⁻¹ of dry weight (DW). Plant capacity to accumulate HM from the soil and rapeseed potential to be used for phytoremediation purposes was evaluated by calculating Bioconcentration factor (BCF) using Equation (1) (Malik et al., 2010).

$$BCF = C_{roots} \div C_{soil} \quad (1)$$

where: C_{roots} is a metal concentration in the roots and C_{soil} is a metal concentration in the soil where plant was grown.

Significance of the obtained average differences was evaluated using *t-Test* (two sample assuming unequal variances with $\alpha = 0.05$) in MS Office Excel.

RESULTS AND DISCUSSION

Soil properties and heavy metal content

Analytical characteristics of the two soils are given in Table 1. Soil type identification was based on granulometric composition and both soils were characterized as sandy loams. Uncontaminated soil exhibited pH 7.4 (slightly alkaline), while contaminated soil had slightly higher pH 8.0 (alkaline). Soil pH is one of the most important factors influencing plant nutrient as well as heavy metal bioavailability. Uptake of most of the nutrients is favoured at pH range 6.5–7.5, while HM, such as Cd, Pb or Zn, are usually less mobile at circumneutral pH. Soils used in this pot experiment exhibited rather low electrical conductivity: 290 µS m⁻¹ for contaminated soil and 245 µS m⁻¹ for uncontaminated one. Soil EC usually have correlations with organic matter content, dissolved ions, soil drainage conditions, soil texture and cation exchange capacity, which in turn affect crop productivity.

Nitrogen (N), phosphorus (P) and potassium (K) are major plant nutrients and growth rate is directly related to them. Composition of NPK in the analysed soils was rather different. Uncontaminated soil contained 2.5 times more of total nitrogen, whereas

mobile phosphorus and mobile potassium content was higher in contaminated soil by 2.6 and 1.7 times respectively.

Table 1. Average values of analytical characteristics of soil samples

Analytes	Contaminated soil	Uncontaminated soil
Soil type	sandy loam	sandy loam
pH	8.0 ± 0.2	7.4 ± 0.2
EC, $\mu\text{S m}^{-1}$	290 ± 5	245 ± 5
Mineral N, mg kg^{-1}	6.5 ± 1.1	16 ± 1.5
Mobile P (as P_2O_5), mg kg^{-1}	921 ± 55	348 ± 23
Mobile K (as K_2O), mg kg^{-1}	227 ± 14	127 ± 8

Concentration of Cd in contaminated soil was $26.8 \pm 0.9 \text{ mg kg}^{-1} \text{ DW}$ and it is nearly 9 times higher than maximum permissible concentration (MPC) ($3 \text{ mg kg}^{-1} \text{ DW}$) and about 335 times higher than in uncontaminated soil ($0.08 \pm 0.05 \text{ mg kg}^{-1} \text{ DW}$). While background concentration for Cd in the soil is only $0.15 \text{ mg kg}^{-1} \text{ DW}$. Concentration of Zn in contaminated soil was $202.6 \pm 7.8 \text{ mg kg}^{-1} \text{ DW}$ and it did not reach MPC ($300 \text{ mg kg}^{-1} \text{ DW}$), but it was 8 times higher than soil background concentration for Zn ($26 \text{ mg kg}^{-1} \text{ DW}$). Zinc concentration in uncontaminated soil was $28.9 \pm 1.5 \text{ mg kg}^{-1} \text{ DW}$ (Lithuanian..., 2004).

Vegetative parameters of rapeseed plants

Vegetative parameters of rapeseed plants measured during the pot experiment at the initial stem growing phase (I), bud formation phase (II), flowering phase (III) and after harvesting (IV) are presented in Figure 1. Rapeseed plants grown on contaminated soil on average developed a slightly longer stem (Fig. 1(a)), however the differences were not statistically significant. Application of fertilizers did not influence stem development.

The longest roots (Fig. 1 (b)) were recorded for rapeseed grown on contaminated soil under the fertilizer addition. Though, the differences between effects of the liquid and solid fertilizers on root length were negligible. Plants from uncontaminated soil provided the shortest main root. Root length is an important parameter when incorporating certain plant species for phytoremediation: the longer the root, the thicker contaminated soil layer can be attenuated.

All plants exhibited the largest dry weight per plant (Fig. 1 (c)) during the flowering phase. Rapeseed from uncontaminated soil had significantly the highest dry weight on average per plant. It was visible during the greenhouse experiment that rapeseed on contaminated soil had fewer and smaller leaves. As shown in Fig. 1 (c) dry weight of rapeseed plants is lower after harvest than in the flowering phase. There are two reasons for this: 1) due to the natural vegetation slowdown, rapeseed lost most of the leaves in the end; 2) dry weight measurements excluded the weight of pods and seeds. The highest dry weight was recorded for plants from uncontaminated soil, whereas fertilization did not have significant effect on average dry weight.

Standard weight of 100 seeds of summer rapeseed cultivar 'Fenja' is 0.39 g (Descriptions..., 2013). It is an important trait when reckoning the future harvest. A hundred seeds matured by plants grown on uncontaminated soil weighted only $0.25 \pm 0.04 \text{ g}$. This gap between Standard weight and results from our experiment might

be due to the rather low phosphorus and potassium content in the soil as no additional macronutrients were added to the uncontaminated soil. Significantly higher weight of hundred seeds was measured for contaminated soil. Furthermore, fertilization using solid form of urea proved to be the most successful as a hundred seeds weighted 0.39 ± 0.02 g.

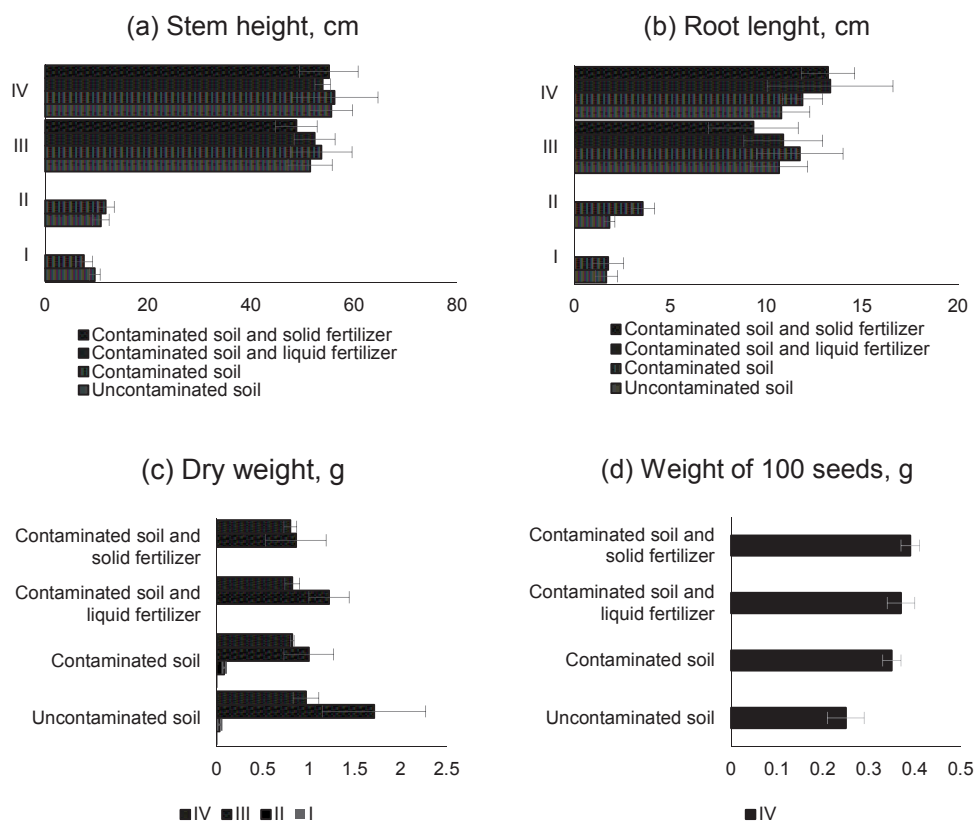


Figure 1. Vegetative parameters of rapeseed: (a) average stem height of a single plant; (b) average root length of a single plant; (c) dry weight of a single plant (pods and seeds excluded); (d) average weight of 100 seeds randomly picked from plants of one bucket. I: initial stem growth phase; II: bud formation phase; III: flowering phase; IV: harvest. Fertilizers applied before phase III.

Cadmium and zinc accumulation in rapeseed tissues

There is no linear reliance on microelements' uptake by plants as it is influenced by many abiotic factors (Kabata-Pendias, 2011). Thus, it is difficult to generalize what HM concentrations in certain plant species are normal or abnormal. On the other hand, it is clear enough that plant cultivated on HM contaminated soil will end up with higher HM concentrations in its tissues.

As shown in Table 2 significantly higher concentrations of Cd were detected in single plant parts when rapeseed was grown on HM contaminated soil in comparison to the rapeseed from uncontaminated soil. Already at the initial stem growth phase rapeseed started to uptake Cd intensively. This can be explained by a rapid breakdown of the

physiological barrier controlling a metabolic absorption of this toxic metal (Kabata-Pendias, 2011). Even in the case of uncontrolled Cd accumulation in tissues, plant may grow without visual toxicity symptoms and show no significant yield loss (Sarwar et al., 2010). Significantly the highest Cd concentrations were found in roots ($15.9 \pm 1.8 \text{ mg kg}^{-1} \text{ DW}$) and stems with remaining leaves ($10.6 \pm 0.5 \text{ mg kg}^{-1} \text{ DW}$) after the harvesting in plants grown on contaminated but fertilizer-untreated soil. Rapeseed accumulated less Cd in stems (by 15%) and roots (by 38%) when nitrogen fertilizers were applied. The subsequent decrease of Cd concentration in roots and stems with leaves might have occurred due to the membrane carrier deficiency as the addition of fertilizers intensified the uptake of nutrients (Dheri et al., 2007). The difference on Cd accumulation in single rapeseed parts between the applications of solid and liquid nitrogen fertilizers was insignificant ($p > 0.05$). Rapeseed pods and, more importantly, seeds exhibited rather low concentrations of Cd. These plant parts turn up lastly, thus the concentrations did not exceed $2.5 \text{ mg kg}^{-1} \text{ DW}$ in our case.

Table 2. Accumulated average concentrations and standard deviations of Cd, $\text{mg kg}^{-1} \text{ DW}$, in rapeseed tissues at different growth phases

Thinning and plant part	Uncontaminated soil	Contaminated soil	Contaminated soil and liquid fertilizer	Contaminated soil and solid fertilizer
I stem and leaves, roots	0.85 ± 0.04	9.50 ± 0.93	-	-
II stem and leaves	0.96 ± 0.02	8.15 ± 0.49	-	-
II roots	0.37 ± 0.07	6.69 ± 1.69	-	-
III stem and leaves	0.46 ± 0.07	5.73 ± 0.47	5.95 ± 0.37	6.57 ± 0.50
III roots	0.27 ± 0.10	9.41 ± 1.54	7.69 ± 1.08	11.85 ± 2.06
III pods with seeds	0.25 ± 0.03	1.44 ± 0.11	0.77 ± 0.25	0.81 ± 0.19
IV stem and leaves	0.45 ± 0.11	10.62 ± 0.44	8.99 ± 0.62	9.11 ± 0.57
IV roots	0.26 ± 0.14	15.97 ± 1.80	10.98 ± 2.78	8.86 ± 2.66
IV pods	0.20 ± 0.001	1.93 ± 0.16	1.49 ± 0.26	1.53 ± 0.27
IV seeds	0.24 ± 0.001	2.20 ± 0.34	1.81 ± 0.03	1.81 ± 0.23

Accumulation of Zn is presented in Table 3. Zinc is an essential micronutrient and its importance for the plant development is beyond the doubt. Although, Zn contamination in soil used for greenhouse experiment did not exceed MPC value, an increased accumulation in plants was detected due to higher than background Zn concentration. Analysing the harvested biomass samples it was found that addition of nitrogen fertilizers decreased Zn concentration in stems and leaves, roots as well as seeds if compared with plants from contaminated but fertilizer-untreated soil. However, there were no significant differences ($p > 0.05$) between applications of liquid or solid fertilizer. Zinc is quite mobile in mildly alkaline environment and therefore it is distributed more uniformly through the plant parts than Cd. Only pods after harvesting contained rather low Zn amounts. While seeds of plants grown in uncontaminated soil exhibited unexpectedly high Zn concentrations—there were no significant differences between the latter and seeds from contaminated but fertilizer-treated soil. The reason behind high Zn accumulation in seeds is that this metal is incorporated in the structure of a variety of enzymes which are so abundant in vegetable oil (Kabata-Pendias, 2011).

Table 3. Accumulated average concentrations and standard deviations of Zn, mg kg⁻¹ DW, in rapeseed tissues at different growth phases

Thinning and plant part	Uncontaminated soil	Contaminated soil	Contaminated soil and liquid fertilizer	Contaminated soil and solid fertilizer
I stem and leaves, roots	33.46 ± 1.47	70.70 ± 7.82	-	-
II stem and leaves	17.70 ± 2.05	55.50 ± 3.08	-	-
II roots	16.47 ± 1.77	50.60 ± 11.12	-	-
III stem and leaves	15.99 ± 0.96	54.89 ± 3.78	57.37 ± 3.94	61.52 ± 4.40
III roots	17.13 ± 0.69	51.92 ± 6.26	57.85 ± 7.99	77.37 ± 11.23
III pods with seeds	23.17 ± 1.82	33.25 ± 2.42	27.60 ± 2.80	27.74 ± 4.56
IV stem and leaves	11.35 ± 2.53	79.04 ± 2.17	65.73 ± 3.97	66.11 ± 4.41
IV roots	12.90 ± 1.81	95.10 ± 9.23	60.03 ± 18.49	51.71 ± 15.29
IV pods	7.66 ± 2.12	9.52 ± 1.75	7.76 ± 1.02	6.40 ± 0.48
IV seeds	57.31 ± 7.98	74.02 ± 6.54	59.31 ± 0.92	59.55 ± 3.28

Cadmium and zinc Bioconcentration factor for rapeseed

Bioconcentration factor as a ratio between a certain heavy metal concentration in roots and soil was calculated for rapeseed plants and is presented in Fig. 2.

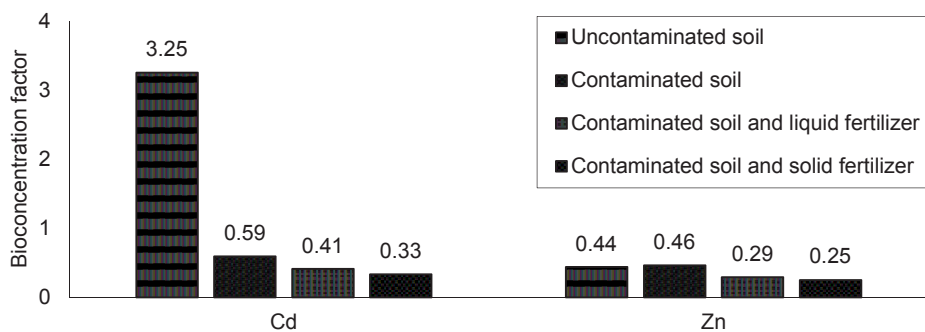


Figure 2. Bioconcentration factors of Cd and Zn for rapeseed.

Plants exhibiting BCF values higher than unity, can be considered as hyperaccumulators (Yoon et al., 2006). Some species in the *Brassicaceae* family, like Indian mustard (*Brassica juncea* L.) or Chinese cabbage (*Brassica rapa* L.) are considered to be effective in phytoextraction process, especially when chelants are used (Brunetti et al., 2011; Ali et al., 2013; Marques et al., 2013). However, in the case of our experiment, rapeseed, being a member of *Brassicaceae* family, exhibited BCF values lower than unity for both Cd and Zn when plants were grown on contaminated soil. This indicates that, usage of rapeseed as Cd or Zn accumulator for intensive phytoextraction is limited, or otherwise alternative HM uptake-enhancing measures should be applied. On the other hand our experiment revealed, that application of nitrogen fertilizers had an inhibitory effect on Cd and Zn bioavailability in rapeseed tissues – Bioconcentration factor values were significantly lower when fertilizers were used. Rapeseed grown on uncontaminated soil exhibited BCF = 3.25 for Cd, it proves aforementioned statement that Cd can easily penetrate biomembrane and be translocated in plant tissues.

Nonetheless, in spite of so high BCF value, Cd concentration in rapeseed tissues from uncontaminated soil remained relatively low.

CONCLUSIONS

Our results showed that despite of higher than background Cd and Zn content in the soil, rapeseed development was similar to the control samples grown on uncontaminated soil. Moreover, rapeseed produced heavier seeds when cultivated on contaminated soil. This is a very important characteristics for oil-bearing plants, such as rapeseed.

Calculation of Bioconcentration factor revealed, that rapeseed is not efficient enough for Cd or Zn phytoextraction purposes. Furthermore, usage of nitrogen fertilizers, despite of its application form (liquid or solid), even more decreased Cd and Zn accumulation in plants. Consequently, this would decrease overall Cd or Zn removal from the contaminated area, but lower accumulation would increase the utilization possibilities of rapeseed biomass for energetic purposes. However, to be able to use fertilizers as an aid for phytoextraction, further research is needed involving different fertilizers as well as application rates.

REFERENCES

- Ali, H., Khan, E. & Sajad, M.A. 2013. Phytoremediation of heavy metals–Concepts and applications. *Chemosphere* **91**, 869–881.
- Bhargava, A., Carmona, F.F., Bhargava, M. & Srivastava, S. 2012. Approaches for enhanced phytoextraction of heavy metals. *Journal of Environmental Management* **105**, 103–120.
- Brunetti, G., Farrag, K., Rovira, P.S., Nigro, F. & Senesi, N. 2011. Greenhouse and field studies on Cr, Cu, Pb and Zn phytoextraction by *Brassica napus* from contaminated soils in Apulia region, Southern Italy. *Geoderma* **160**, 517–523.
- Campbell, J.E., Lobell, D.B., Genova, R.C. & Field, C.B. 2008. The global potential on bioenergy on abandoned agricultural lands. *Environmental Science and Technology* **42**, 5791–5794.
- Descriptions of rapeseed varieties included in the national plant variety list. 2013. http://www.vatzum.lt/uploads/documents/augalu_veisles/2013_raps_veisli_apraai_1.pdf. Accessed 26. 01. 2016. (in Lithuanian).
- Dheri, G.S., Brar, M.S. & Malhi, S.S. 2007. Influence of phosphorus application on growth and cadmium uptake of spinach in two Cd-contaminated soils. *Journal of Plant Nutrition and Soil Science* **170**, 495–499.
- Kabata-Pendias, A. 2011. *Trace Elements in Soils and Plants, 4th Edition*. CRC Press, 548 pp.
- Lithuanian Minister of Health Order on Approval of Lithuanian HN 60:2004 on *Maximum permissible concentrations of dangerous chemicals in soil*. 08. 03. 2004. No. V–114, Vilnius. (in Lithuanian).
- Malik, R.N., Husain, S.Z. & Nazir, I. 2010. Heavy metal contamination and accumulation in soil and wild plants species from industrial area of Islamabad, Pakistan. *Pakistan Journal of Botany* **42**, 291–301.
- Marques, A.P.G.C., Rangel, A.O.S.S. & Castro, P.M.L. 2013. Remediation of heavy metal contaminated soils: phytoremediation as a potentially promising clean-up technology. *Critical Reviews in Environmental Science and Technology* **39**, 622–654.
- Meers, E., Van Slycken, S., Adriaensen, K., Ruttens, A., Vangronsveld, J., Du Laing, G., Witters, N., Thewys, T & ack, F.M.G. 2010. The use of bio-energy crops (*Zea mays*) for

- ‘phytoattenuation’ of heavy metals on moderately contaminated soils: A field experiment. *Chemosphere* **78**, 35–41.
- Nagajyoti, P.C., Lee, K.D. & Sreekanth, T.V.M. 2011. Heavy metals, occurrence and toxicity for plants: a review. *Environmental Chemistry Letters* **8**, 199–216.
- Oves, M., Khan, M.S., Zaidi, A. & Ahmad, E. 2012. Soil contamination, nutritive value, and human health risk assessment of heavy metals: an overview in Zaidi, A. et al. (eds). 2012. *Toxicity of heavy metals to legumes and bioremediation*. Springer–Verlag Wien, 1–28.
- Sarwar, N., Saifullah, Malhi, S.S., Zia, M.H., Naeem, A., Bibi, S. & Farid, G. 2010. Role of mineral nutrition in minimizing cadmium accumulation by plants. *Journal of the Science of Food and Agriculture* **90**, 925–937.
- Witters, N., Mendelsohn, R.O., Van Slycken, S., Weyens, N., Schreurs, E., Meers, E., Tack, F., Carleer, R. & Vangronsveld, J. 2012. Phytoremediation, a sustainable remediation technology? Conclusions from a case study. I: Energy production and carbon dioxide abatement. *Biomass and Bioenergy* **39**, 454–469.
- Yoon, J., Cao, X., Zhou, Q. & Ma, L.Q. 2006. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Science of the Total Environment* **54**, 441–446.