Effect of compost amendment on arbuscular mycorrhiza in relation to bioavailability of heavy metals in contaminated soils

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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 arbuscular mycorrhiza and accumulation of heavy metals in *Lactuca sativa* grown in contaminated soils. To demonstrate the effect of compost the pot experiment was performed. Eight variants of soils with different concentrations of pollutants with and without compost amendment were prepared.
Contaminated soils we used in our experiment come from the Nord France region Noyelles – Godault. Main pollutants were Pb, Cd and Zn. The highest 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. Moreover, we found highest level of root colonization by arbuscular mycorrhiza in compost amended variants where lowest content of heavy metals in plant tissues was observed. But these results are not significant. Based on these results, we conclude that application of organic waste compost has positive effect on immobilization of heavy metals in contaminated soils and also enhances arbuscular mycorrhiza colonization.

Key - Words: heavy metal mobility, contamination, remediation, arbuscular mycorrhiza

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 [1]. 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 [2]. 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 [3]. 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. [4, 5]. Lettuce (*Lactuca sativa L.*) accumulates metals at relatively high internal contents because of the efficient root uptake and subsequent translocation to the shoots [6]. 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 [7].

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 [8]. 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 [9]. 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 [10]. 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 [11, 12].

Mycorrhizal fungi in a metal-polluted soil are the ones which provide a direct link between the soil and roots by interacting with their host plants to form a symbiotic relationship in the contaminated land [13]. Mycorrhizal fungi are commonly found in the soils of most ecosystems. Mycorrhiza can enhance the transfer of scarce nutrient elements to a plant, and facilitate the toxic metal uptake by its host. There is evidence that arbuscular mycorrhiza (AM) fungi can play a role in increasing the tolerance of some plants to toxic metal contamination by developing the metal tolerance of the fungi themselves and binding the metals to polyphosphates within the fungal hyphae implicated [14,15]. The establishment of the mycorrhizal network offers a number of basic advantages to the host plant for the acquisition of mineral nutrients: i) fungal hyphae extend beyond the area of nutrient depletion surrounding the plant's roots; ii) fungal hyphae greatly increase the surface area available for the absorption of nutrients; iii) the hyphae are able to spread into soil pores that are too small for plant roots to enter [15].

According to evidence of C_p ability to enhance heavy metal (HM) immobilization [16, 17, 18] and knowledge of AM function, in this study we want to compare the influence of C_p as AM enhancer with effect of C_p addition on accumulation of HM in *Lactuca sativa*.

Material and Methods

Characterization of samples origin and experimental design

Contaminated soils we used in our experiment come from the Nord France region Noyelles – Godault 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 experiment (tab 1) which was carried out in grow box for 48 d in determined conditions. Day mode was set to 12 h with light intensity of 350 μmol/m·s. Day temperature was 20°C and humidity was 67%, night temperature was 18°C and 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/ha. 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 d the pots were emptied and biomass of roots and leaves and soil were stored separately.

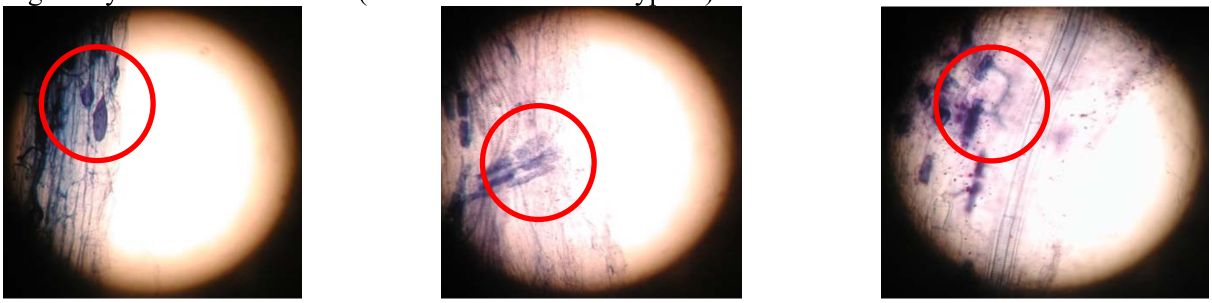
Tab. 1 Pot experiment variants

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)	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)	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)	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)	3x	963.7 + 36.3 g

Mycorrhizal colonization of roots

The percentage of mycorrhizal colonisation was determined in root samples, which were taken from root system of *Lactuca sativa* (from each experimental container). Root samples (3 g fresh weight) were washed in tap water and before processing stored in FAA solution (50% ethanol, acetic acid, formaldehyde). Fixed root samples were washed; cleared and stained according Koske & Gemma [19]. Stained roots were cut into 1.5 cm segments, mounted on microscope slides in glycerol gelatin and evaluate microscopically (200x MA) by Giovanetti & Mosse [20].

Fig. 1 Mycorrhizal characters (vesicle – arbuscula – hyphae)



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

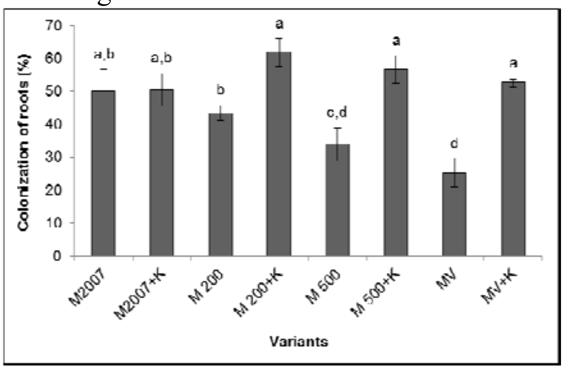
Potential differences in values of mycorrhizal colonization and content of heavy metals in plant biomass (plant biomass) were identified by ANOVA in combination with Tukey´s test (P<0.05). Regression was used for testing relationship between the level of mycorrhizal infection and content of heavy metals in plant biomass.

Results and Discussion

Mycorrhizal colonization of roots

Arbuscular mycorrhiza symbiosis is important mutualistic association that is formed between plant roots and soil fungi. It can be found in majority of crops. Mycorrhizal fungi are able to improve plant nutrition and growth, as well as their resistance to biotic and abiotic stress factors [21].

Fig. 2 Colonization of *Lactuca sativa* roots by AM fungi in soil



AM was observed in all variants and repetitions. The colonization varied within limits 25 – 62 %. The highest value was detected in M200+K variant, the lowest was detected in M700 in comparison with other variants. Figure 2 shows significant differences between equally contaminated variants with and without C_p amendment excluding control variant M2007. There is also shown increasing level of AM colonization in relation with increasing HM concentration. C_p amendment significantly enhanced AM colonization of lettuce roots, what is confirmed by results in Alguacil et al. [21].

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 [8]. Results in Tab. 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 [16, 17, 18]. 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 variants M500 and M700. Zinc is essential element for plants. It is usually found in higher concentrations.

Tab. 2 Heavy metal concentrations in plant tissues, different small letters indicate a significant differences in concentration of individual heavy metals (Cd; Pb and Zn) at level 0.05 (ANOVA; P<0.05; post-hoc Tukey`s test) between individual variants of experiment.

Soil sample	Cd (mg/kg)	±SE	Pb (mg/kg)	±SE	Zn (mg/kg)	±SE
M2007	2.90[a]	0.10	5.85[a]	1.28	82.89[a]	4.75
M2007 + K	0.76[b]	0.09	5.22[a]	0.63	65.13[b]	2.99
M200	10.63[b]	1.07	7.02[a]	0.83	76.96[a]	7.85
M200 + K	6.18[c]	1.54	7.50[a]	0.93	85.31[a]	7.68
M500	8.96[d]	0.54	14.32[b]	1.91	130.66[c]	13.32
M500 + K	6.94[c]	0.83	12.88[b]	1.91	107.28[d]	9.28
M700	18.54[e]	0.501	17.79[c]	1.23	93.99[a]	5.05
M700 + K	15.94[f]	0.61	14.28[b]	0.54	115.74[d]	3.72

The relationship between concentration of heavy metal in plant tissues and colonization of roots by AM

The correlation between HM concentration and AM colonization for Pb and Cd was found. As the bold statistical parameters shows in tables 3 and 4, concentration of HM in plant decreased with increasing degree of AM. Reduced HM uptake, especially at higher soil metal contents, indicates a changed metal tolerance strategy in colonized plants similarly to results of Vogel-Mikuš et al. [22]. Significant correlation for Zn was not found.

Tab. 3 Regression analysis of the relationship between the level of colonization of roots by AM and concentration of cadmium in plant tissues

Parameter	Value
Multiple regression	**0.46932**
R-squared value	0.22026
Statistical power	**6.21467**
Probability	**0.02068**
Standard error	5.35133

Tab. 4 Regression analysis of the relationship between the level of colonization of roots by AM and concentration of lead in plant tissues

Parameter	Value
Multiple regression	**0.50746**
R-squared value	0.25751
Statistical power	**7.63010**
Probability	**0.01137**
Standard error	4,31191

Tab. 5 Regression analysis of the relationship between the level of colonization of roots by AM and concentration of zinc in plant tissues

Parameter	Value
Multiple regression	0.18086
R-squared value	0.03271
Statistical power	0.74397
Probability	0.39770
Standard error	23.59601

Comment for Table 3 – 5: The significant correlation between AM and concentration of heavy metals are shown in bold. The relationship between colonization of roots by AM and concentration of individual heavy metals was always compared for one variants of experiment.

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 correlation of two aspect caused by compost adding. We conclude that compost amendment definitely enhances AM colonization of *Lactuca sativa* roots even in highly contaminated soil. One of the characters of this symbiosis is adapting strategy in stressful environment to protect the host plant. This strategy was confirmed for lead and cadmium, where the significant correlation between AM colonization and reduced HM uptake was observed. There appeared different behaviour of zinc, which was not evidential. 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 bioremediation tool, but at first the pollutant type, level of contamination and the target plant must be considered.

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