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Determination of manganese with crystal violet in plant material in different fertilization

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Abstract. A study in a multiple-factor stationary field experiment with 16 variants NPK on background with and without annual fertilization with manure was carried out at the Agricultural University in Plovdiv, Bulgaria. The manganese content in the roots of radishes, cultivar Red with white tails, was determined by a new extraction-photometric method with Crystal Violet. The ion-associate of manganese (VII) was completely extracted in a single extraction of only 30 s. The molar absorptivity of the associate was $(1.54 \pm 0.05) \times 10^4$ l mol^{-1} cm^{-1}. This indicates the high sensitivity of the given reaction. Manganese has been determined by atomic-absorption method, too, in order to compare the results. It was established that fertilization has an effect upon the manganese content in the roots of radishes. The roots of radishes accumulate the highest level of manganese 116.25 mg/kg in a mineral fertilization N:P:K = 3:2:1 with 40 t/ha manure. The relationship between yield and manganese content in radish roots under the influence of fertilization has been established.

Key words: manganese determination, Crystal Violet, plants, fertilization

INTRODUCTION

The extremely important role of manganese in the growth of plants determined the goal of our study. In the conditions of intensive farming, the problem of trace elements in the soil-plant system attained significant importance.

Manganese is one of the microelements which are actively absorbed by plants. The biological absorption coefficient for manganese is greater than those for the other microelements in this group, namely Zn, Cu, Mo, Ni, Co, Pb. The formation of the plant mass depends on the manganese provision for the plants. Manganese takes part in a number of important physiological and biological processes in the lower and higher plant organisms – in the nitrogen metabolism, photosynthesis, breathing and maintaining the necessary oxidation-reduction conditions in the cell (Sidorovich et al., 1987; Udintseva et al., 1981; Nason, 1952).

The manganese content in the plants varies in a wide range – from one hundred to one thousand parts of the percentage. Among the field crops, beetroot (the leaves) and leguminous plants are characterized with a higher content of manganese, but the content is lower in corn (cereals). Fruit tree species also accumulate different amounts of manganese in their tissues when grown under the same soil conditions.

The level of manganese in plants is usually increased with the progress of the vegetation. Higher levels of manganese were found in plant organs in which the metabolism is more intensive. Experiments on cotton (Nuraliev and Yarovenko, 1980) have shown that manganese accelerates the entrance of phosphorus into the plants and has a positive effect on growth and fruit formation.

Manganese is an important element of nitrogen metabolism in plants. Manganese insufficiency leads to a considerable accumulation of nitrates, disturbance in the protein synthesis in plants and, in some pants, illness (Nicholas, 1961; Bergmann et al., 1976; Heintze, 1966), and causes a decrease in Ca and Mg content (Shkolnik, 1974).

The optimal content of manganese, its critical level and toxic concentration at which the growth is depressed and the yield decreased, have been established for a great number of crops (Potatueva, 1990).

Various methods for determination of manganese have been published. Analyses with some reagents have been used (Kadobayashi, 2004; Tarafder, 2004; Shar, 2003; Balogh, 2003; Alkan, 2003; Winkler, 2003; Biswas, 2003).

The present study is intended to clear up the effect of prolonged fertilization (mineral and organic) on the manganese content in radishes using a new extraction-photometric method with Crystal Violet for determination of manganese (Kostova, in press). We used the triphenylmethane dye Crystal Violet, for the first time, to show that it can be applied to the analysis of manganese in plant material.

MATERIAL AND METHODS

The study was carried out in the 23rd year of a multiple-factor stationary field experiment. The experiment was carried out at 5 levels of fertilization with nitrogen, 5 levels with phosphorus and 4 levels with potassium with 16 variants of NPK, on background with and without annual fertilization of 40 t/ha of manure.

The following scheme was applied:

1	$N_0P_0K_0$	9	$N_{240}P_{240}K_{120}$
2	$N_{120}P_{120}K_0$	10	$N_{240}P_{360}K_{120}$
3	$N_{120}P_0K_{120}$	11	$N_{360}P_{120}K_{120}$
4	$N_0P_{120}K_{120}$	12	$N_{360}P_{240}K_{120}$
5	$N_{120}P_{120}K_{120}$	13	$N_{360}P_{360}K_{120}$
6	$N_{120}P_{240}K_{120}$	14	$N_{240}P_{240}K_{240}$
7	$N_{120}P_{360}K_{120}$	15	$N_{360}P_{360}K_{360}$
8	$N_{240}P_{120}K_{120}$	16	$N_{480}P_{480}K_{360}$

The stationary experiment was set on a strongly leached meadow – cinnamon soil (clay fraction in the 0 – 20 cm layer was from 6.8-6.9 %, the sum of the interchangeable cations ranged from 8.69-7.65 % and that of the interchangeable Ca and Mg – from 5.74 – 7.34 %) with neutral reaction (pH 6.8 to 7.0) with a 2.2 % humus content (by Tyurin).

Soil samples from a 0 – 20 cm deep layer were taken for agrochemical analysis and analysed for mobile P_2O_5 and K_2O - by the Egner-Rhiem method; mineral nitrogen (NH_4-N + NO_3-N) by distillation; pH – potentiometrically in aqueous extract.

The plants were grown by the technology adopted for the region. Yield of roots per hectare was taken into account; dry matter per cent was determined by weight.

For the first 16 years in the stationary field experiment, early tomatoes (1^{st} crop) and late-headed cabbage (2^{nd} crop) were grown; from the 17^{th} – 20^{th} years, medium – early tomatoes with intermediate crop repko were planted in autumn and harvested in spring. Maize for grain was grown in the 21^{st} year, followed in the 22^{nd} year by vegetable marrows, with a second crop of spinach; in the 23^{rd} year, early-headed cabbage and radishes cultivar Red with white tails were grown; the latter crop was used for the present study.

Up to the 16^{th} year, fertilization with the above-mentioned amounts of nutrient elements was applied to each of the cultivated crops, but from the 17^{th} year onwards it was only applied to the first crop.

An analysis for manganese content by variants of averaged root samples was carried out using a new extraction-photometric method with Crystal Violet. Manganese(VII) forms an ion-association complex with triphenylmethane dye Crystal Violet (CV). To remove the possibility of extracting the ion associate, the following different types of organic solvents were tested: hydrocarbons (benzene and toluene), ethers, ketones, alcohols, chloroform, dichlorethane and tetrachlorethane. The studies showed that the associate is best dissolved in 1,2-dichloroethane. Maximum light absorption of the associate is observed at 250 nm. The molar absorbability determined by the Komar-Tolmatchov method (Bulatov and Kalinkin, 1972) is $\varepsilon_{250} = (1.54 \pm 0.05) \times 10^4$ l mol^{-1} cm^{-1}. The sensitivity of the Sandell method (Sandell, 1959) is $W_S = 3.57 \times 10^{-3}$ µg cm^{-2}.

The obtained study results in connection with the method we developed for determination of manganese with Crystal Violet are worked with a computer programme for a two-factor dispersive analysis and multispecified comparative analysis in Duncan's method (Mokreva and Murgova, 1988).

Apparatus

Spectrophotometer VSU with 1-cm light path quartz cells.

Procedure

A wet burning of the plant sample was carried out in which a mixture of sulphuric and nitric acids was used for the oxidation of the organic substance. A portion of 2 g of air-dry plant material was placed into a Kjeldahl flask and moistened with 4 ml distilled water; 5 ml conc. sulphuric acid and 10 ml conc. nitric acid were added. The flask was slightly heated to avoid splashing of the solution decomposition and fuming away of HNO_3. If the oxidation of the organic substance was not completed, HNO_3 was added and heated again. When all the organic material was oxidized, the solution was heated at a higher temperature for 10 min (Vazhenin, 1974). After cooling, the solution was diluted with water and filtered. Portions of 3 ml conc. H_2SO_4, 2 ml conc. H_3PO_4

and 0,1 g potassium periodate were added for oxidation of Mn(II) to Mn(VII). It was heated to boiling point and the temperature was maintained for 10 min (Sandell, 1959). After cooling the solution was diluted with water and filtered, transferred into a volumetric flask of 50 ml and diluted to the mark with distilled water. Aliquot parts of this solution were taken for analysis.

In a separatory funnel of 100 ml are introduced the following solutions: 0.3 ml hydrochloric acid 1.2 M, 0.4 ml Crystal Violet $1x10^{-3}$ M, aliquote of the prepared solution of the plant sample. It is diluted up to a volume of the aqueous phase of 10 ml with distilled water and extracted with 3 ml of 1,2-dichloroethane for 30 s. The organic phase is filtered through a dry paper into a 1 cm cuvette and the absorbance measured at 250 nm. A blank is run in parallel in the absence of a plant sample. A calibration graph is constructed with standards similarly treated.

RESULTS AND DISCUSSION

The mineral fertilization led to considerable changes in manganese content in the radishes. The scheme of fertilization variants is shown in Tables 1 and 2.

Manganese content in the plant material was determined by using a new extraction-photometric method, triphenylmethane dye Crystal Violet, for the first time,.

Taking into account the different fertilization with N, P and K (Variants 2,3, 4) it can be seen that radish accumulate the least manganese 10 mg kg^{-1} in fertilization only with P and K ($N_0P_{120}K_{120}$) (Variant 4). The content of manganese rises to more than 5 times if a nitric fertilizer $N_{120}P_{120}K_0$ (Variant 2) and $N_{120}P_0K_{120}$ (Variant 3) can be used in fertilization.

Table 1. Manganese content in radish (mg kg^{-1} dry mass)

N	Variants NPK	Method with CV Mn mg kg^{-1}	Reliab P= 99 %	RSD* %	AAS Mn mg kg^{-1}	Yield kg ha^{-1}
1	$N_0P_0K_0$	20.00	a	1.2	20.60	6325
2	$N_{120}P_{120}K_0$	52.25	d	0.9	52.00	7480
3	$N_{120}P_0K_{120}$	56.00	b	1.5	55. 20	6325
4	$N_0P_{120}K_{120}$	10.00	b	1.6	9.40	7095
5	$N_{120}P_{120}K_{120}$	15.00	a	1.1	15.90	10314
6	$N_{120}P_{240}K_{120}$	21.75	d	1.4	21.00	10149
7	$N_{120}P_{360}K_{120}$	48.50	c	1.2	47.80	12404
8	$N_{240}P_{120}K_{120}$	59.75	c	1.5	60.30	10039
9	$N_{240}P_{240}K_{120}$	63.2	f	1.4	63.90	8279
10	$N_{240}P_{360}K_{120}$	95.25	a	0.9	94.50	6875
11	$N_{360}P_{120}K_{120}$	20.25	e	1.3	20.90	11964
12	$N_{360}P_{240}K_{120}$	26.00	d	1.5	26.85	9790
13	$N_{360}P_{360}K_{120}$	32.25	b	1.1	33.00	9295
14	$N_{240}P_{240}K_{240}$	30.25	f	1.4	30.00	15261
15	$N_{360}P_{360}K_{360}$	66.25	c	1.2	66.80	11935
16	$N_{480}P_{480}K_{360}$	71.75	a	1.5	71.30	1359

*Relative Standard Deviation for CV method (n = 6)
a, b, c, A degree of reliability

In a constant level of potassium K_{120}, manganese content increased with the increase of the fertilization norm of phosphorus. It is well expressed in the three levels of nitrogen fertilization with N_{120}, N_{240} and N_{360}.

Variants 5,6, 7 with fertilization norm N_{120} comprise 15 mg kg^{-1}, 21.75 mg kg^{-1} and 48.50 mg kg^{-1} Mn dry mass. In fertilization norm N_{240} (variants 8,9,10) the content of manganese increases to 59.75 mg kg^{-1},63.25 mg kg^{-1} and 95.25 mg kg^{-1} with the increasing content of phosphorus.

The above-mentioned subordination can be seen in Variants 11,12, 13 with N_{360} , in which the content of manganese content reaches 20.25 mg kg^{-1}, 26 mg kg^{-1} and 32.25 mg kg^{-1} with the increasing phosphorus content.

The subordination of yield from the content of manganese in fertilization with P_{120} in different levels N и K is a small negative with correlation coefficient. r = - 0.09 (Fig. 1).

A negative correlation (r = -0.46) is obtained between the concentration of manganese and yield in fertilization with N_{120} in different levels of P and K. With the increasing of the concentration of manganese, the yield decreases (Fig. 2).

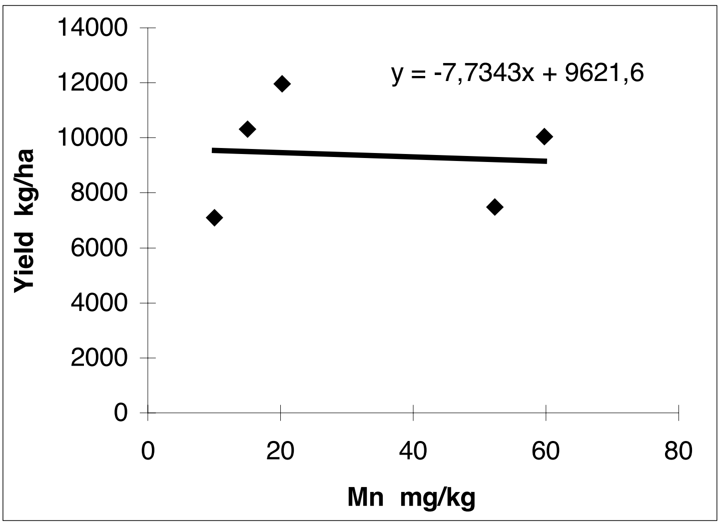

Fig. 1. The subordination between the yield kg ha^{-1} and Mn content – fertilization with P_{120} in different levels N и K; Correlation coefficient r = -0.09

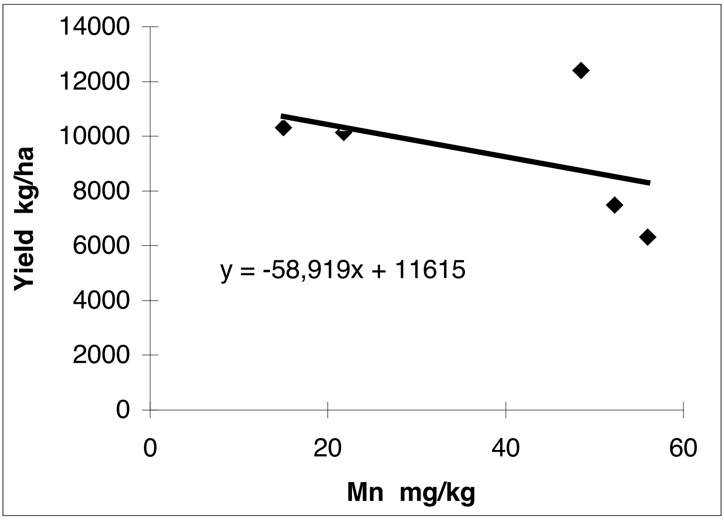

Fig. 2. The subordination between the yield kg ha^{-1} and Mn content – fertilization with N_{120} in different levels P и K; Correlation coefficient r = -0.46

A small negative correlation (r = -0.18) determines the relation between the yield and manganese content in radishes in the variants from 1 to 16 (Fig.3).

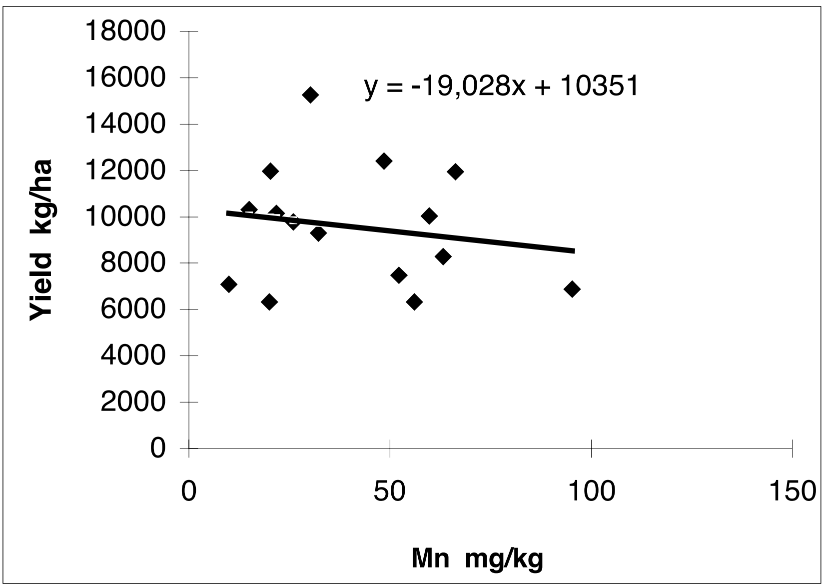

Fig. 3. A general subordination between yield kg ha^{-1} and Mn content in radishes with mineral fertilization; Correlation coefficient. r = -0.18

In the three levels of nitrogen fertilization with N_{120}, N_{240} and N_{360}, the highest content was 95.25 mg kg^{-1} Mn dry mass after fertilization with $N_{240}:P_{360}:K_{120}$ = 2:3:1.

Manganese content in radishes increases with the increasing of the fertilization norm of N, P and K. Thus, for instance, in fertilization with $N_{120}P_{120}K_{120}$ the manganese content in radishes is 15.00 mg kg^{-1} (Variant 5). With the increasing of the fertilization norm of $N_{240}P_{240}K_{240}$, manganese also increases to 30.25 mg kg^{-1} (Variant 14); in a fertilization norm $N_{360}P_{360}K_{360}$, the content of manganese is highest - 66.25 mg kg^{-1} (Variant 15).

In the experiment with mineral fertilization and 40 t ha^{-1} manure, the lowest content of manganese was in the roots of radishes fertilized only with phosphorus and potassium ($N_0P_{120}K_{120}$) – 16 mg kg^{-1} Mn dry mass (Variant 4) (Table 2).

Table 2. Manganese content in radish (mg kg^{-1} dry mass)

N	Variants	NPK + 40 t ha^{-1} manure				
		Method with CV Mn mg kg^{-1}	Reliab. P= 99 %	RSD* %	AAS Mn mg kg^{-1}	Yield kg ha^{-1}
1	$N_0P_0K_0$	17.50	a	1.3	18.00	6189
2	$N_{120}P_{120}K_0$	50.50	b	1.0	51.10	3580
3	$N_{120}P_0K_{120}$	61.50	b	1.5	60 80	1844
4	$N_0P_{120}K_{120}$	16.00	f	1.1	16.70	4319
5	$N_{120}P_{120}K_{120}$	39.50	c	1.2	39.90	3329
6	$N_{120}P_{240}K_{120}$	43.50	d	1.5	44.25	8389
7	$N_{120}P_{360}K_{120}$	28.50	a	1.1	28.85	7869
8	$N_{240}P_{120}K_{120}$	44.50	c	1.5	44.00	10118
9	$N_{240}P_{240}K_{120}$	44.75	f	1.4	44.40	2332
10	$N_{240}P_{360}K_{120}$	38.50	b	1.9	39.20	2407
11	$N_{360}P_{120}K_{120}$	32.75	e	1.2	33.20	2190
12	$N_{360}P_{240}K_{120}$	116.25	e	1.4	116.80	1540
13	$N_{360}P_{360}K_{120}$	56.25	a	1.1	55.80	2064
14	$N_{240}P_{240}K_{240}$	17.50	b	1.9	16.80	3835
15	$N_{360}P_{360}K_{360}$	57.00	c	1.2	57.60	2585
16	$N_{480}P_{480}K_{360}$	57.00	a	1.8	57.85	2009

*Relative Standard Deviation for CV method (n = 6)

a, b, c, A degree of reliability

In the three levels of nitrogen fertilization with manure: N_{120} (variants 5, 6, 7), N_{240} (variants 8, 9, 10) and N_{360} (variants 11, 12, 13) the highest content of manganese is at P_{240} .

Negative correlation (r = -0.71) determines the relation between yield and the manganese content in variants of fertilization with N_{120} and 40 t ha^{-1} manure in different levels P and K (Fig.4). It can be seen from the figure 4 that with the increasing of the concentration of manganese, the yield decreases.

In variants of fertilization with manure and P_{120} in different levels N and K manganese has a positive influence upon yield and the correlation coefficient is r = 0.25 (Fig.5).

The subordination of yield from the content of manganese in radishes with mineral fertilization NPK and 40 t ha^{-1} manure is negative with a small correlation coefficient r = - 0.39 (Fig.6). It can be seen from figure 6 that with the increasing of the concentration of manganese, the yield decreases.

The highest level in a mineral fertilization with 40 t/ha manure was 116.25 mg kg^{-1} Mn dry mass after fertilization with N:P:K = 3:2:1. The experimental data show that this ratio is different if the fertilization has been carried out only with N, P and K.

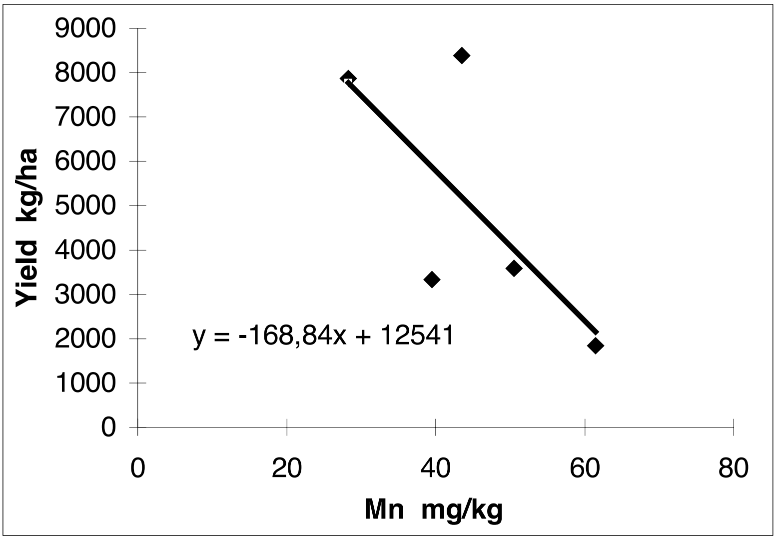

Fig. 4. The subordination between the yield kg ha^{-1} and Mn content – fertilization with N_{120} and 40 t ha^{-1} manure in different levels P and K; Correlation coefficient r = -0.71

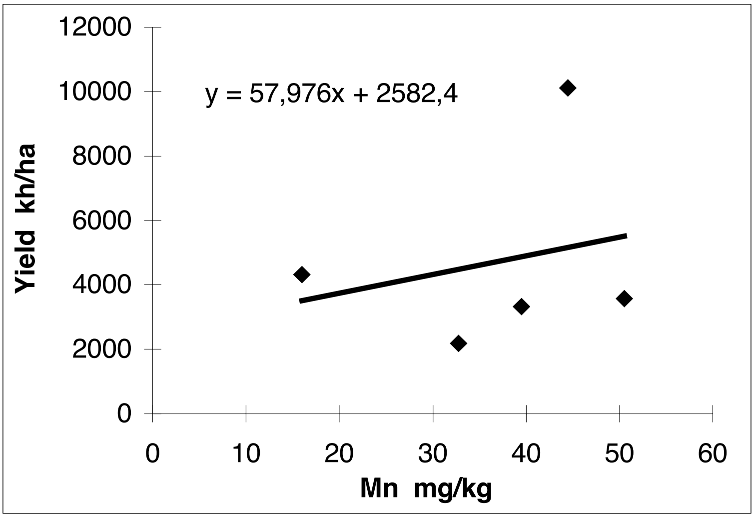

Fig. 5. The subordination between the yield kg ha^{-1} and Mn content – fertilization with P_{120} and 40 t/ha manure in different levels N and K; Correlation coefficient. r = 0.25

It can be assumed that changes in manganese content in the plant roots in this experiment (mineral fertilization with 40 t ha^{-1} manure) were due to the effect of the colloid fractions of the manure upon the mineral nutrition.

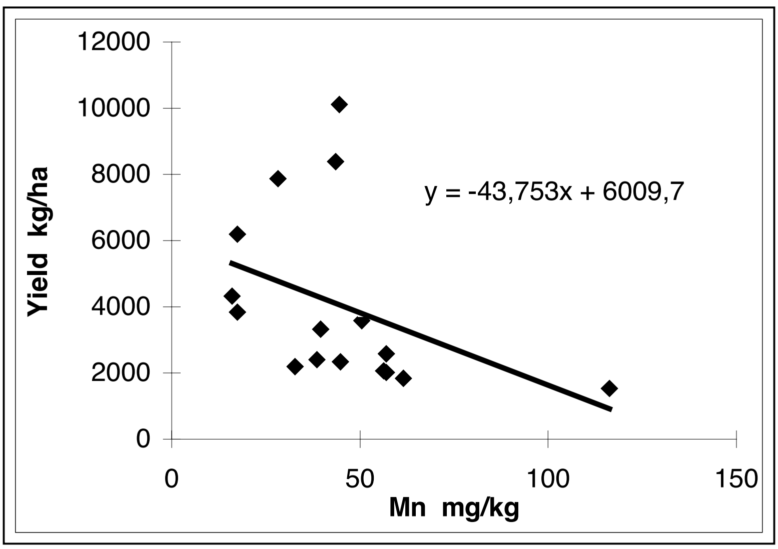

Fig. 6. A general subordination between yield kg ha^{-1} and Mn content in radishes with mineral fertilization NPK and 40 t ha^{-1} manure; Correlation coefficient r = -0.39

The experimental data (Table 2) show that in the three levels of a nitric fertilization the manganese content increases with the increase of the fertilization norm of nitrogen: $N_{120}P_{240}K_{120}$ – 43.50 mg kg^{-1} Mn (Variant 6); $N_{240}P_{240}K_{120}$ – k 44.75 mg kg^{-1} Mn (Variant 9); $N_{360}P_{240}K_{120}$ – 116.25 mg kg^{-1} Mn (Variant 12).

The highest level in a mineral fertilization with 40 t ha^{-1} manure was 116.25 mg kg^{-1} Mn dry mass after fertilization with N:P:K = 3:2:1. The experimental data show that this ratio is different if the fertilization has been carried out only with N, P and K.

It can be assumed that changes in the manganese content in the plant roots in this experiment (mineral fertilization with 40 t ha^{-1} manure) were due to the effect of the colloid fractions of the manure on the mineral nutrition.

To check the method that we propose, a parallel determination of manganese content was carried out by the atomic-absorption method (AAS). The results obtained (Tables 1 and 2) show a satisfactory accuracy of the method for determination of manganese in plant material with triphenylmethane dye Crystal Violet. The relative standard deviation of the method using Crystal Violet is 1.5 % at a statistical reliability of 95%. The experimental data by both methods show that the proposed extraction-photometric method with Crystal Violet can be successfully used for determination of micro quantities of manganese in plant material.

Some changes in the yield of the roots occur under the influence of the fertilization (Table 1). When NPK was individually applied, the highest yield was obtained in the variant 14 where it was fertilized with $N_{240}P_{240}K_{240}$ - 15261 kg ha^{-1}. High yields were also obtained in the variants 7, 11, 15 and 16 where the yield varied from 11359 to12404 kg ha^{-1}. It seems that in these variants (with the exception of variant 11) the yield of the roots decreases with the increase of the manganese content. In the remaining variants of mineral fertilization, a definite connection between the yield obtained and manganese content in the roots was not established.

In the experiment with manure background the yield was lower (Table 2). The lowest yield was in the variant 12 with $N_{360}P_{240}K_{120}$ – 1540 kg ha^{-1}, and the highest – 10118 kg ha^{-1} which was fertilized with $N_{240}P_{120}K_{120}$ variant 8. In these two variants (12 and 8), as manganese content increases, the yield of the roots decreases. It could be concluded therefore that the presence of the highest level of manganese in the roots leads to the lowest yield. After this fertilization with NPK, on the background of manure, again no connection between manganese content and yield obtained was established.

The established changes of the manganese content in the roots and those of the yield obtained by variants could be explained by changes of the agrochemical properties of the soil under the influence of prolonged fertilization.

CONCLUSIONS

A new extraction – spectrophotometric method for the determination of the concentration of manganese with Crystal Violet in plant material was used. The method is simple, sensitive and rapid with satisfactory results and good reproducibility.

Manganese is determined in roots of radishes in different fertilization. When radish is fertilized only with NPK, the manganese content in the roots is increased with the increase of the fertilization norm of phosphorus. In the three levels of nitrogen fertilization with N_{120}, N_{240} and N_{360}, the highest content was 95.25 mg kg^{-1} Mn dry mass after fertilization with N_{240}:P_{360}:K_{120} = 2:3:1. A small negative correlation determines the relation between the yield and the content of manganese in radishes fertilized only with NPK.

The roots of radishes accumulate the highest level of manganese 116.25 mg kg^{-1} in a mineral fertilization N:P:K = 3:2:1 with 40 t ha^{-1} manure. The experimental data show that this ratio is different if the fertilization has been carried out only with N, P and K. The subordination of yield from the content of manganese in radishes with mineral fertilization NPK and 40 t ha^{-1} manure is negative. It can be seen that with the increasing concentration of manganese, the yield decreases.

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