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Effects of Soil Amendments on Metal Uptake, Antioxidant Activities and Production of Bioactive Compounds by Sunflower Sprouts

(Kesan Pindaan Tanah terhadap Pengambilan Logam, Aktiviti Antioksidan dan Penghasilan Bahan Bioaktif oleh Pucuk Bunga Matahari)

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ABSTRACT

The effects of soil amendments on Cd and Zn uptake, bioactive compounds and antioxidant activities in two cultivars (Pacific 33 and Black Oil) of Helianthus annuus *L. were evaluated. Dicalcium phosphate/cattle manure and leonardite/cattle manure mixed in a 1:1 w/w ratio with soil served as soil treatments. The Black Oil cultivar experienced greater metal uptake, biomass production and survival rate compared to the Pacific 33 cultivar. The increased Cd and Zn uptake (>0.2 and >99.4 mg/kg, respectively) in Black Oil cultivar exceeded the regulatory standard for vegetables. Significantly higher levels of bioactive compounds and antioxidant activities were detected in the control treatment for Pacific 33 leaves compared to Black Oil cultivar. Synergistic effects of heavy metals, amendment application and plant genotype are considered key factors in decreased levels of bioactive compounds and antioxidant activities.*

Keywords: Antioxidant activity; bioactive compound; cadmium; Helianthus annuus *L.; soil treatments; zinc*

ABSTRAK

Kesan pindaan tanah terhadap pengambilan Cd dan Zn, sebatian bioaktif dan aktiviti antioksidan dalam dua kultivar (Pasifik 33 dan Minyak Hitam) Helianthus annuus *L. telah dinilai. Baja dikalsium fosfat/lembu dan leonardit/lembu dicampur pada nisbah 1:1 w/w dengan tanah akan dijadikan sebagai rawatan tanah. Kultivar Minyak Hitam mengalami penyerapan logam, pengeluaran biojisim dan kadar kemandirian yang lebih tinggi berbanding kultivar Pasifik 33. Peningkatan pengambilan Cd dan Zn (masing-masing >0.2 dan >99.4 mg/kg) dalam kultivar Minyak Hitam melebihi piawai kawal selia untuk sayur-sayuran. Tahap sebatian bioaktif dan aktiviti antioksidan yang lebih tinggi telah dikesan dalam rawatan kawalan untuk daun Pasifik 33 berbanding Minyak Hitam. Kesan sinergistik logam berat, aplikasi pindaan dan genotip tumbuhan dianggap sebagai faktor utama dalam penurunan tahap sebatian bioaktif dan aktiviti antioksidan.*

Kata kunci: Aktiviti antioksidan; bahan bioaktif; Helianthus annuus *L.; kadmium; rawatan tanah; zink*

INTRODUCTION

Helianthus annuus L. (sunflower) is known to take up substantial quantities of heavy metals from soil with subsequent accumulation in aboveground parts, thus acting as a hyperaccumulator in phytoremediation technology (Dhiman et al. 2017). Key sources of heavy metals to soil include domestic and industrial sectors that release soluble metals into aquatic and soil environments, resulting in possible uptake and accumulation in crop plants and other biota (Tchounwou et al. 2012). In Thailand, adverse effects of metals have been recorded on agricultural land located down-gradient from metal mines (Saengwilai et al. 2017).

Deficiency of a micronutrient in soil results in plant stress and subsequent reductions in antioxidant enzyme activities. Plant stress caused by low soil fertility can also, in some cases, result in increased production of phytochemicals and antioxidant compounds (Ibrahim et al. 2013). Addition of supplemental micronutrients to soil, for example in fertilizers, can stimulate the activities of plant enzymes. Application of zinc (Zn) and copper (Cu) resulted in an increase of 40% in phenolic compounds, 71.5% in flavonoid content and 9.1% protein in rice grain when compared against a control (i.e. without micronutrient treatment) (Panhwar et al. 2015). The use of organic amendments provides a combination of macroand micronutrients which enhances vitamin C content, antioxidant activity, nitrogen and calcium concentrations, and chlorophyll content in herbs and other plants (Hassan et al. 2012).

Studies describing Cd and Zn uptake and accumulation by sunflower sprouts are of value in evaluating fundamental plant physiological processes including production of phytochemicals. Changes in phytochemical (antioxidants, phenolics, and bioactive compounds) production in sunflower tissue caused by Cd exposure can be accurately monitored over short periods (Larpote et al. 2015).

Excessive consumption of Cd by plants can lead to toxicity that subsequently inhibits plant growth and yield. Elevated Cd contents in plants can also result in changes in bioactive compounds and antioxidant activities, and finally, degradation of chlorophyll (Hassan et al. 2005).

Application of organic amendments immobilizes Cd in soil to some extent, resulting in decreased Cd phytoavailability. Questions persist, however, regarding the effects of soil amendments on plant uptake and accumulation of heavy metals by different plant cultivars that can also result in altered antioxidant activities and production of bioactive compounds. Differing results may be a function of soil physicochemical properties as recorded by various studies, and/or be dependent upon plant genotypes (Manquián-Cerda et al. 2016; Xie et al. 2018). A defense mechanism against Cd-induced stress is considered as a plant response that is complementary or alternative to bioactive compounds and antioxidant activity (Manquián-Cerda et al. 2016).

The aims of the present study were to evaluate the effects of soil amendments on growth and Cd and Zn uptake by two sunflower (*H. annuus* L.) cultivars, i.e. Pacific 33 and Black Oil, grown in soil contaminated with both Cd and Zn and assessed for antioxidant activities and contents of bioactive compounds present in sprouts.

Materials and Methods

SOIL PROPERTIES

Cadmium-contaminated paddy soil (designated Cd) was collected from the surface (0-20 cm) from five locations from the Mae Sot District, Tak Province (N16°40′35.9″ E98°37′37.4″), Thailand. Soil material was allowed to air-dry at room temperature and then mixed thoroughly into a composite blend. Soil material low in Cd concentration (designated LCd) was purchased from an agricultural supplier in Nakhonsawan Province.

Soil pH was measured using a pH meter; organic matter (OM) content was analyzed by the Walkley-Black method (Walkley & Black 1934); soil texture by the hydrometer method (Allen et al. 1974); electrical conductivity (EC) by an EC meter; and cation exchange capacity (CEC) by the method of Sparks et al. (1996). Total N was determined by the Kjeldahl method (Black 1965); extractable P by the Bray II method (Bray & Kurtz 1945); and extractable K by atomic absorption spectrophotometry after NH_4OAc extraction (Sparks et al. 1996). Total soil Ca, Mg, Cd, and Zn were extracted using an acid digestion method (Sparks et al. 1996), while extractable Cd and Zn were recovered with 0.005 M diethylenetriamine pentaacetic acid (DTPA) with 0.1 M HCl and 0.01 M $CaCl_2$. All digests and extracts were analyzed for Ca, Mg, Cd and Zn by flame atomic absorption spectrophotometry (FAAS; AAnalyst 200, PerkinElmer®). The detection limits for Ca, Mg, Cd and Zn are 1.5, 0.15, 0.8, and 1.5 µg/mL, respectively.

Leonardite and cattle manure served as an organic amendment for the contaminated soil. Dicalcium phosphate is a source of commercial phosphorus in human and animal foods. Leonardite used in this study was purchased from a commercial source near Mae Moh mine, Lampang Province; cattle manure was obtained from a farm near Mahidol University, Nakhonsawan Campus, and dicalcium phosphate from a factory in Saraburi Province.

EXPERIMENTAL DESIGN

Greenhouse experiments were conducted at Mahidol University, Nakhonsawan Province, Thailand (N15°34′48.1″ E100°08′50.3″). The experiments consisted of two amended soil treatments and a non-amended treatment. Conditions in the greenhouse included temperatures from 27-32 °C, 60-70% relative humidity, 8,446-24,593 lux light intensity, and a 12/12 h photoperiod. Commercial soil (0.75 kg) was packed into plastic containers (22 cm × 30 cm × 5 cm), and Cd-contaminated soil (0.25 kg) was placed on top. Total soil weight in each replicate of the treatment was 1 kg. Amended treatments included dicalcium phosphate/cattle manure (treatment 1; T1) and leonardite/cattle manure (treatment 2; T2) mixed in a 1:1 w/w ratio with soil. Both soil layers (i.e. top and bottom) were mixed with dicalcium phosphate/cattle manure or leonardite/cattle manure at a 20% w/w ratio. Soil at the bottom of the containers was designated LCd, LCd1, and LCd2 (indicating low Cd contents), whereas soil in the top layer was designated Cd, Cd1 and Cd2, as Cd contents were relatively high. T1 and T2 represent soils amended with dicalcium phosphate/cattle manure and leonardite/cattle manure, respectively. Soil without amendment was designated Control. Each treatment had 3 replicates; thus, nine trays comprised one cultivar for all treatments. All containers were arranged in a completely randomized design (CRD) on benches in the greenhouse.

Seeds of two sunflower cultivars (*H. annuus* L.; Pacific 33 and Black Oil) were surface-sterilized with 10% NaOCl for 1 min and subsequently soaked in deionized water (DI water) for 1 day. A total of 2 g sunflower seeds from each cultivar were transferred to the containers. After germination, a combined 100 mL Cd and Zn solution (9 and 10 mg/L from $Cd(NO_3)_2$ and $Zn(NO_3)_2$, Merck®, respectively) was sprayed onto the surface of each treatment every morning and evening for 7 days.

After 7 days, sprouts (shoots) were harvested and washed with DI water several times to remove attached soil particles. Soil material was collected using a plastic spatula. Survival rate of sunflower sprout cultivars was calculated following the formula proposed by Meeinkuirt et al. (2016). Plant and soil material were oven-dried at 60 °C for 3 days prior to determination of weight and metal content.

METAL DETERMINATION OF PLANT AND SOIL MATERIAL

Dried plant material was ground to a fine powder with a mortar machine (IKA; A11 Basic, Japan), sieved through a 2-mm nylon mesh sieve, and weighed. Plant tissue and rhizosphere soil (0.5 g each) were digested in a microwave digestion apparatus (ETHOS One; Milestone Inc.). Extractable Cd and Zn were recovered from soil using 0.005 M diethylenetriamine pentaacetic acid (DTPA) adjusted to pH 5.3 with 0.1 M HCl and 0.01 M $CaCl_2$. Cadmium and Zn concentrations were determined using FAAS. Plant and soil standard reference materials (NIST SRM® 1515 apple leaves and NIST SRM® 2710a Montana soil, respectively) and a blank method were used to evaluate the accuracy and precision of analytical data. Percent recovery for Cd and Zn in plant and soil samples ranged from 90-110%.

BIOACTIVE COMPOUND ANALYSIS

Total phenolics content (TPC) was analyzed by the Folin-Ciocalteu colorimetric method (Abu Bakar et al. 2009), using gallic acid as a standard. The TPC value in each extract was expressed as mg gallic acid equivalents in 1 g of dried sample. Total flavonoids content (TFC) was analyzed following the procedure of Abu Bakar et al. (2009). The TFC value in each extract was expressed as mg rutin equivalents in 1 g of dried sample. All determinations were carried out in triplicate.

ANTIOXIDANT ACTIVITY ANALYSIS

The FRAP (ferric reducing antioxidant power) assay was performed as described by Benzie and Strain (1996). The FRAP value was expressed as μmol $FeSO_4$/g dried sample. DPPH radical scavenging activity was measured following the method of Lim et al. (2007). Each determination was carried out in triplicate.

MEASUREMENT OF CHLOROPHYLL

Plant pigments in dried leaf discs and stems were extracted by 80% acetone following the method of Svec (1991). Supernatants were collected, diluted to 50 mL with 100% acetone, filtered through a 0.45 μm Whatman membrane filter to remove fine particles, and placed into 4 mL screw-top brown vials (Merck™, Germany). Samples were analyzed via high performance liquid chromatography (HPLC) using a Shimadzu LC-20AC, SPD-M20A with a diode array detector, and chromatographic separations were performed on a STR ODS-II (150 × 4.6 mm i.d.) analytical HPLC column. The mobile phase was methanol. Operating conditions were as follows: column temperature, 40 °C; injection volume, 10 L; UV-diode array detection at 220-700 nm.

STATISTICAL ANALYSIS

Statistical significance was determined by one-way ANOVA and least-significant difference (LSD) using *R* version 2.15.1 (R Development Core Team 2012). A *t*-test was used to compare means for independent samples (as data from two sunflower cultivars). The level of statistical significance was expressed at $p < 0.05$.

RESULTS AND DISCUSSION

PLANT GROWTH AND INFLUENCE OF SOIL PROPERTIES

Sprouts of the Black Oil cultivar had a higher survival rate (71.2-86.7%) compared to those of Pacific 33 (44.8-56.3%) in the control and T1 treatments ($p < 0.05$) (Table 1). This implies that the Pacific 33 cultivar was more susceptible to the influence of Cd and Zn. The Black Oil cultivar had a slightly lower survival rate (65.4%) in the T2 treatment compared to the control and T1 treatments (71.2 and 86.7%, respectively) ($p < 0.05$). Survival might also be related to plant genotype and growth stage and to synergistic effects of the metal mixture. Furthermore, differences in percent survival of both cultivars demonstrates that metal stressors and their interactions affect plants differently (Sricoth et al. 2018). De Maria et al. (2013) reported that high soil Cd concentrations (2.5-15 mg/kg) had no effect on overall sunflower growth; however, negative effects from Cd have normally been identified during early growth stages. This is consistent with the significant decrease in survival rate of sunflower sprouts in the current study. The plants did not show visible symptoms of Cd and Zn toxicity as they matured.

Approximately 2.3-5.5 times greater total dry biomass was noted in the Black Oil cultivar in all treatments when compared to Pacific 33 (Table 1); however, growth performance between both cultivars did not differ significantly ($p > 0.05$). Plants in all amended treatments had a lower growth rate when compared with the control; however, differences in percent growth rate were not significant when considering each cultivar and between cultivars ($p > 0.05$). Both total dry biomass and growth rate are considered suitable indicators of plant tolerance when grown in harsh conditions, even for short periods (Saengwilai et al. 2017). The present data could, therefore, be useful for distinguishing different synergistic effects of Cd and Zn when compared between sunflower cultivars. Differential tolerance of plant genotypes exposed to Cd and Zn may depend on plant age and biomass production (Fischer et al. 2017).

Selected physical and chemical properties of the test soils (bottom and top layers) are shown in Table 2. Following application of amendments, soil pH values in LCd, LCd2 and Cd2 were acidic, while the remainder were alkaline. Cattle manure and dicalcium phosphate application resulted in increased soil pH. Many organic compounds enhance metal solubility and phytoavailability, and also affect soil pH, particularly in the rhizosphere (Ghosh & Singh 2005). All tested soils, except for LCd, contained between 3-6% OM, which is considered sufficient for supporting agricultural productivity (Cornell University 2008). Liang et al. (2006) found that increased soil OM content increases CEC. Soil CEC values were high in LCd2 (26.1 cmol/kg) and Cd2 (16.2 cmol/kg). Many reports have indicated that increased soil organic matter and P contents and reduced soil pH influenced metal mobility in soil, and hence bioavailability and uptake by plants (Roberts 2014). Elevated EC values were also considered a key factor in reducing plant survival rate; the T2 (LCd2 and Cd2) treatment had highest EC values (> 2 dS/m) (Table 2). Significantly higher values of total dry biomass and growth rate were recorded in the Black Oil cultivar, particularly in the T1 (LCd1 and Cd1) treatment (Table 1), where the EC values were 0.9 and 0.7 dS/m, respectively. Machado and Serralheiro (2017) reported that EC values should not exceed 2.5 dS/m in agricultural soil; furthermore, plant health deteriorates when using saline water for irrigation, as high EC values may impart detrimental effects to plant growth. Some species, however, can tolerate high soil EC values, for example purslane, asparagus and red beet, which tolerate as much as 6.3, 4.1 and 4.0 dS/m, respectively.

Elevated soil nutrient concentrations were determined in the T1 treatment (691 and 494 mg/kg extractable P in LCd1 and Cd1, respectively; and 6,891 and 5,790 mg/kg total Ca, respectively). These data indicate that dicalcium phosphate is a key source of P and Ca in the test soils. In addition, increased total N and total Mg concentrations were measured in the T2 treatment (LCd2 and Cd2: 0.23 and 0.28% for total N, respectively, and 876 and 625 mg/kg for total Mg, respectively). The elevated macronutrient and micronutrient concentrations should, to some extent, reduce some deleterious effects of metals as they are critical for plant nutrition and growth.

Bottom layer soils (LCd, LCd1, and LCd2) had lower concentrations of Cd and Zn in both total and extractable forms when compared with top layers (Cd, Cd1, and Cd2). The top layers contained in excess of 1 mg/kg Cd, which exceeds agricultural guidelines (Šichorová et al. 2004). Total Zn concentrations in top layers were high (360.6-490.1 mg/kg), whereas levels in bottom layers and extractable Zn concentrations were within average ranges for normal soils (40-120 mg/kg) (Kabata-Pendias 2001). Top layer soils had Cd and Zn concentrations similar to those of contaminated paddy soils in Tak Province (Meeinkuirt et al. 2016).

CADMIUM AND ZINC ACCUMULATION AND ITS EFFECTS ON PLANTS

Cadmium in sunflower tissue ranged from 18.7-27.4 and 20.2-27.7 mg/kg for the Black Oil and Pacific 33 cultivars, respectively, whereas Zn concentrations ranged from 1,178.2-1,735.6 and 1,596.1-1,977.8 mg/kg for Black Oil and Pacific 33, respectively (Figure 1). Highest Cd concentrations were recorded in the control treatments. Cadmium concentrations in sunflower sprouts exceeded the maximum allowable Cd concentrations in food – the acceptable limit value for vegetables is 0.2 mg/kg (FAO-WHO 2016). Zinc concentrations were also high in sunflower sprouts in all treatments and far exceeded the FAO/WHO guideline values of 99.4 mg/kg (Mensah et al. 2009). Elevated Zn accumulation in plants reduces nutrient uptake and can inhibit growth, transpiration and N assimilation due to the tendency of the Zn ion to strongly interact with cellular components (Lin & Aarts 2012). Conversely, however, adequate levels of Zn in plant tissue can reduce Cd toxicity (Adamczyk-Szabela et al. 2020).

TABLE 1. Survival rate, total dry biomass, growth rate, Cd accumulation and uptake and Zn accumulation and uptake of sunflower sprouts (n = 3)

Cultivar	Treatment (T)	Survival rate (%)	Total dry biomass (g)	Growth rate (%)	Cd accumulation (mg/kg)	Cd uptake (mg/plant)	Zn accumulation (mg/kg)	Zn uptake (mg plant)
Black oil	Control	86.7±0.1a#	0.14±0.03a	100.0±0.0a	27.4±5.5a	4.0±1.4a	1735.6±218.6a	252.3±78.6a
	T1	71.2±11.3ab#	0.22±0.05a	99.1±7.1a#	23.3±6.1a	5.3±2.5a#	1250.2±389.2b	287.0±148.6a#
	T2	65.4±8.1b	0.15±0.04a	90.9±9.9a	18.7±4.2a	2.8±0.5a#	1178.1±269.5b	174.9±25.3b#
Pacific 33	Control	56.3±0.9a	0.06±0.02a	100.0±0.0a	27.7±2.1a	1.6±0.6a	1977.8±15.7a	116.6±46.4a
	T1	44.8±8.8a	0.04±0.01b	85.8±9.2a	20.2±4.8a	0.8±0.1b	1596.1±477.4b	63.9±16.7b
	T2	58.4±16.6a	0.04±0.02a	92.9±2.4b	24.1±6.5a	0.8±0.2a	1953.7±587.1a	63.0±13.0b

Cd = cadmium, Zn = zinc, T1 = dicalcium phosphate+cattle manure, T2 = leonardite+cattle manure

Values followed by the same letter are not significantly different ($p > 0.05$). Small letters represent differences in amendment effect when compared between treatments within the same species and sharp (#) represent differences in amendment effect when compared between plant species within the same treatment

TABLE 2. Physicochemical properties of the experimental soils

Parameter	LCd	LCd1	LCd2	Cd	Cd1	Cd2
pH	5.5	7.3	5.0	7.7	7.8	6.2
EC (dS/m)	0.2	0.9	2.0	0.3	0.7	2.1
CEC (cmol/kg)	22.6	17.3	26.1	12.4	8.5	16.2
OM (%)	1.7	3.1	4.5	3.4	3.8	5.5
Sand (%)	14.0	20.0	17.0	49.0	50.0	50.0
Silt (%)	22.6	23.8	22.8	32.8	31.6	28.6
Clay (%)	63.4	56.2	60.2	18.2	18.4	21.4
Soil texture	Clay	Clay	Clay	Loam	Loam	Loam
Total N (%)	0.09	0.16	0.23	0.17	0.19	0.28
Ext P (mg/kg)	38	691	79	53	464	99
Ext K (mg/kg)	351	1406	1172	117	898	927
Total Ca (mg/kg)	3961	6891	5212	5192	5790	662
Total Mg (mg/kg)	658	626	876	383	424	625
Total Cd (mg/kg)	2.0	2.0	1.2	14.1	9.7	11.7
Ext Cd (mg/kg)	0.1	BDL	BDL	2.4	1.6	1.8
Total Zn (mg/kg)	97.6	145.5	67.9	490.1	360.6	403.4
Ext Zn (mg/kg)	4.5	BDL	BDL	31.8	19.1	26.0

L = lower horizon, Cd = cadmium, 1 = cattle manure and dicalcium phosphate, 2 = cattle manure and leonardite, EC = electrical conductivity, CEC = cation exchange capacity, OM = organic matter, Ext = extractable, N = nitrogen, P = phosphorus, K = potassium, Ca = calcium, Mg = magnesium, Cd = cadmium, Zn = zinc, BDL = below detectable limits

The Pacific 33 cultivar experienced lower Cd and Zn uptake when compared to Black Oil, particularly in the amended treatments ($p < 0.05$). Numerous factors are related to metal absorption, uptake and accumulation potential of plants including soil pH, CEC content, nutrient availability, OM content, sesquioxide content, moisture level, temperature, fertilizer application, plant genotype, plant age, choice of crop and seasonal influences (Jung 2008). Elevated OM levels resulted in elevated uptake of Zn and other heavy metals in wheat plants (Rupa et al. 2003). Within plant cells, Zn distribution, mobility and phytoavailability are influenced by metal tolerance mechanisms and metal-activated synthesis of phytochelatins (Kühnlenz et al. 2016).

In this study, Zn concentrations in aboveground plant parts were 8.8-24.1 times above critical toxicity levels (Rout & Das 2003). Zinc is an essential nutrient for plant cell growth and development (i.e. biosynthesis of carbohydrates, chlorophyll formation, auxin metabolism, and root development); recommended Zn concentrations in agricultural soil range from 70-400 mg/kg; however, excessive Zn concentrations can be toxic to plants (Hansch & Mendel 2009).

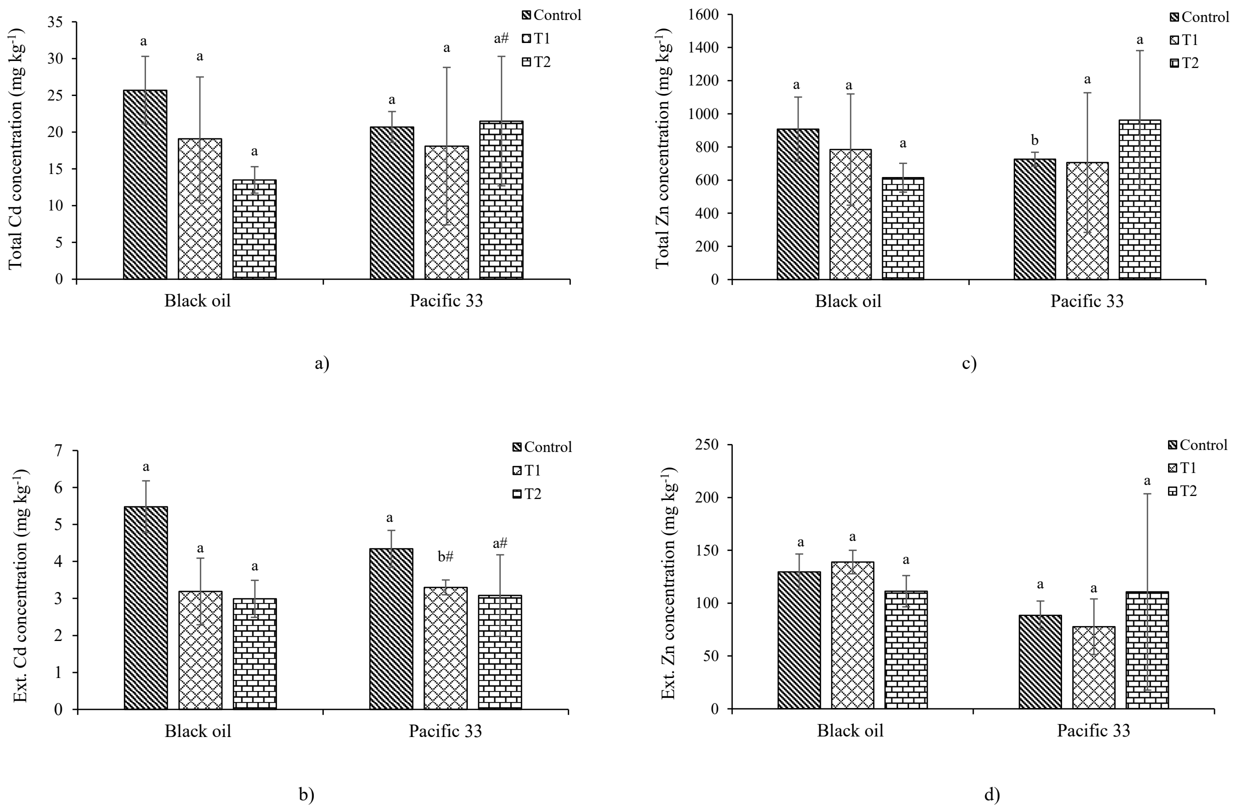

FIGURE 1. Total and extractable Cd and Zn concentrations in the tested soils after plant harvest ($n = 3$). Values followed by the same letter are not significantly different ($p > 0.05$). Small letters represent differences among metal content of all treatments within the same species and sharp (#) represents differences of metal content of the species within the same treatment. T1 = dicalcium phosphate+cattle manure, T2 = leonardite+cattle manure

BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITIES

Higher Chl *a* and *b* contents were measured in sunflower leaves, followed by stems (Table 3). Chlorophyll *a* and *b* were present in the ranges of 2,129.3-6,214.6 µg/g and 2,033.8-6,659.2 µg/g in leaves, respectively, whereas concentrations in stems ranged from 637.9-1,010.8 µg/g and 491.2-797.5 µg/g for Chl *a* and *b*, respectively. Highest Chl *a* and *b* contents were in leaves of the Pacific 33 cultivar in treatment T1, whereas leaf Chl *a* and *b* contents in the T2 treatment were significantly lower ($p < 0.05$) by 2.9 and 3.3 times, respectively. In this study, cattle manure served as a nitrogen source to plants. Nitrogen promotes the formation of the active photosynthetic apparatus by increasing several stromal and thylakoid proteins of the chloroplasts (Bassi et al. 2018). Hao et al. (2012) reported that application of animal manures increased plant growth; however, Cd and Zn accumulation decreased in sunflowers, thereby decreasing phytoextraction potential. Antioxidant levels increased when low quantities of an organic amendment (i.e. tannery sludge) was applied to metal-contaminated soil, whereas high levels of organic amendment decreased activities of antioxidants and enzymes in plants (Singh et al. 2004). Dicalcium phosphate as a phosphorus source increased chlorophyll content and plant biomass production, which is consistent with results from Saengwilai et al. (2017).

Necrosis and chlorosis of leaves were minimal in all plants. Some reports indicate that high soil Cd concentration can affect chlorophyll (Chl) content and the major light-harvesting complex of photosystem II (LHC-II). This effect was observed by significant decreases in Chl *a* and *b* content in the weeds *Chenopodium*, *Cyperus,* and *Digitaria* at 20 mg/kg Cd (Ewais 1997). In Cd-exposed plants net photosynthetic rate declines as a consequence of distorted chloroplast ultrastructure; restricted synthesis of chlorophyll, plastoquinone, and carotenoids; obstructed electron transport; inhibited enzyme activities of the Calvin cycle; and carbon dioxide deficiency in cells (Seregin & Ivanov 2001). Excessive Zn content strongly inhibits enzymatic activities responsible for metabolism, resulting in reduction of photosynthesis and chlorophyll biosynthesis, lipid peroxidation, and decreased antioxidative protection (Khudsar et al. 2008). Numerous studies have reported the toxic effects of Cd to plants, as it generates oxidative stress. Photosynthesis is inhibited due to decreased chlorophyll content in cells, resulting in stunting of plants and ultimately plant death. Increased oxidative stress may be inferred as the key cause of cellular damage. Elevated Cd concentrations in plant tissue have been linked with increased levels of antioxidant enzymes and increased lipid peroxidation (Jibril et al. 2017).

Plant sprouts commonly possess higher nutrient levels, total phenolic (TP) and flavonoid (TF) contents, and antioxidant activity when compared to seeds (Cevallos-Casals & Cisneros-Zevallos 2010*).* Total phenolic acid content was ~2 times higher in sunflower sprouts when compared to seeds (~8-10 and ~3-4 mg/GAE g in sprouts and seeds, respectively). High total flavonoids content (TFC) was measured in sunflower sprouts, whereas sunflower seeds had lower values ~1.8 times (~45-47 and ~25-26 mg/RE g in sprouts and seeds, respectively) (Pająk et al. 2014).

In the present study, TPC values in sunflower sprouts (6.1-9.3 and 5-13 mg/GAE g for Black Oil and Pacific 33 cultivars, respectively) were similar to those of Pająk et al. (2014); however, TFC values were higher than those results or ~82.7-116.4 times and ~73.8-89 times for Black Oil and Pacific 33 cultivars, respectively (Table 4). Many metallic contaminants e.g. Cd and Zn, are common components and/or wastes of industrial and agricultural sectors; plant uptake and accumulation of these contaminants at high quantities result in plant stress. Under such extreme conditions, heavy metal stress inhibits plant photosynthesis and antioxidant activities, interferes with essential nutrient uptake, and leads to reactive oxygen species (ROS) and lipid peroxidation, which finally can lead to death or negatively impact plant growth and development (Chen et al. 2021a, 2021b). Chen et al. (2021a) reported that application of exogeneous plant growth regulators in combined Cd and U greenhouse soils for sunflowers stimulated chlorophyll biosynthesis and activities of antioxidant defense systems. This was somewhat consistent with the present study, since application of soil amendments significantly increased the bioactive compounds and antioxidant activities in the amended treatments for Black Oil cultivar, whereas plants in the control treatment experienced lower performances ($p < 0.05$).

Production of total phenolics increases during plant growth and development, particularly when grown in contaminated soil. Increased phenolics content can protect plants from environmental stresses (Michalak 2006). In this study, TPC and TFC values were significantly different in all treatments for each sunflower cultivar ($p < 0.05$); however, the results did not differ significantly between cultivars ($p > 0.05$). Significant values of TPC occurred in leaves and shoots for the Pacific 33 cultivar (13 and 12.5 mg/GAE g, respectively) in the control treatment. Changes in TPC values were consistent with data for plants grown in copper (Cu)-contaminated media, where a significant increase in TPC was noted with increased Cu concentration (Mamat et al. 2015).

Low TFC values in all treatments of the plants were noted (Table 4). The presence of Cd in the growth media is considered the key cause of decreased TFC values, as flavonoids act as effective metal chelating agents. Under harsh environmental conditions such as those encountered in heavy metal-contaminated soil, flavonoids in plant cells can be oxidized by peroxidase and participate in the H_2O_2-scavenging, phenolic/ascorbate (ASC)/ phenolic peroxidase (POX) system (Michalak 2006). Metal stresses trigger antioxidative systems in plant cells which increase contents of reactive molecules (Jibril et al. 2017).

The content and composition of bioactive compounds in sprouts are a function of physicochemical properties of the soil, storage condition of sprouts, plant aging and variety, and climate (Cevallos-Casals & Cisneros-Zvallos 2010). Substantial TPC and TFC values were measured to some extent in the control (13 mg GAE/g, 622.9 µg RE/g for leaves) for Pacific33 cultivar, and T2 treatment (8.6 m GAE/g and 555.9 µg RE/g) for Black Oil cultivar. Data from the DPPH and FRAP assays showed similar trends; however, slight increases occurred in DPPH values, which are consistent with Kleckerova et al. (2011), who studied the effects of Cd and Zn in maize. Significant values of FRAP and DPPH were determined in Pacific 33 cultivar leaves, particularly in the control (89.7 µmol $FeSO_4$/g for leaves, and 90.7 and 93.1% for leaves and stems, respectively). Xie et al. (2018) found that *Rhima chuanxiong* grown in amended soils had lower antioxidant activities compared to plants grown in heavy metal-contaminated soil alone. This phenomenon could be related to the lower metal uptake and different responses by plant genotypes (Singh et al. 2004). In general, Pacific 33 cultivar had significantly higher levels of antioxidants than did Black Oil cultivar ($p < 0.05$). However, stems of Pacific 33 cultivar in amended treatments had significantly lower antioxidant activities based on FRAP and DPPH assays, compared to Black Oil cultivar ($p < 0.05$). The control treatment contained lower P and higher metals contents when compared to T1 and T2 treatments. The significant values found in Pacific33 leaves may imply metal stress, which results in elevated FRAP and DPPH values. Increased Cd and Zn concentrations in leaves generate reactive oxygen species, i.e. oxidative stress, leading to increases in antioxidant activities, particularly those determined by the FRAP and DPPH assays. Current data regarding the influence of Cd stress in triggering antioxidant activity in tissue based on FRAP assay was consistent with trends found by Jibril et al. (2017).

TABLE 3. Chlorophyll *a* and *b* contents of the tested plants ($n = 3$)

Cultivar	Treatment (T)	Tissue	Chlorophyll *a* (µg/g sample)	Chlorophyll *b* (µg/g sample)
Black oil	Control	leaf	4166.7±5.2a*	2276.8±0.7a*
		stem	878.6±1.5a	648.5±2.7a
	T1	leaf	5903.0±3.4b*	4654.1±2.3b*
		stem	903.3±4.0b#	525.6±0.8b#
	T2	leaf	5334.4±2.9c#*	3821.2±2.6c#*
		stem	909.3±3.2b#	534.6±1.4c
Pacific 33	Control	leaf	4925.8±2.1a*	3912.9±12.9a#*
		stem	1010.8±4.9a#	797.5±3.6a#
	T1	leaf	6214.6±2.5b*	6659.2±9.8b#*
		stem	637.9±1.4b	491.2±2.8b
	T2	leaf	2129.3±1.6c*	2033.8±2.5c*
		stem	813.8±3.4c	583.0±3.5c

T1 = dicalcium phosphate+cattle manure, T2 = leonardite+cattle manure

Values followed by the same letter are not significantly different ($p > 0.05$). Small letters represent differences among chlorophyll content of all treatments within the same species and sharp (#) represents differences of chlorophyll contents of the species within the same treatment. Star (*) represents differences of chlorophyll contents between leaf and stem within the same species

TABLE 4. Bioactive compounds and antioxidant capacity of the sunflower cultivars (sprouts) (n = 3)

Cultivar	Treatment (T)	Tissue	TPC (mg GAE/g)	TFC (µg RE/g)	FRAP (µmol $FeSO_4$/g)	DPPH (%inhibition)
Black oil	Control	leaf	8.5±0.1a	448.8±4.1a	65.0±0.3a	81.3±0.7a
		stem	7.3±0.1a	404.2±5.6a	60.1±0.3a	89.4±0.7a
	T1	leaf	9.3±0.3b	530.1±4.1b*	72.4±0.3b*	90.7±0.7a
		stem	6.1±0.2b	395.3±6.7a	46.5±1.6b#	89.9±0.3b#
	T2	leaf	8.6±0.1a	555.9±3.1c*	75.0±0.8c	84.9±0.5b
		stem	6.7±0.1c	431.7±2.7b	56.7±0.5c#	89.9±0.7a#
Pacific 33	Control	leaf	13.00±0.1a#	622.9±5.6a#	89.7±0.8a#	90.7±1.0a#
		stem	12.5±0.9a#	561.3±3.1a#	88.6±0.7a#	93.1±0.3a
	T1	leaf	10.5±0.1b	616.6±2.7a#	86.1±0.5b*	90.0±0.6a*
		stem	5.0±0.4b	583.5±3.1b#	25.8±0.5b	35.5±1.0b
	T2	leaf	9.3±0.1c	516.6±4.1b	78.3±0.9c*	91.5±0.6a*
		stem	6.6±0.5c	610.4±3.1c#	26.3±0.4b	48.6±0.5c

T1 = dicalcium phosphate+cattle manure, T2 = leonardite+cattle manure, TPC = total phenolics content, TFC = total flavonoids content, FRAP = ferric reducing antioxidant power, DPPH = DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity

Values followed by the same letter are not significantly different (p > 0.05). Small letters represent differences among antioxidant capacity and bioactive compounds of all treatments within the same species. Sharp (#) represents differences of antioxidant capacity and bioactive compounds of the species within the same treatment. Star (*) represents differences of antioxidant capacity and bioactive compounds between leaf and stem within the same species

CONCLUSION

In this study, several key factors affected plant growth performance, heavy metal uptake and accumulation, bioactive compounds and antioxidant activities, including plant genotype and age, heavy metal type, and influence of soil amendment. Lower phytoavailability of the tested heavy metals in amended treatments for Black Oil cultivar was noted when compared to the control treatment. This phenomenon may be linked with soil physicochemical properties such as pH, OM content, CEC, and concentrations of essential nutrients. Synergistic effects of combined soil Cd/Zn may trigger production of elevated bioactive compounds and increase antioxidant activities, particularly in leaves.

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