

Irrigation of *Helianthus annuus* with Pb-polluted water: Improvement of phytoremediation using vermicompost

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Abstract

For investigating the effect of vermicompost (VC) on improving phytoremediation of Pb by sunflower (*Helianthus annuus*) cultivar Lakumka, a factorial experiment was conducted in the pots containing soil or its mixture with 10% VC, in a greenhouse with controlled condition in 2018. After 3 weeks of irrigation with normal water, plants were daily irrigated with high Pb-polluted water (0, 1000, 1500 or 2000 mg L⁻¹) up to 3 more weeks. Then, changes in plant biomass, Pb accumulation in roots and shoots, and absorption of some mineral nutrients were measured. Results showed that the shoot biomass of sunflower was not affected by Pb treatments. Pb accumulation in root was increased in a dose dependent manner, where in plants under 2000 mg L⁻¹ it reached to 31 times more than that of the control. This increase was reinforced by VC so that in Pb+VC-treated plants the bioconcentration factor (BCF) of Pb in roots was higher than those not-amended with VC. However, both plant groups had a good BCF much more than 1. Pb accumulation in leaves of Pb-treated plants showed a significant increase, where VC caused a significant decrease in its accumulation. Effect of VC and Pb treatments on calcium, magnesium and zinc concentration of root and leaf did not follow the regular changes. Overall, sunflower cultivar Lakumka was an appropriate accumulator of Pb. VC can improve Pb phytoremediation by sunflower.

Keywords: Fertilizers, Heavy metals, Lead, Soil contamination, Sunflower

Introduction

Due to the increasing progress of human industrial activities, many pollutants such as heavy metals (HMs) have been released into the water, soil and other parts of the environment (Yongsheng *et al.*, 2011). Lead (Pb) as a HM is the most contaminating HMs in the environment. Plants can absorb Pb from their polluted environment, although it is not an essential nutrient for them (Lamhamdi *et al.*, 2013). It can be highly toxic for plants by disturbing different metabolic processes (Najeeb *et al.*, 2017).

Several remediation techniques have been introduced for removing or deactivating contaminants from the environment that most of them are expensive or inefficient (Abbaspour and Golchin, 2011). In recent years, phytoremediation as an effective remediation method has attracted much attention (Najeeb *et al.*, 2017). Phytoremediation is a biologically cost-effective technology in which living plants are used for stabilizing or removing pollutants from various

environmental components such as water and soil (McComb *et al.*, 2012).

In addition to being a rich nutrient source, organic matters including sewage sludge, composts, manures, and peat can increase removing and/or stabilizing of HMs and then promote phytoremediation process. These amendments can immobilize HMs in polluted soils through adsorption reactions (Abbaspour and Golchin, 2011; Hoehne *et al.*, 2016). Vermicompost (VC) is a nutrient rich and microbiologically active organic fertilizer which is produced from the breakdown of organic matter by the interaction between earthworms and microorganisms due to a nonthermophilic biodegradation (Beykkhormizi *et al.*, 2016). This organic fertilizer has good physical and chemical properties such as large surface area, high porosity, drainage, ventilation, water storage capacity, cation exchange capacity, plant growth regulators, and less soluble salts (Atiyeh *et al.*, 2002; Zuo *et al.*, 2018). The presence of humic and organic materials in VC

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stimulates plant growth better than plant nutrition with mineral fertilizers (Atiyeh *et al.*, 2002).

Suitable plant species for phytoextraction are those that can tolerate a high concentration of HMs and produce a high biomass (McComb *et al.*, 2012). Sunflower (*Helianthus annuus* L.) belonging to Asteraceae (Fozia *et al.*, 2008) is one of the species having these characteristics (Doncheva *et al.*, 2013) and thus can be considered as a good candidate for phytoremediation. The potential of sunflower for phytoremediation of Pb has been reported by researchers (Boonyapookana *et al.*, 2005; Rahman *et al.*, 2013; Winska-Krysiak *et al.*, 2015).

In the present study, the role of VC as a soil amendment in improving phytoremediation of a HM by a relevant suitable plant species, sunflower, was investigated. Moreover, the changes in plant biomass, Pb accumulation in soil and different parts of plant, and the absorption of some mineral nutrients under different levels of Pb or Pb+VC were investigated.

Material and methods

Experimental design: In present study, the interaction effects of different concentrations of Pb and VC on different traits of sunflower was investigated in a greenhouse with controlled condition in 2018, as a factorial experiment based on the randomized complete block design with three replications.

Sunflower seeds were first pre-soaked for 24 hours and then were planted in plastic pots containing 2.5 kg of clay loamy soil (V0) or its mixture with 10% v/v VC (V1). The pots were irrigated with normal water (without treatment), regarding field capacity, to ensure normal growth of the seedlings. They were irrigated daily for three more weeks with different concentrations of Pb including 0 (P0), 1000 (P1), 1500 (P2) and 2000 (P3) mg L⁻¹. P0V0 (neither treated with Pb nor with VC) was considered as control plants. Pb(NO₃)₂ (lead nitrate) was used to prepare different concentrations of Pb after accurate calculations. Six weeks after planting in a greenhouse with 16/8 hrs. photoperiod and 25° C, plants at vegetative stage (6-8 leaf stage) were harvested for investigating the changes in biomass, Pb accumulation, and minerals (Ca, Mg, and Zn) absorption.

Plant and soil analysis: The VC chemical analysis used in this study is presented in Table 1.

The plants were removed from the pots and the shoots were separated from the roots of the plant. The roots were washed by soaking in water-filled buckets. After washing, the root and shoot samples were dried in an oven at 70° C for 48 hrs. Then the dried samples were ground to a fine powder. For acidic digestion, a mixture of nitric acid 65% hydrochloric acid 37% (3: 1) was added to the samples and then the resulting mixture was heated at 115° C. After half an hour, 2 ml of H₂O₂ were added to the mixture. Finally, after filtering, the digested samples were diluted to a final volume of 100 ml with deionized water (Uddin *et al.*, 2016).

Concentration of Pb, and some mineral nutrients including Ca, Mg, and Zn in dilution were determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES). For measuring Pb in the soil of plant growth medium, Atomic Absorption Spectroscopy (AAS) was used after acid digestion with HCl.

Calculation of accumulation factors:

Accumulation factors were calculated using the following equations (Marrugo-Negrete *et al.*, 2016):

$$\text{Bioconcentration Factor (BCF)} = \frac{\text{Pb accumulation in the root}}{\text{Pb accumulation in the soil}}$$

$$\text{Translocation Factor (TF)} = \frac{\text{Pb accumulation in the shoot}}{\text{Pb accumulation in the root}}$$

$$\text{Accumulation Factor (AF)} = \frac{\text{Pb accumulation in the shoot}}{\text{Pb accumulation in the soil}}$$

Statistical analysis: Data were analyzed using Mstat-C software. Significant differences were determined at P≤0.05 and Duncan's multiple range test was used for means comparison.

Result and discussion

Plant biomass: The results showed that none of the Pb concentrations had a significant effect on shoot dry weight (DW) of the sunflower. VC increased DW of the shoot of the control plants (Fig. 1). Inductive role of VC in promoting plant growth and biomass production has been reported in many studies, attributed to its nutrients, biologically active substances, and giving good physical properties to soil (Bachman *et al.*, 2008; Warman and AngLopez, 2010; Lazcano *et al.*, 2011; Singh and Wasnik, 2013; Bellitürk *et al.*, 2015; Zuo *et al.*, 2018).

The mean level of Pb in soil is 27 mg kg⁻¹ (Kabata-Pendias, 2011). If the soil contains more than 100 mg kg⁻¹ Pb, it is considered to be toxic for most plants (Kachout *et al.*, 2012). In present study, in all Pb treatments, both amended with VC or not, the accumulation of Pb in soil was more than this level (table 2). The results showed that with increasing the concentration of Pb treatments, its accumulation in the roots was increased in a dose dependent manner (Fig. 2), so that its amount in the roots of plants treated with 2000 mg L⁻¹ Pb was 31 times more than that of the control plants, i. e., 5133 mg Kg⁻¹ DW. This can be accounted for a high Pb accumulation by a plant species because for most plants the accumulation of a range of 30-300 mg L⁻¹ Pb leads to toxicity (Kabata-Pendias, 2011).

VC significantly increased Pb accumulation in roots of Pb-treated plants, compared to those not amended with VC (Fig. 2). In some literatures it has been reported that presence of some organic matter in soil can limit HMs solubility due to their adsorption and immobilization (Covelo *et al.*, 2004; Duran, 2006; He *et al.*, 2016; Hoehne *et al.*, 2016). However, a high

Table 1- Some chemical properties of vermicompost

Chemical properties	Concentration
Nitrogen (%)	1.54
Calcium (%)	5.2
Magnesium (%)	1.89
Sodium (%)	0.42
Fe (%)	1.52
K ₂ O (%)	1.59
P ₂ O ₅ (%)	2.22
Zinc (mg Kg ⁻¹)	127
Copper (mg Kg ⁻¹)	31.2
Manganese (mg Kg ⁻¹)	488
Lead (mg Kg ⁻¹)	69

Table 2- Pb accumulation in soil, and calculated accumulation factors

Treatment levels		Pb concentration in soil (mg kg ⁻¹)	Accumulation factors		
Pb	VC		Bioconcentration Factor (BCF)	Translocation Factor (TF)	Accumulation Factor (AF)
P0	V0	70.17	2.38	1.00	2.38
	V1	78.73	13.03	0.20	2.59
P1	V0	108.33	12.25	0.22	2.66
	V1	116.76	17.07	0.12	2.00
P2	V0	169.80	16.98	0.31	5.30
	V1	217.53	17.90	0.13	2.37
P3	V0	905.70	5.67	0.18	1.01
	V1	1052.00	5.44	0.07	0.38

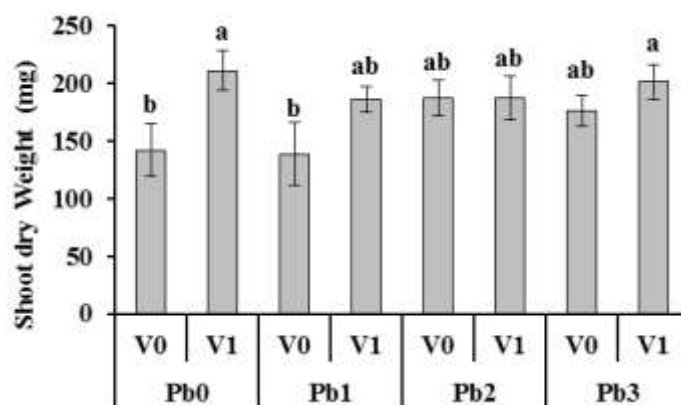


Fig. 1- Interaction effects of VC and Pb on the shoot dry weight of sunflower. V1 and V0 indicate VC-amended or not, respectively. Different levels of Pb (Pb0-4) include concentrations of 0 (Pb0, Pb free), 1000 (Pb1), 1500 (Pb2) and 2000 (Pb3) mg L⁻¹. Different letters on the columns indicate a significant difference ($P \leq 0.05$).

potential of VC for cation exchange capacity (CEC) can be followed by releasing metals leading to their uptake by plant roots (Bellitürk *et al.*, 2015). CEC has an important role in soil fertility. A high CEC indicates a large reserve of minerals (Taiz and Zeiger, 2002). On the other hand, because VC increases plant growth, it finally leads to a higher uptake of these elements by the plant. The plant producing more biomass can absorb more HMs (Bellitürk *et al.*, 2015). A phytoremediation

improvement of cadmium contaminated soil by vermicompost in *Sedum alfredii* has been reported (Wang *et al.*, 2012). Satpathy and Reddy (2013) were also reported an induced increase in the uptake Pb by organic amendment in roots of *Indian mustard*.

Pb accumulation in the leaves of Pb-treated plants was also significant compared to the control, so that its concentration in the plants treated with 2000 mg L⁻¹ Pb was about 5.5 times more than that of the control.

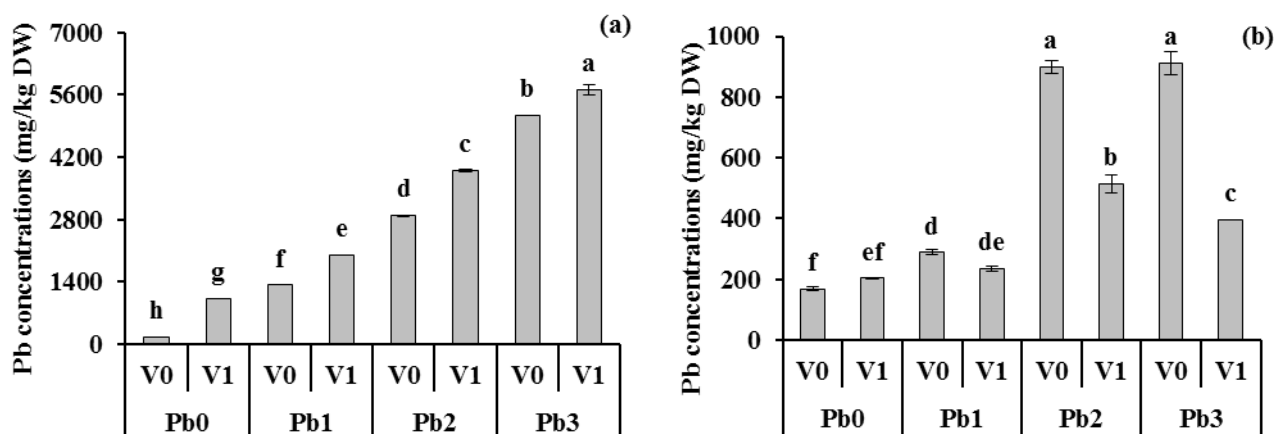


Fig. 2- Interaction effects of VC and Pb on the concentration of Pb in the root (a) and shoot (b) of sunflower. V1 and V0 indicate VC-amended or not, respectively. Different levels of Pb (Pb0-4) include concentrations of 0 (Pb0, Pb free), 1000 (Pb1), 1500 (Pb2) and 2000 (Pb3) mg L⁻¹. Different letters on the columns indicate a significant difference ($P \leq 0.05$). DW: dry weight.

However, VC caused a significant decrease in Pb accumulation in leaves of the plants treated with 1500 and 2000 mg L⁻¹ Pb (Fig. 2). Similar effect of composts has been observed in some investigations (Duran *et al.*, 2006; Eissa, 2015; Hoehne *et al.*, 2016). This may be due to the role of VC in increasing metal complexation in roots. It has been reported that after absorbing of Pb as chelated by fragments of humic acids, its translocation in plant tissues can be limited, leading to its major accumulation in the roots (Duran *et al.*, 2006).

There are several reports that show the sunflower has the ability to accumulate and tolerate high concentrations of Pb. In a similar study, the concentration of Pb was increased 23 fold in the shoot and its concentration on base of dry matter in roots, stems and leaves was 2668, 843 and 3611 mg kg⁻¹, respectively (Boonyapookana *et al.*, 2005). In another study in sunflower treated with different concentrations of Pb, its mean concentration in plant tissues was more than 1000 mg Kg⁻¹ DW (Winska-Krysiak *et al.*, 2015). In another study, a high Pb accumulation (over 1000 mg Kg⁻¹ DW) was observed in shoot of sunflower (Huang *et al.*, 1997). In the case of other plants, there are reports that show the plants have the ability to tolerate and accumulate a high level of Pb in various organs. For instance, *Thlaspi rotundifolium* can accumulate 8200 mg Kg⁻¹ DW of Pb. Plants of *Polycarphaea synandra* F.Muell., *Armeria maritime* (Mill.) Willd. and *Thlaspi alpestre* Jacq. are also able to accumulate Pb up to 1044, 1300 and 2740 mg Kg⁻¹ DW, respectively (Baker and Brook, 1989). *Cyamopsis tetragonoloba* L. and *Sesamum indicum* L. have also been shown to able to tolerate Pb up to 1000 mg Kg⁻¹ (Amin *et al.*, 2018).

Accumulation factors: Plants species with a BCF, TF, and AF greater than one have a high potential for use in the phytoremediation (Marrugo-Negrete *et al.*, 2016). In present study, BCF in Pb-treated sunflower plants was much more than 1. Ignoring the highest Pb level, BCF was increased with increasing dose of Pb

treatment. Pb+VC-treated plants had a higher BCF, compared to those not-amended with VC, except for plants under 2000 mg L⁻¹ Pb (table 2). The higher BCF is due to the positive effect of VC in increasing Pb accumulation in the root. This improving effect is important for phytoremediation because phytoextraction of Pb from the polluted soil is difficult due to its low solubility and formation of stable complexes (Najeeb *et al.*, 2017).

A TF and AF greater than 1 indicate that accumulation in shoots is more than roots and soil, respectively (Marrugo-Negrete *et al.*, 2016). Result showed that TF in plants treated with all levels of Pb (both amended with VC or not) was lower than 1. In Pb-treated plants, VC decreased TF (table 2). In addition to what was discussed in the previous section, this may be due to this fact that VC caused an increase in Pb accumulation in roots while its translocation capacity from roots to shoots was limited. Basically, the translocation of Pb from root to shoot is low (Kabata-Pendias, 2011). In contrast to TF, AF was higher than 1 both in Pb- and Pb+VC-treated plants, except in plants treated with VC+2000 mg L⁻¹ Pb. In Pb-treated plants, those amended with VC had lower AF combined with lower TF (table 2).

Concentration of some minerals in roots and shoots: The results showed that, Ca (Fig. 3 b), Mg (Fig. 3 d), and Zn concentrations (Fig. 3 f) of leaves were not significantly changed by Pb treatments compared to the control. However, the concentrations of these minerals in roots (Fig. 3 a, c, e) significantly increased in the plants treated with 1000 and 1500 mg L⁻¹ Pb, but decreased in those treated with the highest concentration of Pb i.e., 2000 mg L⁻¹, except for Mg. The earlier increase may be due to the induction of metal transporters by Pb led to a higher absorption of ionic metals such as Pb²⁺, Ca²⁺, Mg²⁺, and Zn²⁺. There are different families of plasma membrane transporters in plant cells that non-specifically contribute to the

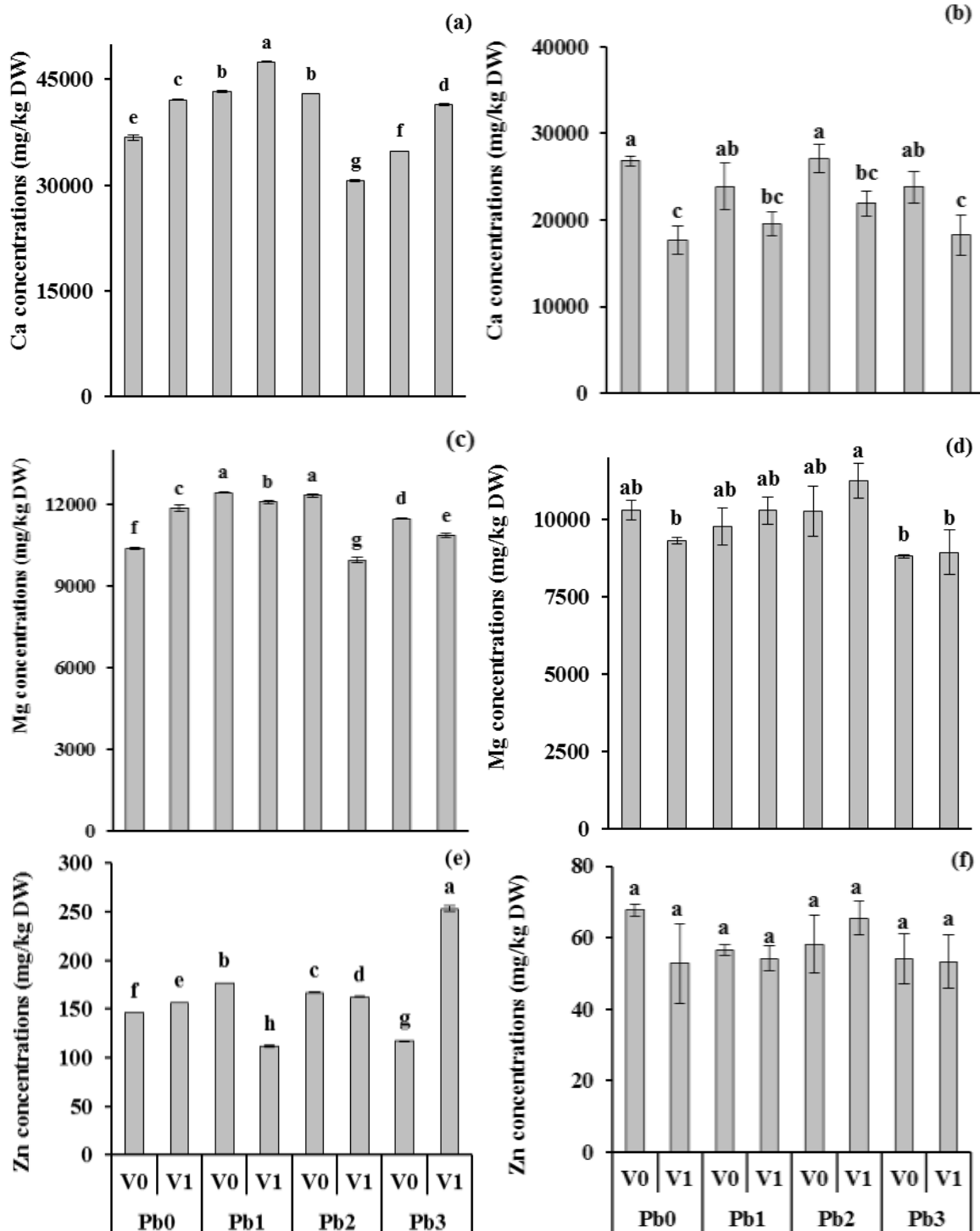


Fig. 3 Interaction effects of VC and Pb on the concentration of Ca, Mg, and Zn in the root (a, c, and e) and shoot (b, d, and f) of sunflower. V1 and V0 indicate VC-amended or not, respectively. Different levels of Pb (Pb0-4) include concentrations of 0 (Pb0, Pb free), 1000 (Pb1), 1500 (Pb2) and 2000 (Pb3) mg L⁻¹. Different letters on the columns indicate a significant difference (P ≤ 0.05). DW: dry weight.

absorption of some metal cations. For example, the ZIP (ZRT/IRT-like protein) family of metal transporters is involved in transporting bivalent cations such as zinc, iron, cadmium and manganese from cell membranes (Komal *et al.*, 2015). The decrease of Ca and Zn in root of the plants treated with the highest concentration of Pb

can be resulted from the competition between mentioned common metal transporters. An excess of a metal ion, such as Pb²⁺, may interfere with the absorption of other similar ions transported by a common transporter (Marschner, 1995). Pb can prevent many ions to bind to their absorption sites on the roots

(Lamhamdi *et al.*, 2013).

ICP-OES results showed that the concentration of Ca, Mg, and Zn in leaves of Pb-treated plants was not significantly changed by VC, except for Ca, compared to those not amended with VC. However, the application of VC caused a significant increase in the amount of these minerals in the roots of the control plants and also those treated with 2000 mg L⁻¹, except for Mg. In contrast, the amount of these minerals in roots of plants treated with 1000 and 1500 mg L⁻¹ Pb (except for Ca concentration of those treated with 1000 mg L⁻¹ Pb) was significantly decreased by VC, compared to those not amended with that (Fig. 3 a, c, e). The interpretation of these non-regular and sometimes contradictory results is difficult. Most notably, while Ca concentration of sunflower plant was in normal range (Fig. 3 a, b), its Mg and Zn concentrations were more than mean concentrations of these minerals in plants (Fig. 3 b-f). Ca concentration of plants varies between 0.1% and more than 5% of DW and depends on the growth conditions, species, and

plant organs (Marschner, 1995). For optimum growth of plants in the vegetative stage the Mg is required in a range of 0.15-0.35% of DW (Marschner, 1995). Finally, leaves usually need 15 to 20 mg Zn Kg⁻¹ DW for normal growth (Yoon *et al.*, 2014).

Conclusion

Sunflower with a high biomass was highly tolerant to Pb, accumulating a large amount of Pb in the roots and shoots. Therefore, it can be considered as an appropriate species for phytoremediation of Pb-polluted soils. VC improved phytoremediation of Pb by sunflower, because the accumulation of Pb in the tissues of plants that were simultaneously treated with Pb and VC was more than its accumulation in plants that were only treated with Pb.

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