# 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 sunflower plant for phytoremediation of contaminated soils in the absence and presence of organic soil amendments (compost and vermicompost). 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 5 and 10%), and vemicompost amendments (added at 5 and 10%). Heavy metal contents in roots, stems, leaves and seeds of sunflower were analysed. Compost and vermicompost application led to effective immobilization of Pb, Zn and Cd mobile forms in soil. A correlation was found between the quantity of the mobile forms and the uptake of Pb, Zn and Cd by the sunflower seeds. Tested organic amendments significantly influenced the uptake of Pb, Cu, Zn and Cd by sunflower plant. Oil content and fatty acids composition were affected by compost and vermicompost amendment treatments. 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. The 10% compost and 5 % vermicompost treatment led to decreased heavy metal contents in sunflower oil bellow the regulated limits. The possibility of further industrial processing will make sunflower economically interesting crops for farmers of phytoremediation technology.*

***Keywords :*** *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 (1). 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 (2) or adverse effect on biological activities, soil structure, and fertility (3). 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 (1,4). 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 (5,6). It is best applied at the sites with shallow contamination of organic, nutrient or metal pollutants (7,8). 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 (9). 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 (10, 11).

The use of crop plants for phytoremediation of contaminated soils has the advantages of their high biomass production and adaptive capacity to variable environments (12, 13). 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 metallic-ferrous soil and can tolerate greater than the used amount of heavy metal or other compounds (14). 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 (15, 16, 17). It was found that sunflower could be successfully employed for decontamination of soils polluted with heavy metals and radionuclides (18). Dushenkov et al. (19) 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. (20) and Jovanovia et al. (21). Apart from the fact that sunflower intensely takes up some heavy metals and radionuclides, it also has high biomass, enabling it to accumulate and extract significant amounts of pollutants from the rhizosphere. Nehnevajova et al. (22) evaluated the potential use of sunflower plants for phytoremediation and reported that sunflower can be used for the phytoextraction of metal-contaminated soils. In contrast, according to Madejon et al. (23) 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 assess the effect of organic additives on the quantity of mobile forms of Pb, Zn, Cd and Cu (ii) to compare the effect of the selected additives on accumulation of heavy metals by the sunflower (*Helianthus annuus L*), (iii) to compare the effect of the selected additives on the oil content and fatty acid composition of sunflower and (iv) to estimate the effect of the introduction of additives on the phytoremediation of contaminated with heavy metals soils.

## Experimental

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 5 and 10%), and vemicompost amendments (added at 5 and 10%). 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 soil (control and soil amended with compost and vermicompost) are shown in Table 1.

**Table 1**. Characteristics of organic amendments and treatment

| Parameter | Compost | Vermicompost | Treatment | | | | |
|---|---|---|---|---|---|---|---|
| | | | Control | 5% compost | 10% compost | 5% vermicompost | 10% vermicompost |
| pH | 6.9 | 7.5 | 5.8 | 6.1 | 6.3 | 6.8 | 7.0 |
| Organic matter (%) | 72.9 | 38.5 | 2.2 | 4.9 | 8.6 | 4.0 | 9.4 |
| Pb(mg/kg) | 12.0 | 32.3 | 876.5 | 894.5 | 900.0 | 879.2 | 931.3 |
| Cu(mg/kg) | 42.2 | 53.3 | 124.8 | 136.0 | 139.4 | 136.3 | 139.0 |
| Zn(mg/kg) | 170.8 | 270.3 | 1430.7 | 1459.8 | 1501.4 | 1483.5 | 1615.3 |
| Cd(mg/kg) | 0.19 | 0.69 | 31.4 | 32.3 | 33.5 | 33.8 | 38.6 |

The soil is characterized by acid reaction (pH 5.8), loamy texture and a moderate content of organic matter (2.2%). The 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 (200 mg/kg Zn, 70 mg/kg Pb, 1.5 mg/kg Cd).
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 (24) with a high biomass producing plant species (25) to remove heavy metals such as zinc or copper from contaminated environment (22, 26).

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, 5% compost, 10% compost, 5% vermicompost and 10 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. The content of crude oil in sunflower was determined by weight after the extraction method. Fatty acid composition was established by gas liquid chromatography.

Total content of heavy metals in soils was determined in accordance with ISO 11466. The mobile heavy metals contents (sometimes referred as the "effective bioavailable metal fraction") in soils were determined by 1 M $NH_4NO_3$ (ISO 19730). The mobilisable heavy metals contents in soils, considered as a "potentially bioavailable metal fraction", were extracted by a solution of AB-DTPA (1 M $NH_4HCO_3$ and 0.005 M DTPA, pH 7.8) (27).
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

**Effect of soil amendments on the mobile forms of Pb, Zn, Cd and Cu**
In many plants there is direct relation between the content of microelements in the soil solution and their uptake by the plants. This relation is most evident with cadmium and less evident with zinc and lead (28). The soil amendments used for phytostabilization may have a significant effect on the mobile forms of Pb, Zn and Cd as a result of sedimentation, absorption and change in the degree of oxidation. The quantity of mobile forms of Pb, Zn, Cd and Cu depended on the soil amendments and the treatment (type and rate). The results presented in Figure 1 showed that the impact of soil amendments on mobile forms of Pb, Zn, Cd and Cu was explicitly expressed and led to their effective immobilization.

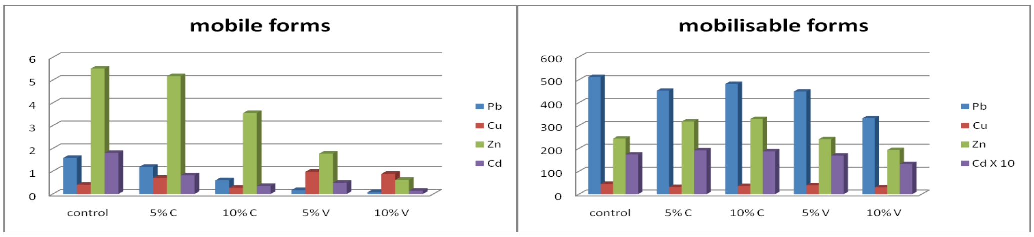



**Figure 1**. Effect of the compost (C) and vermicompost (V) on the quantity of the mobile and mobilisable forms of Pb, Zn, Cd and Cu

The tested amendments decreased $NH_4NO_3$-extractable Pb, Zn, Cd and Cu (mobile fractions) and AB-DTPA – extractable metals (mobilisable fractions). These results can be explained by the fact that acidity is one of the most important factors controlling solubility and adsorption–desorption of metal in soils (10,29). Consequently, the amendments decreased metal mobility/bioavailability mainly because they raised soil pH. Another important factor controlling metal bioavailability is the quantity and quality of the organic matter present (29). Organic amendments that contain a high proportion of humified organic matter can also decrease the mobility of some heavy metals due to the formation of stable chelates (10, 30).

**Effect of organic amendments on the Pb, Zn and Cd accumulation in sunflower**

***Accumulation of Pb, Zn, Cu and Cd by sunflower***

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 Figure 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 284.5 mg/kg, Cu – 19.7 mg/kg, Zn – 551.1 mg/kg and Cd – 18.1 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 – 373.5 mg/kg, Cu – 5.91 mg/kg and Cd – 11.1 mg/kg.

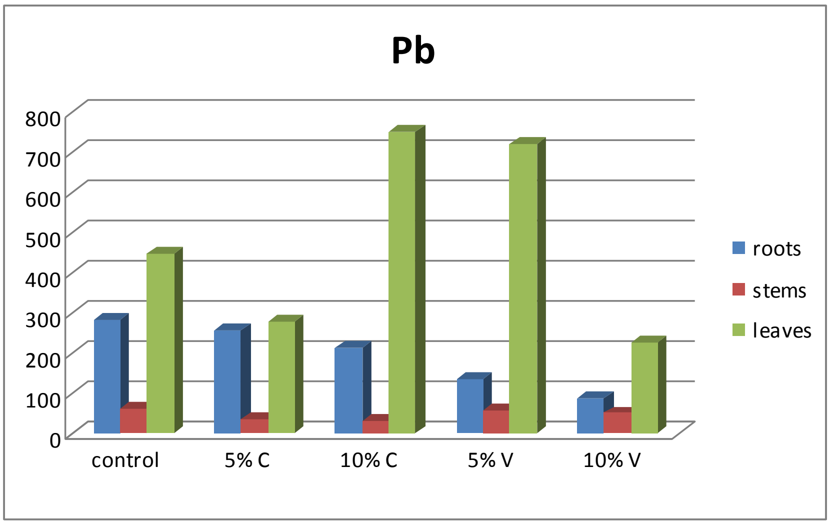
The highest was the accumulation of Pb, Zn, Cu and Cd in the leaves of sunflower, where Pb reached to 449.5 mg/kg, Zn – to 793.1 mg/kg, Cu – to 46.7 mg/kg and Cd - to 206.9 mg/kg. Their stronger accumulation in leaves was probably due to the fact that the leaves of sunflower were covered with short and 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 (31) and by Lombi et al. (32) according to whom Zn, Cu, and Cd were predominantly accumulated in the leaves of sunflower. The obtained results matched well with those from Jadia and Fulekar (33) who found that shoots of sunflower is the major organ of heavy metals accumulation.

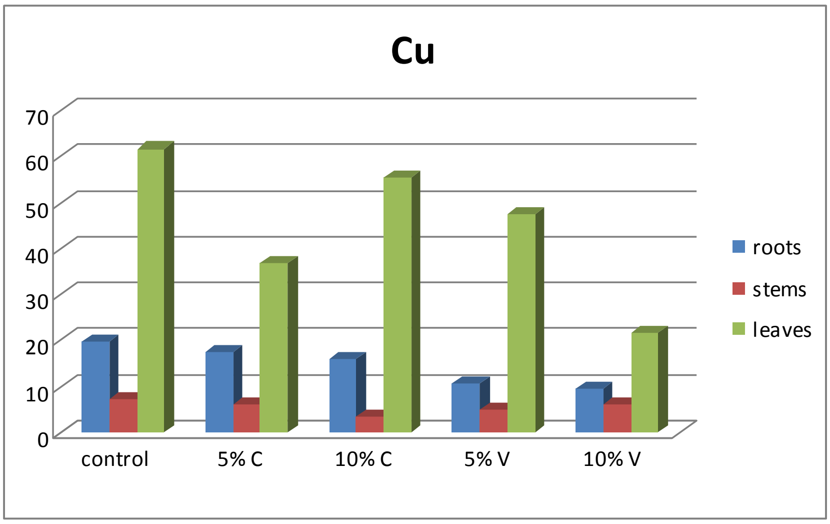
The heavy metal content in the seeds of the sunflower was lower in comparison to that in the roots and leaves. The heavy metal accumulation in sunflower seeds was likely caused by the conductive system. The content of Pb in the seeds of sunflower without amendments reached to 8.3 mg/kg, Zn – 154.9 mg/kg, Cu – 20.3 mg/kg and Cd – 9.6 mg/kg. The contents of Pb, Cu and Zn in the seeds of sunflower were not reached the critical levels of 30 mg/kg Pb, 25 mg/kg Cu 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) (34) and the threshold recommended for human nutrition (1 mg/kg) (35).The results of Chizzola (36) 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. (37) and Korenovska and Palacekova (38), who state that insignificant quantities of Cu, Zn, Pb, and Cd were accumulated in the reproductive organs of rapeseed and sunflower, when grown on soils polluted with heavy metals.

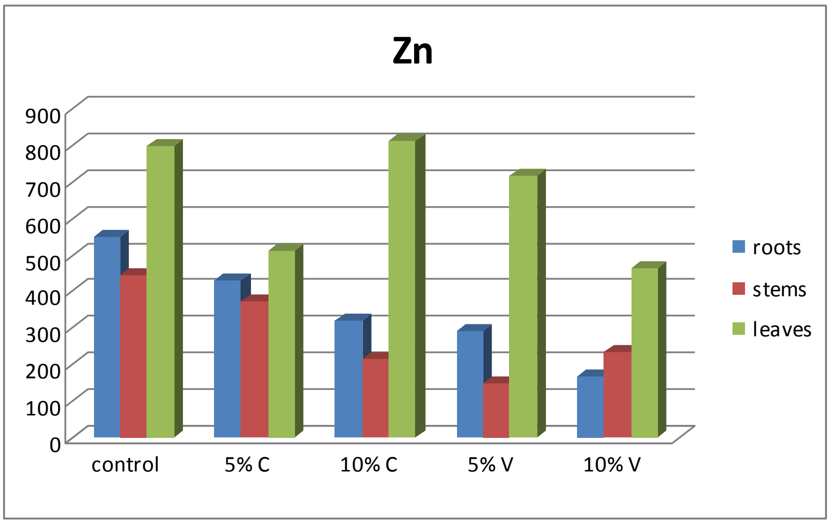
The contents of heavy metals in sunflower oil also were determined. The obtained results showed that the main part of the heavy metals contained in the seeds of sunflower was not transferred in the oil during during the seed processing, due to which their content in the oil was considerably lower. Lead in sunflower oil reached 0.28 mg/kg, Cu to 0.25 mg/kg, Zn to 3.37 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 (0.4 mg/kg Cu and 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 (39) i.e., that the contents of Cu, Fe, and Pb was low and that there was no contents of Cd in the sunflower oil.

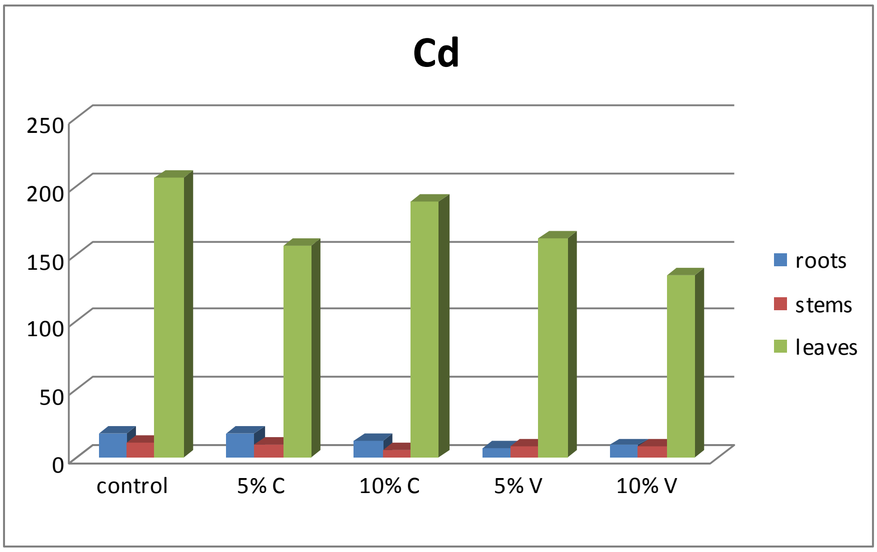
Sunflower meal is the by-product of the oil extraction process. The contents of heavy metals in sunflower meal also were determined. The obtained results showed that the heavy metal content in the sunflower meals was higher compared to that in the seeds. Trace metals observed in the seeds are almost exclusively transferred to meals after seed crushing (40). The content of Pb in the meals of sunflower without amendments reached to 4.1 mg/kg, Zn – 197.8 mg/kg, Cu – 28.5 mg/kg and Cd – 12.5 mg/kg. The contents of Pb, Cu and Zn in the meal of sunflower were not reached the critical levels of 30 mg/kg Pb, 25 mg/kg Cu 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.




Cd
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roots
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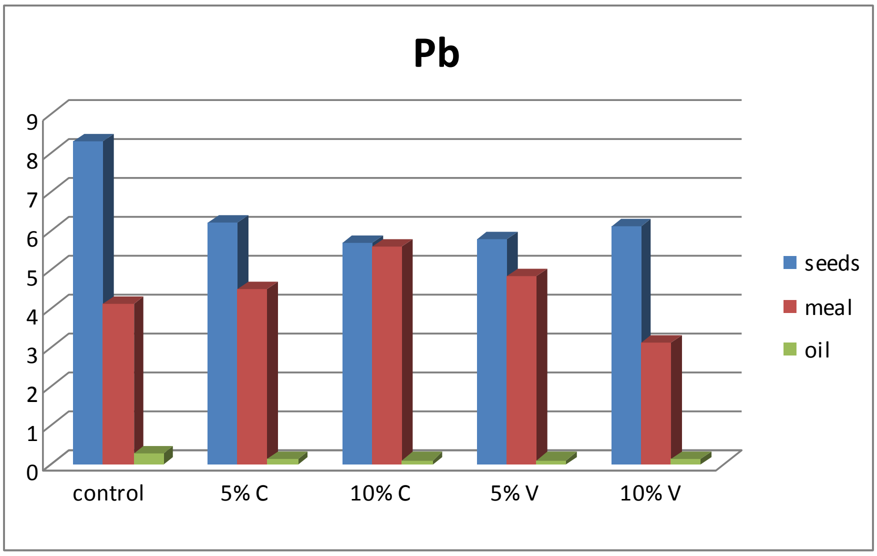
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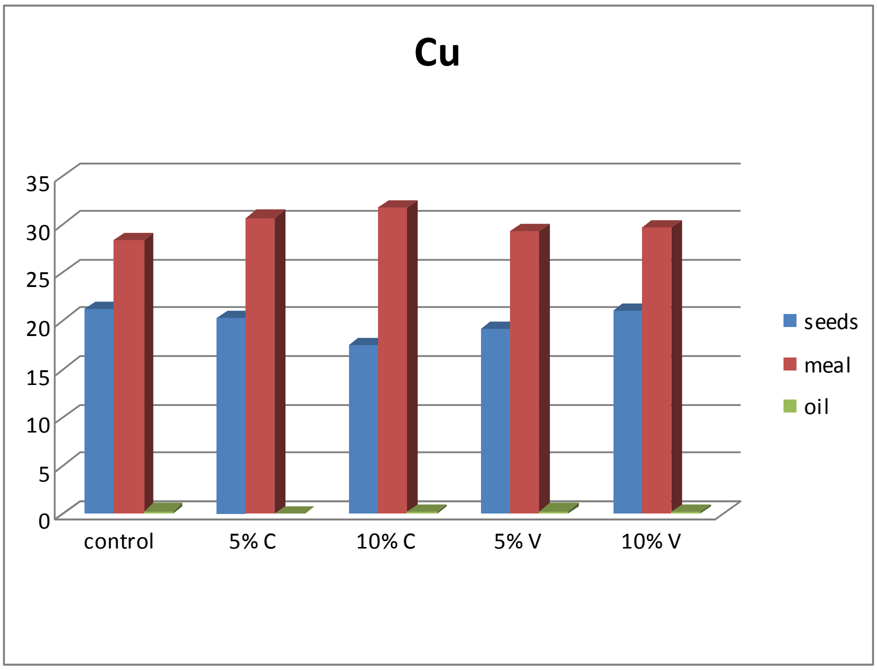
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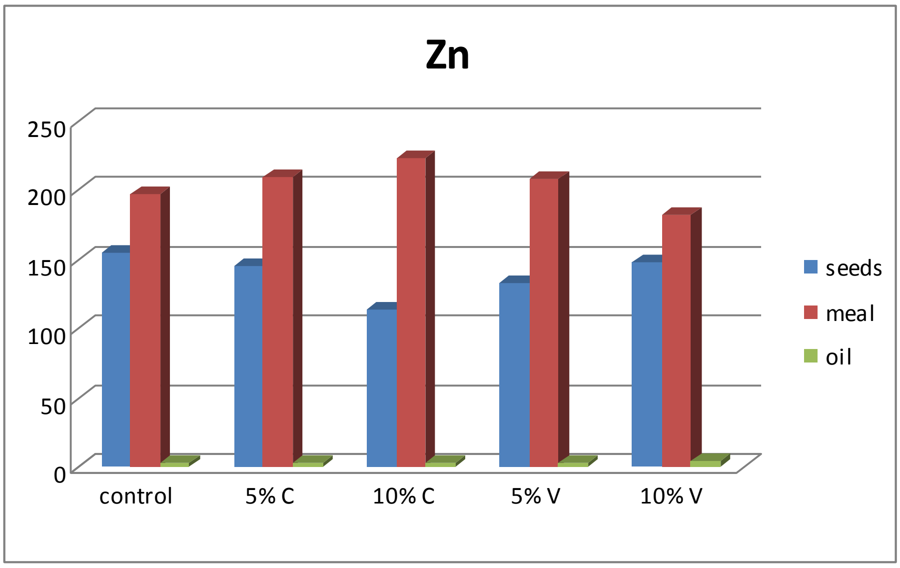


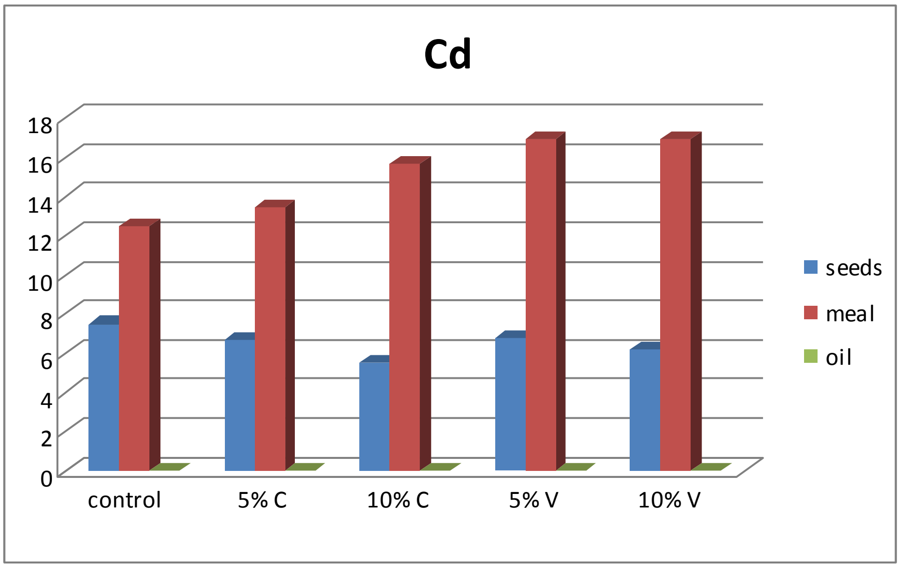


**Figure 2.** Effect of the compost (C) and vermicompost (V) on the quantity of Pb, Zn, Cd and Cu (mg/kg) in sunflower plants

***Organic additives impact***

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 (28).

The results obtained by us showed that Pb, Zn, Cu 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, Cu, 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 compost and vermicompost led to decreased Pb, Cu, Zn and Cd content in the roots and stems of sunflower. When the soil was treated with 10% compost and 5 % vermicompost the contents of Pb in the leaves of sunflower increased (Figure 2).

However, heavy metal contents in seeds of sunflower decreased in the plants treated with all amendments used in the experiments. Impact of organic amendments on Pb, Zn and Cd accumulation in seeds of sunflower depended significantly on their quantity. Increase in the quantity of compost and vermicompost (10%) led to a decrease of Pb content in sunflower seeds to 5.7 mg/kg and 5.8 mg/kg, respectively and these concentrations were below the maximum permissible concentrations for fodder

(30 mg/kg). The application of 10% compost and 10% vermicompost led to a decrease of the Zn content in seeds to 113.9 mg/kg and 117.5 mg/kg, respectively and these concentrations were below the maximum permissible concentrations (300 mg/kg). Cd showed similar tendency. When the soil was treated with 10% compost and 10% vermicompost Cd content in seeds decreased to 5.55 mg/kg and 5.19 mg/kg, respectively and this concentration were above maximum permissible concentrations (0.5 mg/kg) for fodder (41).
Organic amendments significantly reduced heavy metals concentration in sunflower oils and meals, but the effect differed among them. Also, there was a dose effect for amendments. Increase in the quantity of compost and vermicompost (10%) led to a decrease of Pb, Cu and Zn content in sunflower oils. The 10% compost and 5% 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.

***Sunflower oil***

Sunflower oil is a widely consumed product with high nutritional value and significant health benefits. It is known for its superior organoleptic characteristics (taste) and remarkable antioxidant properties; it contains vitamin A, D, and a sufficient amount of vitamin E. The fatty acid composition determines the use of sunflower oil (42, 43). The oil containing a high level of oleic acid is preferred in nutritional use whereas that having higher linoleic content is preferred by paint or fuel industry. Standard sunflower cultivars contain high linoleic acid, moderate oleic acid and low linoloeic acid (44). The fatty acid composition changes depending on genotypes and some other factors such as environmental conditions, planting and harvesting time (45, 46, 47).

Thus, we performed oil analyses to determine the content of aforesaid fatty acids in the present study. The mean palmitic acid ratios were 4.16 %. Very low levels of lauric (0.04 %), stearic (0.08 %) and arachdic (0.36 %) acids were recorded in oil. Thus, the mean saturated fatty acid ratio was found to be 5.47 %. The sunflower oil is high in oleic acid. The oleic acid ratio (90.3 %) was higher than the linoleic acid ratio (3.97 %). Very low levels of palmitoleic (0.01%), α- linolenic (0.01%) and gondoic (0.25%) acids were recorded in sunflower oil. Thus, the mean unsaturated fatty acid ratio was found to be 94.53 %.
Oil content of seeds is known to chance depending on factors like cultivar, soil characteristics and climate (45, 46, 47). Oil content of sunflower cultivars from different production areas of the word was reported as 40- 50 %. Evaluating our results of oil content measurements, it can be established that our results are in accordance with those of previous reports.
Data illustrated in Table 2 showed the effect of compost and vermicompost amendment treatments on oil content of sunflower plants and fatty-acids composition. As shown in Table 2, oil content in seeds and fatty acid composition varied among tested organic amendments significantly. Also, there was a dose effect for amendments. Similar of heavy metal contents in seeds, oil contents were affected significantly by compost and vermicompost amendment treatments in the present study.

**Table 2.** Oil content and fatty acid composition of sunflower oil

| | Control | 5% C | 10% C | 5% V | 10% V | Codex Standard |
|---|---|---|---|---|---|---|
| Oil content, % | 43.1 | 46.9 | 48.1 | 46.4 | 46.0 | 40 - 50 |
| Lauric acid C 12:0 | 0.04 | nd | nd | nd | nd | nd |
| Palmitic acid C 16:0 | 4.16 | 3.67 | 3.75 | 4.0 | 3.95 | 2.6 – 5.0 |
| Palmitoleic acid C 16:1 | 0.01 | nd | nd | 0.09 | 0.1 | nd – 0.1 |
| Stearic acid C 18:0 | 0.08 | 3.32 | 3.65 | 4.09 | 3.38 | 2.9 – 6.2 |
| Oleic acid C 18:1 | 90.3 | 91.91 | 91.64 | 89.01 | 89.15 | 75 – 90.7 |
| Linoleic acid C 18:2 | 3.97 | 1.1 | 0.96 | 1.32 | 2.1 | 2.1 – 17.0 |
| α- Linolenic C 18:3 | 0.01 | nd | nd | nd | nd | nd - 0.3 |
| Arachidic acid C 20:0 | 0.36 | nd | nd | 0.39 | 0.34 | 0.2 -0.5 |
| Gondoic acid C 20:1 | 0.25 | nd | nd | 0.18 | 0.20 | 0.1 – 0.5 |
| Behenic acid C 22:0 | 0.83 | nd | nd | 0.92 | 0.78 | 0.5 -1.6 |
| Saturated:Unsaturated | 5.47:94.53 | 6.99:93.01 | 7.4:92.6 | 9.4:90.6 | 8.45:91.55 | |

In general, compost and vermicompost treatments increased oil content in seeds. When fatty acid composition in the oil is evaluated, linoleic and oleic which are major fatty acids in sunflower oil changed significantly depending on compost and vermicompost treatments. The application of compost led to increased oleic acid in the sunflower oil. When the soil was treated with vermicompost oleic acid slightly decreased. Both organic amendments led to decreased the linoleic acid in the oil.

The other major fatty acids, stearic and palmitic acids were also significantly affected by treatments. Tested organic amendments decreased palmitic acid and increased stearic acid in sunflower oil. The minor fatty acids such as palmitoleic and arachidic were also affected by the treatments. The linolenic acid content (18:3) in sunflower oil was also affected by the treatments. Both amendments reduced the quantity of the linolenic acid in the oil, giving the oil a good oxidative stability.

## Conclusion

1. The tested organic amendments corrected soil acidity and raised soil organic mater and improved soil chemical properties.
2. Organic amendment application led to an effective immobilization of Pb, Zn and Cd mobile forms in soil. A correlation was found between the quantity of the mobile forms and the uptake of Pb, Zn and Cd by the sunflower seeds.
3. Tested organic amendments significantly influenced the uptake of Pb, Cu, 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. The 10% compost and 5 % vermicompost treatment led to decreased heavy metal contents in sunflower oil bellow the regulated limits. The possibility of further industrial processing will make sunflower economically interesting crops for farmers of phytoremediation technology
4. Oil content and fatty acids composition were affected by compost and vermicompost amendment treatments. Compost and vermicompost treatments increased oil content in seeds. Compost treatments increased oleic acid, and decreased linoleic and palmitic acid in sunflower oil.

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