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Effect of Chromium and Cadmium on Growth Parameters and Biochemical Responses in Soil Treated with Compost and Humic Acid

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AC and AM designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors AM, GS and MC managed the literature searches, analyses of the study performed the spectroscopy analysis and author AC managed the experimental process and author AM identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The effects of chromium (0, 100 and 200 ppm) and cadmium (0, 50 and 100ppm) on shoot dry weight, content of chlorophyll, soluble protein content and activity of superoxide dismutase (SOD) and catalase (CAT) were investigated.
Study Design: The experimental design was completely randomized with 3 replications.
Place and Duration of Study: The experiment was carried out in the greenhouse of the University of Shahid Chamran in Ahwaz (Iran), between November 2014 and April 2015.
Methodology: The experiment was carried out in the greenhouse, using soil columns of 20 cm in diameter and 45 cm in depth. The experimental variables were the level of soil contamination with Chromium (0, 100, 200 mg/kg) use $K_2Cr_2O_7$, Cadmium (0, 25, 50 mg/kg) use $Cdcl_2$ and the type of organic substance (compost and humic acid). Each treatment consisted of: [a] control (without heavy metals (T_0) and organic substance); [b] humic acid + T_1(25 mg/kg Cd and 100 mg/kg Cr); [c] compost + T_1(25 mg/kg Cd and 100 mg/kg Cr); [d] humic acid + T_2(50 mg/kg Cd and 200 mg/kg

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Cr); [e] compost + T_2(50 mg/kg Cd and 200 mg/kg Cr). The upper 10 cm of soil was mixed with 40 gr/kg soil compost. The humic acid was a commercial sample from Fluka (product number 35069288) and is used after pretreatment as described by van den Hoop et al. (1999). In short to obtain the soluble fraction of the humic acid, 2.5 g of a Fluka sample were dissolved in 1/1 of water. Seeds of *Zea maize* (single grass 704) were prepared from Seed Research Centre of Karaj, Iran. The seeds were planted in plastic columns. Each column was filled with 14 kg of soil. Prior to filling, soil was mixed with macronutrients (NPK) and heavy metals.

Results: Enhancement of Cr and Cd decrease shoot dry weight and total chlorophyll content of plant in all treatments. Application of organic substance especially humic acid (c) decrease the negative effects of heavy metals. SOD activity increased in treatments, but soluble protein decreased. CAT activity increased in low concentration (T_1) of Cr and Cd with significant effect in reaction of the treatment but in high concentration (T_2) of this elements CAT activity decreased. CAT is susceptible to Cr and Cd than SOD, decrees in its activity pointer its. These results show that aggregation of oxygen species due to oxidative stress of heavy metals in plants cause different respond in treatments.

Conclusion: In conclusion, our results indicated that the exposure of *Zea maize* to Cr and Cd decreased shoot dry weight and pigment content. The present study show that in high level of heavy metals antioxidative system in *Zea maize* was active to survive plant. Decrease in chlorophyll and protein also a signal result for the toxicity of heavy metals. Total Soluble Protein Content decreased in all treatments, indifferently of compound type and Cr and Cd concentration. Decrease in total soluble protein in most treatments suggests that in this condition (stress of heavy metals) synthesis of protein was destruction. SOD activity increased with the increasing concentration of chromium and cadmium. At higher concentration of Cr and Cd the amount of CAT activity decreased. Increase of SOD pointed to its role in antioxidative system in Zea maize.

Keywords: Heavy metals; chlorophyll; soluble protein; superoxide dismutase; catalase.

1. INTRODUCTION

The problem of heavy metal toxicity acquired new dimensions in the industrial era. Besides the beneficial component, the progress of human society had destructive effects on environment, with disastrous repercussions on biological systems [1]. Heavy metals are among the most toxic environmental pollutants, and they pose a particular threat for soils which are the main reservoirs for contamination [2]. Heavy metal pollution contributes to gradual degradation of the soil environment in many parts of the world. It may lead to permanent soil damage, loss of soil fertility and depletion of plant cover [3]. The most important reasons for metal toxicity according to their concentrations and properties are oxidative stress, disruption of the function of pigments, photosynthesis enzymes and electron transport, alteration of membrane permeability due to lipid peroxidation, changes in protein activity and structure owing to high affinity of heavy metals for carboxyl, thioyl and histidyl groups present in catalytic and transport sites and interfering with signal transduction pathways [4]. In addition, a heavy metal excess may stimulate the formation of free radicals and reactive oxygen species, perhaps resulting in oxidative stress. Reactive oxygen species (ROS) are continuously produced at low level during normal metabolic processes [5]. Biological activity levels are determined by various factors, including type of pollutant [6], exposure to pollution, soil pH (and organic carbon content [7]. Organic carbon concentrations are a particularly important indicator because organic matter is a key determinant of soil quality which affects the physical, chemical and biological properties of soil [6]. After wheat and rice, maize (*Zea mays* L.) is the third most important cereal crop grown all over the world in a wide range of climatic condition. Therefore the research was aimed to investigate the effects of chromium and cadmium in growth parameters and biochemical stress in order to study relationship between heavy metal toxicity and responses of them.

2. MATERIALS AND METHODS

The experiment was carried out in the greenhouse of the University of Shahid Chamran in Ahwaz (Iran), using soil columns of 20 cm in diameter and 45 cm in depth. The experimental variables were the level of soil contamination with Chromium (0, 100, 200 mg/kg) use $K_2Cr_2O_7$, Cadmium (0, 25, 50 mg/kg) use $Cdcl_2$ and the type of organic substance (compost and humic acid). Each treatment consisted of: [a] control

(without heavy metals(T_0) and organic substance); [b] humic acid + T_1(25 mg/kg Cd and 100 mg/kg Cr); [c] compost + T_1(25 mg/kg Cd and 100 mg/kg Cr); [d] humic acid + T_2(50 mg/kg Cd and 200 mg/kg Cr); [e] compost + T_2(50 mg/kg Cd and 200 mg/kg Cr). The upper 10 cm of soil was mixed with 40 gr/kg soil compost. The humic acid was a commercial sample from Fluka (product number 35069288) and is used after pretreatment as described by Hoop et al. [8]. In short to obtain the soluble fraction of the humic acid, 2.5 g of a Fluka sample were dissolved in 1/1 of water. Seeds of *Zea maize* (single grass 704) were prepared from Seed Research Centre of Karaj, Iran. The seeds were planted in plastic columns. Each column was filled with 14 kg of soil. Prior to filling, soil was mixed with macronutrients (NPK) and heavy metals. The soil type was a sandy loam and the general properties are shown in Table 1. The experimental design was completely randomized with 3 replications. After two month exposure to heavy metals, the plants were removed and thoroughly rinsed with deionized water and used for chlorophyll and carotenoids measurements. For other biochemical analyses, leaves were detached and immediately frozen in liquid nitrogen and kept at -80ºC.

2.1 Determination of Pigment Content

0.2 g leaves were homogenized with 10 ml of 80% acetone. The extract was centrifuged at 3000 g for 5 min. The upper phase was transferred into a new tube and its absorbance was measured at 663, 646 and 470 nm, respectively, for chlorophyll a, b and carotenoid with acetone 80% as a blank. The chlorophyll a, b and carotenoid content measured according to Lichtenthaler and Wellburn Method [9].

2.2 Protein Estimation

Proteins were estimated by the method of Bradford [10]. Fresh leaves (0.5 g) were homogenized in 1 ml phosphate buffer (pH 7.0). The crude homogenate was centrifuged at 5000 × g for 10 min. Half ml of freshly prepared trichloroacetic acid (TCA) was added and centrifuged at 8000 × g for 15 min. The debris was dissolved in 1 ml of 0.1N NaOH and 5 ml Bradford reagent was added. Absorbance was recorded photometrically at 595 nm (Beckman 640 D, USA) using bovine serum albumin as a standard.

2.3 Determination of Superoxide Dismutase Activity

SOD activity was determined by inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) by superoxide radicals [11]. The assay mixture contained 50 mM phosphate buffer (pH 7), 13 mM methionine, 75 µM NBT, 0.1 mM EDTA and 20 µL of enzyme extract then were brought to a final volume of 3 ml and finally 4 µM riboflavin was added. The test tubes were shaken immediately. The reaction was started by keeping the tubes under 90 mmol m-2 s-1 for 10 min. The absorbance was recorded at 560 nm. One unit of SOD activity is defined as the amount of enzyme required to cause 50% inhibition of nitroblue tetrazolium to farmazan under the assay condition. The specific enzyme activity was expressed as units per mg of protein.

2.4 Determination of Catalase Activity

The activity of catalase was assayed after the method of Chance and Maehly [12] with the following modifications. Five milliliters of the assay mixture for the catalase activity comprised: 300, µmoles of phosphate buffer, pH 6.8, 100 µmoles of H_20_2, and I ml of the twice diluted enzyme extracted. After incubation at 25 C for 1 min, the reaction was stopped by adding 10 ml of 2% (v/v) H_2SO_4 and the residual H_20_2 was titrated against 0.01 N KMnO4 until a faint purple color persisted for at least 15 sec. A control was run at the same time in which the enzyme activity was stopped at "zero" time. One unit of catalase activity is defined as that amount of enzyme which breaks down 1 µmole of H_20_2min under the assay conditions described.

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 Cr (mg/Kg)	Total Cd (mg/Kg)	EC (ds/m)
Soil	sandy loam	0.68	7.8	0.06	12	105	0.023	0.083	2.2
Compost	---	21.3	7.53	1.93	0.65	2.1	Not detected	0.02	2.5

0: Without organic substance, Com: compost, HA: Humic acid

2.5 Statistical Data

Statistical analysis employed SAS windows version 9.1. The significance of differences between variables at $P<0.01$ or $P<0.05$ was checked with a multiple comparison on (LSD) test.

3. RESULTS AND DISCUSSION

3.1 Shoot Dry Weight

The results pertaining to effect of different concentrations of heavy meals on biomass yield of *Zea maize* are depicted in Fig. 1. It was observed that enhancement of Cr and Cd decrease shoot dry weight of plant in all treatments. The concentration of 200 and 50 mg/kg of Cr and Cd proved to be toxic, affecting the plant growth severely. In control, shoot dry weight decreased from 38.6 g to 17.2 g and 14 g with T_0, T_1 and T_2, respectively. Application of organic substance increase shoot dry weight as compared to control. No significant effect observed between compost and humic acid. Shoot dry weight in T_0 and T_1 treatments with organic substance increased about 76.9% and 64% of the control, respectively. T_2 treatment was no significant in use of organic substance. The most common effect of heavy metals toxicity in plants is stunted growth, leaf chlorosis and alteration in the activity of enzymes of various metabolic pathways [13]. In our study, varied concentrations of Cr and Cd affected dry weight of *Zea maize*. The reduction in the shoot dry weight in *Zea maize* could be also due to the suppression of the elongation growth rate of cells, because of an irreversible inhibition exerted by heavy metals on the proton pump responsible for the process [14]. Parameters such as shoot dry weight were used as useful indicator of metal toxicity in plants. In our study, Cr and Cd stress showed a higher decline in these parameters.

3.2 Chlorophyll Content

The effects of heavy metal on chlorophyll derivatives are seen in the Table 2. The amounts of chlorophyll-a, chlorophyll-b and total chlorophyll decrease with increasing chromium and cadmium concentration, compared with their respective controls. Although the decrease in chlorophyll-b is larger than in chlorophyll-a. Exposure to high concentration of Cr (200 mg/kg) and Cd (50 mg/kg) reduced chlorophyll-a significantly to about 68/4% of the control. Application of organic substance especially humic acid (c) decrease the negative effects of heavy metals. Increase in chlorophyll-a observed in both compost and humic acid treatments. Chlorophyll-a in compost and humic acid treatments decreased about 56.1% and 59.7% of the control. Similarly, chlorophyll-b decreased from 1.65 to 1.1 and 0.76 mg/g Fw in control. In all treatment application of compost increase chlorophyll-b (about 51%) rather than humic acid. The chlorophyll ratio, which is used as a stress indicator, increased slightly with increasing metal treatments. In control increase in concentration of Cr and Cd, decrease the chlorophyll ratio. When we use compost in this case the chlorophyll ratio was increased.

Furthermore, the data analysis showed that the sum of chlorophyll a and b content (chlorophyll a+b) was influenced by the higher metal concentrations. In general Total chlorophyll content declined progressively with increasing concentrations of the heavy metal. Chlorophyll content is often measured in plants in order to assess the impact of environmental stress, as changes in pigment content are linked to visual symptoms of plant illness and photosynthetic activity [15].

Heavy metals produce oxyradicals in plants. These radicals cause widespread damage to membranes and associated molecules, including chlorophyll pigments. We suppose that higher concentration of Cr acts indirectly to chlorophyll metabolism (*i.e.* by stimulation the dis-integration of membranes, and enhancing the activity of peroxidase).

3.3 Total Soluble Protein Content

The results of this study showed decrease in soluble protein content in all treatment (Fig. 2). This decrease was low with application of compost and humic acid. The percentage of decrease in control, compost and humic acid with T_2 treatment was 72.2%, 47.6% and 49% as compared to T_0, respectively. Decrease in total soluble protein content under this condition may be caused by increase in protease activity. Application of compost and humic acid increases soluble protein content about 16/6% and 52/7% of the control, respectively. Decrease in total soluble protein in most treatments suggests that in this condition (stress of heavy metals) synthesis of protein was destruction. It is also likely that these heavy metals may have induced lipid peroxidation in *Zea maize* and fragmentation of proteins due to toxic effects of reactive oxygen

species led to reduced protein content [16]. Our studies coincide with Alemzadeh et al. [4] who also reported a decrease in soluble protein content under heavy metal stress.

3.4 SOD Activity

Free radical generation is one the initial responses of plants to stress. Antioxidants enzymes play an important role in the cellular defense strategy against oxidative stress. Considerable changes in SOD activity was observed that depended on the heavy metal concentration (Table 3). At higher concentration of Cr and Cd the amount of SOD activity increased. This increase was about 45% of the control. In T_0 treatment no significant effect observed between organic substances. In fact Application of organic substance decrease procedure of SOD activity enhancement but it was not significant. In compost and humic acid enhancement was observed about 39.1% and 38.9% as compared with control. In high concentration of heavy metals (Cd and Cr) oxygen species increases and synthesis of protein deranged. Increases of SOD enhance resistance of plant opposite heavy metals stress. Plants have conservative mechanisms include enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APx), Guaiacol peroxidase (GPx) and catalase (CAT) against increase of Reactive oxygen species (ROS) [17]. Superoxide dismutase (SOD) with converting O^{2-} to H_2O_2 in cytosol and chloroplast decrease the effect of oxygen species. Increase in SOD activity in all treatments indicate on high accumulation of ROS under heavy metals stress in order to activate antioxidative defense enzymes to inhibit oxygen radical accumulation [18]. Our results be consistent with Dazy et al. [19] and Liu et al. [20].

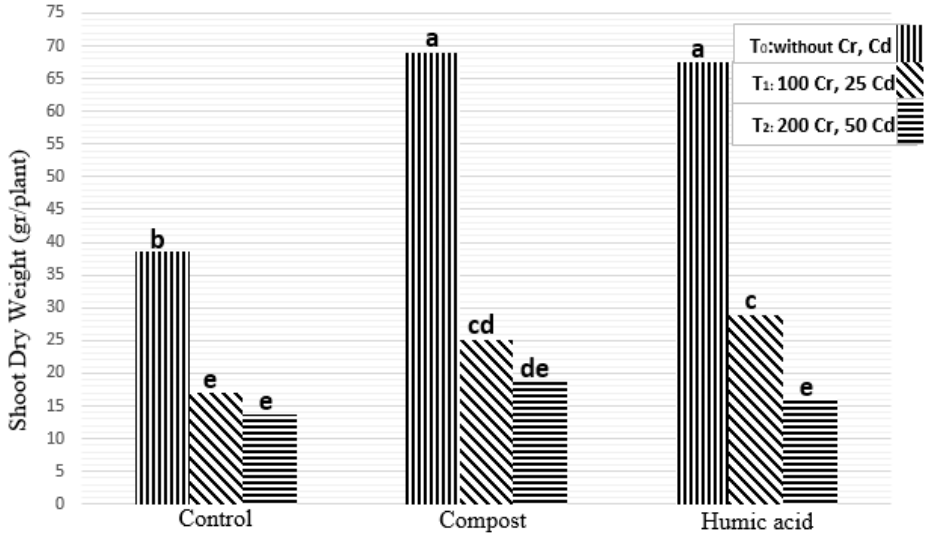

Fig. 1. Effect of different concentrations of Cd and Cr and organic substance on shoot dry weight in Zea maize. Values are means ± SE (n = 3). Similar letters are not significantly different at *P<0.05*, according to Duncan's test

a-e: Letters that indicates difference statistic between mains of parameters

Table 2. Effect of different concentrations of Cd and Cr and organic substance on chlorophyll content in Zea maize

Treatment	Chlorophyll a (mg/g Fw)			Chlorophyll b (mg/g Fw)			Chlorophyll ratio a/b			Total Chlorophyll (a+b)		
OM/	0	Com	HA	0	Com	HA	0	Com	HA	0	Com	HA
T_0	1.52^{c}	1.94^{b}	2.46^{a}	1.65^{d}	2.8^{b}	3.29^{a}	0.92^{a}	0.69^{c}	0.74^{c}	3.17^{c}	4.74^{b}	5.75^{a}
$T_1(Cr_{100}Cd_{25})$	0.94^{d}	1.45^{c}	1.98^{b}	1.1^{e}	2.03^{c}	2.29^{c}	0.85^{b}	0.71^{c}	0.86^{b}	2.04^{d}	3.48^{c}	4.27^{b}
$T_2(Cr_{200}Cd_{50})$	0.48^{e}	0.85^{d}	0.99^{d}	0.76^{f}	0.93^{e}	1.3^{d}	0.63^{d}	0.91^{a}	0.76^{c}	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; a-e: Letters that indicates difference statistic between mains of parameters; 0: without organic substance, Com: compost, HA: Humic acid

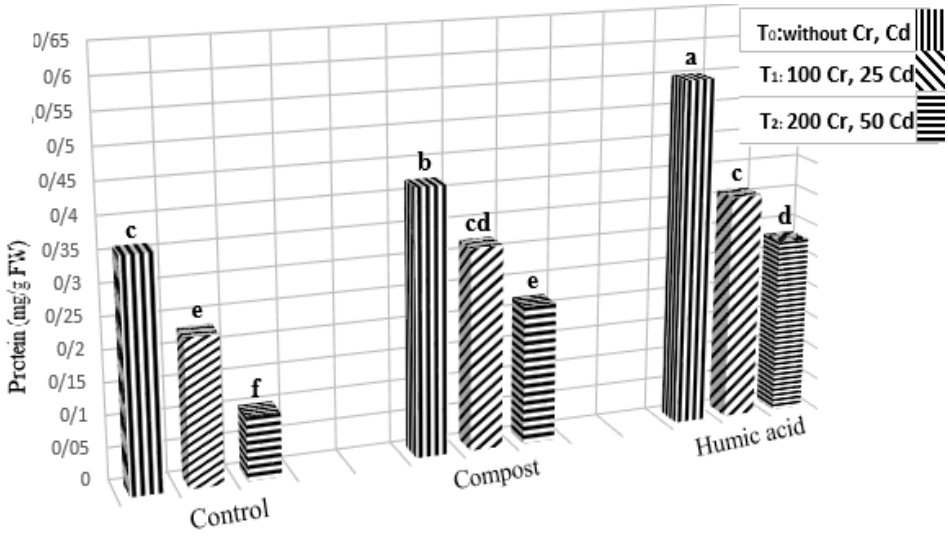

Fig. 2. Effect of different concentrations of Cd and Cr and organic substance on on protein content in leaves. Values are means ± SE (n = 3). Similar letters are not significantly different at *P<0.05*, according to Duncan's test

a-e: Letters that indicates difference statistic between mains of parameters

Table 3. Effect of different concentrations of Cd and Cr and organic substance on enzyme activity in *Zea maize* leaves

Treatment OM/	Superoxide dismutase (U/mg protein)			Catalase (U/mg protein)		
	0	Com.	HA	0	Com.	HA
T_0	605.6^{d}	582^{d}	592.3^{d}	76.8^{d}	65.7^{d}	66.2^{d}
$T_1(Cr_{100}Cd_{25})$	807.4^{b}	637.2^{c}	643^{c}	93.3^{c}	109.3^{b}	124.4^{a}
$T_2(Cr_{200}Cd_{50})$	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; a-e: Letters that indicates difference statistic between mains of parameters; 0: Without organic substance, Com: compost, HA: Humic acid

3.5 CAT Activity

Catalase one of main enzymes in oxidative stress that reaction H_2O_2 to H_2O and O_2. CAT activity increased in low concentration (T1) of Cr and Cd (about 17.2% and 33.3% as compared with control) with significant effect in reaction of the treatment but in high concentration (T2) of this elements CAT activity decreased. In low concentration (T1) of Cr and Cd application of organic substance especially humic acid had positive effect on CAT activity. No significant reaction observed in T2 treatment with organic substance. High concentration of Cd and Cr inhibition catalase activity because in this level CAT lose it power for catalyzing H_2O_2 to H_2O and O_2. These results are in agreement with results of Verma and Dubey [21] and Alemzadeh et al. [4]. In low concentration of Cd and Cr, CAT attendant with SOD decrease the negative effect of ROS. Decline of CAT activity can be result of decrease in its synthesis in plant. CAT is susceptible to Cr and Cd than SOD, decrees in its activity pointer its. These results show that aggregation of oxygen species due to oxidative stress of heavy metals in plants cause different respond in treatments. These results show that aggregation of oxygen species due to oxidative stress of heavy metals in plants cause different respond in treatments.

4. CONCLUSION

In conclusion, our results indicated that the exposure of *Zea maize* to Cr and Cd decreased shoot dry weight and pigment content. The present study show that in high level of heavy metals antioxidative system in *Zea maize* was active to survive plant. Decrease in chlorophyll and protein also a signal result for the toxicity of

heavy metals. Total Soluble Protein Content decreased in all treatments, indifferently of compound type and Cr and Cd concentration. Decrease in total soluble protein in most treatments suggests that in this condition (stress of heavy metals) synthesis of protein was destruction. SOD activity increased with the increasing concentration of chromium and cadmium. At higher concentration of Cr and Cd the amount of CAT activity decreased. Increase of SOD pointed to its role in antioxidative system in Zea maize. When ROS increases, chain reactions start, in which superoxide dismutase (SOD) catalyzes the dismutation of O_2 radicals to H_2O_2.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Olteanu Z, Opric C, Tru E, Zamfirache M. Behavior of antioxidative enzymes and of soluble protein in wheat seedlings after lead-induced stress. Anal Scientific University Alexandra Iona Cuza Genetic Biology Molecular; 2011.
2. Trevisan M, Coppolecchia D, Hamon R, Puglisi E. Potential nitrification, nitrate reductase, and β-galactosidase activities as indicators of restoration of ecological functions in a Zn-contaminated soil. Biology and Fertility of Soils. 2012;48: 923–931.
3. Bartoli G, Papa S, Sagnella E, Fioretto A. Heavy metal content in sediments along the Calore River: Relationships with physical-chemical characteristics. Journal of Environmental Management; 2012;95: 9–14.
4. Alemzadeh A, Rastgoo L, Tale A, Tazangi S, Eslamzadeh T. Effects of copper, nickel and zinc on biochemical parameters and metal accumulation in gouan, *Aeluropus littoralis*. Plant Knowledge Journal. 2014; 3:31-38.
5. Arora A, Sairam RK, Srivastava G. Oxidative stress and antioxidative system in plants. Curr Sci. 2002;82:1227-1238.
6. Wyszkowski M, Ziółkowska A. Content of polycyclic aromatic hydrocarbons in soils polluted with petrol and diesel oil after remediation with plants and various substances. Plant, Soil and Environment. 2013;59:287–294.
7. Barančíková G, Makovníková J. The influence of humic acid quality on the sorption and mobility of heavy metals. Plant, Soil and Environment. 2003.49: 565–571.
8. Hoop M, Leeuwen H, Cleven R. Chim Anal Acta.1990;141-232.
9. Lichtenthaler H, Wellburn AR. Determination of total carotenoids and chlorophyll ‘a’ and ‘b’ of leaf extracts in different solvents. Biochem. Soc Trans. 1983;603:591-592.
10. Bradford M. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248–254.
11. Dhindsa RS, Plumb-Dhindsa P, Thorpe TA. Leaf senescence correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. Exp Bot. 1981;32:93-101.
12. Chance B, Maehly A. Assay of catalase and peroxidases. Methods enzymol. 1955; 2:764-775.
13. Kim YJ, Kwon Y, Jeong S. Combined effects of copper, cadmium, and lead upon *Cucumis sativus* growth and bioaccumulation. Sci. Total Environ. 2004; 32685–93.
14. Wojcik M, Vangronsveld J, Tukiendorf A. Cadmium tolerance in *Thlaspi caerulescens*. I. Growth parameters, metal accumulation and phytochelatin synthesis in response to cadmium. Environ. Exp. Bot. 2005;53:151–161.
15. Parekh D, Puranik RM, Srivastava HS. Inhibition of chlorophyll biosynthesis by cadmium in greening maize leaf segments. Biochemie Physiologie der Pflanzen. 1990; 186:239–242.
16. Palma JM, Sandalio LM, Javier F, Romero- Puertas MC, McCarthy I, Del Rio LA. Plant proteases protein degradation and oxidative stress: Role of peroxisomes. Plant Physiol. Biochem. 2002;40:521–530.

17. Groppa MD, Tomaro ML, Benarides MP. Polyamines and heavy metal stress: The antioxidant behavior of spermine in cadmium and copper treated wheat leaves. Biometals. 2007;20:185-195.
18. Alemzadeh A, Rastgoo L. Biochemical responses of Gouan (*Aeluropus littoralis*) to heavy metals stress. AJCS. 2011;4: 375-383.
19. Dazy M, Masfaraud JF, Férard JF. Induction of oxidative stress biomarkers associated with heavy metal stress in *Fontinalis antipyretica Hedw*. Chemosphere. 2009;75:297–302.
20. Liu D, Zou J, Meng Q, Zou J, Jiang W. Uptake and accumulation and oxidative stress in garlic (*Allium sativum L.*) under lead phytotoxicity. Ecotoxicology.2009;18: 134–143.
21. Verma S, Dubey RS. Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Sci. 2003;164:645-655.

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