EFFECTS OF VARIOUS CHELATORS ON THE UPTAKE OF Cu, Pb, Zn AND Fe BY MAIZE AND INDIAN MUSTARD FROM SILTY LOAM SOIL POLLUTED BY THE EMISSIONS FROM COPPER SMELTER

Anna Karczewska*, Bernard Gałka, Cezary Kabała, Katarzyna Szopka, Karolina Kocan and Kamila Dziamba

Wrocław University of Environmental and Life Sciences, Institute of Soil Sciences and Environmental Protection, Grunwaldzka 53, 50357 Wrocław, Poland

ABSTRACT

Three chelating compounds: EDTA, EDDS, and histidine, were tested as potential agents to mobilize Cu and three other metals: Pb, Zn, and Fe from polluted soil collected from arable land in the vicinity of copper smelter. Total concentrations of Cu and Pb in soil were: 395 and 212 mg/kg, respectively, and considerably exceeded soil quality standards. In extraction tests, Cu was mobilized with the following efficiency: EDTA > EDDS > histidine. Therefore, all those chelators were chosen for a pot experiment to examine induced phytoextraction of Cu by maize (*Zea mays*) and Indian mustard (*Brassica juncea*). Chelators were applied at the rate of 1.0 mmol/kg, and histidine was additionally used at doubled rate (2.0 mmol/kg). Experiment was carried out with two different watering regimes. EDTA and EDDS caused significant increase of Cu uptake from soils, but Cu concentrations in biomass were far below those required for efficient soil remediation. Moreover, Cu leaching from soil was much more intensive than plant uptake. Histidine did not prove to be an efficient chelator to induce intensive uptake of Cu by tested plants. Plant uptake of Pb, Zn and Fe was only poorly affected by chelate application, so was also the process of their leaching.

KEYWORDS: phytoextraction, leaching, EDDS, EDTA, histidine, heavy metals

INTRODUCTION

Soils in the surroundings of metal smelters are usually considerably polluted with heavy metals. Polish smelters Legnica and Głogów, the largest European producers of metallic copper in the last decades of 20th century, have caused serious soil contamination with Cu and Pb, as well as - to a lower extent - with several other metals [1, 2]. In the vicinities of smelters, there are the zones, where the concentrations of Cu and Pb in soils significantly exceed those determined in Polish law as soil quality standards [3]. According to the law [4], soil remediation should remove excessive amounts of pollutants in a possibly environmental-friendly way.

Phytoextraction has recently become a popular and widely recommended technology for in-situ remediation of heavy metal-contaminated soils. A number of papers have been published that provided proofs that the solubility of metals in soil, and their subsequent uptake by plants and translocation in shoots may be considerably enhanced by addition of synthetic chelators, such as EDTA [5, 6]. In more recent papers, however, various disadvantages of chelate-induced phytoextraction were stressed, e.g. low metal extraction rates [7, 8], persistence of synthetic chelators like EDTA in soil environment, as well as the risk of groundwater contamination [9-11]. Therefore, various strategies have been suggested to raise the efficiency of metal uptake and at the same time to reduce the risk of environmental pollution. The approaches to obtain those effects included the use of natural, easily biodegradable, compounds such as low molecular weight organic acids [9, 12, 13] or EDDS [14-17], lowering the concentrations of chelators or splitting dosages [18], addition of acrylamide hydrogels, clay minerals or apatite mixtures in order to improve soil sorption properties [14], as well as application of slow-release coated EDTA granules [19] and leachate recirculation [20].

Obviously, the risk of metal leaching depends on the persistence of chelates in the environment, soil moisture conditions and the time to pass between application of chemicals and the first heavy rainfall causing accelerated leaching of easily soluble compounds. This paper presents the results of a greenhouse experiment in which we examined the effects of induced phytoextraction applied to decontaminate the soil polluted by the emissions from copper smelters. Three different chelating agents were examined: synthetic EDTA and two potentially biodegradable compounds EDDS and histidine that proved to be effective in Cu and Pb extraction from that soil. The efficiency of metal uptake by two plant species: maize (*Zea mays*) and Indian mustard (*Brassica juncea*), as well as the amounts of metals leached from soil were tested in two various watering regimes.

MATERIAL AND METHODS

Soil Origin and Properties

The soil used in the experiment was collected from arable field situated close to the border of former protection zone of copper smelter Legnica, SW Poland. Basic properties of soil were determined with standard methods [21]. The soil had a texture of silty loam, and contained 6% of clay fraction (<0.002 mm), 67% of silt (0.002-0.05 mm), and 27% of sand (0.05-2.0 mm). Organic matter content was 2.9%, and pH 6.8 Total concentrations of metals in soil, measured after acid digestion in the mixture of concentrated nitric and perchloric acids (HNO_3 + $HClO_4$), as well as potentially and actually soluble metals (determined in the extractions with 1M HCl, 1:10, m:v, and with 1M ammonium nitrate, 1:25, m:v), are presented in the Table 1.

TABLE 1 – Total and extractable metals in soil, mg/kg

Form of metal	Cu	Pb	Zn	Fe
Total (digestion in HNO_3 + $HClO_4$)	395	212	115	8700
Potentially soluble (extracted with 1M HCl)	332	201	89	293
Actually soluble (extracted with 1M NH_4NO_3)	300	26	64	23
Water soluble	1.2	0.4	0.6	1.5

Extractability Tests

Extractability of metals, determined with the use of various chelators, was examined prior to the pot experiment. On the basis of similar experiments described in the literature, the following chelating agents were chosen and tested in batch extractions: EDTA, EDDS, tartaric acid, citric acid, glycine and histidine. Soil samples were shaken end-over-end for 6 hours with the solutions containing chelating agents in concentrations of 0.1, 0.5, and 2.0 mmol/L (m/v: 1/10), that corresponded with the ratios: 1.0, 5.0, and 20 mmol/kg. Higher chelate to soil ratios were not examined, because several experiments proved that in such cases, most of chelated Cu and Pb would rather be leached than taken up by plants [22]. The extracts were analysed for Cu, Pb, Zn and Fe using flame AAS (Philips PU 9100 X). The results of extractability tests let us choose three most effective chelating agents to be used a pot experiment.

Pot Experiment

The experiment was carried out in a university greenhouse in Wrocław. Each of 48 plastic pots was filled with 5 kg of soil, placed on a 5 cm deep bottom gravely layer, in which leachates were collected throughout the experiment. Occasionally, when necessary, the leachates were removed from the bottom zone with manual suction system, and analysed for Cu, Pb, Zn and Fe. At the beginning of experiment, soil was moistured and fertilized. Soil analysis proved its high fertility, therefore ammonium nitrate was the only fertilizer applied at the rate 1.0 g N per pot. Maize (*Zea mays* L.) var. Claudia, and Indian mustard (*Brassica juncea*) var. Rota, were used as experimental plant, as many authors recommend these two species as the most suitable for phytoremediation, because of their high production of underground biomass, easy harvest-ability, as well as very good heavy-metal tolerance and accumulation [6, 15, 17, 19].

With respect to the results of extractability tests, EDTA, EDDS, and histidine, i.e. three most efficient chelates with various biodegradability, were chosen for a pot experiment to induce metal uptake by plants. At the stage of plant pre-maturity, the chelates, in the form of 0.025 M water solution, were spread onto soil surface at the rate 1.0 mmol/kg (and in the case of histidine, additionally at doubled rate, i.e. 2.0 mmol/kg). The doses of chelates were splitted into two parts applied within 2 days, according to the indications given in the literature [23, 24]. 15 days after the second rate of chelators was applied, the plants were harvested, weighted, dried and examined on metals concentrations. Thereafter, the experiment was continued with two different watering regimes: normal (N)), that simulated dry weather within first two weeks, with occasional rain fully retained in soil, and wet (W) with repeated heavy rainfalls. Accordingly, soil in the pots with wet watering regime was leached with distilled water four times: on the first, third, 14th, and 50th day after the application of chelators (series I, II, III, IV), whereas and in a normal watering regime leaching was performed only twice: on 14th and 50th day (series III and IV). The volume of leaching water was adjusted to the mass of wet soil in pots and on original water field capacity, and was calculated to obtain 200 mL of leachates in normal watering regime and 500 mL in wet regime. In between, soil was watered to maintain its moisture at 60-80% of field capacity, to enable possible biodegradation of chelators. The volumes of leachates collected from the pots could not be precisely measured, therefore, the amounts of metals leached in the experiment were calculated on the basis of rough assessment.

RESULTS AND DISCUSSION

Extractability Tests

EDTA proved to be the most efficient extractant to mobilize Cu and Pb, which was confirmed in the tests with different rates (Table 2). At the highest chelator to soil ratio (5 mmol/kg), citric and tartaric acids were more effective in mobilizing Cu than were aminoacids, most likely because of simultaneous effect of soil acidification. Differently, at lower chelator to soil ratios, Cu was mobilized better by aminoacids than by low molecular organic acids; and the order of mobilizing efficiency was as follows:

TABLE 2 – Total amounts of metals extracted from soil with various chelating agents applied at the rates of 1, 5, and 20 mmol/kg

Chelating agent	Rate, mmol/kg	The amounts extracted, mg/kg		
		Cu	Pb	Zn
EDTA	1	64	8.9	0.9
	5	148	26.3	2.2
	20	280	65.5	16.5
EDDS	1	42	2.1	0.7
	5	77	2.8	0.8
	20	155	4.7	0.8
Histidine	1	6.3	0.5	0.6
	5	24.5	0.5	0.9
	20	75.3	1.6	1.0
Glycine	1	3,2	0.8	0.8
	5	11.9	1.5	1.0
	20	30.2	1.8	1.0
Citric acid	1	2,5	0.6	1.2
	5	8.5	1.4	3.2
	20	125	8.5	15.2
Tartaric acid	1	2.6	0.6	1.1
	5	14.7	1.8	2.5
	20	155	13.0	18.6

TABLE 3 – Biomass of plants in the pot experiment, expressed as dry mass.

Chelating agent	Maize				Indian mustard			
	Biomass of plant shoots per pot, g d.m.							
	min	max	mean	SD	min	max	mean	SD
O (control)	21.3	37.1	29.1	7.2	43.1	48.0	45.6	2.8
EDTA	27,3	37,1	30.8	4,8	36.2	44.4	41.1	3.8
EDDS	27.6	42.8	33.3	6.1	41.7	44.4	43.2	1.4
Histidine	28.2	39.8	33.1	4.9	42.8	47.8	44.9	2.1

EDTA > EDDS > histidine >> glycine > citric and tartaric acids. Beside EDTA, none of the other chelators examined, appeared to be highly effective in extraction of Pb, and Zn, and the amounts of those metals released by extraction were not much higher than their water soluble forms. However, if considering the fact that Pb and Zn do not contribute as much as does Cu, to the problem of soil pollution in the surroundings of copper smelter Legnica, the choice of the most appropriate chelators was based on the results of Cu extraction. Therefore, three chelating agents were chosen to be applied in a pot experiment: EDTA, EDDS, and histidine, the latter used in two different rates.

Pot Experiment

Plant Growth

The growth of both plant species was assessed as satisfactory (Figure 1). Throughout the whole experiment, the plants indicated only slight symptoms of Cu toxicity such as interveinal foliar chlorosis and white lesions [25]. These effects were much stronger in the case of maize, and became more intensive after application of EDTA and EDDS. Some leaves started to wilt 3-4 days after application of chelating solution, however this effect was not as strong as that observed when the plants were grown in sandy soils, which was described in another paper [26]. The biomass of harvested plants did not depend on the kind of chelating agent applied. The mean biomass of maize shoots was 32.1 g d.m. per pot, and the mean biomass of Indian mustard shoots was 43.7 g d.m. (Table 3).

FIGURE 1 – General view of the pot experiment – the plants directly before application of chelating agents

Metal Uptake

Application of 1 mmol/kg EDTA and EDDS caused significantly enhanced uptake of Cu and Pb in comparison with control plots, whereas the same dosage of histidine did not affect plant uptake of those metals. Histidine applied in a doubled rate (2 mmol/kg) caused slight but significant increase of Cu and Pb uptake from soil at normal watering regime, however this effect was much poorer than that observed for EDTA and EDDS (Figure 2). For technical reasons, the histidine was applied in a double rate only in the plots with normal watering regime, and therefore the effects of its doubled rate on plant uptake in wet conditions, fostering metal leaching, has not been checked. EDDS appeared to be slightly, and insignificantly, more effective than was EDTA in enhancing the uptake of Cu by both plant species examined (Figure 2 and 3). The mean Cu concentrations in the shoots of maize with normal watering regime in the plots with EDTA, EDDS and control were: 40.2, 41.8, and 8.1 mg/kg d.m., respectively (Figure 2). Analogous concentrations of Cu in the shoots of Indian mustard grown in the pots with normal watering regimes were even higher, with the mean values: 68.7, 54.5, and 18.6 mg/kg d.m. Soil moisture proved to be a crucial factor maintaining the uptake of Cu by maize after application of EDTA and EDDS. Cu concentrations in the shoots of maize grown in the pots with wet watering regime, were much higher that those with normal watering (Figure 3), and reached the mean values of 362 and 390 mg/kg d.m., in the plots with EDTA and EDDS, respectively. Those values might be considered as promising from the point of view of effective Cu phytoextraction, however, as it will be shown further, the simultaneous process of Cu leaching in wet plots was intensive, undoubtedly indicating high ecological risk intrinsically associated with application of chelators under natural conditions, when the weather might be unpredictable.

The scheme of our experiment did not involve examination of Indian mustard in wet conditions, therefore we can only expect that the results would be similar to those obtained for maize. The relationships between Cu uptake by Indian mustard and its leaching in wet conditions should be checked in a further study. Despite the fact that Cu uptake and translocation to maize shoots increased considerably after application of chelators, particularly with EDDS, Cu concentrations in the biomass were still far lower than those required for fast and efficient soil remediation. Calculated loss of metals from soils due to plant uptake are presented in the Table 4. The highest reduction of total Cu, obtained in the case of EDDS at wet watering regime, was 2.46 mg/kg, i.e. below 1% of original Cu concentration in soil. Much higher reduction in Cu concentration resulted at the same time from soil leaching, which will be discussed further.

Pb concentrations in plant shoots were much lower than concentrations of Cu, and far lower than those reported in the literature [6]. EDTA was highly more efficient than was EDDS in supporting Pb uptake from soil, both by maize and by Indian mustard (Figure 2 and 3). The high-

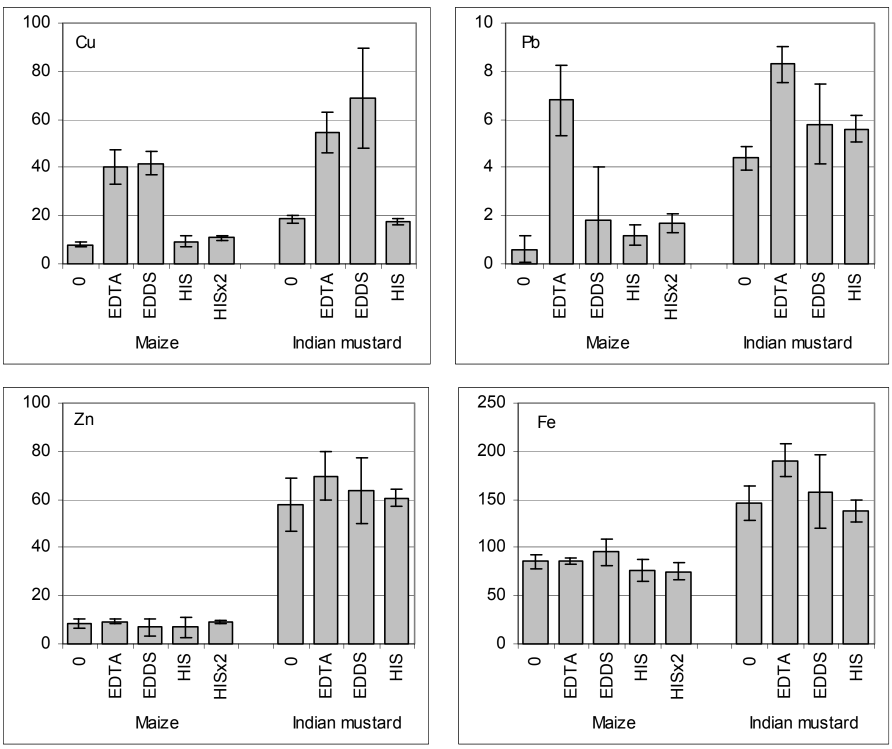

FIGURE 2 – Concentrations of metals in maize and Indian mustard with "normal" watering regime, mg/kg d.m.

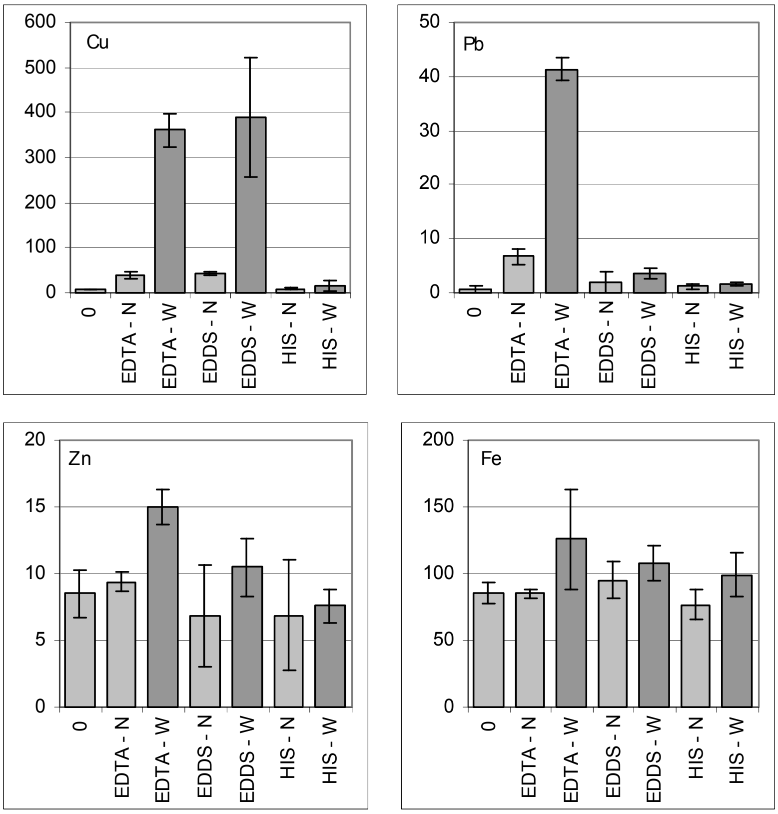

FIGURE 3 – Concentrations of metals in maize – comparison of plots with "normal" (N) and "wet" (W) watering regime, mg/kg d.m.

TABLE 4 - Maximum concentrations of metals in leachates. Indicated are the concentrations, mg/L, and the plots in which they were obtained.

Plots Watering regime	Serie	Cu		Pb		Zn		Fe	
		Concentr. mg/L	Plot	Concentr. mg/L	Plot	Concentr. mg/L	Plot	Concentr. mg/L	Plot
0 Normal	III	0.47	Im*-0	0.03	M*-0	0.12	Im - 0	3.1	Im - 0
	IV	0.20	Im - 0	0.40	M - 0	0.19	M - 0	0.33	Im - 0
Im / M Normal	III	204	Im-EDDS	6.6	Im-EDTA	34.0	Im-EDTA	5.8	M-EDDS
	IV	45.6	Im-EDDS	4.4	M-EDTA	67.0	M-EDTA	2.3	M-EDTA
Maize Wet	I	75.4	EDDS	6.5	EDTA	9.7	EDTA	0.8	EDTA
	II	64.3	EDDS	3.8	EDTA	8.7	EDTA	0.8	EDTA
	III	129	EDDS	7.4	EDTA	14.9	EDTA	3.6	EDTA
	IV	107	EDTA	6.8	EDTA	109	EDTA	2.1	EDTA

* Im - Indian mustard, M – maize

est Pb concentrations in plant shoots (with the mean value of 41.3 mg/kg d.m.) were obtained in the plots with EDTA and wet regime. In all other plots, Pb concentrations in plant shoots remained below 10 mg/kg d.m.

Differently from the effects obtained in the case of Cu and Pb, plant uptake of Zn and Fe was practically unaffected by application of chelating agents (Figure 2 and 3). Slightly increased uptake of Zn and Fe by plants was observed in the plots with EDTA, which was statistically significant for Zn and maize at wet regime, and for Fe and Indian mustard at normal watering. The differences with control plots remained in those cases below 50%.

Risk of Metal Leaching

The concentrations of metals in leachates collected 4 times in a wet regime, and 2 times in the normal watering scheme, indicate high risk of metal leaching caused by application of chelating agents, particularly of EDTA and EDDS. The concentrations of metals in leachates obtained from untreated soils (control plots) did not exceed the values of 0.5 mg/L Cu and Pb, 0.2 mg/L Zn and - incidentally - 3.1 mg/L Fe. Application of chelating agents, in particular EDTA and EDDS, caused a dramatic increase of Cu concentrations in leachates, and the amounts of other metals leached from soils were also considerable (Table 4). Maximum concentrations of particular metals, measured in leachates were: 204 mg/L Cu, 7.4 mg/L Pb, 109 mg/L Zn, and 5.8 mg/L Fe. The concentrations of all metals in the leachates obtained 1 day and 3 days after application of chelators were much lower than those obtained after 14 and 50 days (Table 4), that might be caused by the slow processes of water front movement in soils, by secondary sorption of released complexes and by the concurrence with plant uptake. Extremely high concentrations of Cu in the leachates collected from the pots with EDDS and EDTA indicate high risk of Cu leaching. The changes in Cu concentrations in leachates from various plots are shown in Figure 4. The highest Cu leaching within the first 14 days after application of chelators (series I, II, III) was observed in the plots with EDDS. After 50 days (series IV), the highest Cu concentrations were measured in the leachates from the plots with EDTA, whereas Cu concentrations in the leachates from EDDS-amended plots were much lower. This effect was also observed in our previous experiment [26], and in the light of literature may be explained by EDDS biodegradation [14, 27]. This hypothesis will be checked in a further study.

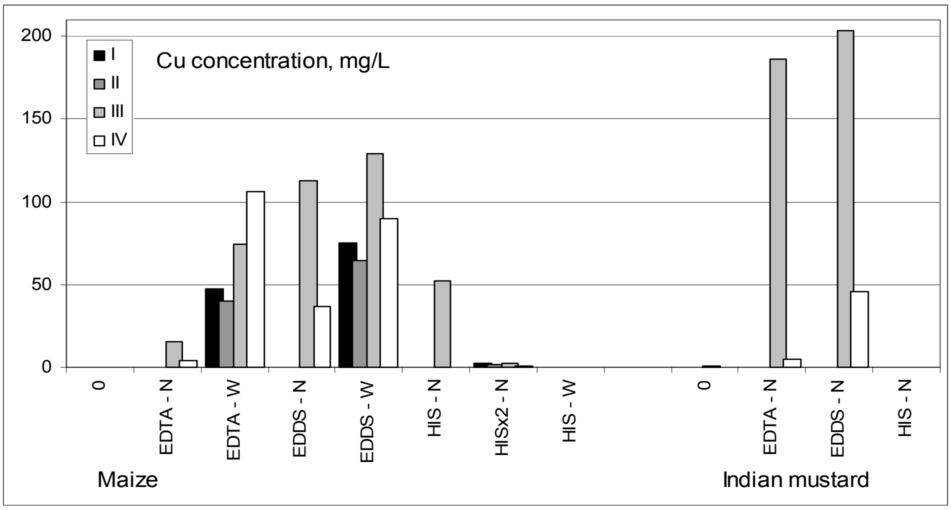

FIGURE 4 – Cu concentrations in leachates obtained in the series I, II (wet regimes), and III, IV (all watering regimes). Detailed description in the text.

TABLE 5 - Estimated decrease in soil concentration due to metal uptake by plants and leaching, mg/kg.

	Cu		Pb		Zn		Fe	
	Plant uptake	Leaching	Plant uptake	Leaching	Plant uptake	Leaching	Plant uptake	Leaching
				Maize - normal watering regime				
0	0.05	0.02	0.00	0.02	0.05	0.01	0.50	0.00
EDTA - N	0.24	0.79	0.04	0.28	0.06	2.81	0.51	0.11
EDDS - N	0.29	5.99	0.01	0.07	0.05	0.38	0.67	0.30
HIS - N	0.06	2.09	0.01	0.02	0.05	0.01	0.50	0.02
HISx2 - N	0.07	0.01	0.01	0.02	0.06	0.01	0.50	0.01
				Maize - wet regime				
EDTA - W	2.28	26.8	0.26	2.46	0.09	14.2	0.79	0.68
EDDS - W	2.46	35.9	0.02	0.57	0.07	0.65	0.68	0.32
HIS - W	0.11	0.74	0.01	0.12	0.05	0.02	0.67	0.02
				Indian mustard - normal watering regime				
0	0.17	0.03	0.04	0.01	0.53	0.01	1.33	0.14
EDTA - N	0.45	7.63	0.07	0.33	0.57	3.33	1.57	0.09
EDDS - N	0.59	9.97	0.05	0.05	0.55	0.02	1.37	0.08
HIS - N	0.16	0.03	0.05	0.14	0.54	0.01	1.24	0.09

Calculated amounts of metals taken up by plants in various experimental plots as referred to those leached from soils are set together in Table 5. The amounts of Cu and Pb leached from soils were much higher than those removed by plants.

CONCLUSIONS

The amounts of Cu and Pb leached from soils in the plots with EDTA and EDDS were at least by ten fold higher than those removed by plants. This effect referred to both plant species examined and to both watering regimes. EDDS proved to be more effective in inducing Cu uptake by maize and Indian mustard than was EDTA. However, relatively high uptake of Cu by plants caused by EDDS application was associated with intensive Cu leaching from soils. Cu leaching after application of EDTA was quite intensive as well. Additionally, EDTA caused considerable leaching of Zn, which might in a short time lead to Zn deficiency in soil. In neither of plots, the concentrations of Cu and Pb in plant shoots reached the values expected for successful phytoextraction. Histidine appeared to be of practically no importance in supporting Cu phytoextraction. Increased metal solubility in EDDS- and EDTA-treated soils indicated that soluble metal chelates remained in soil for at least several weeks, and that their leaching should be considered as an intrinsic effect of the method. The comparison of plant uptake and leaching effects indicate that in our experimental conditions the role played by plants in the processes of soil decontamination was negligible, and the main mechanism of Cu removal was leaching, which in fact does not need the presence of plants and might be carried out as purely technical operation (soil washing), and not a biological treatment. However, the studies on induced phytoextraction should be continued with the chelators slowly released to soils or applied in small doses within the whole season of plant growth.

ACKNOWLEDGEMENTS

This research was supported by the Polish Ministry for Science and Education (formerly the Committee for Scientific Research), in the frame of the Project No. 2 PO6S 062 28.

We are grateful to Prof. Zofia Spiak for supervision and scientific assistance of the pot experiment, and to the staff of the Chair of Plant Nutrition involved in the experiments for their technical support.

REFERENCES

[1] Karczewska, A. (1996) Chemical speciation and fate of selected heavy metals in soils strongly polluted by copper smelters. In: Geochemical approaches to environmental engineering of metals; Reuther R., Ed.; Springer: Berlin, 55-79.

[2] Szerszeń, L., Chodak, T. and Kabała, C. (1999) Monitoring of trace metals concentrations in soils in the surroundings of copper smelters Głogów and Legnica. Zeszyty Problemowe Postępów Nauk Rolniczych 467, 2, 405-412 (in Polish).

[3] Decree of the Ministry of Environment on Soil and Land Quality Standards, dated 9.09.2002 (Official Journal of Laws Dz.U. No. 176, Item 1453)

[4] Act on Environmental Protection dated 27.07.2001 (Official Journal of Laws Dz.U. No.62, Item 627 with further amendments)

[5] Blaylock, M.J. and Huang, J.W. (2000) Phytoextraction of metals. In: Phytoremediation of toxic metals. Using plants to clean up the environment; Raskin I., Ensley B.D., Eds.; John Wiley & Sons: New York, 53-70.

[6] Huang, J.W., Chen, J. and Cunningham, S.D. (1997) Phytoremediation of lead contaminated soils: Role of synthetic chelates in lead phytoextraction. Environmental Science and Technology 31; 800-805.

[7] Kayser, A., Wenger, K., Keller, A., Attinger, W., Felix, H.R., Gupta, S.K. and Schulin, R. (2000) Enhancement of phytoextraction of Zn, Cd and Cu from calcareous soil: The use of NTA and sulfur amendments. Environmental Science and Technology 34; 1778–1783.

[8] Puschenreiter, M., Stöger, G., Lombi, E., Horak, O. and Wenzel, W.W. (2001) Phytoextraction of heavy metal contaminated soils with *Thlaspi goesingense* and *Amaranthus hybridus*: Rhizosphere manipulation using EDTA and ammonium sulfate. Journal of Plant Nutrition and Soil Science 164; 615–621.

[9] Lombi, E., Zhao, F.J., Dunham, S.J. and McGrath, S.P. (2001) Phytoremediation of heavy metal-contaminated soils: natural hyperaccumulation versus chemically enhanced phytoextraction. Journal of Environmental Quality 30; 1919–1926.

[10] Romkens, P., Bouwman, L.., Japenga, J. and Draaisma, C. (2002) Potential and drawbacks of chelate-enhanced phytoremediation of soils. Environmental Pollution 116; 1, 109-121.

[11] Wu, L.H., Luo, Y.M., Xing, X.R. and Christie, P. (2004) EDTA-enhanced phytoremediation of heavy metal contaminated soil with Indian mustard and associated potential leaching risk. Agriculture, Ecosystems and Environment, 102; 307-318.

[12] Wu, L.H., Luo, Y.M., Christie P. and Wong M.H. (2003) Effects of EDTA and low molecular weight organic acids on soil solution properties of a heavy metal polluted soil. Chemosphere 50; 819–822.

[13] Nascimento da, C.W.A., Amarasiriwardena, D. and Xing B. (2006) Comparison of natural organic acids and synthetic chelates at enhancing phytoextraction of metals from a multi-metal contaminated soil. Environmental Pollution 140; 114-123.

[14] Kos, B. and Leštan, D. (2003) Influence of a biodegradable ([S,S]-EDDS) and nondegradable (EDTA) chelate and hydrogel modified soil water sorption capacity on Pb phytoextraction and leaching. Plant and Soil 253; 403–411.

[15] Luo, C., Shen, Z. and Li, X. (2005) Enhanced phytoextraction of Cu, Pb, Zn and Cd with EDTA and EDDS. Chemosphere 59; 1–11.

[16] Luo, C., Shen, Z., Baker, A.J.M. and Li, X. (2006) A novel strategy using biodegradable EDDS for the chemically enhanced phytoextraction of soils contaminated with heavy metals. Plant and Soil 285; 67–80.

[17] Meers, E., Ruttens, A., Hopgood, M.J., Samson, D. and Tack, F.M.G. (2005) Comparison of EDTA and EDDS as potential soil amendments for enhanced phytoextraction of heavy metals. Chemosphere 58; 1011-1022.

[18] Schmidt, U. (2003) Enhancing phytoextraction: The effect of chemical soil manipulation on mobility, plant accumulation, and leaching of heavy metals. Journal of Environmental Quality 32; 1939–1954.

[19] Li, H., Wang , Q., Cui, Y., Dong, Y. and Christie P. (2005) Slow release chelate enhancement of lead phytoextraction by corn (*Zea mays* L.) from contaminated soil - a preliminary study. Science of the Total Environment 339; 179-187.

[20] Madrid, F., Liphadzi, M.S. and Kirkham, M.B. (2003) Heavy metal displacement in chelate-irrigated soil during phytoremediation. Journal of Hydrology 272; 107–119.

[21] Tan Kim H. (2005) Soil sampling, preparation, and analysis. CRC Press.

[22] Karczewska A. and Pańczuk D. (2004): Preliminary estimation of phytoextraction as a method for soil remediation in copper smelters protection zones. Zeszyty Problemowe Postępów Nauk Rolniczych 502, 831-838 (in Polish).

[23] Fischer, K. and Bipp, H.P. (2002) Removal of heavy metals from soil components and soils by natural chelating agents. Water, Air and Soil Pollution 138; 271–288.

[24] Wenzel, W.W., Unterbrunner, R., Sommer, P. and Sacco, P. (2003) Chelate-assisted phytoextraction using canola (*Brassica napus* L.) in outdoors pot and lysimeter experiments. Plant and Soil 249; 83–96.

[25] Reichman S. M. (2002) The responses of plants to metal toxicity: a review focusing on copper, manganese and zinc. Australian Minerals and Energy Environment Foundation: Melbourne.

[26] Karczewska A., Orłów K., Kabała C., Szopka K. and Gałka B. (2007) Copper and lead uptake by maize in the greenhouse experiment on enhanced phytoextraction used to remediate soils polluted by copper smelters. Communications in Soil Science and Plant Analysis, in press

[27] Evangelou M.W.H., Ebel M. and Schaeffer A. (2007). Chelate assisted phytoextraction of heavy metals from soil. Effect, mechanism, toxicity, and fate of chelating agents. Review. Chemosphere 68; 989–1003.

Received: October 31, 2008
Accepted: February 11, 2009

CORRESPONDING AUTHOR

Anna Karczewska
Wrocław University of Environmental and Life Sciences
Institute of Soil Sciences and Environmental Protection
Grunwaldzka 53
50357 Wrocław
POLAND

E-mail: anna.karczewska@up.wroc.pl

FEB/ Vol 18/ No 10a/ 2009 – pages 1967 - 1974