The heavy metal availability in long-term polluted soils as affected by EDTA and alfalfa meal treatments

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ABSTRACT

A 38-day incubation experiment was carried out in order to evaluate the response of plant-available portions of heavy metals in long-term contaminated arable and grassland soils on addition of ethylenediaminetetraacetic acid (EDTA) and alfalfa (*Medicago sativa* L.) meal. Soils with different soil management (arable and grassland) from the vicinity of a lead smelter were used in the experiment. Readily available heavy metal fractions of Cd, Pb, Zn and Cu increased in the presence of EDTA at the beginning of experiment. The increase of heavy metal availability was higher in the arable soil with lower content of soil organic carbon than in the grassland soil. Addition of EDTA increased content of K_2SO_4-extractable carbon which remained higher throughout the overall time of experiment. During the first part of the experiment, the alfalfa meal addition decreased the available metal concentrations in the EDTA-treated grassland soil whereas no effect of alfalfa meal was observed in EDTA-treated arable soil.

Keywords: contaminated soils; smelter; chelates; organic substrates; incubation

The sources of heavy metals as the old smelters affect seriously the surrounding environment and the agricultural production in the area could exceed the permitted limits due to possible entering of trace elements into the food chain. The total heavy metal cannot be measure of their availability for plants as only free metal ions are considered as being the most toxic. Metals that are complexed with organic compounds may be less available due to the formation of metal-enriched organic particles, and thus an increase of stabilization and sequestration of metals in the soil (Zhang et al. 2001). In addition, long-term permanence of toxic metals in soil increases binding to clay particles (Kamitani et al. 2006) or to Fe/Mn oxides or carbonate complexes (Reddy et al. 2010) and therefore decreases their biological availability.

The phytoavailability of toxic elements can be increased by the use of synthetic chelators (Kos and Leštan 2004). Among these chelators, the ethylenediaminetetraacetic acid (EDTA) is studied as it was found to be efficient in increasing the availability of metals for phytoextraction (Wenzel et al. 2003). EDTA was found to be very efficient in desorbtion of Pb from soils (Komárek et al. 2007, Neugschwandtner et al. 2008). However, EDTA is able to extract a portion of the organic- and sulphide-bound metal fractions, which are usually less available to plants (Cao et al. 2008).

Addition of alfalfa meal improved soil microbial characteristics in EDTA-treated soils (Mühlbachová 2009). Alfalfa meal effects on metal mobility in soil tended to be small and often not significant (Quian et al. 2011). However, the decrease on exchangeable Pb fractions and a different effect on Cd fraction depending on soil pH was noted (Abbaspour et al. 2007).

The aim of this research was to determine changes of concentrations of readily available heavy metal fractions after EDTA addition into long-term contaminated soils in the area near the lead smelter at Příbram (Czech Republic) in an incubation

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experiment. The effects of addition of alfalfa meal together with EDTA were studied to elucidate whether the stimulation of soil biological activity can additionally affect the mobile portions of investigated heavy metals in soil.

MATERIAL AND METHODS

Sampling site. The sampling area was situated in the vicinity of a lead smelter in Příbram, Czech Republic operating since 1786, and it originally worked lead ores. Since 1972 the smelter processes secondary lead and cadmium sources. Since 1982 the emissions decreased 300–500 times due to installation of a 98% efficient dust separator and a 160 m stack; prior to that, no efficient dust separation method was used (Šichorová et al. 2004). The studied soils belong among Cambisols.

Soils contaminated with heavy metals from the areas using two different agricultural practices (CM-A: arable soil with normal agricultural cultivation; CM-G: permanent grassland soil) were chosen for the experiment. Soil cores 10 cm in diameter and 0–20 cm in depth were taken from each sampling site, making up 3 subsamplings in a 2 m distance from a central point. The soil characteristics are shown in Table 1.

Incubation experiment. Seven days before the start of the experiment, three replications of each treatment (1 kg of soil on an oven-dry basis) were placed into the 3-L plastic containers, covered with tightly-fitting lids. The soils were pre-incubated at 40% water holding capacity (WHC) at 28°C and with a jar of 25 mL 1 mol/L NaOH (to take up the evolved CO_2), and with distilled water at the bottom of the container.

At the beginning of the experiment, the following experimental design was used in the same manner for both soils CM-A and CM-G: Control – without any added chelators or alfalfa meal; Alfalfa 1 – alfalfa meal containing 1000 μg C/g soil; Alfalfa 2 – alfalfa meal containing 2000 μg C/g soil; EDTA – 6 mmol Na_2EDTA/kg soil; EDTA + Alfalfa meal 1 – 6 mmol Na_2EDTA/kg soil and alfalfa meal containing 1000 μg C/g soil; EDTA + Alfalfa 2 – 6 mmol Na_2EDTA/kg soil and alfalfa meal containing 2000 μg C/g soil. Na_2EDTA used for the experiment is ethylenediaminetetraacetic acid disodium salt ($C_{10}H_{14}Na_2O_2 \cdot 2\ H_2O$). The experimental soils were during the experiment regularly adjusted to 50% WHC, daily aerated to ensure a sufficient oxygen supply and incubated under the above-described conditions. The incubation continued for 38 days.

Analytical methods. The soil samples were air-dried at 20°C, ground in a ceramic mortar and passed through a 2-mm plastic sieve. The total metal concentrations were determined in 0.5 g subsamples after microwave assisted wet digestion in a mixture of 8 mL of HNO_3, 5 mL of HCl, and 2 mL of HF (Ethos 1, system, MLS GmbH, Leutkirch, Germany). A certified reference material RM 7001 Light Sandy Soil (Analytika s.r.o., Prague, Czech Republic) was used as a quality assurance of the results. The mobile fractions of heavy metals were determined from moist samples taken from incubation containers in 1 mol/L NH_4NO_3 extract (1:2.5 w:v) after 2 h of shaking and subsequent centrifugation for 10 min at 4000 rpm (Pruess et al. 1991). Supernatants

Table 1. Basic physico-chemical soil characteristics, total and NH_4NO_3-extractable metal contents in the experimental soils

	Soil	
	CM-A	CM-G
Soil type	Cambisol	Cambisol
Soil use	arable	grassland
Particle size distribution (%)		
Clay	38	40
Silt	20	29
Sand	42	31
CEC ($mmol_{(+)}$/kg)	171	212
pH (H_2O)	6.24	5.45
C_{org} (%)	1.77	5.82
Available nutrients – Mehlich III (mg/kg)		
P	33	55
K	92	112
Ca	2703	2983
Total heavy metal contents (mg/kg)		
Cd	4.06	12.1
Cu	35.2	22.9
Pb	1138	3221
Zn	255	384
NH_4NO_3-extractable heavy metal contents (mg/kg)		
Cd	0.18	0.12
Cu	0.03	0.04
Pb	0.62	1.44
Zn	3.84	1.04

were kept at 6°C before measurement. The total heavy metal content of the soil digests and 1 mol/L NH_4NO_3 extracts were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, Varian VistaPro, Mulgrave, Australia), with axial plasma configuration.

Total organic carbon (TOC) was determined spectrophotometrically according to Sims and Haby (1971). For determination of soil microbial biomass connected organic matter were the soil samples extracted with 0.5 mol/L K_2SO_4 for 30 min at the ratio of 1:4 w:v (Vance et al. 1987). For determination of carbon in the extracts digestion in a mixture of H_2SO_4 and $Cr_2K_2O_7$, followed by titration of the excess dichromate with $(NH_4)_2Fe(SO_4)_2 \cdot 6\ H_2O$ was applied.

Statistical analysis. The statistical analyses were carried out using the Statistica 9.0 software (StatSoft Inc., Tulsa, USA), with the results expressed as the mean values. For all figure data, the standard deviation was determined, which are visualized as the vertical bars. The multiple-way ANOVA Duncan's test determined the significant differences of the data obtained during the incubation experiment.

RESULTS AND DISCUSSION

The NH_4NO_3-Cd concentrations increased up to 3.5 mg Cd/kg in the soil CM-A and up to 1.7 mg Cd/kg in the soil CM-G with the EDTA treatment at day 3 of incubation and despite to decrease in the further period of incubation remained significantly higher in comparison with the control soils and both alfalfa meal treatments (CM-A soil – 0.26 mg Cd/kg, CM-G soil – 0.11 mg Cd/kg) (Figure 1). The simultaneous addition of EDTA and both doses of alfalfa meal increased NH_4NO_3-Cd similarly as only EDTA treatment in the CM-A soil. In the CM-G soil, the NH_4NO_3-Cd concentrations remained higher in EDTA-treatments with both alfalfa meal doses during latter phases of incubation than in simple EDTA-treatment.

The alfalfa meal treatments in the soil CM-A increased 2 and 4 times the NH_4NO_3-Cu concentrations in Alfalfa1 and Alfalfa2 treatment, respectively (Figure 1). The EDTA-treatment inA creased NH_4NO_3-Cu concentrations in the soil CM-A 362 times and in the soil CM-G 24 times in comparison with control soils. NH_4NO_3-Cu concentrations in EDTA treatments with Alfalfa1 and Alfalfa2 treatments increased in the soil CM-A 325 times and 123 times in reference to corresponding alfalfa meal treatments without EDTA. Only a fifteen and sixteen fold increase of NH_4NO_3-Cu concentrations was observed in treatments with EDTA together with both alfalfa meal additions in the soil CM-G. The decrease of NH_4NO_3-Cu concentrations in all EDTA and EDTA + alfalfa treatments was observed throughout the incubation period.

The treatment of CM-A soil with EDTA increased the NH_4NO_3-Pb concentrations 686 times in the CM-A soil and 203 times in the CM-G soil at day 3 of incubation. Heavy metal concentrations in control and alfalfa meal-treated soils remained very low at about 0.6–1 mg Pb/kg in the CM-A soil and 1.08–1.44 mg Pb/kg in the CM-G soil (Figure 1). Alfalfa meal additions into the soil CM-A did not affect significantly the EDTA treatment. The decrease of NH_4NO_3-Pb concentrations till the 24th day of incubation was observed in all treatments with EDTA in the soil CM-A. The simultaneous alfalfa meal addition into the soil CM-G decreased Pb concentrations in EDTA treatments during the first 10 days of incubation (Figure 1).

The EDTA treatment increased the NH_4NO_3-Zn concentrations in the CM-A soil up to 42–45 mg Zn/kg and up to 32 mg Zn/kg in the CM-G soil. In the soil CM-A, the NH_4NO_3-Zn concentrations in all EDTA treatments decreased since the third day to the end of incubation. In the CM-G soil increased to 21 and 19.7 mg Zn/kg for EDTA + Alfalfa 1 and EDTA + Alfalfa 2 treatments at day 3 of the incubation. Contrariwise to simple EDTA treatment, the addition of alfalfa meal maintained the Zn concentrations relatively stable during the incubation in the soil CM-G.

Komárek et al. (2007) and Neugschwandtner et al. (2008) found EDTA to be very efficient in releasing heavy metals, particularly Pb, in the area of the Příbram smelter. In the present study, EDTA was very efficient in releasing heavy metals from the experimental soils CM-A and CM-G, and significantly increased the concentrations of the available metal fractions during the experiment. Despite to higher total heavy metal concentrations, the extractability of readily available metal fractions in the soil CM-G with higher organic carbon content was lower after EDTA treatment possibly due to existence of organo-mineral complexes (Lair et al. 2007) and metal-enriched organic particles (Kao et al. 2006).

The effect of alfalfa meal addition on element mobility differed between the soils CM-A and CM-G. Addition of alfalfa meal into the CM-A soil did not affect significantly the heavy metal

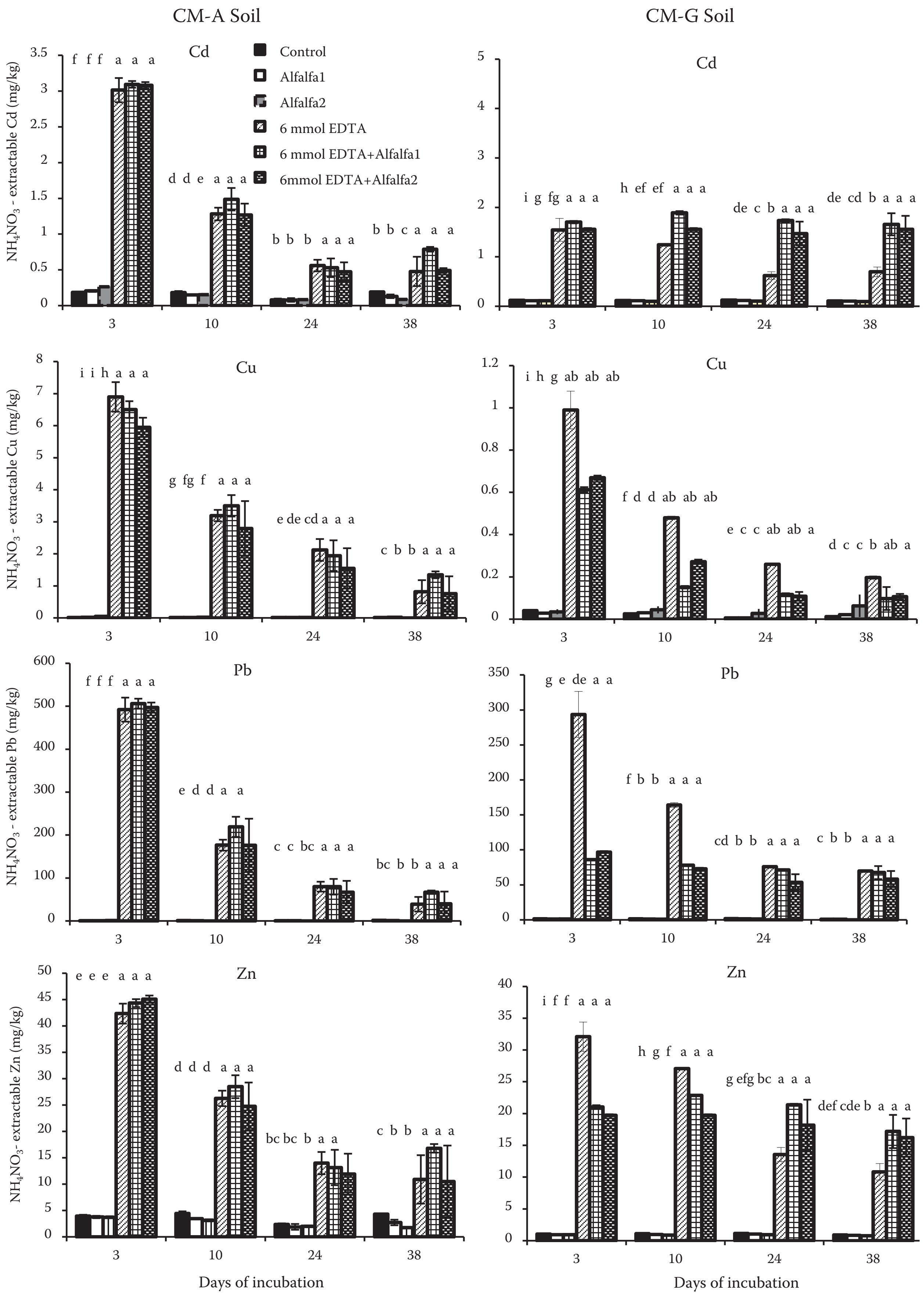

Figure 1. NH_4NO_3-extractable Cd, Cu, Pb, and Zn in the soils CM-A and CM-G during the incubation experiment. Vertical bars indicate the standard deviation error. The letters show the statistical differences among the data after a multiple Duncan's ANOVA test

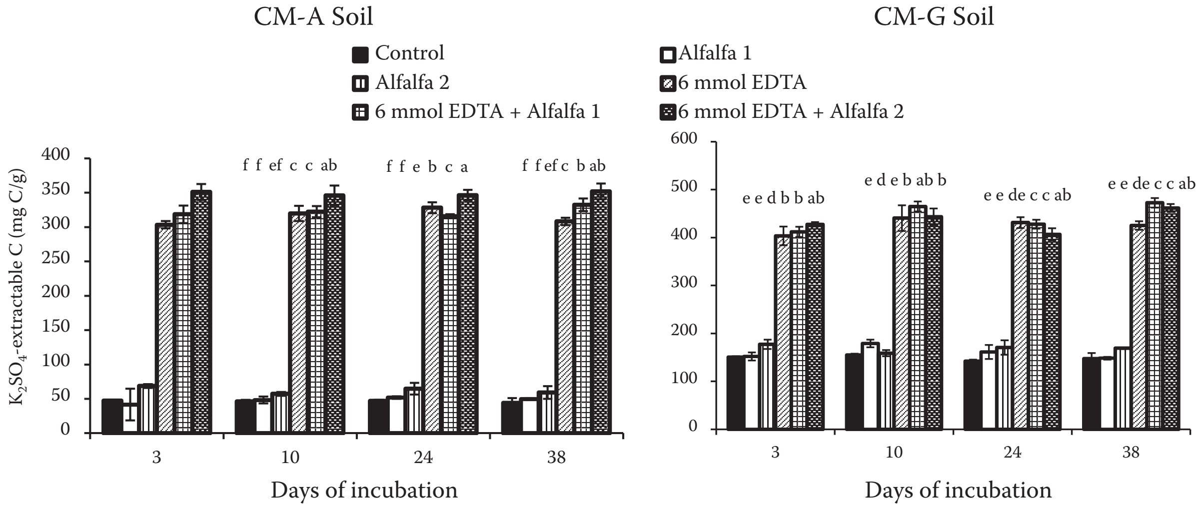

Figure 2. K_2SO_4-extractable C during the incubation experiment in the soils CM-A and CM-G. Vertical bars indicate the standard deviation error. The letters show the statistical differences among the data after a multiple Duncan's ANOVA test

availability in EDTA treated variants. On the contrary, in the EDTA-treated soils CM-G decreased during the first 10 days of the experiment alfalfa meal, with exception of Cd, the concentrations of NH_4NO_3-heavy metal fractions. The experimental soils differed in the organic matter content. The distribution of heavy metals between the soil solution and sorbed phase could be described as a function of organic carbon content (Cappuyns et al. 2006) and this could be one of reasons of lower heavy metal extractability after alfalfa meal addition together with EDTA-treatment in the soil CM-G. In addition, Abbaspour et al. (2007, 2008) showed that alfalfa meal addition into soils decreased Pb solubility and Cd availability was observed only in the acid and neutral soils due to formation of organic-Pb complexes, pH changes or increase in Cl concentrations and thereby with formation of Cd and Pb chlorides.

The non-fumigated portion of K_2SO_4-extractable C method represents the C background of classical fumigation-extraction method for microbial biomass determination (Vance et al. 1987) and was used for enhancement of mobile C fraction by Mühlbachová (2011). In our case it increased immediately after its addition to the soils, and remained similar in both studied soils throughout the incubation (Figure 2). The alfalfa meal treatment affected significantly the K_2SO_4-extractable concentrations of C in the soil CM-A, and the last day of incubation also in the soil CM-G. Compared to EDTA, however, the alfalfa meal presence resulted in lower increase of K_2SO_4-extractable C in soils. Wang et al. (2007) found elevated concentrations of dissolved organic carbon in EDTA-treated soils and Meers et al. (2008) assessed degradation of chelators by measuring dissolved organic carbon. EDTA contains carbon, and possibly a portion of the carbon in the K_2SO_4 solution of our experiment could derive from EDTA, as no increase of K_2SO_4-extractable C was observed in the control soils and it was also significantly higher than in alfalfa meal-treated soils.

The extractability of heavy metals with NH_4NO_3 increased after EDTA-treatment of soils and differed between arable and grassland soil. The addition of alfalfa meal into EDTA-treated soils did not affect the extractability of elements in the arable CM-A soil. The addition of organic substrates together with EDTA was useful particularly in a soil with lower organic carbon content. The use of organic substances should be therefore taken in consideration in soils in which chelators are used for phytoremedial purposes.

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