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A CARRYOVER EFFECT OF THE CHELATING AGENTS EDTA AND EDDS APPLIED TO SOILS ON THE UPTAKE OF COPPER AND IRON BY MAIZE IN THE SECOND YEAR OF A POT EXPERIMENT

Anna Karczewska, Bernard Gałka, Karolina Kocan

Institute of Soil Science and Environmental Protection
Wrocław University of Environmental and Life Sciences

Abstract

A pot experiment was established in 2006 to examine whether induced phytoextraction can remove copper from soils polluted with emissions from copper smelters. Two soils tested in the experiment were sand and loam in texture, and contained 620 and 510 $mg \cdot kg^{-1}$ Cu, respectively. Maize was used as a testing plant. Two chelating agents: EDTA and biodagradable EDDS were introduced into the soils at the rates of 0.2; 0.5 and 1.0 $mmol \cdot kg^{-1}$ to intensify the process of Cu phytoextraction. The results that have been already published indicated that application of both chelators caused increase in Cu uptake by plants in 2006, although the concentrations of Cu in biomass were far below those required for effective phytoextraction. Additionally, both chelators caused intensive leaching of metals from soils. This paper focuses on the carryover effects observed in the subsequent year, 2007, certainly caused by the application of chelators. EDTA, particularly when applied at the highest rate, caused considerable deterioration of plant growth, reduction of plant yields and increased uptake of Cu by plants grown in both soils. The plants showed unquestionable effects of copper phytotoxicity. In the plots where EDDS was applied, a small decrease in yield was observed in the case of plants grown on sandy soil, whereas in plants grown on loamy soil a significant increase in plant yields and decrease in Cu concentrations in shoot biomass occurred compared with the control plots. Cu and Fe concentrations in plant shoots were positively correlated with each other, and the plants with the highest concentrations of Cu also contained the highest concentrations of Fe. Application of the chelating agents, particularly EDTA, in 2006 caused a long-lasting increase of Cu and Fe solubility

dr hab. Anna Karczewska prof. nadzw., Institute of Soil Science and Environmental Protection, Wrocław University of Environmental and Life Sciences, 50-357 Wrocław, ul. Grunwaldzka 53, e-mail: Anna.karczewska@up.wroc.pl

in soil, and plant uptake of those elements in 2007 correlated positively with soil concentrations of soluble metal forms, extracted with 1 mol·dm^{-3} NH_4NO_3 and 0.01 mol·dm^{-3} $CaCl_2$.

Key words: soil, phytoextraction, complexing, chelator, EDTA, EDDS, copper, iron.

NASTĘPCZY WPŁYW SUBSTANCJI CHELATUJĄCYCH EDTA I EDDS NA POBRANIE MIEDZI I ŻELAZA PRZEZ KUKURYDZĘ W DRUGIM ROKU DOŚWIADCZENIA WAZONOWEGO

Abstrakt

W doświadczeniu wazonowym założonym w 2006 r., z zastosowaniem metody indukowanej fitoekstrakcji do usuwania Cu z gleb zanieczyszczonych emisjami hut miedzi, testowano 2 gleby: piaszczystą i gliniastą, zawierające odpowiednio 620 i 510 mg·kg^{-1} miedzi. Rośliną testową była kukurydza. W celu zintensyfikowania fitoekstrakcji w 2006 r. wprowadzono do gleby 3 różne dawki (0,2; 0,5 i 1,0 mmol·kg^{-1}) substancji kompleksujących: EDTA i łatwo biodegradowalnego EDDS. Opublikowane już wyniki wskazują, że w 2006 r. oba chelatory spowodowały wzrost pobrania Cu przez kukurydzę, jednak zawartość Cu w biomasie była daleko niższa od wymaganej dla skutecznej fitoekstrakcji. Dodatkowo oba odczynniki spowodowały intensywne wymywanie metali z gleb. W niniejszej pracy przedstawiono następcze efekty zastosowania substancji chelatujących do gleb w kolejnym roku doświadczenia, 2007. Zastosowane środki wpłynęły na plonowanie kukurydzy w roku następnym. EDTA, szczególnie w najwyższej dawce, spowodował na obu glebach pogorszenie wzrostu roślin i zmniejszenie plonu, wzrost koncentracji Cu w biomasie oraz silne objawy toksyczności Cu u kukurydzy. W wariantach z EDDS na glebie piaszczystej stwierdzono nieznaczne obniżenie wielkości plonu kukurydzy, a na glebie pyłowo-gliniastej – wzrost plonu roślin i zmniejszenie koncentracji Cu w częściach nadziemnych w porównaniu z wariantami kontrolnymi. Stężenia Cu i Fe w biomasie wykazywały wzajemną dodatnią korelację, a rośliny w wariantach o najwyższej koncentracji Cu zawierały też najwyższe stężenia Fe w biomasie. Zastosowane w 2006 r. chelatory, szczególnie EDTA, spowodowały długotrwały wzrost rozpuszczalności Cu i Fe w glebie. Pobranie obu pierwiastków z gleby przez rośliny w 2007 r. było dodatnio skorelowane z zawartością w glebie łatwo rozpuszczalnych form tych pierwiastków, ekstrahowanych roztworami 1 mol·dm^{-3} NH_4NO_3 i 0,01 mol·dm^{-3} $CaCl_2$.

Słowa kluczowe: gleba, fitoekstrakcja, kompleksowanie, chelatory, EDTA, EDDS, miedź, żelazo.

INTRODUCTION

Although soils polluted with heavy metals occur in Poland only on a local scale, effective and environmental-friendly soil cleaning methods are needed to enable removal of excessive amounts of pollutants from soils. Phytoextraction is considered as a potential method ofr decontamination of heavy metal-polluted soils. In particular, induced hyperaccumulation, in which chelating agents are used to mobilize metals from soil solid phase, has attracted scientists' attention in recent decades. First papers in which the results of induced phytoextraction were presented opened promising prospects for future use of this method (BLAYLOCK 2000, SCHMIDT 2003). They were, however, followed by many others stressing drawbacks of the method rather than its advantages (ROMKENS et al. 2002, EWANGELOU et al. 2007). Despite this, experiments on induced phytoextraction continue with various chelating agents different in their biodegradability and applied in different rates (KOS, LESTAN 2003, MEERS et al. 2005, NASCIMENTO DA et al. 2006).

The results presented in this paper refer to a pot experiment conducted in 2006-2007, in which EDTA and easily biodegradable EDDS were used for induced phytoextraction of copper from soils polluted with emissions from copper smelters. The effects of Cu removal from soils by plant uptake, which enables us to assess the phytoextraction efficiency in 2006, have already been published (KARCZEWSKA et al. 2008), and this paper focuses on carryover effects caused by chelate application in the first year,on plant growth, biomass yield and Cu and other elements uptake by maize in the following year. The plants grown in 2007 showed typical symptoms of Cu toxicity, which can be interpreted as physiological results of Fe deficiency (KABATA-PENDIAS 2001). Therefore, particular attention is given to Cu and Fe uptake by maize.

MATERIAL AND METHODS

Soil material used in the experiment was collected from the surface layer of soils in the vicinity of copper smelters in Legnica (soil L) and Głogów (soil G). Both soils contained enhanced concentrations of heavy metals, in particular copper. The texture of soil L corresponded to sandy loam and the soil contained 510 $mg \cdot kg^{-1}$ Cu whereas soil G, with the texture of loamy sand, contained 510 $mg \cdot kg^{-1}$ Cu. Basic properties of both soils are presented in Table 1. Maize (*Zea mays* L., var. Blaskj) was used in 2006 as the experimental plant. At the stage of plant pre-maturity, two chelates: EDTA and

Table 1

The properties of soils used in the experiment

Soil	Texture*	Percentage of grains with diameter		C org %	pH	CEC $cmol(+) \cdot kg^{-1}$	Cu total concentration $mg \cdot kg^{-1}$
		<0.02 mm	<0.002 mm				
G	loamy sand	15	2	0.73	6.7	6.2	510
L	sandy loam	26	6	0.95	6.6	7.8	620

*Soil textural groups and their symbols – acc. to Polish Society of Soil Sciences (2008)

EDDS were spread over the soil surface at the rates of 0.2, 0.5 and 1.0 mmol·kg^{-1}. The numbers: 1, 2 and 3 stand for those rates in the descriptions of experimental plots (Table 2, Figures 1-3). The experiment was continued with two different watering regimes, simulating „normal" weather with occasional rain (N) and „wet" with repeated heavy rainfalls (W). The design of the experiment is presented in Table 2. Under the wet regime, soil was leached with distilled water 6 times, i.e. 2, 5, 14. 28, 50, and 100 days after chelate application, while under the normal regime – 4 times:

Table 2

Design of experiment – soil from Głogów (G). For the soil from Legnica, the same design was used, with letter L instead of G in all descriptions and symbols

Experimental plot	Chelating agent	Chelator rate	Watering regime
G/0/0/N	---	0	N - normal
G/0/0/W			W - wet
G/EDTA/1/N	EDTA	1 – low (0.2 mmol·kg^{-1})	N - normal
G/EDTA/1/W			W - wet
G/EDTA/2/N		2 – medium (0.5 mmol·kg^{-1})	N - normal
G/EDTA/2/W			W - wet
G/EDTA/3/N		3 – high (1.0 mmol·kg^{-1})	N - normal
G/EDTA/3/W			W - wet
G/EDDS/1/N	EDDS	1 – low (0.2 mmol·kg^{-1})	N - normal
G/EDDS/1W			W - wet
G/EDDS/2/N		2 – medium (0.5 mmol·kg^{-1})	N - normal
G/EDDS/2/W			W - wet
G/EDDS/3/N		3 – high (1.0 mmol·kg^{-1})	N - normal
G/EDDS/3/W			W - wet

after 14, 28, 50, and 100 days. The volume of leaching water ranged from 100 to 750 cm^3 per pot, and was adjusted to obtain 200 cm^3 of leachates under the normal regime and 500 cm^3 under the wet regime. The volume of water applied depended on the maize growth and properties of the root system. Seven days after the chelate application, aerial parts of the plants were harvested. Thereafter, as well as after all the leaching treatments, the soils' moisture was kept to enable chelate biodegradation. On the basis of metal concentrations in the leachates and their volumes, the amounts of leached metals were estimated. The estimation showed that considerable amounts of Cu, up to 5 percent of total Cu, were leached from soils, and

Fig. 1. Growth of maize in the year 2007: a – general view of the experiment, b – soil G, the plots with the maximum rate of EDTA and EDDS applied in 2006 (pot 67: G/EDTA/3/N, pot 71: G/EDTA/3/W, pot 91: G/EDDS/3/N, pot 95: G/EDDS/3/W), c – soil L, the plots with the maximum rate of EDTA and EDDS applied in 2006 – the pots 167, 171, 191 i 195 analogous to those with soil G: L/EDTA/3/N, L/EDTA/3/W, L/EDDS/3/N, L/EDDS/3/W

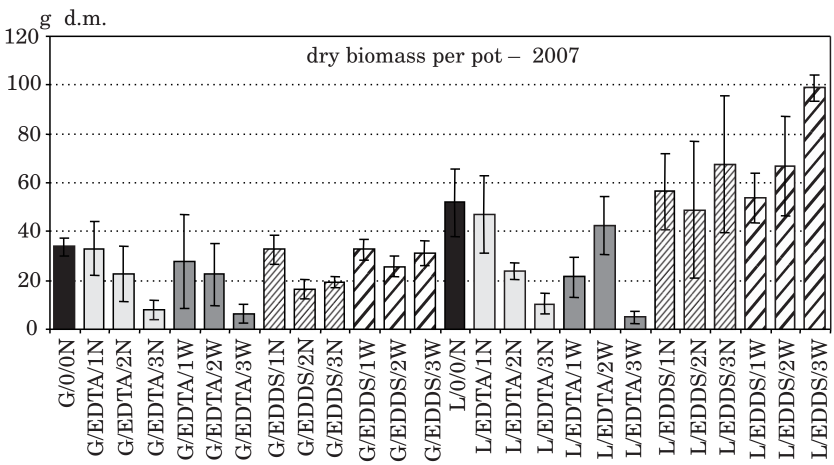

Fig. 2. Dry biomass of maize harvested in 2007 in various experimental plots, g d.m. per pot

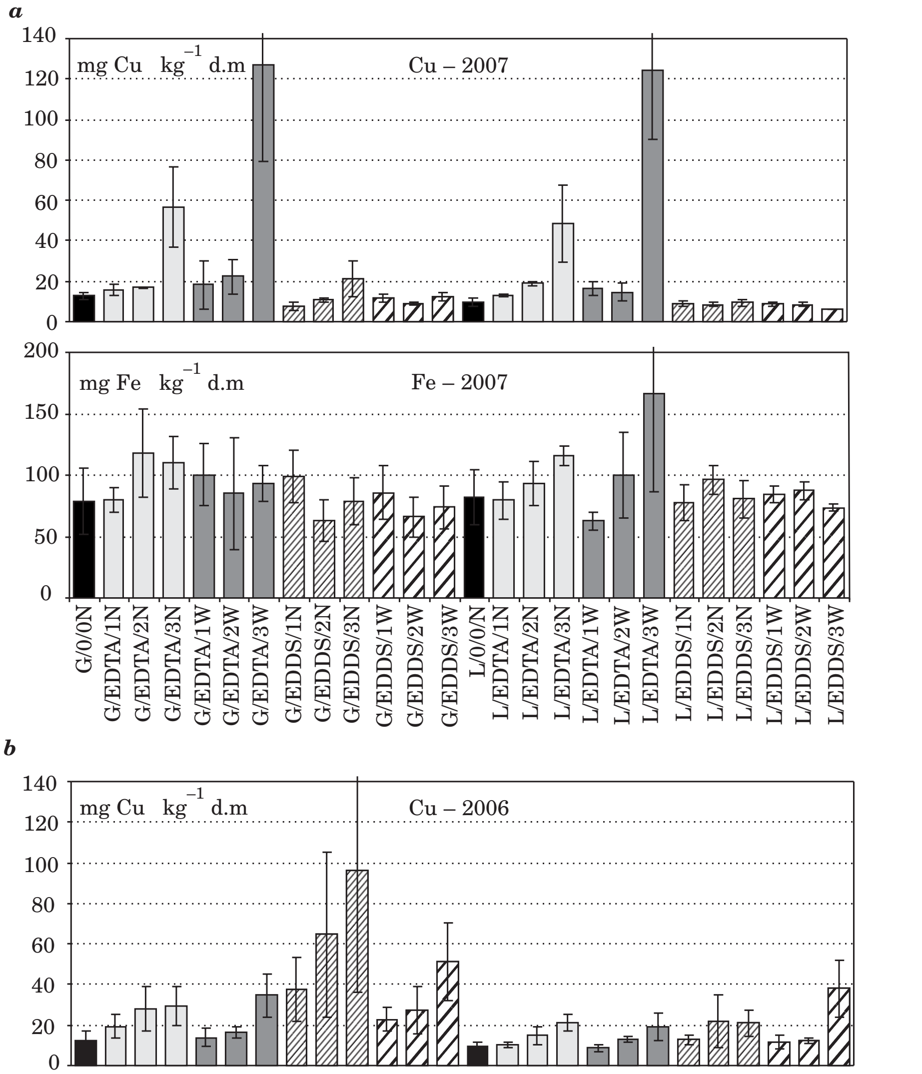

Fig. 3. Cu and Fe concentrations in dry biomass of maize: *a* – in 2007, *b* – compared with Cu in maize in 2006 (based on Karczewska et al. 2008)

the result depended on the kind and rate of a chelating agent. The highest amounts of Cu were leached from the pots with EDDS applied at the highest rates. A more detailed description of the results obtained in 2006 was presented by Karczewska et al. (2008). Figure 2b shows the mean concentrations of copper in maize biomass in 2006.

In the following year, maize was used again as an experimental plant. Before sowing, soil samples were collected and analyzed for available nutrients. Additionally, necessary fertilization with macro- and micronutrients was applied. Furthermore, easily soluble forms of Cu and Fe were analyzed in soils by extraction with 1 mol·dm^{-3} NH_4NO_3, 0.01 mol·dm^{-3} $CaCl_2$ and water (KARCZEWSKA 2002). During plant vegetation, soil moisture was maintained in the range of 60-90% of field capacity. When the plants reached the stage of maturity, their aerial parts were cut, weighed, dried and analyzed for Cu and Fe concentrations. Additionally, maize roots were collected, washed thoroughly with distilled water, dried, weighted and analyzed for Cu and Fe. The plant material was dry ashed at the temperature 450°C, and then dissolved in nitric acid (OSTROWSKA et al. 1991). Cu and Fe were determined in solutions by flame AAS. The results obtained for soils amended with the chelating agents were compared with control plots without chelate addition. Correlation coefficients were determined between Cu and Fe concentrations in plant material and in soil extracts.

RESULTS AND DISCUSSION

Plant growth differed considerably among experimental plots, as it is show in the photo (Figure 1) and confirmed by the data on plant biomass (Figure 2). Plant growth on soil G was much poorer than that on soil L, and related yields of biomass differed significantly. Mean biomass of maize grown in 2007 in control plots, without chelate additions, was 33.7 g d.m. per pot in the case of soil G (G/0/0/N) and 51.8 g d.m. in the case of soil L (L/0/0/N). Application of EDTA in 2006 resulted in a considerable, statistically significant reduction of plant yield in 2007, particularly in the plots with the highest EDTA rate (Figures 2 and 3). The plants in those plots indicated very strong signs of Cu toxicity, with typical symptoms such as interveinal foliar chlorosis, necrotic leaf tips and margins, leaf wilting from edges towards central part of the leaf, as well as changes in the root system (REICHMANN 2002). In those pots where EDDS as a biodegradable chelator (KOS, LESTAN 2003, MEERS et al. 2005) was applied in 2006, different effects were observed on maize grown on soils G and L. In the plots with soil G, plant growth and biomass yield were slightly reduced in comparison with control plots, whereas in the plots with soil L, the growth of maize was affected positively, which means that maize yield increased significantly and Cu concentrations in leaves decreased when compared with control plots (Figures 2 and 3). This effect was particularly distinct in the plots with the wet watering regime (W) applied in 2006. At this stage of research, it is difficult to say what mechanism was responsible for such dependence on soil watering. Presumably, the most soluble Cu forms were intensively leached, which was followed by biodegradation of a chelating agent, which therefore did not cause any further Cu mobilization from soil. The ranges of soluble Cu and Fe in soils, determined before maize was sown, at the beginning of season 2007, as well as at the end of the season, after plant harvest, are shown in Table 3. The amounts of Cu extracted from soils with 1mol·dm^{-3} NH_4NO_3 were higher than those extracted with 0.01 mol·dm^{-3} $CaCl_2$, although the relationships between the plots were similar. The highest concentrations of soluble Cu were found in soils treated with high rates of EDTA, whereas in the plots with EDDS, the concentrations of soluble Cu were lower even then those in control plots (Table 3). Similarly, the highest concentrations of soluble Fe were found in 2007 in soils amended with EDTA. Comparison of soluble Cu in soils before the season 2007 and after plant harvesting indicates that Cu extractable with 0.01 mol·dm^{-3} $CaCl_2$ decreased significantly in both soils, by 40 percent on average, whereas in the case of Cu extractable with 1 mol·dm^{-3} NH_4NO_3, a similar decrease was confirmed only in soil L. There were no significant differences between the amounts of soluble Fe extractable from soils before and after plant cultivation.

Table 3

Soluble forms of Cu and Fe, extracted from soils with 1 mol·dm^{-3} NH_4NO_3 and 0.01 mol·dm^{-3} $CaCl_2$, mg·kg^{-1}

Plots	Value	Soil G				Soil L			
		Cu		Fe		Cu		Fe	
		the forms extracted with:							
		NH_4NO_3	$CaCl_2$	NH_4NO_3	$CaCl_2$	NH_4NO_3	$CaCl_2$	NH_4NO_3	$CaCl_2$
Before the season 2007									
Control		36.5	2.4	0.3	0.7	16.5	2.2	0.3	0.7
EDTA	min.	20.9	3.7	1.0	0.2	12.7	5.0	0.3	0.5
	max.	35.6	12.9	2.0	0.8	36.4	21.2	3.1	1.9
EDDS	min.	20.7	2.7	0.3	0.3	12.4	2.1	0.3	0.5
	max.	24.2	4.8	1.6	0.4	15.7	5.2	1.3	0.6
After maize harvesting in 2007									
Control		20.7	1.8	0.1	0.3	16.5	1.5	0.2	0.0
EDTA	min.	17.6	2.4	0.0	0.0	11.6	3.1	0.0	0.0
	max.	24.4	8.5	0.1	2.4	17.7	10.8	0.0	1.0
EDDS	min.	17.7	2.0	0.0	0.0	9.8	1.4	0.0	0.0
	max.	26.2	2.2	0.0	0.5	10.9	2.5	0.0	1.4

Copper concentrations in dry biomass of aerial parts of maize were significantly negatively correlated with plant yields (Table 4). The highest Cu concentrations, above 120 mg·kg^{-1} d.m., were found in the plants with the poorest growth and the strongest symptoms of Cu toxicity, i.e. in the plots with the highest EDTA rate and the wet watering regime: G/EDTA/3/W and L/EDTA/3/W, as it is shown in the diagram (Figure 3).

Table 4

Correlation coefficients between Cu and Fe concentrations in dry biomass, the biomass of maize shoots, and concentrations of soluble forms of metals in soils

Parameter		Metal concentrations in plant shoots ($mg \cdot kg^{-1}$ d.m.)	
		Cu	Fe
Plant yield (fresh biomass), g per pot		-0.680**	-0.450*
Plant yield (dry biomass), g d.m. per pot		-0.564**	-0.380
Cu in maize roots, $mg \cdot kg^{-1}$ d.m.		0.044	*x*
Cu in maize shoots, $mg \cdot kg^{-1}$ d.m.		*x*	-0.143
Fe in maize shoots, $mg \cdot kg^{-1}$ d.m.		0.640**	*x*
Soluble forms of elements (Cu or Fe) as determined in extraction with:	1 $mol \cdot dm^{-3}$ NH_4NO_3	0.697**	0.684**
	0.01 $mol \cdot dm^{-3}$ $CaCl_2$	0.715**	0.674**
	water	0.666**	-0.268

Correlations significant at: * $p = 0.05$; ** $p = 0.01$

Neither the analysis of Fe concentrations in the aerial parts of maize, nor the relationships between Cu and Fe concentrations in biomass confirmed any reduced uptake of Fe from soils by those plants that took up very high amounts of Cu. Under the conditions of the experiment, Cu and Fe concentrations in dry biomass of maize were positively correlated ($r = 0.640$, $p = 0.01$) – Table 4. Such relationship does not necessarily mean that there is no antagonistic effects of Cu on Fe activity at the level of cell physiology. The results of the experiment proved, however, that Fe concentrations in plant biomass increased together with increasing concentrations of Cu.

It was also proven that the concentrations of Cu and Fe in the aerial parts of maize were positively correlated with the concentrations of soluble forms of those elements present in soils, extractable with both 1 $mol \cdot dm^{-3}$ NH_4NO_3 and 0.01 $mol \cdot dm^{-3}$ $CaCl_2$, which was confirmed by high correlation coefficients ($r > 0.67$, $p = 0.01$), as indicated in Table 4. On the contrary, the concentrations of Cu and Fe in maize roots did not depend on any variable of the experiment (soil, rate of chelating agent or watering regime). Copper and iron concentrations in the aerial parts of maize did not correlate with concentrations of those elements in plant roots, either (Table 4).

CONCLUSIONS

1. EDTA applied to support copper phytoextraction from polluted soil caused long-lasting carryover effects such as raised copper phytotoxicity and its increased leachability in the subsequent year. Similar effects were not observed after application of easily biodegradable EDDS, therefore further research on induced phytoextraction of Cu from polluted soils should be carried out with biodegradable chelators.

2. Under the conditions of chelate-induced Cu phytoextraction, Cu and Fe solubility in soils increased, and plant uptake of those elements correlated well with soil concentrations of their easily soluble forms.

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