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ПОТЕНЦИАЛ НА СЛЪНЧОГЛЕД (*HELIANTHUS ANNUUS* L.) ЗА ФИТОРЕМЕДИАЦИЯ НА ЗАМЪРСЕНИ С ТЕЖКИ МЕТАЛИ ПОЧВИ
POTENTIAL OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) FOR PHYTOREMEDIATION OF SOILS CONTAMINATED WITH HEAVY METALS

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Abstract

A field study was conducted to evaluate the efficacy of the sunflower plant for phytoremediation of contaminated soils in the absence and presence of organic soil amendments (compost and vermicompost). Tested organic amendments significantly influenced the uptake of Pb, Zn and Cd by the sunflower plant. The compost and vermicompost treatments significantly reduced the heavy metals concentration in the sunflower seeds, meals and oils, but the effect differed among them. There was a dose effect of the amendments as well. The 40 t/da compost and 20 t/da treatment led to decreased heavy metal contents in the sunflower oil below the regulated limits. The possibility of further industrial processing will make sunflower an economically interesting crop for farmers applying the phytoremediation technology.

Key words: phytoremediation, heavy metals, organic amendments, sunflower.

INTRODUCTION

Heavy metal contamination of agricultural soils is a worldwide problem. The remediation of metal contaminated sites often involves expensive and environmentally invasive and civil engineering based practices (Marques et al., 2008). A range of technologies such as fixation, leaching, soil excavation, and landfill of the top contaminated soil ex situ have been used for the removal of metals. Many of these methods have high maintenance costs and may cause secondary pollution (Haque et al., 2008) or adverse effect on biological activities, soil structure, and fertility (Pulford and Watson, 2003). Phytoremediation is an emerging technology, which should be considered for remediation of contaminated sites because of its cost effectiveness, aesthetic advantages and long term applicability (Marques et al., 2008; Chaney et al., 1997). This technology can be defined as the efficient use of plants to remove, detoxify or immobilize environmental contaminants in soils, waters or sediments through the natural, biological, chemical or physical activities and processes of the plants (Cuira et al., 2005; Lone et al., 2010). It is best applied at the sites with shallow contamination of organic, nutrient or metal pollutants (Schnoor, 1997; Yang et al., 2005). This plant based technique is essentially an agronomic approach and its success depends ultimately on agronomic practices applied at the site. Addition of organic matter amendments, such as compost, fertilizers and wastes, is a common practice for immobilization of heavy metals and soil amelioration of contaminated soils (Clemente et al., 2005). Organic amendments are able to improve soil physical, chemical and biological properties by: (i) raising the pH, (ii) increasing the organic matter content, (iii) adding essential nutrients for plant growth, (iv) increasing the water holding capacity, and (v) modifying heavy metals bioavailability (Walker et al., 2003, 2004; Angelova et al., 2013).

The use of crop plants for phytoremediation of contaminated soils has the advantages of their high biomass production and adaptive capacity to variable environments (Komarek et al., 2007; Fassler et al., 2010). However, to succeed they must be tolerant to the contaminants and be capable of accumulating significant concentrations of heavy metals in their tissues. Additionally, crops could make the long time-periods for decontamination more acceptable, economically and environmentally.

If the contaminated biomass may be further proceed for added value products (not only concentrated on deposits of hazardous wastes), then such fact represents an improvement of economical efficiency of phytoremediation technology. Industrial plants, i.e. energy crops or crops for bio-diesel production, are therefore the prime candidates as plants for phytoremediation. The use of energy and/or bio-diesel crops as plants for phytoremediation would give contaminated soil a productive value and decrease remediation costs.

Researchers have observed that some plant species are endemic to metallicferrous soil and can tolerate greater than the used amount of heavy metal or other compounds (Peralta et al., 2001). Plants such as Indian mustard (*Brassica juncea*), Corn (*Zea mays* L.) or sunflower (*Helianthus annuus* L.) show high tolerance to heavy metals and therefore, are used in phytoremediation studies (Schmidt, 2003; Tang et al, 2003; Pilon Smith, 2005). It was found that sunflower could be successfully employed for decontamination of soils polluted with heavy metals and radionuclides (Adler,1996). Dushenkov et al. (1995) found in the laboratory that within 24 h roots of sunflower plants were able to substantially reduce the levels of Cd, Cr (VI), Cu, Mn, Ni, Pb, Sr, U (VI), and Zn in water, bringing metal content close to or below the discharge limits. The ability of sunflower to accumulate uranium (U) was reported by Salt et al. (1998) and Jovanovic et al. (2001). Apart from the fact that sunflower intensely takes up some heavy metals and radio-nuclides, it also has high biomass, enabling it to accumulate and extract significant amounts of pollutants from the rhizosphere. Nehnevajova et al. (2005) evaluated the potential use of sunflower plants for phytoremediation and reported that sun-flower can be used for the phytoextraction of metal-contaminated soils. In contrast, according to Madejon et al. (2003) the potential of sunflower for phytoextraction is very low.

Cited results suggest that sunflower may be suitable for remediation of soils and waters polluted with heavy metals and radionuclides.

The aim of this experiment was to compare the effect of organic soil amendments (compost and vermicompost) applied to the soil on the accumulation of heavy metals by the sunflower (*Helianthus annuus* L), as well as the possibilities to use the plant for phytoremediation of heavy metal contaminated soils.

MATERIALS AND METHODS

The experiment was performed on an agricultural field contaminated by the Non-Ferrous-Metal Works near Plovdiv, Bulgaria. The field experimental was a randomized complete block design containing five treatments and four replications (20 plots). The treatments consisted of a control (no organic amendments), compost amendments (added at 20 t/daa and 40 t/da), and vemicompost amendments (added at 20 t/da and 40 t/da). Plot size was 24 m^2 (3 m x 8 m). The soil was excavated from each plot and combined and mixed with amendments a 6 week before sunflower planting.

Characteristics of soils and organic amendments are shown in Table 1. The soils used in this experiment were slightly acidic, with moderate content of organic matter and essential nutrients (N, P and K) (Table 1). The pseudo-total content of Zn, Pb and Cd is high (1430.7 mg/kg Zn, 876.5 mg/kg Pb and 31.4 mg/kg Cd, respectively) and exceeds the maximum permissible concentrations (320 mg/kg Zn, 100 mg/kg Pb, 2.0 mg/kg Cd).

Table 1. Characteristics of the soil and the organic amendments used in the experiment

Parameter	Soil	Compost	Vermicompost
pH	6.5	6.9	7.5
EC, dS/m	0.2	0.2	2.2
Organic C,%	2.22	40.50	21.43
N Kjeldal,%	0.24	2.22	1.57
C/N	9.25	18.24	13.65
Pseudo-total P, mg/kg	642	12653.9	10210.8
Pseudo-total K, mg/kg	5517.5	6081.7	10495.1
Pseudo-total Pb, mg/kg	876.5	12.0	32.3
Pseudo-total Zn, mg/kg	1430.7	170.8	270.3
Pseudo-total Cd, mg/kg	31.4	0.19	0.69

The test plant was sunflower (*Helianthus annuus* L). It was selected because it is known that sunflower is the fast-growing deep-rooted industrial oil crop (Prasad, 2004; Tahsin and Yankov, 2007) with a high biomass producing plant species (Zhuang et al., 2005) to remove heavy metals such as zinc or copper from contaminated environment (Nehnevajova et al., 2005).

Sunflower seeds were sown in each plot, between row and within row distances were 70 and 20 cm, respectively. Each hole was 7 cm deep, containing 3 seeds. After sunflower had grown for 15 days, the sunflower was thinned to one plant per hole.

Upon reaching commercial ripeness, the sunflower plants were gathered. Five plants per treatment (control, 20 t/da compost, 40 t/da compost, 20 t/da vermicompost and 40 t/da vermicompost) where chosen at random for analysis. Roots were excavated and separated from the adhering soil by washing. Shoots were divided immediately into stems, leaves and sunflower heads. The samples were packed into plastic bags and immediately transported into the laboratory. Here, they were well washed with tap water, cut into pieces, and then oven-dried for 78 hours at 60 ^{0}C. The oil from sunflower was derived under laboratory conditions through an extraction method with Socksle's apparatus. The contents of heavy metals (Pb, Zn and Cd) in the plant material (roots, stems, leaves and seeds) and in the oils and meals of sunflower were determined by the method of the dry mineralization.

Total content of heavy metals in soils was determined in accordance with ISO 11466. The mobile heavy metals contents were extracted by a solution of AB-DTPA (1 M NH_4HCO_3 and 0.005 M DTPA, pH 7.8) (Soltanpour and Schwab, 1997).

To determine the heavy metal content in the plant and soil samples, inductively coupled emission spectrometer (Jobin Yvon Horiba "ULTIMA 2", France) was used.

RESULTS AND DISCUSSION

Humic acids from organic amendments tend to form complexes that are different for each metal and also depend on soil conditions such as pH, cation exchange capacity and clay mineral fraction (Barancikova and Macovnikova, 2003). Organic matter not only forms complexes with these metals but it also retains them in exchangeable forms, affecting each metal differently. Some metals are bound and rendered unavailable while others are bound and readily available (Kononova, 1996). The results of the previous study (Angelova et al., 2013) indicated that enrichment of soil with organic matter could reduce the content of bioavailable metal species as a result of complexation of free ions of heavy metals. This is indicative of heavy metals immobilisation by humic substances from compost and vermicompost application. Obtained results appear that verify the function of humic acid in improving phytoremediation efficiency of soils contaminated with heavy metals; and potential environmental availability of metals may be controlled by soil organic amendments.

***Accumulation of Pb, Zn and Cd by sunflower without* organic amendments**

The results for the influence of the organic additives on the accumulation and distribution of Pb, Zn and Cd in the sunflower plants are presented in Table 2. Considerably lower values were established in the roots of sunflower compared to the above-ground parts of sunflower. The content of Pb in the roots of sunflower without amendments reached to 255.9 mg/kg, Zn – 431.6 mg/kg and Cd – 17.7 mg/kg. The obtained results could be explained with the anatomic and biologic peculiarities of the sunflower plants. Sunflower plants have strong taproots, from which deeply-penetrating lateral roots develop, with a strong ability to uptake the nutrients.

The heavy metals contents in the stems of the sunflower were considerably lower compared to those in the root system, which showed that their movement through the conductive system was strongly restricted. The content of Pb in the stems of sunflower without amendments reached to 60.9 mg/kg, Zn – 334.4 mg/kg and Cd – 11.1 mg/kg.

The highest was the accumulation of Pb, Zn and Cd in the leaves of sunflower, where Pb reached to 461.3 mg/kg, Zn – to 793.1 mg/kg, and

Table 2. Content of Pb, Cd and Zn (mg/kg) in sunflower (without organic amendments)

Element	Roots x±sd	Stems x±sd	Leaves x±sd	Seeds x±sd	Oil x±sd	Meal x±sd
Pb	255.9±2.5	60.9 ±0.8	461.3±3.6	6.3±0.2	0.126±0.005	3.3±0.2
Cd	17.7±1.3	11.1±0.5	138.8±2.1	7.5±0.1	nd	12.2±0.1
Zn	431.6±5.3	334.4±2.8	793.1±6.8	116.5±2.0	2.99±0.4	187.1±2.1

Cd - to 138.8 mg/kg. Their stronger accumulation in leaves was probably due to the fact that the leaves of sunflower were covered with short and rough pappus, which contributed to the fixing of the aerosol pollutants and for their accumulation. The results corresponded with those obtained by Eckhardt and Khanal (1999) and by Lombi et al. (1998) according to whom Zn and Cd were predominantly accumulated in the leaves of sunflower. The obtained results matched well with those from Jadia and Fulekar (2008) who found that shoots of sunflower is the major organ of heavy metals accumulation.

The heavy metal content in the seeds of the sunflower was lower in comparison to that in the roots and leaves. The heavy metal accumulation in sunflower seeds was likely caused by the conductive system. The content of Pb in the seeds of sunflower without amendments reached to 6.3 mg/kg, Zn – 116.9 mg/kg and Cd – 7.5 mg/kg. The contents of Pb and Zn in the seeds of sunflower were not reached the critical levels of 30 mg/kg Pb and 300 mg/kg Zn recommended for livestock. However, the Cd accumulated in quantities considerably above the proposed maximum levels tolerated by livestock (0.5 mg/kg Cd) (Chaney, 1989) and the threshold recommended for human nutrition (1 mg/kg) (Hapke, 1996).The results of Chizzola (1998) who reported that in the seeds of sunflower the highest values were for Cd were confirmed. Our results were in contradiction with the found from Kastori et al. (1998) and Korenovska and Palacekova (2000), who state that insignificant quantities of Zn, Pb, and Cd were accumulated in the reproductive organs of rapeseed and sunflower, when grown on soils polluted with heavy metals.

The contents of heavy metals in sunflower oil also were determined. The obtained results showed that the main part of the heavy metals contained in the seeds of sunflower was not transferred in the oil during during the seed processing, due to which their content in the oil was considerably lower. Lead in sunflower oil reached 0.126 mg/kg, Zn to 2.99 mg/kg, and the content of Cd is below the limits of detection of the apparatus. The contents of Zn and Cd in the sunflower oil were lower then the accepted maximum permissible concentrations (10.0 mg/kg Zn). Although the contents of heavy metals in the oil was lower compared with the seeds, the quantities of Pb in the sunflower oil, were higher than the accepted maximum permissible concentrations (0.1 mg/kg Pb). The results matched well with those of Anonymous (1997) i.e., that the contents of Cu, Fe, and Pb was low and that there was no contents of Cd in the sunflower oil.

Sunflower meal is the by-product of the oil extraction process. The contents of heavy metals in sunflower meal also were determined. The obtained results showed that the heavy metal content in the sunflower meals was higher compared to that in the seeds. Trace metals observed in the seeds are almost exclusively transferred to meals after seed crushing (Darracq et al., 2004). The content of Pb in the meals of sunflower without amendments reached to 3.3 mg/kg, Zn – 187.1 mg/kg, and Cd – 12.2 mg/kg. The contents of Pb and Zn in the meal of sunflower were not reached the critical levels of 30 mg/kg Pb and 300 mg/kg Zn recommended for livestock. However, the Cd accumulated in quantities considerably above the proposed maximum levels tolerated by livestock (0.5 mg/kg Cd).

The distribution of the heavy metals in the organs of the sunflower has a selective character that in sunflower decreases in the following order: leaves > roots> stems > seeds. The main part of Pb accumulates in the leaves (59%) and a very small amount is contained in the seed (1%). Analogous results are obtained for Zn and Cd, where the main part is accumulated in the leaves (47 and 79%, respectively). The seeds contain only 2% of the total assimilated Cd of sunflower plants. The content of Zn in the seeds is significantly higher - 7% of the total amount of Zn.

***Organic additives impact* on the Pb, Zn and Cd accumulation in sunflower**

According to the literature the content of organic substance in soil has a significant impact on uptake and translocation of heavy metals in soil and their uptake by plants. Zn, Pb and Cd are adsorbed on organic matter, which generate stable forms and lead to their accumulation in organic horizons of soil and peat (Kabata Pendias, 2001).

The results obtained by us showed that Pb, Zn and Cd uptake by sunflower plants depended on the soil amendments and treatment (type and rate). The application of compost and vermicompost significantly influenced the uptake of Pb, Zn and Cd by the tested plant. Changes in heavy metals content in sunflower organs were rather complex. Impact of organic amendments on heavy metals accumulation in organs of sunflower depended significantly on their quantity. The application of 20 t/da of compost leads to increased the content of Pb in the roots and stems of sunflower, while the addition of 40 t/da leads to lower the contents of heavy metals. The application of vermicompost led to decreased Pb, Zn and Cd content in the roots and stems of sunflower (Figure 1).

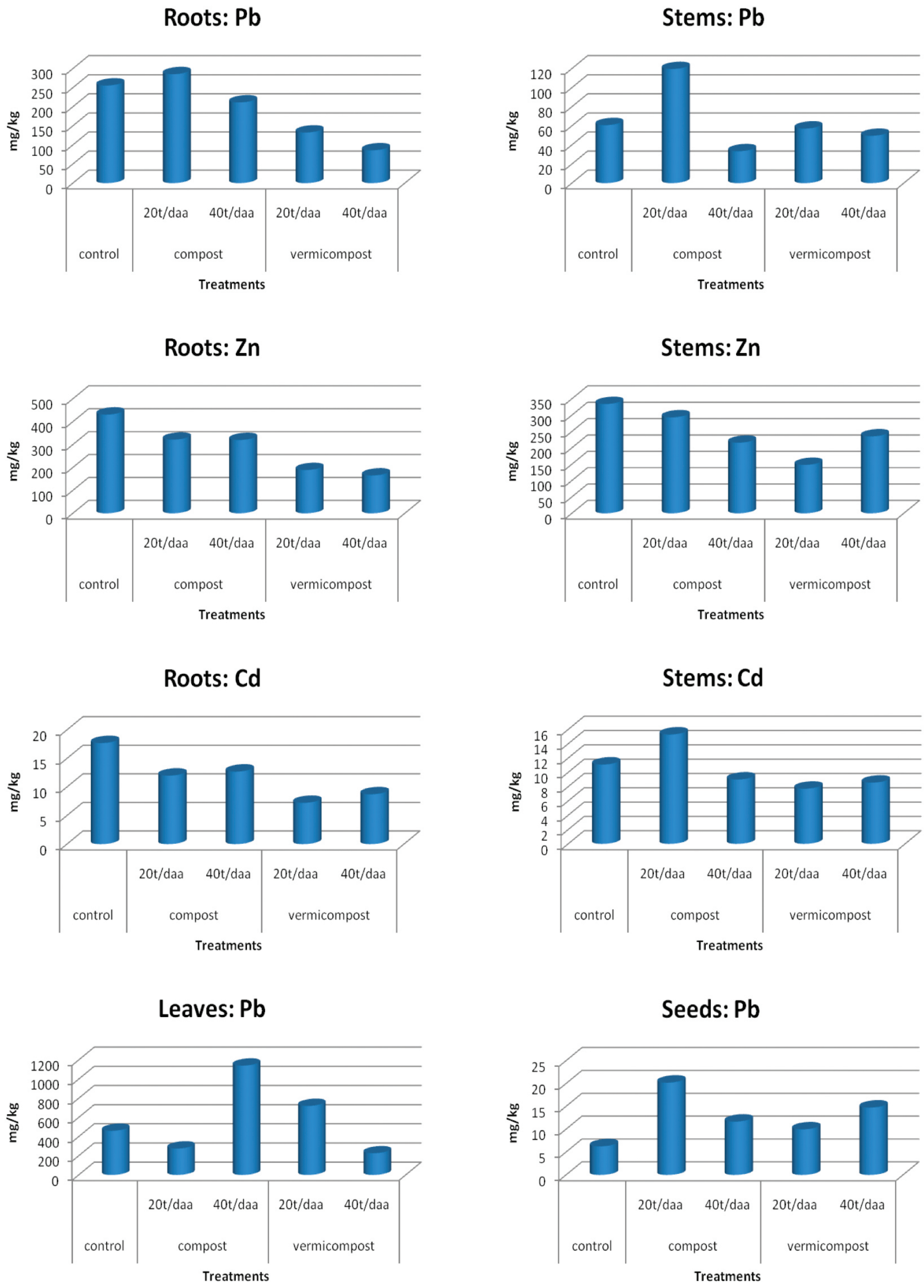

Fig. 1a. *Effect on the organic amendments (compost and vermicompost) on the content of Pb, Cd and Zn (mg/kg) in vegetative and reproductive organs of sunflower plants, oil and meal*

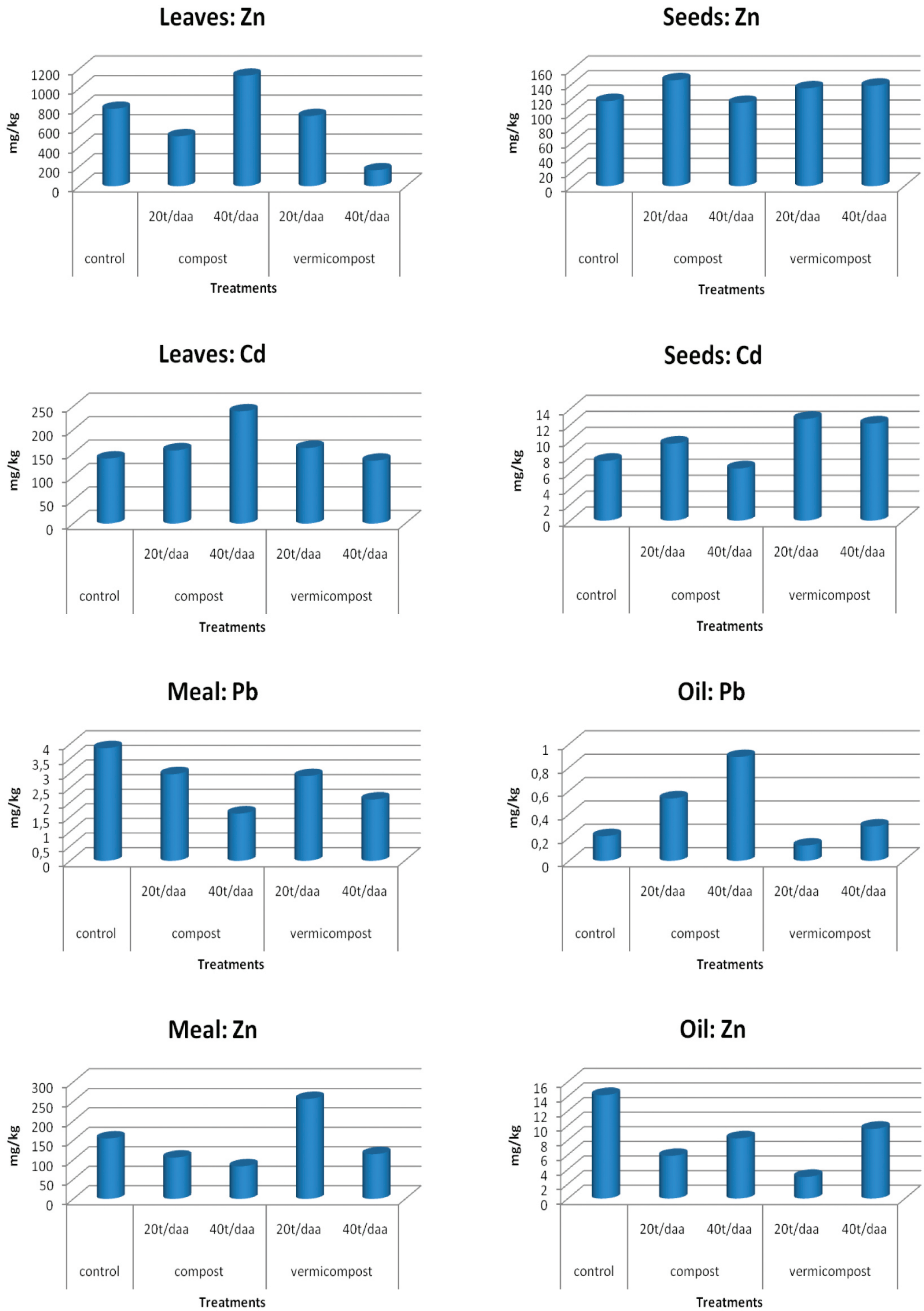

Fig. 1b. *Effect on the organic amendments (compost and vermicompost) on the content of Pb, Cd and Zn (mg/kg) in vegetative and reproductive organs of sunflower plants, oil and meal*

The application of 20 t/da of compost leads to decreased the content of heavy metals in the leaves, while the addition of 40 t/da leads to increased their content. The application of 40 t/da of vermicompost leads to increased content of Pb and Cd in the leaves of sunflower, while the addition of 20 t/da leads to decreased their content. Addition of vermicompost leads to an increase in Zn content in the leaves, as this decline is less pronounced on the treatment with 20 t/da vermicompost. It has been found relationship between the content of heavy metals in the leaves of sunflower and quantity of the vermicompost (Figure 1a, b).

The influence of organic amendments on the accumulation of Pb in the seeds depends on their quantity. Increasing the content of Pb in the seeds compared to the control (sunflower without amendments) is strongly expressed, as in the treatments with compost content of Pb is increased by 6.3 mg/kg to 20.3 mg/kg (20 t/da compost) and 11.7 mg/kg (40 t/da of compost). Similar results were obtained for Pb in the treatments with the 40 t/da vermicompost where Pb content is increased to 14.8 mg/kg and these concentrations were below the maximum permissible concentrations for fodder (30 mg/kg) (Hapke, 1994). Increasing the content of Cd in seeds compared to control is evident in the treatments with vermicompost (up to 12.2 mg/kg - 12.8 mg/kg). It has been not found relationship between the content of heavy metals in the seeds of sunflower and quantity of the vermicompost. The influence of compost on the content of Cd is not unidirectional. Reducing the content of Cd in seeds compared to control only occurs in treatments with 40 t/da of compost (up to 6.6 mg/kg) and this concentration were above maximum permissible concentrations (0.5 mg/kg) for fodder (Hapke, 1994). Similar results were obtained for Zn. The application of vermicompost leads to an increase in the Zn content of the seeds to 133.8 mg/kg - 137.5 mg/kg. Reducing the content of Zn in the seeds compared to control only occurs in the treatments with the 40 t/da of compost (to 113.9 mg/kg) and these concentrations were below the maximum permissible concentrations (300 mg/kg) (Hapke, 1994).

The influence of organic amendments on the content of Pb and Zn in the oil is not unidirectional and depends on their quantity. Addition of compost leads to increased content of Pb in the oil, this increase was greater in the treatments with 40 t/da of compost (0.28 mg/kg). Application of 40 t/da vermicompost led to decreased Pb in the oil until 0.106 mg/kg (within the maximum permited concentration of 0.1 mg/kg in oil), while the addition of 20 t/da vermicompost had no significant impact. Application of 40 t/da of compost and 40 t/da vermicompost reduces Zn content in the oil (to 2.62 mg/kg and 2.85 mg/kg respectively), while in the treatments with 20 t/da of compost and vermicompost rises slightly (3.36 mg/kg - 3.37 mg/kg, respectively). In all treatments, however, Zn content in the oil is lower than the maximum permitted concentration of oil from plant origin (10 mg/kg).

The 40 t/da vermicompost addition was especially effective for the reduction of Pb content in sunflower oils bellow the regulated limits (0.1 mg/kg Pb), and oil can be used for human consumption**.**

The application of the compost and vermicompost leads to an increase in the content of Pb in the meal compared with the control. Increasing the amount of the amendment (40 t/da compost) increased the content of Pb to 5.58 mg/kg and these concentrations are lower than the maximum concentration limits for the feed of the animals (30 mg/kg). Addition of 20 t/da of compost and vermicompost leads to a slight increase in Zn content in the meal respectively to 210.3 mg/kg and 209.3 mg/kg, and these values are lower than the maximum permissible concentrations of forage (500 mg/kg). Treatments with 40 t/da of compost and 40 t/da vermicompost there are no significant influence over the content of Zn in the meal compared to control.

CONCLUSIONS

1. The distribution of the heavy metals in the organs of the sunflower has a selective character that in sunflower decreases in the following order: leaves > roots> stems > seeds.

2. Tested organic amendments significantly influenced the uptake of Pb, Zn and Cd by sunflower plant. The compost and vermicompost treatments significantly reduced heavy metals concentration in sunflower seeds, meals and oils, but the effect differed among them. Also, there was a dose effect for amendments.

3. The 40 t/daa vermicompost treatment led to decreased Pb content in sunflower oil bellow the regulated limits (0.1 mg/kg Pb), and oil can be used for human consumption**.**The possibility of further industrial processing will make sunflower economically interesting crops for farmers of phytoremediation technology.

4. Sunflower is a plant which is tolerant to heavy metals and can be successfully used in the phytoremediation of heavy metal contaminated soils. The processing of seeds to oil and using the obtained oil for nutritional purposes will greatly reduce the cost of phytoremediation.

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