EFFECT OF COMPOST AMENDMENT ON HEAVY METALS TRANSPORT TO PLANT

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Abstract: Concentration of heavy metals in environment has been significantly affected by human since last century. This work presents the analysis of the influence of compost amendment on heavy metals transport to the plant Lactuca sativa L. grown in contaminated soils. To demonstrate the effect of compost, a pot experiment was performed. Eight variants of soils with different concentrations of pollutants with and without compost amendment were prepared. The contaminated soils we used in our experiment come from the Nord France region Noyelles-Godault. Main pollutants were Pb, Cd and Zn. The decrease of heavy metals content in plants was observed by the simultaneous applications of compost to contaminated soils, from 10% to 50% in comparison with the variants without compost amendment. The BCF (bioconcentration factor) gives a clear view on reduced uptake of HM (heavy metal) by plant. Based on these results, we conclude that application of organic waste compost has positive effect on immobilization and bioavailability of heavy metals.

Key Words: heavy metal mobility, contamination, remediation, arbuscular mycorrhizal

INTRODUCTION

Concentration of heavy metals (HM) in environment has been significantly affected by human since last century. Contamination caused by metals is mainly associated with mining, industrial activities, chemical application such as pesticides and waste production (He et al. 2005). Soil pollution results dominantly from emission of fumes and smoke, which is followed by dry or wet deposition. Heavy metals remain in soil and may retard growth of plants or of soil microorganisms, may be transferred into the plant tissue and via food chain may endanger the human health (Kleckerová et al. 2013). In addition, many metal-polluted soils are also characterized by negative properties such as poor nutrient availability, a lack of soil structure, low organic matter (OM) content, high salinity and/or acid pH (Adriano 2001). Edible plants grown in contaminated soils may accumulate elevated levels of metals that may, when consumed, increase exposures to humans. For example, crops like lettuce, spinach, carrot, radish, and zucchini have been shown to accumulate increased levels of potentially toxic metals such as Mn, Pb, Fe, Zn, Cu, etc. (Ferri et al. 2012, Hooda 1997). Lettuce (*Lactuca sativa* L.) accumulates metals at relatively high internal contents because of the efficient root uptake and subsequent translocation to the shoots (Peijnenburg et al. 2004). Lettuce is also considered a good indicator species for derivation of critical soil Cd concentrations, which generally are used in a first-tier risk assessment (Swartjes 2011).

A conventional method of treatment of contaminated soil suffers from recognizable drawbacks and may involve some level of risk. Bioremediation is a natural process which relies on bacteria, fungi, and higher plants to alter contaminants and environmental conditions as these organisms carry out their normal life functions and can be enhanced by adding organic amendments to soils (Park, Lamb 2011). The addition of organic amendments, such as agroindustrial wastes and composts (C_p) from different origins to contaminated soils can act on a great variety of processes, leading to improvements in physico-chemical soil properties and fertility status and even altering the heavy metal distribution in the soil (Bernal et al. 2007). Thus, high-quality C_p, rich in biologically stable and humified organic matter, non-phytotoxic and showing low concentrations of heavy metals, should be used in reclamation of polluted soil and help to reduce the mobility, the (phyto)availability and toxicity of pollutants and, at the same time, increase soil fertility in order to improve plant development (Kidd et al. 2009). Mechanisms for enhanced bioremediation of heavy metal(loid)s by organic amendments include: immobilization, reduction and rhizosphere modification. Addition of organic amendments (especially humified) to soils increases the immobilization of metal(loid)s through adsorption reactions. The organic amendment-induced retention of metal(loid)s is attributed to an increase in surface charge and the presence of metal(loid) binding compounds (Clark et al. 2007, Gondar, Bernal 2009).

Adsorption of heavy metals strongly depends on soil pH, ion exchange capacity, redox potential and also proportion of silicate clays, organic matter and Fe and Mn oxides (Park, Lamb 2011). When soil pH increases, H^+ dissociates from functional groups such as carboxyl, phenolic, hydroxyl, and carbonyl functional groups, thereby increasing the affinity for metal cations (Bolan et al. 2003). The general order of affinity of heavy metals on organic matter is as follows $Cu^{2+} > Hg^{2+} > Cd^{2+} > Fe^{2+} > Pb^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+} > Zn^{2+} > As^{(V)} > As^{(III)}$.

According to Farrel et al. 2010, Liu et al. 2009 and Herwijnen et al. 2007, where evidence of C_p ability to enhance heavy metal (HM) immobilization was proved, in this study we want to evaluate effect of C_p addition on HM transport into Lactuca sativa L.

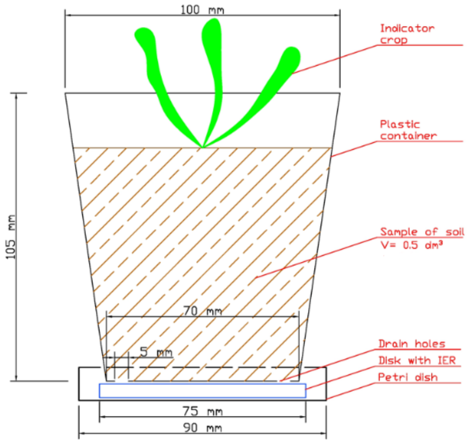
MATERIAL AND METHODS

Characterization of samples origin and experimental design

Contaminated soils used in our experiment come from the Nord France region Noyelles-Godault (50°25'37.7"N 3°00'47.9"E) where a lead smelter called Metaleurop has been under activity for more than one hundred years. Main soil pollutants were Pb, Cd and Zn.

Samples are top soils taken at 0–25 cm deep from different distance of smelter. For each soil many point samplings were realized to cover the entire plot and to constitute large amounts (more than 50 kg). There were formed three soil samples with different level of Pb contamination: M200 (200 ppm), M500 (500 ppm), M700 (800 ppm). At laboratory, samples were air-dried, and then sieved to pass through a 10 mm mesh. Prior to use, they were stored in plastic container in a dry (not humid) chamber. From these representative samples, subsamples were prepared according to the CSN ISO 11464 standard. Our hypotheses were tested by pot (Figure 1) experiment (Table 1) which was carried out in grow box for 48 days in determined conditions. Day mode was set to 12 h with light intensity of 350 $\mu mol \cdot m^{-1} \cdot s^{-1}$. Day temperature was 20°C and humidity was 67%, night temperature was 18°C and night humidity was 71%. Each type of soil was placed into pot in three repetitions without C_p amendment and in three repetitions with C_p amendment. Also control (non-contaminated) sample variants were set. C_p was obtained from the Central Composting Plant in Brno which is registered for agriculture use in the Czech Republic. The C_p amendment represented dose of 50 $t \cdot ha^{-1}$. The indicator plant lettuce was seeded next. During cultivation the pots were watered three times a week with 60 ml of demineralised water. After 48 days the pots were emptied and biomass of roots and leaves and soil were stored separately.

Figure 1 Proportions of experimental pot containing soil sample and monitoring plant



Soil samples analysis

The soil samples collected from the pot experiment were air-dried, crushed to pass through a 2 mm stainless steel sieve, and then passed through a 250-µm sieve with an ultracentrifugal mill (ZM 200), and then stored in polypropylene bottles.

Table 1 Pot experiment variants set for grow box

Soil sample	Characteristic	Repetitions	Amount of substrate in pot (soil + compost)
M2007	Non-contaminated, control sample	3x	900 g
M2007 + K	M2007 with compost amendment (50 t · ha^{-1})	3x	863.7 + 36.3 g
M200	Soil contaminated with approx. 200 ppm Pb	3x	900g
M200 + K	M200 plus compost amendment (50 t · ha^{-1})	3x	863.7 + 36.3 g
M500	Soil contaminated with approx. 500 ppm Pb	3x	900g
M500 + K	M500 plus compost amendment (50 t · ha^{-1})	3x	863.7 + 36.3 g
M700	Soil contaminated with approx. 800 ppm Pb	3x	1000g
M700 + K	M700 plus compost amendment (50 t · ha^{-1})	3x	963.7 + 36.3 g

Pseudototal Cd, Pb and Zn concentration

Pseudototal Cd, Pb, and Zn concentrations in all soil samples (Table 2) were obtained by Hot Block system-assisted digestion (Environmental Express® SC100, Charleston, SC, USA) and determined by flame atomic absorption spectrometry (FAAS, AA-6800, Shimadzu, Japan): 300 mg of soil samples were digested in a mixture of 1.5 ml HNO_3 (70%) and 4.5 ml HCl (37%). Quality control was based on the use of blanks and the internal reference material (Pelfrêne et al. 2015). The mean recovery rates (%) in reference soil material are 92.2% (Cd), 101.7% (Pb), and 101.7% (Zn).

Table 2 Heavy metals pseudo-total concentrations in soil samples

	M2007	M200	M500	M700	C_p
Cd (mg · kg^{-1})	0.22 ± 0.06	3.80 ± 0.55	9.70 ± 0.70	14.13 ± 1.40	0.89 ± 0.27
Pb (mg · kg^{-1})	20.10 ± 3.02	214.50 ± 23.53	531.6 ± 45.70	730.60 ± 67.40	1.85 ± 0.71
Zn (mg · kg^{-1})	61.50 ± 1.60	330.3 ± 43.32	583.50 ± 49.30	999.76 ± 87.80	3139.06 ± 27.54

Heavy metal concentrations in plant tissues

In the laboratory, aboveground parts of lettuce were washed in three successive baths of osmotic water. Excess water on these plant organs was blotted by a clean paper towel before cutting them into small pieces. The belowground organs were washed thoroughly with tap water to remove the soil particles.

Rhizomes were separated from roots with scissors. Both organs were rinsed in three successive RO water baths, and then cut into small pieces. All samples were oven-dried at 40°C, and then ground and sieved to 250 µm using a knife mill (GM200) for leaves and roots, and an ultracentrifuge mill (ZM200) for stems and rhizomes. Sample digestion was realized by adding 5 ml of 70% HNO_3 (Baker Analyzed Reagent) in a tube (50 ml Digestion Cup) containing 300 mg of plant powder. The tube was covered with a watch glass and heated at 80°C on the hot block (HOT BLOCK Environmental Express) for 1 hour under the hood box. After cooling, 5 ml of 30% H_2O_2 (Baker Analyzed Reagent) were added to the digest, and the mixture was again heated at 80°C for 3 hours. After cooling, the volume was adjusted to 25 ml with double-distilled water and filtered (0.45 µm acetate membrane filters, Minisart).

Filtrates were stored at 4°C before Cd, Pb, and Zn determination by atomic absorption spectrophotometry (AA-6800, Shimadzu).

Quality control for chemical extraction and digestion was performed by including blanks, internal and certified (INCT-PVTL-6) reference materials. The mean recovery rates in the reference material are 97.0% (Cd), 107.3% (Pb), and 104.9% (Zn). The residual moisture of the dried plant samples was measured by weighing a sample (≤ 10 g) before and after passage in an oven at 105°C (ISO 11465) and was used to apply the moisture correction factor so as to express results on dry weight (DW) basis.

Statistical analysis

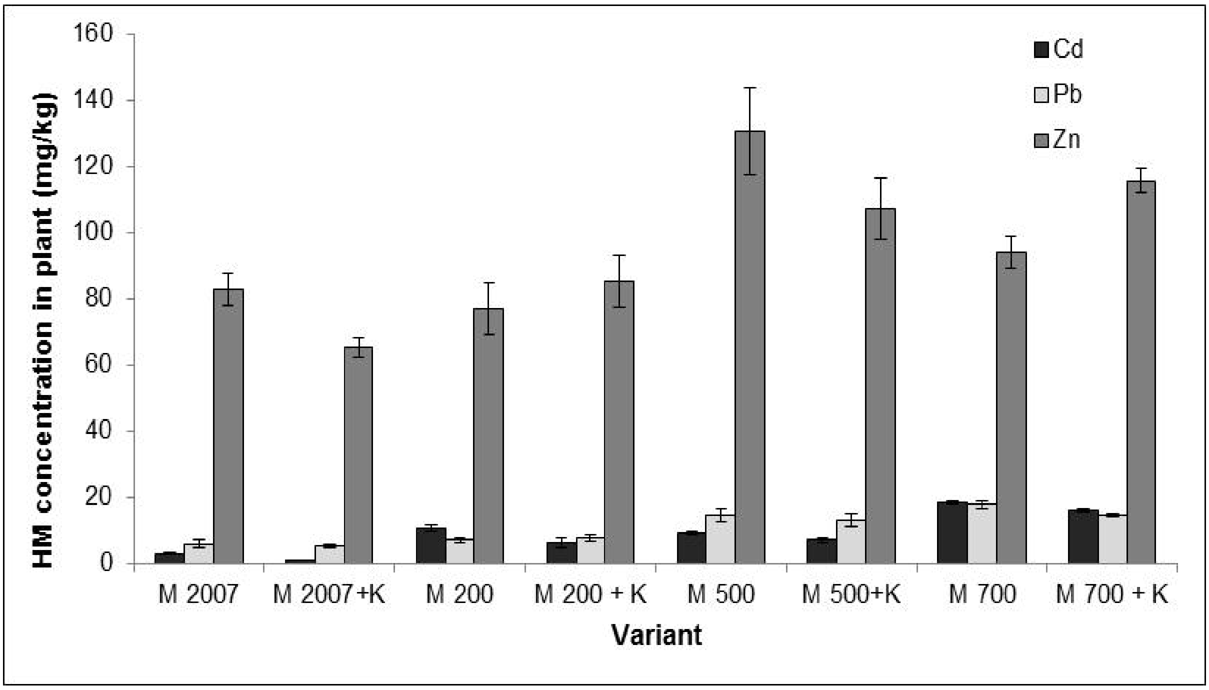
Potential differences in values of pseudo-total concentrations of heavy metals and their plant biomass content were identified by ANOVA in combination with Tukey´s test (P<0.05).

RESULTS AND DISCUSSION

Heavy metal concentration in plant tissues

The soil immediately surrounding plant roots (rhizosphere) is a modified microbiologial and chemical environment due to plant-soil-microbe interactions. The changes in soil chemistry due ‘ to soil amendment and plant growth can therefore influence the transformation, mobility and bioavailability of metals (Park, Lamb 2011). Results in Figure 2 present lower HM uptake in C_p amended variants. The significant differences are observed between all of amended and non-amended variants for Cd as we expected due to (Farrel et al. 2010, Liu et al. 2009, Herwijnen et al. 2007). There are also significant differences between variously contaminated variants and control for Cd. The same differences are observed in variants with high Pb contamination, but these are not significant excluding M700 variant. The different results were found for Zn, where C_p amendment enhanced Zn uptake, especially in highly contaminated variant M700. Zinc is essential element for plants. It is usually found in higher concentrations.

Figure 2 Heavy metal concentrations in plant tissues obtained after the experiment, (Cd; Pb and Zn) at level 0.05 (ANOVA; P<0.05; post-hoc Tukey`s test) between individual variants of experiment



Bioconcentration factor

The bioconcentration factor (BCF) represents a ratio of metal content in plant and soil content (Waterlot et al. 2013).

This parameter allows evaluating plant ability to transfer heavy metals from soil to tissues. As Table 3 shows in compost amended variants was BCF lower in the most of cases (Cd, Pb), suggesting positive compost effect to reduction of HM uptake by plants.

Table 3 Bioconcentration factor values

Variant	Bioconcentration factor		
	Cd	Pb	Zn
M 2007	8.48	0.26	1.70
M 2007+K	1.84	0.22	0.99
M 200	2.64	0.03	0.24
M 200+K	1.51	0.03	0.27
M 500	0.89	0.03	0.25
M 500+K	0.69	0.02	0.22
M 700	1.13	0.02	0.10
M 700+K	1.01	0.02	0.13

CONCLUSION

Nowadays trends of bioremediation are heading to using compost as reclamation substrate on heavy metal contaminated areas. The aim of this experiment was evaluation of compost amendment on HM uptake. We conclude that compost amendment definitely enhances HM immobilization and subsequently bioavailability of cadmium and lead. Lower concentrations of those two metals were found in plants grown on compost amended variants. There appeared different behaviour of zinc. The reason of different zinc behaviour could be higher affinity to forming chelates with organic compounds, which are readily available for plant. We find compost suitable as a bioremediation tool, but at first the pollutant type, level of contamination and the target plant must be considered.

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