ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 4–5 2010

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EFFECT OF SOIL CONTAMINATION BY LEAD, NICKEL AND CADMIUM AND VA-MYCORRHIZAL FUNGI ON YIELD AND HEAVY METAL CONCENTRATION IN ROOTS AND ABOVEGROUND BIOMASS OF OAT

WPŁYW SKAŻENIA GLEBY OŁOWIEM, NIKLEM I KADMEM ORAZ VA-MIKORYZY NA PLONOWANIE I ZAWARTOŚĆ METALI CIĘŻKICH W KORZENIACH I CZĘŚCIACH NADZIEMNYCH OWSA

Abstract: An exact two-factorial pot experiment was conducted in an environmental test chamber at the Institute of Plant Nutrition (Justus-Liebig-Universität) in Giessen, Germany. Experimental factor 1 was soil contaminated by Pb, Ni and Cd, and experimental factor 2 was plant infection by VA and mycorrhizal fungi the introduction of cadmium, nickel and lead into the soil system.

Soil contamination by heavy metals at a level of 50 mg Ni, 10 mg Cd and 100 mg Pb per kg of soil significantly decreased the weight of roots and aboveground parts of oat plants. Nickel, cadmium and lead were accumulated primarily in the roots, and their translocation to the aboveground parts was limited. This suggests that plants possess effective mechanisms involved in the detoxification of heavy metals in the roots.

Root infection by mycorrhizal fungi had no significant effect on the concentrations of nickel, cadmium and lead in the roots and aboveground parts of plants, or on oat yield. Nickel and cadmium were readily absorbed by the roots. The nickel and cadmium content of roots was high in contaminated than in natural soil, reaching 440.6 mg $\cdot$ kg^{-1} d.m. and 110.9 mg $\cdot$ kg^{-1} d.m., respectively. Lead was found to be quite immobile. Following the introduction of the largest amount of this heavy metal into the soil system, Pb content reached 18.5 mg $\cdot$ kg^{-1} d.m. in the roots and only 3.4 mg $\cdot$ kg^{-1} d.m. in the green matter of oat.

Plants grown in soil contaminated by nickel, cadmium and lead had significantly smaller length of roots (by 47 % on average), compared with plants grown in soil with a natural heavy metal content.

Keyword: nickel, cadmium, lead, soil contamination, VA-mycorrhiza, oat

Progressing industrialization and urbanization are the main causes of increased concentration of trace elements in the natural environment. This process poses a growing threat for living organisms. Industrial emissions of large quantities of Pb, Zn, Cd, Cu and Ni and the widespread application of various types of industrial and

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municipal waste for fertilizing purposes have an adverse effect on the soil and vegetation. Soil is the key element in the natural nutrient cycle and the main link in the food chain, which is why the growing accumulation of trace elements in the soil system poses a serious threat.

Plants have developed defense mechanisms protecting them from the toxic effect of trace elements. As part of those mechanisms, plants limit the uptake of toxic elements and maintain toxin contents in the tissue at a low level due to changes in the selectivity of cytoplasmatic membranes, removal of excess ions from cells, secretion of compounds that can complex with metals in the soil system, and immobilization of metals in tissues followed by their detoxification [1, 2].

Substrate toxicity may also be minimized by mycorrhizal association where heavy metals are immobilized in mycorrhizal mycelium structures. This mechanism inhibits the transport of toxic elements into plant tissues and supports plant growth even in strongly contaminated environments. However, mycorrhizal fungi have different tolerance to various trace elements. While some mycorrhizal fungal strains effectively protect plants against high concentrations of heavy metals, other fungal species die out in contaminated soil. Therefore, plant inoculation should be preceded by careful selection of the appropriate mycorrhizal fungi [3–6].

The objective of this study was to determine the effect of soil contamination by lead, nickel and cadmium, and the influence of VA-mycorrhizal fungi on the yield and heavy metal content of the roots and aboveground parts of oat plants.

Material and methods

An exact two-factorial pot experiment was conducted in an environmental test chamber at the Institute of Plant Nutrition (Justus-Liebig-Universität), Giessen, Germany. Experimental factor 1 was the introduction of cadmium, nickel and lead into the soil system, and experimental factor 2 was plant invasion by vesicular-arbuscular mycorrhizal fungi (VAM). The experiment comprised four treatments (each replicated four times): 1 – control treatment comprising soil with a natural heavy metal content, 2 – soil contaminated by Cd, Ni and Pb, 3 – soil infected by VAM, 4 – soil infected by VAM and contaminated by Cd, Ni and Pb.

Pots were filled with 3 kg of air-dry soil with granulometric composition of medium silty loam. The investigated soil was characterized by a low content of available potassium and phosphorus and a high content of available magnesium. Soil pH, determined in 1 mol $\cdot$ dm^{-3} KCl, was 6.1. Heavy metal content, determined after soil decomposition in *aqua regia*, was as follows: 0.66 mg Cd, 37.9 mg Ni and 12.0 mg Pb $\cdot$ kg^{-1} of soil. Prior to the establishment of the experiment, soil was roasted for 24 hours at 80 ^{o}C to eliminate natural mycorrhizal fungi and pathogens.

Heavy metals were introduced into the soil system in the form of $3CdSO_4 \cdot 8H_2O$, $NiSO_4 \cdot 6H_2O$ and $Pb(NO_3)_2$ in the amount of 10 mg Cd, 50 mg Ni and 100 mg Pb $\cdot$ kg^{-1} of soil. Pre-sowing rates of 0.25 g N in the form of NH_4NO_3, 0.45 g P and 0.57 g K in the form of KH_2PO_4 were introduced to each pot.

The applied VAM infectious material was "Lekadan" bentonite containing VA-mycorrhizal spores (*Glomus* spp., T6 isolate) with infected roots. After 5 days of soil incubation with fertilizers, 100 cm^3 of the above infectious material was mixed with 200 cm^3 of experimental soil and placed in pots at a depth of 3 cm. Oat seeds were sown and 15 oat plants were left per pot after thinning. During the spring growing season, soil moisture was maintained at a level of 60 % maximum capillary water capacity. Top-dressing with 0.285 g N in the form of NH_4NO_3, 0.225 g P and 0.285 g K in the form of KH_2PO_4 per pot was additionally applied. Plants were harvested after 50 days of growth, at the first node stage. Roots were sampled from the soil to determine their weight, length, severity of VAM infection as well as Cd, Ni and Pb contents. Root length was measured with the use of a Fa. Comair (Australia) root length scanner.

Dried and pulverized plant samples (roots and aboveground parts separately) were wet mineralized in a mixture of HNO_3, $HClO_4$ and H_2SO_4 (40:10:1). Heavy metals in plant material and in the soil were determined by atomic absorption spectrometry, using a Perkin Elmer 5000 spectrometer. Plants from each pot were analyzed separately. The degree of VA infection in roots was determined with the use of a binocular and by the gridline intersect method involving the prior staining of infected roots with trypan blue [7].

The results were statistically verified by an analysis of variance for a two-factorial pot experiment in a completely randomized orthogonal design. The significance of differences between the mean values of all treatments was estimated by Tukey's test.

Results and discussion

Soil contamination by cadmium, nickel and lead at a level of 10 mg Cd, 50 mg Ni and 100 mg Pb $\cdot$ kg^{-1} of soil led to a significant drop in the weight of roots and the aboveground parts of oat plants (Table 1). This dependency was observed in treatments without VAM as well as in treatments where the infectious material had been applied.

Table 1

Effect of soil contamination by Pb, Ni and Cd and plant infection by VA mycorrhizal fungi on dry mass of roots and aboveground parts of oat

VAM	Dry mass [g $\cdot$ pot^{-1}]					
	Roots			Aboveground parts		
	Heavy metals		Mean for VAM	Heavy metals		Mean for VAM
	–	+		–	+	
– VAM	4.49	1.60	3.05	11.13	6.80	8.97
+ VAM	4.70	1.70	3.20	11.08	6.75	8.92
Mean for heavy metals	4.59	1.65		11.10	6.78	
Value of F function	F_{HM} – ***	F_{VAM} – n.s.	$F_{interac.}$ – n.s.	F_{HM} – ***	F_{VAM} – n.s.	$F_{interac.}$ – n.s.

Explanation for Tables: F_{HM}, F_{VAM}, $F_{interac.}$ – value of F function for heavy metals, VA-mycorrhiza and interaction, respectively; *** – significant at p = 0.001; n.s. – nonsignificant.

The weight of roots in soil contaminated by heavy metals was on average 64 % lower in comparison with the roots from uncontaminated soil, while a 39 % drop in the weight of the green matter of oat was observed.

Root infestation with mycorrhizal fungi did not significantly affect the weight of roots and aboveground parts of oat plants in soil with a natural Cd, Ni and Pb content and in soil contaminated by those heavy metals. Published sources make numerous references to the beneficial effect of mycorrhizal fungi on plant yield in soils with an increased heavy metal content [4, 8–10]. In a study conducted by Kiepas and Iwaniuk [11], the weight of the roots and shoots of grass and ribwort plantain grown in zinc contaminated soil increased significantly after a mycorrhizal infection. The above response was not reported in an experiment involving lucerne and white clover. Mycorrhizal strains differ in their ability to immobilize heavy metals and, consequently, in their ability to reduce soil toxicity. Mycorrhizal fungi isolated from soil substrates contaminated by heavy metals are more effective in immobilizing toxic elements, thus protecting the host plant. Further research is required to identify and characterize different morphotypes of mycorrhizal fungi to select strains which are most effective in bioremediation [3–5, 9, 12].

The application of VA-mycorrhizal infectious material significantly contributed to the infestation of oat roots by arbuscular fungi (Table 2).

Table 2

Effect of soil contamination by Pb, Ni and Cd and plant infection by VA mycorrhizal fungi on root length colonization by VAM and root length

VAM	Root length colonization by VAM [%]			Root length [metres per pot]		
	Heavy metals		Mean for VAM	Heavy metals		Mean for VAM
	–	+		–	+	
– VAM	10.2	9.4	9.8	747.7	365.7	556.7
+ VAM	48.1	43.2	45.6	739.0	420.8	579.9
Mean for heavy metals	29.2	26.3		743.3	393.3	
Value of F function	F_{HM} – n.s.	F_{VAM} – ***	$F_{interac.}$ – n.s.	F_{HM} – n.s.	F_{VAM} – ***	$F_{interac.}$ – n.s.

The average rate of fungal infection in treatments with a starter inoculum reached 45.6 %, and in treatments where the infectious material was not applied – only 9.8 %. It should be noted that the obtained level of infestation by mycorrhizal fungi was unsatisfactory – it should exceed 60 % in an effective symbiotic relationship. Soil contamination by Ni, Cd and Pb did not significantly minimize root colonization by arbuscular fungi.

In soil contaminated by heavy metals, total root length was reduced by nearly 50 % (Table 2), which was the main cause of the drastic drop in the yield of the green matter of oats. The application of VA-mycorrhizal infectious material did not decrease the toxic effect of heavy metals on root development.

The introduction of 10 mg Cd, 50 mg Ni and 100 mg Pb · kg^{-1} of soil significantly increased the content of those metals in both the roots and the aboveground parts of oat plants (Table 3, 4 and 5).

Table 3

Effect of soil contamination by Pb, Ni and Cd and plant infection by VA mycorrhizal fungi on Cd concentration in roots and aboveground parts of oat

VAM	Cd content [mg Cd · kg^{-1} d.m.]					
	Roots			Aboveground parts		
	Heavy metals		Mean for VAM	Heavy metals		Mean for VAM
	–	+		–	+	
– VAM	0.75	119.76	60.26	0.92	28.29	14.61
+ VAM	0.75	102.04	51.40	0.85	27.57	14.21
Mean for heavy metals	0.75	110.90		0.88	27.93	
Value of F function	F_{HM} – ***	F_{VAM} – n.s.	$F_{interac.}$ – n.s.	F_{HM} – ***	F_{VAM} – n.s.	$F_{interac.}$ – n.s.

Table 4

Effect of soil contamination by Pb, Ni and Cd and plant infection by VA mycorrhizal fungi on Ni concentration in roots and aboveground parts of oat

VAM	Ni content [mg Ni · kg^{-1} d.m.]					
	Roots			Aboveground parts		
	Heavy metals		Mean for VAM	Heavy metals		Mean for VAM
	–	+		–	+	
– VAM	10.34	462.50	236.42	4.18	82.62	43.40
+ VAM	13.00	418.68	215.84	3.39	69.55	36.47
Mean for heavy metals	11.67	440.59		3.79	76.08	
Value of F function	F_{HM} – ***	F_{VAM} – n.s.	$F_{interac.}$ – n.s.	F_{HM} – ***	F_{VAM} – n.s.	$F_{interac.}$ – n.s.

Table 5

Effect of soil contamination by Pb, Ni and Cd and plant infection by VA mycorrhizal fungi on Pb concentration in roots and aboveground parts of oat

VAM	Pb content [mg Pb · kg^{-1} d.m.]					
	Roots			Aboveground parts		
	Heavy metals		Mean for VAM	Heavy metals		Mean for VAM
	–	+		–	+	
– VAM	1.47	19.59	10.53	0.60	3.20	1.90
+ VAM	1.55	17.44	9.50	0.60	3.63	2.12
Mean for heavy metals	1.51	18.52		0.60	3.42	
Value of F function	F_{HM} – ***	F_{VAM} – n.s.	$F_{interac.}$ – n.s.	F_{HM} – ***	F_{VAM} – n.s.	$F_{interac.}$ – n.s.

In comparison with the control treatment, the concentrations of cadmium, nickel and lead in the roots of oat plants grown in contaminated soil increased 150-fold, 38-fold and 12-fold, respectively. Oat roots accumulated particularly high quantities of nickel (440.6 mg Ni $\cdot$ kg^{-1} dry matter on average) and cadmium (110.9 mg $\cdot$ kg^{-1} dry matter). According to Wusheng et al [13], the roots of garlic plants grown in a soil system heavily contaminated with cadmium also accumulated large amounts of this metal (more than 600 mg $\cdot$ kg^{-1} d.m.). The lead content of roots was the lowest (18.5 mg Pb $\cdot$ kg^{-1} d.m.) although the highest quantities of this metal had been introduced to contaminate the soil. This indicates that lead is relatively immobile in soil.

The aboveground parts of oat plants which were harvested in treatments contaminated by heavy metals also had a significantly higher nickel, cadmium and lead content in comparison with plants grown in soil with natural heavy metal contents. Yet the noted differences were not as profound as those observed in the roots. The content of cadmium, nickel and lead in the aboveground parts of oat plants grown in soil contaminated by heavy metals increased approximately 30-fold, 20-fold and 6-fold in comparison with uncontaminated treatments. The green matter of oat accumulated high quantities of nickel (76 mg $\cdot$ kg^{-1} d.m. on average) and cadmium (28 mg $\cdot$ kg^{-1} d.m.). The lead content of the aboveground parts was the lowest (3.4 mg Pb $\cdot$ kg^{-1} d.m.) despite the fact that the highest quantities of this metal had been introduced to contaminate the soil.

The obtained results point to relatively high mobility of nickel and cadmium. Those trace elements were easily absorbed from the soil into the roots, as shown by their high concentrations in the roots of oat plants grown in contaminated soil. Other authors also emphasize the dangerous implications of soil contamination by cadmium [13–15]. This metal is quite mobile in soil [16] and it is readily absorbed by roots. Cadmium is also easily transported from the roots to the aboveground parts of plants. This metal has the highest biological enrichment index in comparison with other trace elements [14].

The translocation of lead, cadmium and nickel from the roots to the aboveground parts of oat plants was limited – the content of those metals in the green matter of oat was fivefold lower than in roots grown in soil with increased heavy metal contents. The above suggests that oat plants possess effective mechanisms involved in the detoxification of lead, nickel and cadmium in roots. This observation is consistent with the findings of other authors [13, 15, 17–19] who have also noted much higher heavy metal levels in the roots than in the aboveground parts of plants grown in soils contaminated with those trace elements. Plants grown in a contaminated environment develop adaptive or defense mechanisms. Roots produce slime containing high quantities of polygalacturonates which bind toxic metals. Owing to this defense mechanism, heavy metals are immobilized in or outside root cells [1].

The mobility of heavy metals in soil and the rate of their translocation to the aboveground parts of plants vary significantly. Nickel and cadmium have shown greater mobility in comparison with lead. This observation is supported by the findings of other authors [14, 18, 20, 21]. Although the highest quantities of lead were introduced into the soil system for the purpose of contamination, lead contents were less likely to increase in the roots and aboveground parts of oat plants in comparison with nickel and cadmium. The fact that lead is strongly fixed by clay minerals, Fe-Mn concretions, Fe-Mn hydroxides and organic substances, and is precipitated in the form of carbonates and phosphates, visibly inhibits Pb uptake by plants [1].

Plant infection by mycorrhizal fungi had no significant effect on Cd, Ni and Pb accumulation in plants. The morphotypes of mycorrhizal fungi differ significantly with regard to their metal detoxification ability [8, 22, 23]. According to Diaz et al [9], lead accumulation in plants is determined by both the type of mycorrhizal fungi and the element concentration in the soil system. VAM fungal strains isolated from contaminated soil often limit heavy metal translocation to the aboveground parts, thus increasing plant tolerance to high concentrations of toxic elements [3–5, 24, 25].

Conclusions

1. Soil contamination by heavy metals at a level of 10 mg Cd, 50 mg Ni, and 100 mg Pb per kg of soil significantly decreased the weight of the roots and aboveground parts of oat plants.

2. Nickel, cadmium and lead were accumulated primarily in the roots, and their translocation to the aboveground parts was limited. This suggests that oat plants possess effective mechanisms involved in the detoxification of heavy metals in the roots.

3. Cadmium and nickel were easily absorbed by oat roots from soil contaminated by those metals. The content of cadmium in roots growing in contaminated soil increased approximately 150-fold, and the content of nickel – 38-fold.

4. Lead was found to be relatively immobile in comparison with cadmium and nickel, as shown by very low Pb contents in plants in reference to the level of soil contamination by lead.

5. Root infection by VAM fungi had no significant effect on the weight of roots and aboveground parts of oat plants, and it did not inhibit excessive accumulation of lead, nickel and cadmium in soil contaminated by those metals.

Acknowledgments

The author would like to thank Professor dr. h.c. W. Höfner for his support and for enabling the author to carry out this study at the Institut für Pflanzenernährung Justus-Liebig-Universität Giessen.

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WPŁYW SKAŻENIA GLEBY OŁOWIEM, NIKLEM I KADMEM ORAZ VA-MIKORYZY NA PLONOWANIE I ZAWARTOŚĆ METALI CIĘŻKICH W KORZENIACH I CZĘŚCIACH NADZIEMNYCH OWSA

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Abstrakt: Badania wykonano jako dwuczynnikowe doświadczenie wazonowe przeprowadzone w klimatyzowanej komorze Instytutu Żywienia Roślin Uniwersytetu Justusa Liebiga w Giessen. Pierwszym czynnikiem doświadczalnym było zanieczyszczenie gleby kadmem, niklem i ołowiu, zaś drugim infekcja roślin grzybami arbuskularnymi (VAM).

Zanieczyszczenie gleby metalami ciężkimi na poziomie 50 mg Ni, 10 mg Cd i 100 mg Pb na kg gleby istotnie zmniejszyło masę korzeni i części nadziemnych owsa. Nikiel, kadm i ołów kumulowane były przede wszystkim w korzeniach, a ich transport do części nadziemnych został silnie ograniczony. Sugeruje to istnienie sprawnych mechanizmów detoksykacji metali ciężkich w korzeniach, uruchamianych przez samą roślinę. Infekcja korzeni grzybami mikoryzowymi nie różnicowała istotnie plonów roślin oraz nie wpłynęła znacząco na koncentrację niklu, kadmu i ołowiu w korzeniach i częściach nadziemnych.

Nikiel i kadm przenikał do korzeni z dużą łatwością. W glebie skażonej zawartość niklu i kadmu w korzeniach była duża i wynosiła 440,6 mg Ni $\cdot$ kg^{-1} s.m. i 110,9 mg Cd $\cdot$ kg^{-1} s.m. Ołów okazał się natomiast bardzo mało ruchliwym pierwiastkiem. Pomimo zastosowania dużej ilości tego metalu w celu zanieczyszczenia gleby, zawartość Pb w korzeniach wynosiła 18,5 mg $\cdot$ kg^{-1} s.m. i tylko 3,4 mg $\cdot$ kg^{-1} s.m. zielonki owsa.

Rośliny uprawiane na glebie zanieczyszczonej niklem, kadmem i ołowiem miały znacznie mniejszą długość korzeni w porównaniu z roślinami uprawianymi na glebie o naturalnej zawartości metali ciężkich.

Słowa kluczowe: nikiel, kadm, ołów, zanieczyszczenie gleby, VA-mikoryza, owies