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THE EFFECT OF ORGANIC AMENDMENTS ON UPTAKE ON HEAVY METALS IN SAFFLOWER (*CARTHAMUS TINCTORIUS L.*)

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ABSTRACT - The effects of organic soil amendments (compost and vermicompost) on uptake of heavy metals (Pb, Cd, Zn and Cu) in safflower (Carthamus tinctorius L.) were studied. Field experiments with randomized complete block design with five treatments (control, compost amendments added at 5 and 10% , and vemicompost amendments added at 5 and 10%) were carried out. Heavy metal contents in roots, stems, leaves and seeds of safflower 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 safflower. Compost and vermicompost treatments were effective organic amendments and reduced heavy metals in safflower seeds, oils and meals. Tested organic amendments increased linoleic acid and palmitic acid, and decreased oleic acid in safflower oil.

Keywords: safflower, heavy metals, organic amendments

INTRODUCTION

Safflower (*Carthamus tinctorius* L., Asteraceae) an annual oilseed crop has been cultivated on small plots in the world. Safflower is one of the alternative oil crops, particularly in dry land due to tolerance to cold, drought and salinity stress [1,2]. According to hull types of seeds, the seed oil content ranges from 20 % to 45 %. The oil is high in linoleic acid, an unsaturated fatty acid that aids in lowering the cholesterol level in the blood. In addition, this oil is used in soft margarines and as salad oil besides being used raw for edible purposes [3]. Because of rapid drying, the oil is in high demand in paint and emulsion industries [4].

Addition of organic matter amendments, such as compost, fertilizers and

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wastes, is a common practice for immobilization of heavy metals and soil amelioration of contaminated soils [5]. 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 [6,7].

The use of crop plants for phytoremediation of contaminated soils has the advantages of their high biomass production and adaptive capacity to variable environments [8,9]. 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.

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 safflower (*Carthamus tinctorius L.*), (iii) to compare the effect of the selected additives on the oil content and fatty acid composition of safflower and (iii) to estimate the effect of the introduction of additives on the phytoremediation of contaminated with heavy metals 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 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 safflower 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	Vermi-compost	Treatment				
			Control	5% compost	10% compost	5% vermi-compost	10% vermi-compost
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 saflower (*Carthamus tinctorius L.*). Safflower seeds were sown in each plot; between row and within row distances were 60 and 20 cm, respectively. Each hole was 5–6 cm deep, containing 3 seeds. After safflower had grown for 15 days, the safflower was thinned to one plant per hole.

Upon reaching commercial ripeness, the safflower plants were gathered and the concentrations of Pb, Cu, Zn and Cd in their different parts (roots, stems, leaves and seeds) were determined. The oil from safflower 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 safflower were determined by the method of the dry mineralization. The content of crude oil in safflower 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) [10].

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 [11]. 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.

The tested amendments decreased NH4NO3-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 [6,12]. 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 [12]. 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 [6,13].

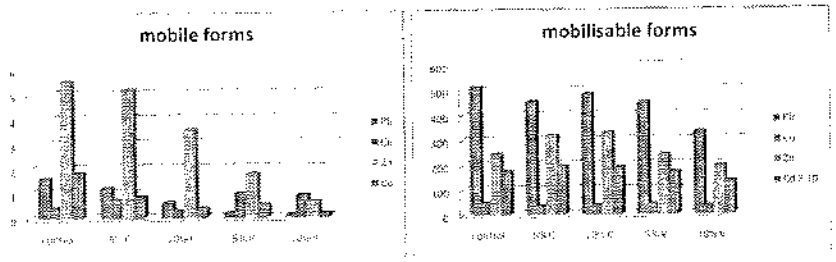

Figure 1. Effect of the compost (C) and vermicompost (V) on the quantity of the mobile and mobilisable forms of Pb, Zn, Cd and Cu

Effect of organic amendments on the Pb, Zn and Cd accumulation in safflower
Accumulation of Pb, Zn, Cu and Cd by safflower

The results for the influence of the organic additives on the accumulation and distribution of Pb, Zn and Cd in the safflower plants are presented in Figure 2. Considerably lower values were established in the roots of safflower compared to the above- ground parts of safflower. The content of Pb in the roots of safflower without amendments reached to 142.8 mg/kg, Cu – 28.9 mg/kg, Zn – 436.0 mg/kg and Cd -52.4 mg/kg. Safflower developed a thickened taproot that can extend down to 2-3 m, with a strong ability to uptake the nutrients.

The heavy metals contents in the stems of the safflower 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 safflower without amendments reached to 86.3 mg/kg, Zn – 207.5 mg/kg, Cu – 19.9 mg/kg and Cd – 29.4 mg/kg.

The highest was the accumulation of Pb, Zn, Cu and Cd in the leaves of safflower, where Pb reached to 580.5 mg/kg, Zn – to 651.9 mg/kg, Cu – to 34.9 mg/kg and Cd - to 148.1 mg/kg. Their stronger accumulation in safflower was probably due to the fact that the leaves of safflower were waxy, shiny oval, spiny-edged leaves alternate around the stem, which contributed to the fixing of the aerosol pollutants and for their accumulation.

The heavy metal content in the seeds of the safflower was lower in comparison to that in the roots and leaves.The heavy metal accumulation in safflower seeds was likely caused by the conductive system. The content of Pb in the seeds of safflower without amendments reached to 3.57 mg/kg, Zn – 109.6 mg/kg, Cu – 14.67 mg/kg and Cd – 2.0 mg/kg. The contents of Pb, Cu and Zn in the seeds of safflower 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) [14].

The contents of heavy metals in safflower oil also was determined. The obtained results showed that the main part of the heavy metals contained in the seeds of safflower was not transferred in the oil during during the seed processing, due to which their content in the oil was considerably lower. Lead in safflower oil reached 0.21 mg/kg, Cu to 0.64 mg/kg, Zn to 14.18 mg/kg, and the content of Cd is below the limits of detection of the apparatus. Although the contents of heavy metals in the oil was lower compared with the seeds, the quantities of Pb, Cu, and Zn in the safflower oil, were higher than the accepted maximum permissible concentrations (0.1 mg/kg Pb, 0.4 mg/kg Cu and 10.0 mg/kg Zn).

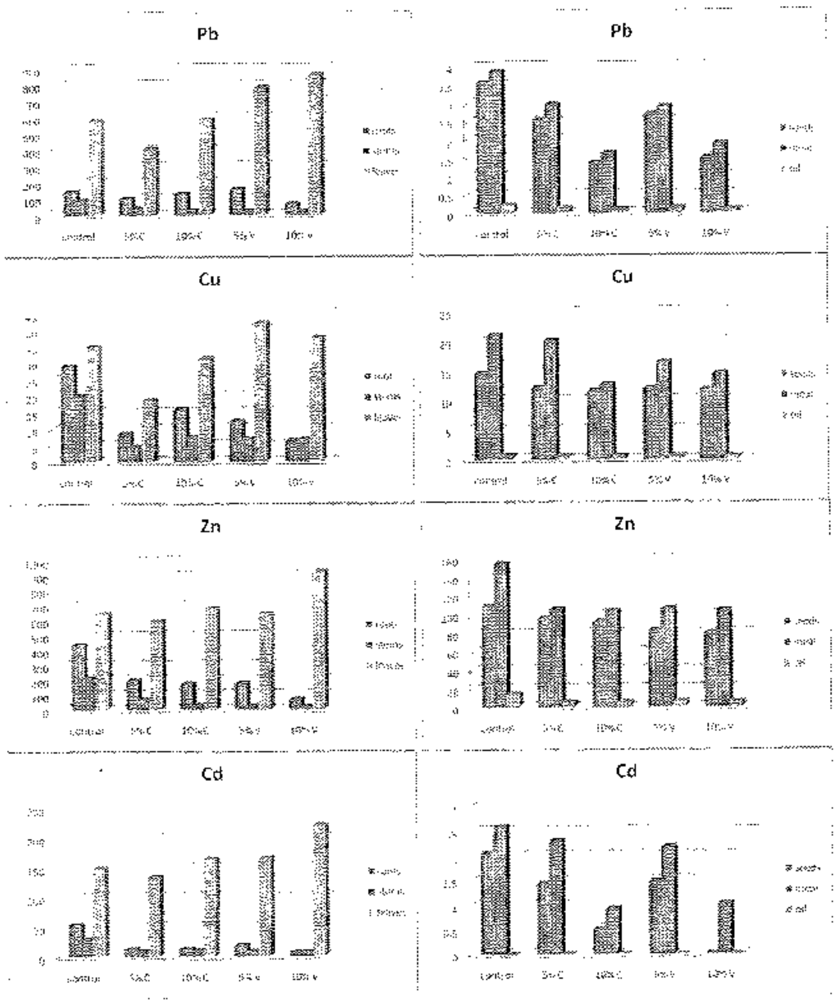

Figure 2. Effect of the compost (C) and vermicompost (V) on the quantity of Pb, Zn, Cd and Cu (mg/kg) in safflower plants

The by-product of the extraction of safflower oil is a grayish tan to brown meal that exhibits flakes or shreds of whitish safflower hulls. Heavy metal content in the safflower meals was higher compared to that in the seeds. Trace metals observed in the seeds are almost exclusively transferred to meals after seed crushing [15]. The content of Pb in the meals of safflower without amendments reached to 3.86 mg/kg, Zn – 155.2 mg/kg, Cu – 21.08 mg/kg and Cd – 2.58 mg/kg. The contents of Pb, Cu and Zn in the meal of safflower 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 safflower has a selective character that in safflower decreases in the following order: leaves > roots> stems > seeds.

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 [11].

The results obtained by us showed that Pb, Zn, Cu and Cd uptake by safflower 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 safflower organs were rather complex. Impact of organic amendments on heavy metals accumulation in organs of safflower 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 safflower. When the soil was treated with 10% compost and 10 % vermicompost heavy metal contents in the leaves of safflower increased (Fig.2).

However, heavy metal contents in seeds of safflower 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 safflower depended significantly on their quantity. Increase in the quantity of compost and vermicompost (10%) led to a decrease of Pb content in safflower seeds to 1.37 mg/kg and 1.5 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 72.4 mg/kg and 80.45 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 0.5 mg/kg, and this concentration were within maximum permissible concentrations (0.5 mg/kg) for fodder [14].

Organic amendments significantly reduced heavy metals concentration in safflower 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, Zn and Cd content in safflower oils and meals. The 10% compost and 10% vermicompost addition was especially effective for the reduction of Pb and Zn content in safflower oils bellow the regulated limits (0.1 mg/kg Pb and 10.0 mg/kg Zn, respectively), and oil can be used for human consumption.

3.2.3. Safflower oil

Safflower oil quality is high due to its fatty acids composition [16]. As known, the fatty acid composition of vegetable oil is a main factor affecting on its commercial uses. Standard safflower oil contains about 5-8 % palmitic acid, 2-3 % stearic acid, 8-20 % oleic acid, and 68-83 % linoleic acid [17].

Safflower oil naturally contains the lowest levels of total saturated fatty acids among cultivated oil crops [18]. Oil quality is a significant concern of consumers, particularly for the contents of oleic and linoleic acids which are proven as healthy sources of oil for human body. Safflower is thought to be one of the highest quality vegetable oils and its oil consists of mainly palmitic, stearic, oleic and linoleic acids [19,20]. Thus, we performed oil analyses to determine the content of aforesaid fatty acids in the present study. The mean stearic acid and palmitic acid ratios were 1.91 and 6.04 %, respectively. Very low levels of lauric (0.09 %) and arachdic (0.32 %) acids were recorded in oil. Thus, the mean saturated fatty acid ratio was found to be 8.2 %. The safflower oil is high in linoleic acid. The oleic acid ratio (14.93 %) was lower than the linoleic acid ratio (76.22 %). Very low levels of C18:3 (0.08%), C20:1 (0.13%) and C22:1 (0.20%) acids were recorded in safflower oil. Thus, the mean unsaturated fatty acid ratio was found to be 91.8 %.

Oil content of seeds is a very important economic trait for safflower cultivars and considered one of the most important factors affecting the success of safflower introduction in new areas [21]. Oil content is known to chance depending on factors like cultivar, soil characteristics and climate [22]. Oil content of safflower cultivars from different production areas of the word was reported as 23.86- 40.33 % [23], 26.72-35.78 % [2], 26.3-28.5 % [24] and 31.3-36.3 % [25]. Evaluating our results of oil content measurements, it can be established that our results are in accordance with those of previous reports.

Table 2. Oil content and fatty acid composition of safflower oil

	Control	5% C	10% C	5% V	10% V	Codex Standard
Oil content, %	27.15	24.87	20.86	28.59	27.35	
Lauric acid C 12:0	0.09	0.09	0.10	0.10	0.10	nd
Palmitic acid C 16:0	6.04	6.83	6.54	6.66	6.57	5.3 – 8.0
Palmitoleic acid C 16:1	0.07	0.08	0.1	nd	nd	nd – 0.2
Stearic acid C 18:0	1.91	1.88	1.73	1.90	1.87	1.9 – 2.9
Oleic acid C 18:1	14.93	14.03	14.45	14.44	14.48	8.4 – 21.3
Linoleic acid C 18:2	76.22	76.37	76.46	76.64	76.70	67.8 -83.2
α- Linolenic C 18:3	0.07	0.08	0.08	nd	nd	nd - 0.1
Arachidic acid C 20:0	0.32	0.30	0.33	0.25	0.28	0.2 -0.4
Eicosenoic acid C 20:1	0.15	0.13	nd	nd	nd	0.1 – 0.3
Erucic acid C 22:1	0.20	0.20	0.21	nd	nd	nd -1.0
Saturated:Unsaturated	8.0:92.0	8.8:91.2	8.5:91.5	8.7:91.3	8.5:91.5	

Data illustrated in Table 2 showed the effect of compost and vermicompost amendment treatments on oil content of safflower 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. In general, vermicompost treatments increased oil content in seeds when compared to the control. All treatments increased linoleic acid and palmitic acid, and decreased oleic acid when compared to the control.

CONCLUSIONS

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 safflower seeds.
3. Tested organic amendments significantly influenced the uptake of Pb, Cu, Zn and Cd by safflower plant. The compost and vermicompost treatments significantly reduced heavy metals concentration in safflower seeds, meals and oils, but the effect differed among them. Also, there was a dose effect for amendments. The 10% compost and 10% vermicompost treatment led to decreased heavy metal contents in safflower oil bellow the regulated limits. The possibility of further industrial processing will make safflower economically interesting crops for farmers of phytoremediation technology
4. Oil content and fatty acids copmosition were affected by compost and vermicompost amendment treatments. Vermicompost treatments increased oil content in seeds. Tested organic amendments increased linoleic acid and palmitic acid, and decreased oleic acid in safflower oil.

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