Original article

Role of antioxidant enzymes and plant performances towards heavy metal stress in ornamental sunflower by vermicompost implementation

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Summary

Almost hyperaccumulators have a short growth period and some are edible crops with low-biomass. So, in this study, the potential of ornamental sunflower to remedy contaminated soil was evaluated. The experiment was set in as factorial arrangements in a completely randomized design where the treatments were 3 concentrations vermicompost (0, 15, 30 kg m⁻²), 3 Pb concentrations (0, 400, 600 ppm) and 4 Cd concentrations (0, 5, 10, 15 ppm). The oxidative enzyme activities were greatly influenced by the presence of vermicompost and various contaminant levels. Treatment of Pb and Cd without vermicompost application, increased superoxide dismutase and catalase activity 27- and 12-folds, respectively, in comparison with control, but in the presence of both metal treatments accompany with 30 kg m⁻² vermicompost, enzyme activity was increased 15 times in superoxide dismutase and 9 times in catalase more than the control. The addition of vermicompost amendments has been shown to increase soil microbial activity. The results show that the translocation factor was not greater than 1 in ornamental sunflower at none of the studied treatments, but the biological concentration factor was more than 1. Our results indicated that ornamental sunflower showed tolerance to Pb and Cd contamination and *Helianthus annuus* 'Sungold' was more favorable for Pb uptake compared to Cd.

Keywords

ornamental plant, lead, cadmium, SOD, CAT, organic amendment

Significance of this study

What is already known on this subject?

- Nowadays, soil contamination by heavy metals has posed a serious challenge in the industrial area soils worldwide. Phytoremediation is a promising approach for soil improvement which removes heavy metals. Most of the research works in the field of phytoremediation have been so far focused on vegetables and forage crops which may bring about the risk of heavy metals entrance in the food chain.

What are the new findings?

- Ornamental sunflower (*Helianthus annuus* 'Sungold') as a non-edible plant was chosen based on their high biomass and fast growth rate. The presence of organic matter in the soil has beneficial influences and improves its phytoremediation properties. The oxidative enzyme activities were greatly influenced by the presence of vermicompost and various Pb and Cd concentrations. Ornamental sunflower showed tolerance to Pb and Cd contamination and it was more favorable for Pb uptake compared to Cd.

What is the expected impact on horticulture?

- *Helianthus annuus* 'Sungold' showed tolerance toward Pb and Cd contamination and this ornamental plant was more favorable for Pb and Cd uptake. Therefore, it can be recommended for the remediation of soil co-contaminated in landscape of polluted area.

Introduction

Nowadays, soil contamination by heavy metals has posed a serious challenge in the industrial area soils worldwide. Heavy metal contamination of the soil is different from the air or water contaminations as heavy metals won't easily leach away upon their accumulation in the soil, giving rise to their higher durability in soils rather than any other parts of the biosphere (Lasat, 2002). Human activities have resulted in extensive soil contamination by lead (Pb) and cadmium (Cd). High concentrations of heavy metals will decrease the nutrients uptake, prevent enzymatic activity and induce oxidative stress (changes in the enzymes involved in the antioxidant defense system) (Majer et al., 2002; Agrawal

and Sharma, 2006). The antioxidative system uses the antioxidant enzymes (mainly including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and glutathione reductase (GR) to scavenge the reactive oxygen species (ROS) and thereby, prevent oxidative damage (Sandalo et al., 2009; Aibibu et al., 2010). Heavy metals can greatly threaten the plant and human health cycles upon their entrance into the soil. In this regard, a huge deal of attention has been paid to control and manage the heavy metals content of the soils to prevent their entrance into the food chain and groundwater (Farouk et al., 2011). Phytoremediation is a promising approach for soil improvement which removes heavy metals through absorption (Ghori et al., 2016). As one of the phytoremediation technologies, phytoextraction involves the transportation of the heavy metal from the polluted water

source or terrestrial source to the phyto-biomass. This is a biological process for the decontamination of heavy metal (Singer et al., 2007). Each type of contaminant requires a different phytoremediation process which may include a wide variety of plants. Various plant species can hyper-accumulate the heavy metals in their tissues. 45 families of plants are able to accumulate heavy metals (Ullah et al., 2015). Most of these plants suffer from several deficiencies, for instance, hyperaccumulator plants have short growth duration and are low-biomass producers. On the other hand, high-biomass plants are generally sensitive to high concentrations of heavy metals and have less ability to transport metals from roots to shoots (Muszynska and Hanus-Fajerska, 2015). Thus, researches aimed at identifying the native hyperaccumulators, and non-edible plants (such as ornamental plants) with high biomass production rate during the growth period and elevated metals uptake and root-shoot transport have been increased.

Most of the research works in the field of phytoremediation have been so far focused on vegetables and forage crops which may bring about the risk of heavy metals entrance in the food chain. In this regard, studies on tolerance, absorption, and accumulation of heavy metals by different plant species sound crucial. Ornamental plant sunflower (*Helianthus annuus* 'Sungold') belongs to the family of Asteraceae. It is grown in a wide range of soil types as a bedding plant outside, pot flower and cut-flower.

Previous studies have revealed the high tolerance of sunflower (as an edible crop) against heavy metals (Pilon-Smits, 2005; Chirakkara and Reddy, 2015; Rizwan et al., 2016; Govarthanana et al., 2018). So, in this study, in addition to the ornamental characteristics of sunflower, its potential ability to improve the contaminated soil will be investigated. For this purpose, the role of vermicompost in Pb and Cd absorption by ornamental sunflower and the effects of heavy metals contamination on antioxidant characteristics and phytoremediation indices were addressed.

Materials and methods

Soil and vermicompost sampling, characterization, and processing

The physicochemical parameters of soil samples were analyzed by standard methods and their organic carbon (OC), pH, EC, lime, cation exchange capacity (CEC), total nitrogen and total phosphorus, transferable potassium, total nitrogen, soil texture, and extractable heavy metals (Cd and Pb) were determined. The results of physical and chemical analyses of the studied soils are presented in Table 1. The concentrations of Pb and Cd were below the detection limit of the applied instrument. The cow vermicompost with min-

imum heavy metal content was used as the organic fertilizer whose analysis results are listed in Table 1. The soil sample was mixed with CdCl₂ at 0, 5, 10, 15 ppm and Pb(NO₃)₂ at 0, 400, 600 ppm. Then it was equilibrated in a greenhouse for one month at 60% water holding capacity (WHC) prior to the experiment. The polluted soil was filtered through a 2-mm stainless steel sieve. The vermicompost was then added in different treatments (0, 15, 30 kg m⁻²). The plastic pots were filled with 5 kg soil and different proportions of vermicompost to soil were added: concentrations of 0, 50, and 100 g per pot.

Greenhouse experiments

Seeds of ornamental sunflower (*Helianthus annuus* 'Sungold') were obtained from Hem Zaden BV. Greenhouse pot culture experiments were conducted to study the effect of vermicompost on Cd and Pb phytoremediation potential (uptake) of the ornamental sunflower. The heavy metal-contaminated soil-vermicompost was employed as the potting media. The seeds were sown in soil-vermicompost media and irrigated with distilled water. To prevent the loss of nutrients and trace elements from the pots, plastic trays were placed under each pot and the leached fluid was returned to the corresponding pots. After two weeks, the seedlings were thinned to one plant per pot and grown. Distilled water was added as needed.

Harvest and determination of heavy metals in plant and soil

After 60 days of cultivation, the plants were gently removed from the pots. They were then cut into roots and shoots, washed with tap water, and then with deionized water to remove any residual soil or dust and dried at room temperature. Shoot and roots samples were dried in an oven at 60°C for 48 h and their dry matter was measured. The plant materials were ground by an electric grinder into fine powders. They were then kept for further measurement of heavy metals. 0.5 g of the sieved plant samples were digested with a mixture of HCl/HNO₃ (3:1, v/v). Pb and Cd contents (ppm) of the digested dry plants were determined using atomic absorption spectrophotometry (AAS, Perkin Elmer) as described by Lindsay and Norvell (1978). The soil was digested in order to determine the Pb and Cd content by an atomic absorption spectrophotometer (Sabien et al., 2004) as well. In this study, phytoremediation factors of Pb and Cd were also assessed. The ability of ornamental sunflower for heavy metal accumulation and upwards translocation was determined by calculating the tolerance index (TI), biological concentration factor (BCF), biological accumulation coefficient (BAC), and translocation factor (TF) (Martinez-Sanchez et al., 2012; Giri and Patel, 2012).

TABLE 1. Some physiochemical properties of experimental soil and vermicompost fertilizer.

Soil		Vermicompost	
pH	7.3	pH	8
EC (dS ⁻¹)	1.1	EC (dS ⁻¹)	6.9
Organic carbon (%)	0.1	Organic carbon (%)	21.4
Soil texture	Loamy	Total N (%)	1.5
Lime (%)	8	Available Zn (mg kg ⁻¹)	125.17
Pb (mg kg ⁻¹)	ND	Available P (mg kg ⁻¹)	1.23
Cd (mg kg ⁻¹)	ND	Pb (mg kg ⁻¹)	3.4
CEC (c mol 100 g ⁻¹ soil)	13.3	Cd (mg kg ⁻¹)	1.1

Measurement of antioxidant enzymes and microbial respiration

The total SOD activity was evaluated by measuring its ability to inhibit the photochemical reduction of nitrotriazolium blue chloride (NBT), as described by Rios-Gonzales et al. (2002). The CAT activity was also measured by H_2O_2 -consumption as previously described by Aebi (1984). SOD and CAT activities were determined by monitoring the decline in the absorbance at 420 nm and 240 nm by spectrophotometer, respectively. The basal soil microbial respiration was determined as evolved CO_2 using the method developed by Alef and Nannipieri (1995).

Design and statistical analysis

The experiment was set in as factorial arrangements in a completely randomized design (CRD) ($3 \times 3 \times 4$) where the treatments were the 3 concentrations vermicompost, 3 Pb concentrations and 4 Cd concentrations. Each treatment consisted of three replicates for statistical purposes. All the results were statistically analyzed using the SAS program (v. 9.1). Means of three replicates were subjected to one-way ANOVA. Duncan's Multiple Range Test (DMRT) was also used to compare the mean values and determine the significance of statistical differences in treatments at $p \leq 0.05$. The obtained data were reported as mean plus the standard deviation ($X \pm S.D.$).

TABLE 2. Effect of vermicompost amendment on biomass, antioxidant activity and microbial respiration in ornamental sunflower under lead (Pb) and cadmium (Cd) stress. Values are mean $\pm$ SD of three replicates. In each column, numbers followed by the same letter are not significantly different according to Duncan's test ($P < 0.05$).

Vermi-compost (kg m ⁻²)	Pb (ppm)	Cd (ppm)	FW (g) [†]	DW (g)	SOD (U mg ⁻¹ protein)	CAT (U mg ⁻¹ protein)	CO (mg C-CO ₂ kg ⁻¹ soil)
0	0	0	0.72 $\pm$ 0.45fghi	0.49 $\pm$ 0.29efghi	0.08 $\pm$ 0.005vw	0.1 $\pm$ 0.005uv	1.11 $\pm$ 0.01z ^{††}
0	0	5	0.66 $\pm$ 0.36fghi	0.43 $\pm$ 0.22efghi	0.31 $\pm$ 0.02r	0.41 $\pm$ 0.02q	4.24 $\pm$ 0.02s
0	0	10	0.32 $\pm$ 0.11ghi	0.22 $\pm$ 0.10hig	1.21 $\pm$ 0.01h	1.11 $\pm$ 0.01k	5.0 $\pm$ 0.05q
0	0	15	0.83 $\pm$ 0.70fghi	0.51 $\pm$ 0.43efghi	1.38 $\pm$ 0.02f	1.49 $\pm$ 0.01g	6.11 $\pm$ 0.02l
0	400	0	0.15 $\pm$ 0.13i	0.09 $\pm$ 0.07i	0.13 $\pm$ 0.01u	0.32 $\pm$ 0.01rst	1.5 $\pm$ 0.03x
0	400	5	0.08 $\pm$ 0.007i	0.05 $\pm$ 0.002i	0.52 $\pm$ 0.01o	0.7 $\pm$ 0.03o	5.1 $\pm$ 0.01p
0	400	10	0.12 $\pm$ 0.02i	0.08 $\pm$ 0.01i	1.62 $\pm$ 0.02d	2.05 $\pm$ 0.01d	6.3 $\pm$ 0.04k
0	400	15	0.49 $\pm$ 0.17ghi	0.30 $\pm$ 0.01fghi	1.91 $\pm$ 0.01b	2.21 $\pm$ 0.01c	7.5 $\pm$ 0.03fg
0	600	0	0.64 $\pm$ 0.64fghi	0.38 $\pm$ 0.40fghi	0.28 $\pm$ 0.005	0.44 $\pm$.03q	2.1 $\pm$ 0.02v
0	600	5	0.32 $\pm$ 0.13ghi	0.19 $\pm$ 0.07hi	0.72 $\pm$ 0.02n	0.98 $\pm$ 0.03l	5.8 $\pm$ 0.02m
0	600	10	0.39 $\pm$ 0.27ghi	0.22 $\pm$ 0.15hi	1.81 $\pm$ 0.01c	2.28 $\pm$ 0.02b	7.1 $\pm$ 0.03h
0	600	15	0.29 $\pm$ 0.15ghi	0.16 $\pm$ 0.08hi	2.18 $\pm$ 0.0a	2.41 $\pm$ 0.03a	8.29 $\pm$ 0.02e
15	0	0	0.51 $\pm$ 0.25ghi	0.28 $\pm$ 0.13fghi	0.04 $\pm$ 0.00xy	0.17 $\pm$ 0.01v	1.3 $\pm$ 0.02y
15	0	5	5.46 $\pm$ 0.81a	3.08 $\pm$ 0.44a	0.21 $\pm$ 0.01t	0.31 $\pm$ 0.01st	4.93 $\pm$ 0.02r
15	0	10	5.21 $\pm$ 1.65ab	3.10 $\pm$ 0.89a	0.72 $\pm$ 0.02n	0.91 $\pm$ 0.01m	5.34 $\pm$ 0.03o
15	0	15	3.22 $\pm$ 2.41bcde	1.80 $\pm$ 1.35bcde	1.04 $\pm$ 0.03k	1.11 $\pm$ 0.01k	6.40 $\pm$ 0.02k
15	400	0	1.5 $\pm$ 0.82efghi	0.86 $\pm$ 0.42defghi	0.08 $\pm$ 0.00vw	0.22 $\pm$ 0.02u	1.91 $\pm$ 0.01w
15	400	5	1.2 $\pm$ 0.9efghi	0.71 $\pm$ 0.50defghi	0.32 $\pm$ 0.01r	0.51 $\pm$ 0.01p	5.49 $\pm$ 0.01n
15	400	10	5.1 $\pm$ 4.0ab	3.09 $\pm$ 2.47a	0.96 $\pm$ 0.01l	1.30 $\pm$ 0.01i	6.71 $\pm$ 0.02j
15	400	15	1.4 $\pm$ 0.41efghi	0.87 $\pm$ 0.47defghi	1.32 $\pm$ 0.02g	1.83 $\pm$ 0.03e	8.45 $\pm$ 0.04d
15	600	0	4.02 $\pm$ 1.62abcd	2.33 $\pm$ 0.95abc	0.10 $\pm$ 0.005	0.33 $\pm$ 0.05rs	2.42 $\pm$ 0.03v
15	600	5	5.42 $\pm$ 1.32a	3.20 $\pm$ 0.82a	0.49 $\pm$ 0.01	0.72 $\pm$ 0.2o	6.13 $\pm$ 0.01l
15	600	10	1.95 $\pm$ 0.4defghi	1.14 $\pm$ 0.28cdefghi	1.15 $\pm$ 0.04	1.49 $\pm$ 0.06g	7.45 $\pm$ 0.04g
15	600	15	0.26 $\pm$ 0.13hi	0.15 $\pm$ 0.07	1.52 $\pm$ 0.02uv	2.04 $\pm$ 0.01d	10.20 $\pm$ 0.09b
30	0	0	2.19 $\pm$ 1.1defghi	1.26 $\pm$ 0.68cdefghi	0.01 $\pm$ 0.005y	0.08 $\pm$ 0.01w	1.60 $\pm$ 0.01x
30	0	5	4.94 $\pm$ 0.27abc	2.88 $\pm$ 0.22ab	0.20 $\pm$ 0.005t	0.22 $\pm$ 0.01u	5.22 $\pm$ 0.01po
30	0	10	1.39 $\pm$ 1.10efghi	0.77 $\pm$ 0.61defghi	0.52 $\pm$ 0.02o	0.75 $\pm$ 0.02o	5.71 $\pm$ 0.01m
30	0	15	2.22 $\pm$ 1.6defghi	1.25 $\pm$ 0.95	0.07 $\pm$ 0.01vw	0.81 $\pm$ 0.03n	7.59 $\pm$ 0.01f
30	400	0	3.57 $\pm$ 1.90abcde	2.04 $\pm$ 1.11abcd	0.05 $\pm$ 0.005wx	0.2 $\pm$ 0.005uv	2.13 $\pm$ 0.01v
30	400	5	2.92 $\pm$ 2.15cdef	1.61 $\pm$ 1.17bcdefg	0.34 $\pm$ 0.02r	0.35 $\pm$ 0.005r	5.70 $\pm$ 0.01m
30	400	10	2.65 $\pm$ 1.10defg	1.51 $\pm$ 0.63cdefgh	0.81 $\pm$ 0.01m	1.13 $\pm$ 0.03	6.94 $\pm$ 0.02i
30	400	15	2.60 $\pm$ 0.51defgh	1.44 $\pm$ 0.26cdefghi	1.11 $\pm$ 0.01j	1.35 $\pm$ 0.04	9.55 $\pm$ 0.05c
30	600	0	2.07 $\pm$ 1.0defghi	1.23 $\pm$ 0.61cdefghi	0.08 $\pm$ 0.005vw	0.28 $\pm$ 0.00t5	2.71 $\pm$ 0.02t
30	600	5	1.44 $\pm$ 0.54efghi	0.84 $\pm$ 0.32defghi	0.39 $\pm$ 0.01q	0.49 $\pm$ 0.01p	6.30 $\pm$ 0.02
30	600	10	0.84 $\pm$ 0.30fghi	0.48 $\pm$ 0.16efghi	0.97 $\pm$ 0.02l	1.25 $\pm$ 0.01j	7.60 $\pm$ 0.04f
30	600	15	2.97 $\pm$ 0.36cdef	1.67 $\pm$ 0.28bcdef	1.20 $\pm$ 0.03h	1.73 $\pm$ 0.02f	12.13 $\pm$ 0.1a

[†] FW: Fresh weight; DW: Dry weight; SOD: superoxide dismutase enzyme; CAT: catalase enzyme; CO: microbial respiration.

^{††} Values followed in each column by the same letter were not significantly different at 5% level.

Results

The amount of biomass produced during phytoremediation is the most prominent characteristic in this technique. The results of this experiment showed that the application of different levels of vermicompost in the soil polluted by cadmium and lead enhanced the fresh and dry weight of ornamental sunflower during the cultivation period. The application of vermicompost at the concentration of 15 kg m⁻² led to the highest of fresh (5 g) and dry (3 g) weights of ornamental sunflower during the cultivation period in the soil contaminated with 0 ppm and 600 ppm lead and 5 ppm cadmium. Conversely, an increase of Pb and Cd concentrations in the soil resulted in a reduction in biomass production. The least biomass production (0.05–0.15 g) was measured in the

treatment containing 400 ppm lead and 0–10 ppm cadmium and the one with no vermicompost amendment. The effect of cadmium concentrations on the decrease of the dry weight was more noticeable than that of lead (Table 2).

Moreover, oxidative enzyme activities were greatly influenced by the presence of vermicompost and various contaminant levels. CAT and SOD activities were decreased as a result of vermicompost enhancement, but the presence of cadmium and lead in the polluted soil caused abiotic stress and induced the CAT and SOD activity. The maximum activity of both enzymes was measured in the treatment containing the highest concentration of metal contaminants, i.e., 600 ppm lead and 15 ppm cadmium and without vermicompost amendment (2.18 for SOD and 2.41 for CAT U mg⁻¹ protein).

TABLE 3. Effect of vermicompost amendment on BAC, BCF and TF parameters for lead in ornamental sunflower under lead (Pb) and cadmium (Cd) stresses. Values are mean $\pm$ SD of three replicates. In each column, numbers followed by the same letter are not significantly different according to Duncan's test ($P < 0.05$).

Vermicompost (kg m ⁻²)	Pb (ppm)	Cd (ppm)	BAC Pb [†]	BCF Pb	TF Pb
0	0	0	0.00 $\pm$ 0.00p	0.00 $\pm$ 0.00l	0.00 $\pm$ 0.00o ^{††}
0	0	5	0.00 $\pm$ 0.00p	0.00 $\pm$ 0.00l	0.00 $\pm$ 0.00o
0	0	10	0.00 $\pm$ 0.00p	0.00 $\pm$ 0.00l	0.00 $\pm$ 0.00o
0	0	15	0.00 $\pm$ 0.00p	0.00 $\pm$ 0.00l	0.00 $\pm$ 0.00o
0	400	0	0.68 $\pm$ 0.005ghi	1.49 $\pm$ 0.02bcde	0.45 $\pm$ 0.01fgh
0	400	5	0.67 $\pm$ 0.03hij	1.45 $\pm$ 0.01hij	0.46 $\pm$ 0.01efg
0	400	10	0.69 $\pm$ 0.01efgh	1.45 $\pm$ 0.008hij	0.47 $\pm$ 0.01cde
0	400	15	0.69 $\pm$ 0.009defgh	1.43 $\pm$ 0.02j	0.48 $\pm$ 0.002bc
0	600	0	0.71 $\pm$ 0.01abcde	1.51 $\pm$ 0.01abcd	0.46 $\pm$ 0.004defg
0	600	5	0.71 $\pm$ 0.01abcd	1.52 $\pm$ 0.03ab	0.46 $\pm$ 0.005defg
0	600	10	0.72 $\pm$ 0.02a	1.53 $\pm$ 0.01a	0.47 $\pm$ 0.01cdef
0	600	15	0.66 $\pm$ 0.02ij	1.44 $\pm$ 0.01ij	0.45 $\pm$ 0.01fgh
15	0	0	0.00 $\pm$ 0.00p	0.00 $\pm$ 0.00l	0.00 $\pm$ 0.00o
15	0	5	0.00 $\pm$ 0.00p	0.00 $\pm$ 0.00l	0.00 $\pm$ 0.00o
15	0	10	0.00 $\pm$ 0.00p	0.00 $\pm$ 0.00l	0.00 $\pm$ 0.00o
15	0	15	0.00 $\pm$ 0.00p	0.00 $\pm$ 0.00l	0.00 $\pm$ 0.00o
15	400	0	0.65 $\pm$ 0.01jk	1.46 $\pm$ 0.02fghij	0.45 $\pm$ 0.01hi
15	400	5	0.68 $\pm$ 0.006fghi	1.45 $\pm$ 0.01ghij	0.47 $\pm$ 0.02def
15	400	10	0.71 $\pm$ 0.01abc	1.45 $\pm$ 0.009hij	0.49 $\pm$ 0.004ab
15	400	15	0.70 $\pm$ 0.008cdefg	1.39 $\pm$ 0.01k	0.50 $\pm$ 0.01a
15	600	0	0.67 $\pm$ 0.009ij	1.51 $\pm$ 0.02abcd	0.44 $\pm$ 0.0006ij
15	600	5	0.67 $\pm$ 0.003ij	1.43 $\pm$ 0.01ij	0.46 $\pm$ 0.003efg
15	600	10	0.70 $\pm$ 0.003bcdef	1.46 $\pm$ 0.004fghij	0.48 $\pm$ 0.002bcd
15	600	15	0.72 $\pm$ 0.01ab	1.52 $\pm$ 0.03abc	0.47 $\pm$ 0.006cde
30	0	0	0.00 $\pm$ 0.00p	0.00 $\pm$ 0.00l	0.00 $\pm$ 0.00o
30	0	5	0.00 $\pm$ 0.00p	0.00 $\pm$ 0.00l	0.00 $\pm$ 0.00o
30	0	10	0.00 $\pm$ 0.00p	0.00 $\pm$ 0.00l	0.00 $\pm$ 0.00o
30	0	15	0.00 $\pm$ 0.00p	0.00 $\pm$ 0.00l	0.00 $\pm$ 0.00o
30	400	0	0.57 $\pm$ 0.01l	1.46 $\pm$ 0.03ghij	0.39 $\pm$ 0.01k
30	400	5	0.58 $\pm$ 0.02l	1.49 $\pm$ 0.03bcdef	0.39 $\pm$ 0.01k
30	400	10	0.57 $\pm$ 0.01l	1.48 $\pm$ 0.01defgh	0.38 $\pm$ 0.01k
30	400	15	0.64 $\pm$ 0.006k	1.49 $\pm$ 0.02cdefg	0.43 $\pm$ 0.008j
30	600	0	0.45 $\pm$ 0.0040	1.50 $\pm$ 0.009abcd	0.30 $\pm$ 0.004
30	600	5	0.47 $\pm$ 0.003n	1.47 $\pm$ 0.01efghi	0.32 $\pm$ 0.005lm
30	600	10	0.46 $\pm$ 0.001no	1.49 $\pm$ 0.02bcde	0.31 $\pm$ 0.003mn
30	600	15	0.50 $\pm$ 0.004m	1.53 $\pm$ 0.02a	0.32 $\pm$ 0.006l

[†] BAC: biological accumulation coefficient; BCF: biological concentration factor; TF: translocation factor.

^{††} Values followed in each column by the same letter were not significantly different at 5% level.

Heavy metal contaminations also affected the biological quality of rhizosphere. So, the increase of the vermicompost, lead, and cadmium enhanced the impacts on microbial respiration in soil. Maximum microbial respiration was observed in the soil containing 30 kg m⁻² vermicompost, 600 ppm lead, and 15 ppm cadmium (12.13 mg C-CO₂ kg⁻¹ soil) (Table 2).

Phytoremediation indices were also influenced by the vermicompost content of the soil (Tables 3 and 4). By increase of vermicompost, the BAC for cadmium and lead was

decreased, but increasing this organic matter just enhanced the BCF for cadmium. The metals concentration was also effective on these two factors. BAC factor for both lead and cadmium were increased upon enhancement of the metal content of the soil. In the case of BCF, different results were obtained. Increasing the lead and cadmium concentration in soil caused an increase and a decrease in BCF, respectively. Elevation of cadmium concentration from 0 ppm to 15 ppm, just caused a reduction in BCF of the lead. In other words,

TABLE 4. Effect of vermicompost amendment on BAC, BCF, TF and Ti parameters for cadmium in ornamental sunflower under lead (Pb) and cadmium (Cd) stresses. Values are mean $\pm$ SD of three replicates. In each column, numbers followed by the same letter are not significantly different according to Duncan's test ($P < 0.05$).[†]

Vermicompost (kg m ⁻²)	Pb (ppm)	Cd (ppm)	BAC Cd	BCF Cd	TF Cd	Ti
0	0	0	0.00 $\pm$ 0.00k	0.00 $\pm$ 0.00n	0.00 $\pm$ 0.00m	1.00 $\pm$ 0.00efgh ^{††}
0	0	5	0.89 $\pm$ 0.13bc	2.48 $\pm$ 0.2cde	0.35 $\pm$ 0.02ghi	0.86 $\pm$ 0.45efgh
0	0	10	0.57 $\pm$ 0.01fg	1.57 $\pm$ 0.03jk	0.36 $\pm$ 0.01ghi	0.45 $\pm$ 0.20gh
0	0	15	0.80 $\pm$ 0.05de	2.05 $\pm$ 0.04i	0.39 $\pm$ 0.01def	1.03 $\pm$ 0.87efgh
0	400	0	0.00 $\pm$ 0.00k	0.00 $\pm$ 0.00n	0.00 $\pm$ 0.00m	0.18 $\pm$ 0.14h
0	400	5	0.89 $\pm$ 0.07bc	2.24 $\pm$ 0.10fgh	0.39 $\pm$ 0.01def	0.11 $\pm$ 0.005h
0	400	10	0.55 $\pm$ 0.02fgh	1.50 $\pm$ 0.01kl	0.36 $\pm$ 0.01fgh	0.16 $\pm$ 0.03h
0	400	15	0.85 $\pm$ 0.02cd	2.05 $\pm$ 0.03i	0.41 $\pm$ 0.01cd	0.60 $\pm$ 0.03fgh
0	600	0	0.00 $\pm$ 0.00k	0.00 $\pm$ 0.00n	0.00 $\pm$ 0.00m	0.77 $\pm$ 0.80fgh
0	600	5	0.98 $\pm$ 0.01a	2.05 $\pm$ 0.06i	0.48 $\pm$ 0.02a	0.38 $\pm$ 0.15gh
0	600	10	0.56 $\pm$ 0.02fgh	1.57 $\pm$ 0.03jk	0.36 $\pm$ 0.01ghi	0.44 $\pm$ 0.31gh
0	600	15	0.94 $\pm$ 0.02ab	2.03 $\pm$ 0.03i	0.46 $\pm$ 0.004ab	0.32 $\pm$ 0.17gh
15	0	0	0.00 $\pm$ 0.00k	0.00 $\pm$ 0.00n	0.00 $\pm$ 0.00m	0.57 $\pm$ 0.27fgh
15	0	5	0.75 $\pm$ 0.10e	2.93 $\pm$ 0.00a	0.25 $\pm$ 0.03k	6.16 $\pm$ 0.88a
15	0	10	0.50 $\pm$ 0.02hij	1.56 $\pm$ 0.00jk	0.32 $\pm$ 0.01j	6.20 $\pm$ 1.78a
15	0	15	0.95 $\pm$ 0.01ab	2.27 $\pm$ 0.01fg	0.41 $\pm$ 0.008cd	3.60 $\pm$ 2.70bcde
15	400	0	0.00 $\pm$ 0.00k	0.00 $\pm$ 0.00n	0.00 $\pm$ 0.00m	1.72 $\pm$ 0.85defgh
15	400	5	0.90 $\pm$ 0.05bc	2.41 $\pm$ 0.19ed	0.37 $\pm$ 0.05efg	1.42 $\pm$ 1.00defgh
15	400	10	0.51 $\pm$ 0.009hij	1.53 $\pm$ 0.01jkl	0.33 $\pm$ 0.01ij	6.19 $\pm$ 4.94a
15	400	15	0.85 $\pm$ 0.03cd	2.13 $\pm$ 0.03hi	0.40 $\pm$ 0.009de	1.75 $\pm$ 0.94defgh
15	600	0	0.00 $\pm$ 0.00k	0.00 $\pm$ 0.00n	0.00 $\pm$ 0.00m	4.66 $\pm$ 1.91abc
15	600	5	0.93 $\pm$ 0.04ab	2.27 $\pm$ 0.10fg	0.41 $\pm$ 0.003d	6.41 $\pm$ 1.65a
15	600	10	0.59 $\pm$ 0.003f	1.65 $\pm$ 0.05j	0.36 $\pm$ 0.01ghi	2.28 $\pm$ 0.57cdefgh
15	600	15	0.90 $\pm$ 0.01bc	2.03 $\pm$ 0.04i	0.44 $\pm$ 0.005bc	0.30 $\pm$ 0.14gh
30	0	0	0.00 $\pm$ 0.00k	0.00 $\pm$ 0.00n	0.00 $\pm$ 0.00m	2.53 $\pm$ 1.037cdefgh
30	0	5	0.51 $\pm$ 0.04hij	2.64 $\pm$ 0.22b	0.19 $\pm$ 0.003l	5.76 $\pm$ 0.44ab
30	0	10	0.44 $\pm$ 0.01j	1.24 $\pm$ 0.04m	0.35 $\pm$ 0.02ghi	1.55 $\pm$ 1.22defgh
30	0	15	0.94 $\pm$ 0.04ab	2.52 $\pm$ 0.05bcd	0.37 $\pm$ 0.01efg	2.51 $\pm$ 1.90cdefgh
30	400	0	0.00 $\pm$ 0.00k	0.00 $\pm$ 0.00n	0.00 $\pm$ 0.00m	4.08 $\pm$ 2.22abcd
30	400	5	0.80 $\pm$ 0.04de	2.58 $\pm$ 0.02bc	0.31 $\pm$ 0.02j	3.22 $\pm$ 2.34bcdef
30	400	10	0.45 $\pm$ 0.02ij	1.41 $\pm$ 0.02l	0.32 $\pm$ 0.02j	3.02 $\pm$ 1.26cdefg
30	400	15	0.80 $\pm$ 0.01de	2.35 $\pm$ 0.06ef	0.33 $\pm$ 0.003hij	2.89 $\pm$ 0.53cdefgh
30	600	0	0.00 $\pm$ 0.00k	0.00 $\pm$ 0.00n	0.00 $\pm$ 0.00m	2.46 $\pm$ 1.22cdefgh
30	600	5	0.98 $\pm$ 0.02a	2.14 $\pm$ 0.10ghi	0.45 $\pm$ 0.02ab	1.68 $\pm$ 0.64defgh
30	600	10	0.53 $\pm$ 0.008fgh	1.42 $\pm$ 0.01l	0.37 $\pm$ 0.004efg	0.96 $\pm$ 0.32efgh
30	600	15	0.84 $\pm$ 0.01cd	2.23 $\pm$ 0.02fgh	0.37 $\pm$ 0.007efg	3.34 $\pm$ 0.57

[†] BAC: biological accumulation coefficient; BCF: biological concentration factor; TF: translocation factor; Ti: tolerance index.

^{††} Values followed in each column by the same letter were not significantly different at 5% level.

BCF and BAC for lead were influenced by different concentrations of cadmium rather than lead. Maximum BCF and BAC for lead (0.72) were recorded at the presence of high concentrations of lead (600 ppm) and 10 ppm cadmium without vermicompost amendment. Also, the highest of BAC for cadmium (0.98) was found in the treatment containing 600 ppm lead and 5 ppm cadmium with no vermicompost amendment. The presence of 5–15 ppm cadmium along with low concentrations of lead (0–400 ppm) enhanced the BCF for ornamental sunflower (Tables 2 and 3). As the amount of vermicompost increased, the TF was reduced; but the elevation of metals content in different treatments resulted in a rising trend in this phytoremediation index for both lead and cadmium. The soil amended with 15 kg m⁻² vermicompost led to the maximum value of TF for lead (0.5) in the treatment containing 400 ppm lead and 15 ppm cadmium. The highest value of TF for cadmium (0.48) was also measured in the treatment involving no vermicompost addition to the soil contaminated by 600 ppm lead and 5 ppm cadmium (Tables 3 and 4). The increase of vermicompost augmented the heavy metal tolerance index, but the elevation of heavy metal concentration caused a decreasing trend of TI. Treating soil with different concentrations of cadmium (up to 10 ppm) at constant vermicompost and lead contents increased the TI followed by a decline. The highest tolerance index (6.41) was recorded in the treatment involving 15 kg m⁻² vermicompost, 600 ppm lead and 5 ppm cadmium (Table 4).

Discussion

The application of vermicompost significantly increased the biomass of *Helianthus annuus* 'Sungold'. Ornamental sunflower was chosen in this experiment based on their high biomass, fast growth rate and its ability to eliminate heavy metals from contaminated soils. The presence of organic matter in the soil has beneficial influences on the soil and improves its physical and biochemical properties. Thus, vermicompost can increase plant growth and biomass yields (Fu et al., 2011). Sunflower growth and yield were also affected by the stress of the heavy metal (e.g., Pb and Cd). High concentrations of Pb and Cd inhibited the growth, reduced biomass production and caused characteristic visible effects similar to those reported by other workers in different plant species (Alaboudi et al., 2018). The decrease in dry matter production as a result of metal stress is in agreement with the earlier studies on plants other than sunflowers (Pandey and Pathak, 2006; Ryser and Sauder, 2006). Decline of sunflower dry and fresh matter as a result of increased Pb and Cd content of the soil can be due to the adverse effects of the heavy metals on the ornamental sunflower roots and its translocation into the shoot system, which may reduce the competence of dry matter synthesis and leaf photosynthesis and decrease the dry matter accumulation of the plant. These results were also reported in *Helianthus annuus* by Zadeh et al. (2008) and Chhotu et al. (2008).

High concentrations of Pb and Cd reduced the absorption of the nutrients, preventing the enzymatic activity and inducing oxidative stress, which will alter the enzymes involved in the antioxidant resistance system (Majer et al., 2002; Agrawal and Sharma, 2006). Comparison of the traits showed a significant increase in the CAT and SOD activities in the *Helianthus annuus* 'Sungold', with an increase of the heavy metals concentration. Such an increase in both enzymes was significantly different from the controls in the treatment involving 600 ppm Pb and 15 ppm Cd. The maximum responses of these enzymes (2.18% and 2.41% for SOD and CAT, respec-

tively) were observed at the maximum concentrations of lead (600 ppm) and cadmium (15 ppm). In the absence of vermicompost, a 27-fold and 12-fold increase was observed in SOD and CAT enzymes, respectively at the presence of lead and cadmium (600 ppm of Pb and 15 ppm of Cd) when compared to the control. For the same metal concentration, the use of 30 kg m⁻² vermicompost resulted in 15- and 9-fold increase in SOD and CAT activities, compared to the controls. Oxidant enzymes play a key role in the defense system of the plants against the heavy metal-induced oxidative stresses (Weckx and Clijsters, 1996). Enzymes such as CAT and SOD can deactivate the reactive oxygen species produced in the cells (Mishra et al., 2006; Nair et al., 2015). The increase in the CAT activity could be explained by the adaptive mechanism of the plants to maintain hydrogen peroxide (H₂O₂) of the cells at a steady level (Mishra et al., 2006). Oxidative stresses induce the production of oxygen free radicals, such as superoxide radicals (O⁻), H₂O₂ and hydroxyl radicals (OH) and free radicals hydroxyl respond resulting in the fat peroxidation (Chen et al., 2007). In the present study, CAT and SOD activity was significantly increased in leaves of Pb- and Cd-treated plants compared to the controls, which perhaps indicates the breakdown of H₂O₂ and toxic peroxides by heavy metal accumulation. Increase of CAT and SOD activity in response to Cd and Pb was reported in *Vigna radiate* L. Wilczek (Anjum et al. 2008), *Jatropha curcas* L., (Shu et al., 2012), *Pisum sativum* L. (Rodríguez-Serrano et al., 2006), and *Morus alba* (Tewari et al., 2013) as well. In contrast, addition of vermicompost significantly reduced the activity of CAT and SOD enzymes. The protective effect of vermicompost application might be due to its ability to prevent Pb and Cd transfer to the aerial organs, adjust the heavy metals stress effect, and inhibit oxygen-centered free radical generation, thereby preventing the initiation of ROS formation (Aravind and Prasad, 2005). It has been also reported that modulation of the antioxidant response may be the main task of fungal symbiont in metal tolerance contribution in the mycorrhizal symbioses as a biotic component of vermicompost (Garg and Kaur, 2013).

Apparently, vermicompost application significantly affected microbial respiration, especially in the Pb-contaminated. The addition of organic amendments has been shown to increase the soil microbial activity (Chander and Joergensen, 2002). As the microbial role of soil is essential in the nutrient turnover and plant nutrition, further research is required to assess the changes in the subsurface microbial activities. Our results generally agree with Farrell et al. (2009), who found that compost amendment can significantly affect the remediation strategy in metal-contaminated soils.

The BAC, BCF, and TF have been usually used to evaluate the translocations of heavy metals into the plant's tissues. The BCF and TF reflect the plant's potential to tolerate and accumulate heavy metals in their organs as well as their ability to phytoextraction (Barbafieri et al., 2017). TF values greater than 1 indicate the heavy metal translocation from root to the aerial organs (Galal and Shehata, 2015). The results showed that the TF was not greater than 1 in ornamental sunflower at any of the studied treatments, but the BCF exceeded 1 in the examined samples, with the exception of the pots free from Pb and Cd. Therefore, this species is unsuitable for Pb and Cd phytoextraction due to TF < 1 and BCF > 1 (Sidhu et al., 2017), but ornamental sunflower can be a proper candidate for phytostabilization of Pb and Cd at the examined concentrations. The TF factor decreased with the application of 15 to 30 kg m⁻² vermicompost compared to others. This finding indicated that Pb and Cd concentrations in the shoot of

ornamental sunflower decreased with the addition of vermicompost due to a decline in the extractable Cd content of the soil. This could be attributed to the heavy metals bound by organic ligands in vermicompost (Wei et al., 2011; Wang et al., 2013). The organic amendment led to an effective immobilization of some heavy metals such as Pb, Cu, Zn and Cd in soil (Angelova et al., 2016). Furthermore, the extended roots of ornamental sunflower and intensive dispersion of roots into the soil decreased the leaching via stabilization; hence immobilizing and concentrating Pb and Cd in the roots. The results agreed with those obtained by Angelova et al. (2016) who found that the organic amendments led to decreased Pb and Cu content in the potato peel and tubers. They also observed that at higher Cd concentrations, the amount of added amendment was not sufficient to immobilize all the available Cd. The presence of organic carbon increases the cation exchangeability of the soil which retains the nutrients assimilated by plants (Yobouet et al., 2010). Increasing the amount of organic matter in the soil helps to minimize the absorption of heavy metals by the plants (Fijalkowski et al., 2012). Organic amendments to contaminated soils (containing labile elements) reduced the overall bio-availabilities of the metals due to sorption processes (Brown et al., 2003).

The obtained results showed that ornamental sunflower can accumulate Pb and Cd in its tissues. However, the accumulation of Pb in the plant shoot was more favorable than Cd. Considering the potential for Pb phytoextraction, sunflower exhibits promising characteristics, as previously reported by other authors (Seth et al., 2011; Kacálková et al., 2014; Sewalem et al., 2014; Alaboudi et al., 2018; and Pilon-Smits, 2005). Computed TI was higher than 1 in the ornamental sunflower grown in the vermicompost-containing Pb- and Cd-contaminated soil. TI values were above one or approximately one; confirming the non-toxicity of heavy metals on the plant biomass. Gondek (2008) found that composts and vermicompost increased the tolerance characteristics of the plants cultivated in Cd-contaminated soils, which is consistent with the results of this study.

H. annuus 'Sungold' plant showed tolerance toward Pb and Cd contamination and this ornamental plant was more favorable for Pb uptake compared to Cd. Therefore, it can be recommended for the remediation of Pb- and Cd-contaminated soils.

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