HEAVY METAL UPTAKE PATTERNS BY SPINACH (Spinacia oleracea L.) GROWN ON CONTAMINATED DIFFERENT SOIL TYPES

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Abstract

Heavy metals have different behaviour on crop plants, depending on several factors such as metal type, the quantity and mixture of metals in soil and soil type. Both pedological and agrochemical soil characteristics can influence heavy metals mobility and bioavailability, with notable consequences on plant growth. This study follows the accumulation of heavy metals and growth of spinach plants (Spinacia oleracea L.) on three different soil types (chernozem, fluvisol, chromic luvisol- acc. WRB-SR, 1998), each soil type having six variants (1 untreated soil + 5 contaminated soils) with different concentrations of heavy metals. The contaminations were made with five mixtures of heavy metals (Cd, Pb, Ni, Co, Cu and Zn) in quantities ranging near reference values to values that exceeds intervention threshold for sensitive soils, according to Ord. 756/1997 (Cd from 3 to 20 ppm, Pb from 30 to 150 ppm, Ni from 25 to 200 ppm, Co from 20 to 100 ppm, Cu from 20 to 200 ppm and Zn from 100 to 700 ppm). Soil and plant analysis for heavy metals determination were made using ICP-MS technique. The experimental results showed a significant difference on growth parameters of spinach plant and on heavy metals bioaccumulation between untreated and high contaminated soils variants, and between soil types, respectively.

Key words: bioaccumulation, heavy metals, soils, Spinacia oleracea L.

INTRODUCTION

Nowadays, soil degradation (e.g., erosion, loss of organic matter, sealing, pollution) is an increasing problem all over the world. Contamination by heavy metals is considered as one of the main threats due to their potential bio-systems accumulation in through contaminated water, soil and air (Pauget et al., 2015). A better knowledge of heavy metal sources, their accumulation in the soil and the effect of their presence on plant system is a issue for studies fundamental on risk assessment (Salawu et al., 2015).

All soils contain very low levels of heavy metals, naturally. The presence of them in soil does not necessarily mean contamination. The concentration of metals in uncontaminated soil is most often related to the geology of the parent material from which the soil was created (McLean & Bledsoe, 1992). Metals found in the aqueous phase of the soil are prone to movement with soil water and can be transported to groundwater by leaching processes. Metals, unlike some toxic organic compounds, cannot be degraded. But most of them can be transformed by chemical processes into more or less stable compounds that can reduce their toxicity and mobility. The immobilization of metals by adsorption and precipitation processes can prevent both accumulation in plants and leaching in groundwater. Even though they are toxic in large quantities, some heavy metals are essential for the normal growth of plants, and for some of them their biological role is still difficult to establish.

Most heavy metals are hardly soluble, with low mobility. Many times, metals are found in the soil as residual forms, due to strong bonds with organic and inorganic ligands. For example, Pb and Cu are found mostly in complexed form, while Zn and Cd are found in ionic form in the soil solution (Shahid, 2017).

There is a dynamic equilibrium in soil between liquid and solid phases. When the concentration of an element exceeds the equilibrium state, then it interacts with other elements in the soil, changing the normal balance. This balance depends on the nature of the soil, structure, chemical composition, organic matter, pH and many other characteristics (Butnariu, 2012). Soil reaction (pH) has been described in many studies as one of the main factors influencing the mobility of heavy metals in soil. Hou et al. (2019) found that the available content of Cu, Zn and Pb was negatively correlated with soil pH, while organic matter (OM) content was positively associated with Zn availability. Another study on the influence of pH on heavy metal mobility showed that the Cd, Cu and Zn depends on soil pH and the decrease from the value of 8.1 to 7.2 lead to a larger content of Cd, Cu and Zn (Vrancuta et al., 2019).

The solubility of heavy metal compounds, especially minerals, oxides or carbonates depends on the pH value. In general, the decreasing tendency of the pH leads to release of cations and an increase of the pH leads to release of free anions (Król et al., 2020).

The amount of available soil OM significantly influences metal bioavailability since it is considered one of the most important soil constituents that retains heavy metals (Aigberua, 2018). Organic matter appears to have the greatest capacity for sorption of trace elements in cationic form. Humic substances contain a large number of complexation centers, therefore they behave as a natural multiligand system (Violante et al., 2010).

Many studies report on the interactions between different heavy metals and their competition for binding sites. For some adsorption sites, cationic heavy metals are preferentially adsorbed instead of the major cations (Mg, Ca) and anionic metals are preferentially adsorbed over major anions (SO4²⁻, NO3⁻) (McLean & Bledsoe, 1992). Trace metals also will compete with each other for adsorption sites. The presence of other cations, either major elements or heavy metals, can significantly affect the mobility of a particular element. Some studies have looked at interactions between heavy metals, but have generally grouped two or a maximum of three elements. The Cu, Zn and Cd interaction was observed by Kuo & Baker (1980). Ueno et al. (2004), Zemanová et al. (2014) and many more. Other interactions were studied between Ni-Cd (Davari et al., 2014), Zn-Ni-Cu (Hossain et al., 2009) and many more combinations.

This study aims to determine the accumulation of various heavy metals in spinach plants, using increasing concentrations starting from normal soil values, to values that exceed the norms described in the legislation in force. Three types of soils with different characteristics are subjected to the same contamination conditions, in order to observe their influence on the accumulation of heavy metals.

MATERIALS AND METHODS

Soil Characterization, Sampling and Processing Three types of soil from different regions of Romania were selected for this study. The soilswere taken from three representative areas for the type of soils, as presented in Table 1.

able 1. The soils used experiment	
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Code	Туре	Region	Photo
S1	Chernozem	Near Fundulea (Calarasi) GMS 44°29'34.0"N 26°29'47.2"E	
S2	Chromic luvisol	Moara Domneasca (Ilfov) GMS 44°29'57.7"N 26°15'09.7"E	
S3	Fluvisol	Near Belciug (Prahova) GMS 44°48'57.8"N 26°15'26.9"E	

Some of the pedological and agrochemical properties of these soils, at the time of collection, are presented in Table 2.

Table 2. Soil characteristics

Characteristic	S1	S2	S3
pH	6.53	6.45	7.8
EC (µS/cm)	64.53	102.23	200.03
Organic carbon (%)	2.06	1.61	2.48
Organic matter (%)	3.55	2.77	4.27
Total N (%)	0.17	0.16	0.25
Bulk density (g/cm ³)	1.20	1.39	1.26
Total porosity (%)	48.38	44.37	49.31
Soil compaction (%)	3.23	-12.34	12.56

The soil was collected from the areas indicated in the previous paragraph, from a depth of 0-20 cm (rhizosphere), in polyethylene bags to keep its moisture, until the end of the collecting period. Excessive soil drying has been avoided, to be easily processed for the following steps. After collecting the entire required amount, the soils were cleaned of plant remains, stones and other impurities, crushed by hand and then sifted through a 7x5 mm mesh horticultural sieve. Each type of soil was homogenized and kept in polyethylene bags until the entire quantity was conditioned.

Greenhouse experiment

For the establishment of the spinach crop, 3liter pots were used. The vessels were covered in polyethylene bags to prevent the loss of excess water in case of over-irrigation. Loss of water means loss of soluble salts from the soil, leading to a decrease of heavy metals concentration. The pots were placed on plates, numbered, coded and then filled with 3 kg of soil (Figure 1). The vessels were placed in one of the USAMV Bucharest greenhouses.

The biological material was represented by spinach seeds (*Spinacia oleracea* L.), Matador variety. Six soluble salts were used, Cd(NO₃)₂•4H₂O, Pb(CH₃COO)₂•3H₂O, Ni(NO₃)₂•6H₂O, Co(NO₃)₂•6H₂O,

Cu(NO₃)₂•3H₂O, Zn(CH₃COO)₂•2H₂O as soluble form of Cd, Pb, Ni, Co, Cu and Zn.



Figure 1. Preparing experimental pots

Five mixtures were prepared, with different concentrations of each metal, resulting in five variants of treatments, denoted as C1, C2, C3, C4, C5. The concentrations were chosen to cover as much as possible the whole interval, from reference value to values that exceed intervention threshold for sensitive use of soils, as described by Romanian regulation (Order 756/1997). Untreated soils were used as control variants (C0). It must be taken into account that the initial soils had their specific load of heavy metals, to which was added the amounts established in the experiment, resulting in the quantities in Table 3.

Metal	Initial amount of heavy metals in control (C0) (ppm)	C1 (ppm)	C2 (ppm)	C3 (ppm)	C4 (ppm)	C5 (ppm)
Cd	Vi	Vi+3	Vi+5	Vi+10	Vi+15	Vi+20
Pb	Vi	Vi+30	Vi+50	Vi+75	Vi+100	Vi+150
Ni	Vi	Vi+25	Vi+75	Vi+100	Vi+150	Vi+200
Со	Vi	Vi+20	Vi+30	Vi+50	Vi+75	Vi+100
Cu	Vi	Vi+ 20	Vi+60	Vi+100	Vi+150	Vi+200
Zn	Vi	Vi+100	Vi+300	Vi+450	Vi+600	Vi+700

Table 3. Concentrations of heavy metals used for soil contamination on each variant

Five concentrations were used, along with the initial soil used as control, resulting in six treatment variants for each soil type (Table 4). Having three types of soil, six variants of concentrations and four replicates, resulted in a total of 72 pots, respectively 216 kg of conditioned soil.

After preparing the pots, the contaminations were performed with the previously prepared solutions, at the second irrigation, after 24

hours from the first. The first irrigation was carried out using an amount of water of approximately 10% of the amount of soil, 300 ml of water/pot. The amounts of metals calculated for each concentration were dissolved in 300 ml of distilled water and added to each pot. The seeds were planted 48 hours after contamination, after the soil solution was stabilized.

Table 4. Experimental se	cheme
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		Soil type										
		Chernoz	zem (S1)		Chromic luvisol (S2)			Fluvisol (S3)				
Replicates Conc.	Rep 1	Rep 2	Rep 3	Rep 4	Rep 1	Rep 2	Rep 3	Rep 4	Rep 1	Rep 2	Rep 3	Rep 4
Control C0	S1C0R1	S1C0R2	S1C0R3	S1C0R4	S2C0R1	S2C0R2	S2C0R3	S2C0R4	S3C0R1	S3C0R2	S3C0R3	S3C0R4
C1	S1C1R1	S1C1R2	S1C1R3	S1C1R4	S2C1R1	S2C1R2	S2C1R3	S2C1R4	S3C1R1	S3C1R2	S3C1R3	S3C1R4
C2	S1C2R1	S1C2R2	S1C2R3	S1C2R4	S2C2R1	S2C2R2	S2C2R3	S2C2R4	S3C2R1	S3C2R2	S3C2R3	S3C2R4
C3	S1C3R1	S1C3R2	S1C3R3	S1C3R4	S2C3R1	S2C3R2	S2C3R3	S2C3R4	S3C3R1	S3C3R2	S3C3R3	S3C3R4
C4	S1C4R1	S1C4R2	S1C4R3	S1C4R4	S2C4R1	S2C4R2	S2C4R3	S2C4R4	S3C4R1	S3C4R2	S3C4R3	S3C4R4
C5	S1C5R1	S1C5R2	S1C5R3	S1C5R4	S2C5R1	S2C5R2	S2C5R4	S2C5R4	S3C5R1	S3C5R2	S3C5R3	S3C5R4

Before planting, soil samples were taken from each pot, from a depth of 0-5 cm. Five seeds were planted per pot at the depth specified by the seed producer. The planting took place at the beginning of April, and after the 45-day vegetation period, all the plants were harvested. After harvesting, the final soil samples were taken from each pot, from a depth of 0-5 cm, for further analysis.

Soil and plant analysis

Sample preparation and analyses were carried out at the Research Center for Studies of Food and Agricultural Products Quality, USAMV Bucharest.

The soil samples were dried at room temperature, ground with a laboratory soil grinder and sifted through a 250 μ m sieve, in order to prepare for elemental analysis.

After harvesting the spinach samples, they were weighed and measured.

The plant samples were dried to constant mass, ground with the laboratory grinder into fine powder and kept in exicators until analysed.

Heavy metal analysis was performed using ICP-MS technique (Agilent 7700 system). To perform this analysis, the soil samples were subjected to acid digestion with aqua regia (65% HNO3 and 37% HCl in 3 to 1 proportion). An amount of 0,1 g of each sample was placed in digestion tubes, adding 6 ml of HNO₃ and 2 ml HCl. The digestion was accomplished using a microwave system (Ethos Up), at 180°C for 15 minutes. For digestion plant samples. the was accomplished using 0.1 g of dried sample, 8 ml HNO3 and 2 ml H2O2. The digestion tubes were subjected to the same digestion program. The following heavy metals were studied: Cd, Cu, Ni, Pb, Co and Zn.

The obtained data were processed using IBM SPSS statistical software. Duncan's Multiple Range Test at P \leq 0.05 level was used for significance determination between groups of means of the six variants used in the experiment and also between the three soil types. The bars in the charts represent the means \pm SE of each variant. The same letters above each bar means that they are not significantly different.

RESULTS AND DISCUSSIONS

Heavy metals behaviour in soils

Regarding the initial content of heavy metals, excepting Ni that exceeds the alert threshold, all values are slightly above the reference values according to the order 756/1997 for all three types of soil (Table 5).

Table 5. The amount of heavy metals (HM) in initial soils (C0)

	HM content (mg/kg)									
Metal	S1 C0	S2 C0	S3 C0							
Cd	0.23	0.32	0.31							
Pb	16.40	21.89	20.70							
Ni	106.32	105.21	168.29							
Со	10.22	10.33	12.43							
Cu	17.68	19.24	27.46							
Zn	55.53	60.64	98.73							

After contamination, the quantities increased in accordance with the established level of contamination. The contamination was managed by irrigation water; therefore, it is possible that some differences of HM content may occur in the collected soil samples.

The initial contamination level (I) is presented in the Figure 2, alongside the values obtained from soil samples collected after the harvest of the spinach crop (F).



Figure 2. The total amounts of heavy metals in pot soils at the beginning (I) and at the end (F) of the experiment (mg/kg)

As can be seen, excepting the control variant (C0), the level of contamination decreased significantly from the C1 concentration, with the same tendency to the C5 concentration. In

the table below (Table 6) are presented the dropping rates for the heavy metal quantities for each variant.

		HM qua	antity redu	ction betwe	en initial and	l final samp	les (%)	Average HM
Concentration	Soil type	Ni	Со	Cu	Zn	Cd	Pb	loss by each soil type and contamination (%)
	S1	8.72	-0.46	1.09	14.72	7.19	-11.15	3.35
C0	S2	8.54	5.28	-9.36	5.51	27.57	-9.07	4.74
	S3	14.19	1.82	-3.29	19.67	15.23	2.152	8.29
	S1	20.69	56.19	54.02	54.84	62.11	55.01	50.48
C1	S2	25.32	57.40	68.57	59.52	72.73	32.94	52.75
	S3	25.15	63.65	51.23	59.24	70.18	50.91	53.39
	S1	25.72	39.29	49.27	42.58	40.63	38.67	39.36
C2	S2	30.59	41.63	60.18	47.14	51.90	46.49	46.32
	S3	28.81	49.44	44.68	50.22	44.026	30.73	41.32
	S1	16.23	36.17	51.19	38.56	34.43	44.20	36.80
C3	S2	45.35	53.09	39.46	53.59	31.95	18.66	40.35
	S3	30.93	57.81	70.24	60.98	61.04	42.27	53.88
	S1	35.17	36.86	42.52	46.48	24.49	17.08	33.77
C4	S2	36.96	46.19	8.21	48.48	26.13	-52.76	18.87
	S3	28.60	53.09	24.74	47.74	35.74	-0.94	31.49
	S1	46.10	47.38	28.18	41.81	33.61	23.57	36.77
C5	S2	34.08	36.46	68.72	32.89	21.08	56.16	41.56
	S3	22.89	42.58	26.29	41.74	24.44	9.54	27.91
Average loss fo	r each	26.89	40.21	37.55	42.544	38.03	21.91	
metal (%)							

Table 6. The reduction rates between initial and final pot soil samples, at 0-5 cm depth (%)

Considering that the sampling was done at the depth of 0-5 cm, it means that the heavy metals

migrated to the base of the pots, with the application of successive irrigations carried out

during the vegetation period. This leaching process is dependent on the characteristics of the soil, which makes the modelling efforts very difficult.

There is a significant difference between C0 HM leaching and any of contaminated variants (C1-C5). This means that in the case of low concentrations of heavy metals, they are bound in stable compounds and adsorbed in the soil structure. When the concentration increases, these elements are in excess of the possible bonds and compete with the major elements such as Ca, Mg, Na, K. If they form insoluble compounds, the physical adsorption and chemisorption processes occurs pH dependent and only temporarily reduce the concentration of heavy metals.

Important amounts of heavy metals can be retained in the crystal lattice of silicates, depending of soil type, and are practically harmless to the plant.

As can be seen, there are differences on heavy metals loss, both between soil types and between metal types. It can be noted that the lowest loss was experienced by lead and the highest loss was attributed to zinc. All these differences are generated by the soil characteristics, and they lead to a different response to the plant. This response is influenced by both the type of soil and the remaining amounts of active metals and possibly toxic to plants (Figure 3).



Figure 3. Spinach development on each concentration and soil type (from left to right: S1<C0 to C5>; S2<C0 to C5>; S3<C0 to C5>)

In a previous study, it was shown that the same amounts of heavy metals as those used in this experiment have a significant effect on germination and viability of spinach plants. At the maximum concentration, the viability of seedlings tends to zero, after just ten days (Mot et al., 2019).

Regarding the growth in the pots, after 45 days of vegetations, the following amounts of dried plant were obtained for each concentration and soil type (Table 7).

Table 7. The total dried mass harvested from each soil type and concentration

Soil	Dried mass of plant (g)									
Туре	C0	C0 C1 C2 C3 C4 C5								
S1	1.9	3	1.3	0	0	0				
S2	2.4	3.1	5.2	0	0	0				
S3	24.6	18.8	18.7	19.6	7.2	8.3				

Apart from the fact that much smaller amounts of plants were obtained from the soils S1 and S2, at the concentrations above C2 the plants did not even survive.

Surprisingly, and the total amounts of heavy metals in S3 are slightly higher, the plants have grown much better, even at high concentrations, including C5, in which germination has been inhibited.

One of the important factors that generated this behaviour is pH. After some authors, this indicator is the main factor that controls the adsorption of cations in the soil.

It has been established that heavy metals form hardly soluble compounds in alkaline media (S3 soil, pH=7,8) and can form precipitates of metal oxides, hydroxides, carbonates and phosphates. Also, the stability of metal complexes derived from organic ligands increases with pH, thus binding heavy metals into stable compounds.

Regarding the accumulation of heavy metals by spinach leaves, this differs both depending on the type of soil and the used concentration.

Cadmium accumulation

For the same used concentration, a significant difference can be observed in the accumulation of cadmium in different type of soils. Although higher amounts of cadmium were determined in S3 soil, it was not absorbed by the plant. For equivalent concentrations it was observed that the control variant (C0) accumulated an amount 77.72% lower than S1 and 67.7% lower than S2. Also, at concentrations C1 and C2, the spinach accumulated significantly smaller amounts on S3 soil compared to S1 and S2 soils (Figure 4). However, cadmium did not cause the plant death at higher concentrations, in S1 and S2 soils. As proof, in the case of S3 soil, the plants developed up to a concentration of 37.64 mg/kg, reached at C5 level of contamination, almost twice compared to accumulation on S1 and S2 at C2 concentration.

As another observation, the accumulation of Cd for both S1 and S2 at C2 concentration did not exceed the value from C1 concentration. This behaviour may be due both to the existence of a competition of cadmium with other elements, and to the fact that the absorption was inhibited by certain physiological processes.



Figure 4. Cd accumulation by spinach on each soil type and concentration (mg/kg)

Lead accumulation

Lead accumulation was rather constant on all soils and concentrations, with no significant differences, with two exceptions. The control variant on S3 soil had the lowest concentration in plant leaves, and the accumulation at C5 concentration level, on S3 as well. It should also be mentioned that the amount of heavy metals accumulated by the plant in soil C3 at level C5 does not differ from the amount accumulated by the plant at level C2 in soils S1 and S2 (Figure 5).



Figure 5. Pb accumulation by spinach on each soil type and concentration (mg/kg)

The results obtained for lead accumulation were somewhat to be expected. Many studies

have found that immobilizing lead leads to low absorption, due to the rapid reaction with the present anions, anions that form hardly soluble compounds. Also, in soils where the soil reaction exceeds pH 6, lead is easily adsorbed by clays or precipitated as carbonates. Here is the case of S3 with alkaline reaction, where the accumulation of lead in plants was lower compared to other soils.

The same observation as on cadmium accumulation, the accumulation of Pb for both S1 and S2 at C2 concentration were lower than C1 concentration, but this is due to the fact that the existence of a high concentration of salts favored the reaction of lead with the anions from soil solution forming insoluble compounds.

Nickel accumulation

The accumulation of nickel in spinach plants had a totally different pattern than lead and cadmium. If at low values of soil concentration, no significant difference was observed in the absorption of this metal, with the increase of soil concentration the absorbed amount increased rapidly on S1 and S2. Comparing the value from S3, C2 concentration with values from S1 and S2 with the same C2 concentration, it is observed that the nickel absorption was multiplied by 11.07 and 13.85 respectively in the case of these two soils. The nickel accumulation on S3 gradually increased with soil concentration, but not at the rate of the other soils (Figure 6).



Figure 6. Ni accumulation by spinach on each soil type and concentration (mg/kg)

Cobalt accumulation

The cobalt accumulation follows the same pattern as nickel. At low concentrations as

control variants, there were no significant differences between soil types. If the concentration was increased, there were significant differences in absorption between soil types, even from the C1 concentration (Figure 7).



Figure 7. Co accumulation by spinach on each soil type and concentration (mg/kg)

As nickel, at C2 concentrations, the quantity of absorbed heavy metals on S1 and S2 was multiplied by 15,68 and 21,40 respectively.

Copper accumulation

The copper absorption did not show significant differences between similar concentrations on S1, S2 and S3 soils, excepting the control variant, when the absorption was greatly influenced by the initial load of heavy metals (Figure 8).



Figure 8. Cu accumulation by spinach on each soil type and concentration (mg/kg)

At greater concentrations (C3, C4, C5), there is a significant increase in copper accumulation in soil S3 compared to S1 and S2. Considering the contamination with a much larger amount of zinc compared to copper, it is possible that its absorption was limited by the zinc abundance. However, the level of copper absorbed by the plant is below the level required for normal growth. Certainly, the increased level of the other metals inhibited the absorption of copper, resulting in a deficiency in this element.

Zinc accumulation

The zinc accumulation, as well as nickel and cobalt, was significantly higher in S1 and S2 soils, compared to S3, at all tested concentrations (Figure 9).



Figure 9. Zn accumulation by spinach on each soil type and concentration (mg/kg)

As some studies show, the soil pH has a great influence on zinc solubility. With the decrease of the pH value, the zinc solubility increases, becoming available in large quantities for plant absorption (Lindsay, 1979). Is the case of S1 and S2 soils, which showed a much higher availability of zinc compared to S3.

Also, some study showed antagonistic interaction of zinc with different elements: Zn-Fe, Zn-Cu, Zn-Ca (Vrînceanu et al., 2010). Therefore, following the absorption of copper on the three soils, a relationship of inverse proportionality can be observed in relation to zinc accumulation.

As an overview, Figure 10 shows the total amount of heavy metals accumulated in the spinach plants that have reached maturity. As can be seen, the amount of absorbed heavy metal keeps the proportionality of the contamination amounts.



Figure 10. An overview with whole amount of heavy metals accumulated by spinach plants (mg/kg)

CONCLUSIONS

Except for the control variants, all soils contaminated with all concentrations underwent heavy metal leaching processes, most likely due to excessive amounts of elements that did not find free binding positions, with an average loss over 40%.

The alkaline reaction and the high value of soil OM on fluvisol (S3) have made it possible to grow plants even in conditions of severe soil contamination, due to the capacity of releasing cations and to the high level of binding of heavy metals in complex forms.

The main factor that influenced the absorption of heavy metals by spinach plants was the amount of metal added to the soil. Therefore, zinc was the most accumulated metal, due to massive added amounts (of up to 700 mg/kg).

Soil pH influenced the accumulation of lead in plants, which can be observed in soil with alkaline pH (7.8). The amount of lead was much lower compared to soils with acidic pH.

With an increased abundance of heavy metals in the soil, copper could not be accumulated in the required quantities, even if the amount in the soil was above normal.

By the total amount of plant sample collected, small amounts of heavy metals (below the alert threshold) had a positive influence on plant growth. This is due to the fact that the physiological needs of the plant were covered with microelements, and the amounts of unnecessary elements (Cd, Pb) were not large enough to cause increased toxicity to the plant.

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