Alleviation of Cadmium Toxicity to Maize by the Application of Humic acid and Compost

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Abstract: The influence of compost and humic acid on cadmium (Cd) concentration in shoot and root as well as growth parameters (root and shoot dry weight, chlorophyll content) and activities of antioxidant enzymes of maize in contaminated soil were investigated. The experimental variables were the level of soil contamination with Cadmium (0, 25, 50 mg cd/kg soil) and the type of organic substance (compost and humic acid). The study was laid out in a factorial randomized complete design. Results indicated that enhancement of Cd concentration in soil decreased root and shoot dry weight, chlorophyll content and relative growth rate of plant. Growth parameters declined progressively with increasing concentrations of cadmium. Application of organic substance especially humic acid decreased the negative effects of Cd. Humic acid was more effective than compost as Cd concentration in root and shoot was concerned. Increase in Cd concentration in shoots can be attributed to the high mobility of this element in plant. Organic substances usage enhanced transportation index of Cd. Cd toxicity created oxidative stress in plant that consequently antioxidant enzymes were activated. Increasing of Cd concentration enhanced superoxide dismutase and catalase activity. Noticeable point it was the stop of catalase activity at high level of Cd.
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1. Introduction

Heavy metals are among the most toxic environmental pollutants, and they impose a particular threat for soils which are the main reservoirs for contaminant (Khan, et al., 2009; Macedo, et al., 2008; McBride, 2002). Some of heavy metals are essential to human beings, animals and higher plants, for example Zn, Ni, while others like Cd, Cr and Pb are toxic (Kabata, A; Mukherjee B.A, 2007). The most important reason for metal toxicity are oxidative stress, disruption of the pigments function and changes in protein activity (alemzadeh et al, 2014). Plants are equipped with antioxidative defense systems to eliminate or reduce the oxidative damage (Arora, et al., 2002). The plant antioxidant network consist of antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APx), Guaiacol peroxidase (GPx) and catalase (CAT) (Michalak, 2006). It was well established that in high internal concentrations Cd disturbed almost all physiological processes in plants (Gilvanise, et al., 2014). The availability of heavy metals for plants is governed by several soil factors such as pH, cation exchange capacity, organic matter content and adsorption by clays (Macedo, et al., 2008; Karaca, 2004). Applications of organic matter provide many benefits to agricultural soil, including increased ability to retain moisture, a better nutrient-holding capacity, better soil structure and high level of microbial activity (Pizzeghello, et al., 2013). Soil organic matter has been of particular interest in studies of heavy metal mobility in soils, because of tendency of transition metal cations to form solution complexes with organic ligands (Angelova, et al., 2013). Some researchers believe that addition of organic matter amendments, such as compost, humic acid and wastes, is a common practice for mobilization of heavy metals of contaminated soils. They showed that amendment of contaminated soils with organic matter enhanced bioavailability and mobility of heavy metals (Khan, et al., 2000; Pizzeghello, et al., 2013; Yildirim, 2007) while others indicated that organic substances have the capacity to bind metal ions and could thus be used for stabilization of heavy metals (Janos, et al., 2010; Topcuoglu, 2012; Zhang, et al., 2013;). Thus, the effects of organic substances on the behavior of Cd in soil and plant need more investigation. One of the important strategic crop in Iran is maize (Zea mays L.). Maize can accumulate heavy metals when grown in contaminated soil (Keawsringam, et al., 2015). The aim of the present study was to investigate and clarify the influence of organic substances (compost and humic acid) on the growth parameters and antioxidant enzymes activity of maize plant in a contaminated soil (with Cd).

2. Materials and Methods

2.1. Experimental design. The experiment was carried out in the greenhouse of the Shahid Chamran University of Ahvaz (Iran), using soil columns of 20 cm in diameter and 45 cm deep. The experimental variables were the level of soil contamination with

Cadmium (0, 25, 50 mg cd/kg soil) and two levels of each organic substance (compost (0, 40 g/kg soil) and humic acid (0, 5 mg)). Each treatment consisted of: [a] no heavy metal (T_0) + no organic substance (M_0); [b] compost (M_1) + T_1 (25 mg cd/kg soil); [c] humic acid (M_2) + T_1 (25 mg cd/kg soil); [d] compost (M_1) + T_2 (50 mg cd/kg soil); [e] humic acid (M_2) + T_2 (50 mg cd/kg soil). The treatments were laid out in a factorial completely randomized design (CRD) with 3 replicates. The upper 10 cm of soil was mixed with 40 gr compost/kg soil. The humic acid was a commercial sample from Fluka and used after pretreatment as described by Hoop (1990). Maize seeds (single grass 704) were collected from Seed Research Centre of Karaj, Iran. Prior to column filling, soil mixed with required amount of Cd solution then incubated for one month fallowed by application of NPK as per soil test. The soil texture was sandy loam and the general properties are shown in Table (1). The seeds were planted in plastic columns containing 14 kg of soil. Up to harvest received proper operations. At the end of the experiment, plants were harvested by cutting the shoots from the soil surface and washed with deionized water. Plant roots were separated from the soil and washed with water until free of soil and then washed three times with deionized water. Shoots and roots of plant were dried in an oven at 65º C for 72 h and then weighting in fallowed by powdering the samples.

Table 1. Physical and chemical properties of soil and compost.

Properties	texture	TOC* (%)	pH	Total N (%)	Total P (mg/Kg)	Total K (mg/Kg)		Total Cd (mg/Kg)	EC (ds/m)
Soil	sandy loam	0/68	7.8	0.06	12	105		0.043	2.2
Compost	---	21.3	7.53	1.39	0.65	2.1		0.02	4.5

TOC: Total organic carbon

2.2. Determination of heavy metals. The metal content in plant was analyzed by atomic absorption spectroscopy (Mireles, 2014). Heavy metal content in soil column was measured in three depth (0-15, 15-30 and 30-45 cm) as previously described by Chapman and Pratt (1961).

2.3. Chlorophyll content. The chlorophyll content measured according to Lichtenthaler and Wellburn Method (1983).

2.4. Transportation index. The transportation index (Ti) gives the shoot/ root heavy metal concentration and depicts the ability of the plant to translocate the metal species from roots to shoot (Marchiol, et al., 2004). It is calculated as follows:

Ti = heavy metal in shoot (mg/kg)/heavy metal in roots (mg/kg)

2.5. Relative growth rate. The RGR for maize plant, based on the following equation:

$RGR = (\ln W_2 - \ln W_1) / (t_2 - t_1)$, where W_2 and W_1 represent total plant dry weights at times t_1 and t_2, respectively (Lutts et al., 2004).

2.6. Superoxide dismutase (SOD) and Catalase (CAT) activity. SOD activity was determined by inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) by superoxide radicals (Dhindsa et al., 1981) and the activity of catalase was assayed after the method of Chance and Maehly (1955).

2-7.Statistical analysis. Statistical analysis employed SAS windows version 9.1. The significance of differences between variables at P<0.05 was checked with a multiple comparison on Duncan's test.

3. Results and Discussion

3.1. Concentration of Cd in soil column

Application of organic substances compered to control led to increased available cd concentration in all treatments (Fig. 1). Highest concentration of Cd recorded in depth 30-45 cm for M_2T_2 treatment. It was clear that application of organic substances enhanced Cd concentration by depth. At T_1 treatment, application of organic substances with no significant difference between compost and humic acid decreased Cd concentration in 0-15cm but increased in lower depths. In M_1T_2 treatment and depth 30-45cm, Cd concentration increased about 74 and 20% compare to depth 0-15 and 15-30cm respectively. Cadmium movement in soil column was more pronounced by humic acid then compost. In general over the test period, the concentration of cadmium decreased in 0-15 cm and accumulation of Cd was happened in the depth 30-45 cm. Therefore application of organic substances enhanced mobility of Cd. Increased heavy metals amount such as Pb, Cd and Ni by compost have been reported (Ben Achiba, et al., 2009). Enhancement in Cd availability and mobility eventually was due to multiple functional groups of organic substances (Zhang, et al., 2013) that can interacted with metal ions (Cd). This finding is similar to results in the literature (Strobel, et al., 2005; Meers, et al., 2005; McBride, 2002; Gray, et al., 1999) showing that Cd concentration in soils was influenced by soil properties such as organic substances.

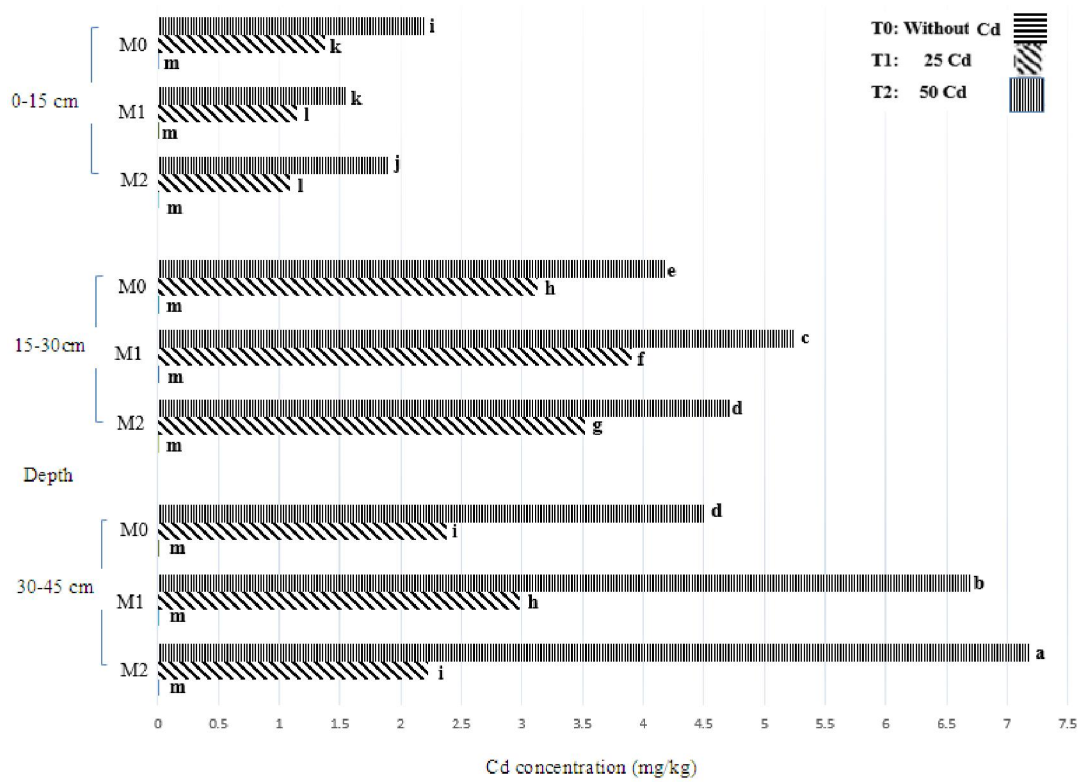

Figure 1. Effect of different concentrations of Cd and type of organic substance on concentration of Cd in soil column. Values are means ± SE (n = 3). Similar letters are not significantly different at *P<0.05*, according to Duncan's test. M0: without organic substance, M1: compost, M2: Humic acid.

3.2. Cd concentration in shoot and root

Data of table.2 indicated that Cd concentration in shoots and roots of plant (received Cd treatments) enhanced with application of organic substances. At T_0 treatment, no significant difference was observed in the Cd concentration of maize shoots and roots. humic acid in combination with T_1 and T_2 was more effective than compost as increasing Cd concentration in root and shoot was concerned. Increase in Cadmium concentration in maize plant can be attributed availability and mobility of this element in soil (Fig. 1). At M_1T_2 and M_2T_2 treatments, Cd concentration increased by 26% and 132% in shoot and 4.5% and 80% in root as compared to control (M_0T_2) respectively. These results suggest that soil organic substances can effectively increase Cd concentration in maize plant in polluted soils. Furthermore, root Cd concentration exceeded that of shoot. M_2T_1 and M_2T_2 treatments increased cd concentration in root by 100 and 80% compared to control (M_0T_2), respectively. It was hypothesized that roots could play an important role in metal retention by preventing an excessive and toxic accumulation in shoots (Orrono, D and Lavado, R. 2009). Cadmium accumulation in root of maize plant than shoot can be due to connecting of Cd in cation exchange sites of roots (Zeng, et al., 2011) and creating complexes with ligands containing –S group (Sulfhydryl) (Topcuoglu, 2012; Park, et al., 2012) which led to sediment and accumulation of cd in apoplast of root.

Table 2. Accumulation of Cd in shoots and roots and transportation index of maize.

Cadmium Treatment (mg/kg)	Cd in shoots (mg/kg dw)			Cd in roots (mg/kg dw)			Transportation index (Ti)		
	M_0	M_1	M_2	M_0	M_1	M_2	M_0	M_1	M_2
0 (T_0)	0.08^e	0.07^e	0.07^e	0.09^e	0.08^e	0.08^e	0.88^a	0.87^a	0.87^a
25 (T_1)	8.5^d	10.4^d	16.2^b	14.3^d	15.4^d	28.4^b	0.6^b	0.67^b	0.57^{bc}
50 (T_2)	13.3^c	16.7^b	30.9^a	24.1^c	25.2^c	45.1^a	0.53^c	0.69^b	0.68^b

Values represent mean (n = 3). Means in the same column followed by the same letters are not significantly different (*P<0.05*), according to Duncan's test. M_0: without organic substance, M_1: compost, M_2: Humic acid.

3.3. Transportation index (Ti)

Maximum Ti of Cd with no significant different between organic substances was observed in T_0 treatment (Table 2). Compared to respective T_0 treatment increment of soil Cd concentration decreased Ti significantly. Such an increment in Cd concentration in soil (fig.1) or in root (Table 2) will activate mechanisms of retention of Cd in root and causes hindrance of its translocation. Minimum amount of Ti was observed in M_0T_2. Even though no significant different was observed in Ti recorded for application of organic substances but increasing level of Cd concentration from T_1 to T_2 in combination with M_1 and M_2 increased Ti. The decreasing trend in T_0 to T_1 and increasing trend from T_1 to T_2 in combination with M_1 and M_2 treatment indicated that eventually increasing Cd concentration in root reduces Cd retention capacity which in turn increase Cd up ward translocation. Such reduction in Cd retention capacity might be due to neutralization of negative charges in apoplast (Evangelou, et al., 2004) or limited capacity of phytochelation production of plant cells. As a consequence, the translocation of Cd from root to shoot is an important factor affecting concentration of this metal in aerial tissues (Haliru, et al., 2009). The difference between root and shoot Cd concentrations indicates an important restriction of the internal transport of Cd from roots to shoot, resulting in higher root concentrations rather than translocation to shoots. (Turan, M; Angin, I. 2004).

3.4. Chlorophyll content

The effects of Cd on chlorophyll content show that increasing Cd concentration decreased content of chlorophyll compared to control (Table 3). Application of organic substance especially humic acid (M_2) decrease the negative effects of heavy metals. The data analysis showed that chlorophyll content was influenced by the higher metal concentrations. In general chlorophyll content declined progressively with increasing concentrations of Cd. At M_1T_2 and M_2T_2 treatments, chlorophyll content increased by 43.5% and 84.6% as compared to control (M_0T_2) respectively. These results indicated that soil organic substances can decreases negative effect of Cd in chlorophyll content of maize plant. Soil organic substances may exert several effects on plant functions such as improving uptake of macro and micro elements, supplying cell growth and photosynthesis (Ouni et al. 2014). However, this result suggesting some increase in chlorophyll hydrolysis due to the metal accumulation. As a consequence, the reduction of chlorophyll in Cd treated plant related to inhibition of its biosynthesis (Parekh, et al., 2009). Also Cd disturbed chlorophyll molecules integration in stable complexes (Yildirim, et al., 2007). Heavy metals produce oxyradicals in plants. These radicals cause widespread damage to membranes and associated molecules, including chlorophyll pigments (Mireles, et al., 2004).

Table. 3: Effect of different concentrations of Cd and type of organic substance on root and shoot dry weight, Relative growth rate and Chlorophyll content of maize.

Cadmium Treatment (mg/kg)	Root dry weight (gr)			Shoot dry weight (gr)			Relative growth rate (mg/day)			Chlorophyll content		
	M_0	M_1	M_2	M_0	M_1	M_2	M_0	M_1	M_2	M_0	M_1	M_2
0 (T_0)	7.4 ^{b}	9.1^a	9.2^a	37.6^b	69^a	67.5^a	58.8^b	69.5^a	69.1^a	3.17^c	4.74^b	5.75^a
25 (T_1)	3.5^d	5.5^c	5.1^c	17^e	24.9^{cd}	28.8^c	44.2^d	50.9^c	53.6^c	2.04^d	3.48^c	4.27^b
50 (T_2)	2.5^e	5^c	4.7^c	13.7^e	18.8^d	16.1^e	40.2^e	46^d	43.1^d	1.24^e	1.78^d	2.29^d

Values represent mean (n = 3). Means in the same column followed by the same letters are not significantly different (*P<0.05*), according to Duncan's test. M_0: without organic substance, M_1: compost, M_2: Humic acid.

3.5. Root dry weight

Table 3 showed that increasing levels of Cd significantly influenced the dry weight of roots. Dry weight of roots decreased with the incrimination of cadmium concentration. This result indicated that cadmium can toxify root and restrict its growth. The highest among root dry weight with no significant difference was recorded in M_1T_0 and M_2T_0. Although no significant difference was observed between effects of organic substances (compost and humic acid) compared to respective control their application significantly enhanced dry weight of roots. M_1T_1 and M_1T_2 treatments improved root dry weight by 52% and 92% as compare to respective M_0 treatment, respectively. Compost and humic acid positively increased root dry weight of maize and contracted negative effect of Cd in root. Increasing level of Cd application led to increment of Cd concentration in plant (Table 2) and followed by disruption in the synthesis of chlorophyll which restricted plant growth. The symptoms of phytotoxicity were expressed more clearly in roots (Gilvanise, et al., 2014) because of the significantly higher Cd accumulation in them (table.2). In plants, the presence of Cd affects absorption, transport and use of macronutrients and trace elements (Jiang, et al., 2005) also root after germination has no barrier to protect against heavy metal stress (Salati, et al., 2010). Due to these effects, the presence of Cd inhibit plant growth.

3.6. Shoot dry weight

It is generally accepted that high concentrations of heavy metals can cause plant injury. In this experiment, increment of Cd concentration decreased shoot dry weight of plant in all treatments (table.3). Application of organic substance significantly increased shoot dry weight of plants. Organic substance enhance plant growth significantly due to the increasing cell membrane permeability, respiration, oxygen and phosphorus uptake and supplying root cell growth (Pizzeghello, et al., 2013). No significant difference was observed between compost and humic acid effect on shoot dry weight receiving similar Cd concentration. At M_1T_0 and M_2T_0 treatments shoot dry weight increased by 83.5% and 79.5% compare to M_0T_0, respectively. The most common effect of heavy metals toxicity in plants is stunted growth (Park, et al., 2011), leaf chlorosis (Parsafar, et al., 2013) and alteration in the activity of enzymes of various metabolic pathways (Kim, et al., 2004; Mohammad, et al., 2013). In fact heavy metal causes decline in plant biomass by disturbance in photosynthesis, respiration and other metabolic process (Pizzeghello, et al., 2013). It has been reported that reduction in the shoot dry weight in maize plant could also be due to the suppression of cell elongation (Xiaoli, et al., 2007), because of an irreversible inhibition exerted by heavy metals on the proton pump responsible for metabolic process (Wojcik, et al., 2005). Parameters such as shoot dry weight were used as useful indicator of metal toxicity in plants. In this study, Cd stress showed a higher decline in shoot dry weight. Growth of the upper plant parts is more sensitive to heavy metal compared to roots.

3.7. Relative growth rate (RGR)

The adverse effect of Cd on plant growth was accompanied by decrease in RGR. The RGR value of maize plant significantly declined due to the increase in the concentration of Cd (table. 3). In this study it has been established that Cd in M_0T_2 inhibits RGR by 32% compare to M_0T_0. Indeed reduction of plant dry weight led to decrement of RGR value. Although no significant difference observed between RGR recorded for compost and humic acid, application of organic substances increased relative growth rate of maize. This response in M_1 and M_2 treatments suggest that maize plant recovered (to some extend) after an initial shock of heavy metal. Although improving heavy metal soil application, promoted their concentration in plant followed by chlorophyll content syntheses disruption and dwindling plant growth to reduces dry matter production and RGR. Nevertheless the effect of high concentration of cadmium in plant was contracted by organic substances specially that of humic acid. Heavy metals stress causes a considerable decrease in growth rate (Zeliha, et al., 2011).

3.8. Antioxidant enzymes

Table 4 shows the changes in the Superoxide dismutase and Catalase activity under different concentrations of Cd. Increase in level of Cd application as compared to respective control enhanced SOD activity. As expected, higher concentration of Cd (50 mg) had greater impact on SOD. At T_1 and T_2, application of organic substances with no significant difference between them decreased SOD activity. M_1T_1 and M_2T_1 treatments compare to M_0T_1 decreased SOD activity by 21% and 20%, respectively. However, increased SOD activity is known to conform oxidative stress tolerance. Heavy metal excess may stimulate the formation of free radicals and reactive oxygen species, perhaps resulting in oxidative stress (Alemzadeh, et al., 2014). Antioxidants enzymes play an important role in the cellular defense strategy against oxidative stress. Superoxide dismutase (SOD) with converting O_2^- to H_2O_2 in cytosol and chloroplast decrease the effect of oxygen species (Wang, et al., 2008). In fact enhancement of SOD activity is reaction of plant against heavy metals stress. Those results supported by finding of Dazy et al. (2009) and Liu et al. (2009). Catalase one of antioxidant enzymes that breakdown H_2O_2 to H2O and O2. CAT activity enhanced at low concentration of Cd (T_1) and declined under high concentration of Cd (50 mg). At T_0 and T_2, no significant difference was observed between effect of each organic substances (compost and humic acid). In M_1T_2 and M_2T_2 treatments CAT activity decreased about 36% and 31% as compare to M_0T_2, respectively. In toxic level of Cd (T_2) either CAT syntheses ceased (Michalak, A. 2006) or lose its ability to breakdown H_2O_2 to H_2O and O_2. CAT is sensitive to Cd than SOD, decrease in its activity pointer its. The decrease in the activity of catalase enzyme indicate that CAT plays only a minor role in detoxifying H_2O_2 due to CAT inhibition by O_2^- (Alemzadeh, et al., 2014).

Table 4. Effect of different different concentrations of Cd and type of organic substance on enzyme activity in Zea maize leaves.

Cadmium Treatment (mg/kg)	Superoxide dismutase (U/mg protein)			Catalase (U/mg protein)		
	M_0	M_1	M_2	M_0	M_1	M_2
0 (T_0)	605.6[d]	582[d]	592.3[d]	76.8[d]	65.7[d]	66.2[d]
25 (T_1)	807.4[b]	637.2[c]	643[c]	93.3[c]	109.3[b]	124.4[a]
50 (T_2)	878.6[a]	809.5[b]	823.2[b]	44.07[e]	28.2[f]	30.8[f]

Values represent mean (n = 3). Means in the same column followed by the same letters are not significantly different (P<0.05), according to Duncan's test. M_0: without organic substance, M_1: compost, M_2: Humic acid.

4. Conclusion

In conclusion, those results indicated that the exposure of maize plant to Cd decreased root and shoot dry weight, chlorophyll content and relative growth rate. Application of organic substance significantly increased shoot and root dry weight of maize. Moreover, concentrations of Cd in shoot and root of plant was increased by application of organic substances. The results suggest that roots of maize plant are efficient barriers to Cd translocation to the above ground plant parts. However low transport of Cd to shoots may be due to saturation of root metal uptake, when internal metal concentrations are high. In general, the increase in Cd concentration in plant was consistent with the metal concentration in soil. Application of compost increased Cd concentration in lower depths. This finding indicates that there is a close relationship between soil properties, especially soil organic matter and metal accumulation in plants. Increasing of Cd concentration enhanced SOD and CAT activity. Next we should focus on the effects of functional groups of organic substances on metal uptake, organic chelate amended aspect must be tested in the field under different site-specific conditions and on multi-heavy metal contaminated soil.

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