Journal of Medicinal Plants and By-products (2016) 1: 15-22

Original Article

Effect of Cadmium on Germination Characters and Biochemical Parameters of Two Iranian Ecotypes of Cumin (*Cuminum cyminum* L.)

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Article History: Received: 1 June 2015/Accepted in revised form: 18 September 2015

Abstract

Cadmium (Cd), being a highly toxic metal pollutant of soils, it inhibits root and shoot growth and yield production, affects nutrient uptake and homeostasis. It is frequently accumulated by agriculturally important crops and then enters the food chain with a significant potential to impair animals and human's health. Therefore, a study was conducted to evaluate the effects of various Cd levels (0 as control, 300, 450, 600, 750 and 1050 µM) on some growth and biochemical parameters of two Iranian ecotypes of cumin (*Cuminum cyminum*) seedlings. The results revealed that seed germination, root growth, chlorophyll content and total soluble protein of both ecotypes decreased significantly with increase in metal concentration. The proline showed an increase in lower concentrations of Cd but at higher concentrations it decreased. The present results allow us to conclude that the cumin plants adversely affected by cadmium toxicity. Decrease in the seed germination percentage, root growth, chlorophyll and protein content may be considered as circumstantial evidence for the toxicity of cadmium. The present study demonstrated that under cadmium stress, *C. cyminum* underwent biochemical changes to survive under high concentrations of this metal. Increase in metal chelate components (proline) proves this fact. It can be concluded that Isfahan ecotype was superior to Khorasan ecotype in most of the measured parameters and it can be suggested that Isfahan ecotype is more tolerant to Cd stress than Khorasan ecotype.

Keywords: Cadmium, *Cuminum cyminum*, Heavy metal toxicity, Physiological responses

Abbreviations
Cd: Cadmium, DMSO: Dimethylsulphooxide, TCA: Trichloroacetic acid, Chl: Chlorophyll, ROS: Reactive oxygen species, FW: Fresh weight

Introduction

Pollution of environment by toxic metals arises as a result of various industrial activities and has turned these metal ions into major health issue. Although several adverse effects of the toxic metals have been known for a long time, exposure to heavy metals continues and is even increasing in some parts of the world in particularly in less developed countries [1].

Cadmium (Cd) is a highly toxic heavy metal that enters into the environment mainly by industrial processes and phosphate fertilizers. It can be reached high levels in agricultural soils and easily accumulates in plants. Cd ions are taken up readily by the plant roots and translocated to the shoots [2]. Excessive Cd induces complex changes in plants at genetical, biochemical and physiological levels, leading to phytotoxicity. The presence of Cd at

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higher concentrations in the soil damages root tips, reduces nutrients and water uptake, impairs photosynthesis and inhibits growth of the plants [3]. Furthermore, Cd directly or indirectly induces reactive oxygen species (ROS), which affect the redox status of the cell and cause oxidative damage to proteins, lipids, and other biomolecules [4]. Cd damages the nucleoli in cells of the root tip, alters the synthesis of RNA, inhibits ribonuclease activity and inhibits the DNA repair mechanism [5].

Cumin (*Cuminum cyminum* L.) belongs to the family Apiaceae is a small annual and herbaceous plant. Cumin is naturally found in Iran, Turkey, India, Pakistan, Argentina, China, CentralAmerica and other regions. Its fruit known as cumin seed, is most widely used for culinary and medicinal purposes. It is generally used as a food additive, popular spice, and flavoring agent in many cuisines. Cumin seeds have also been widely used in traditional medicine for the treatment of several health disorders and diseases, such as toothaches, dyspepsia, diarrhea, epilepsy, and jaundice. These medicinal benefits have generally been ascribed to its rich content and potent action of active constituents such as terpenes, phenols, and flavonoids [6]. The aim of the present study was to assess the effects of different concentrations of Cd on growth and biochemical attributes of two Iranian ecotypes of cumin seedlings in order to contribute to the understanding of *C. cyminum* adaptation to heavy metals.

Material and Methods

Plant Materials, Seed Germination and Root Growth Measurement

Two Iranian ecotypes of cumin (*C. cyminum*) seeds, namely Isfahan and Khorasan were used in this study that kindly provided by Dr. Mohammadinejad, Department of Agronomy and Plant Breeding, Shahid Bahonar University of Kerman, Iran. The seeds were surface sterilized by immersion in 70% ethanol for 3 min., followed by stirring in sodium hypochlorite (5% chlorine) for 20 min. The surface sterilized seeds were rinsed 3 times with sterile distilled water for 5 min. The seeds were placed in Petri dishes (90-mm diameter) on filter paper (Whatman # 42) and were treated with 5mL solutions of 300, 450, 600, 750 and 1050 µM Cd (in the form of $CdCl_2.2H_2O$ of high purity 98% of Merck Chemicals, Germany). The control treatment was supplied with distilled water. The Petri dishes were kept at room temperature (28±2ºC) under four 40-Watt tube lights. The experiment was concluded after 7 days of exposure to Cd and various growth indices such as seed germination and root growth were measured. Inhibitory rate (%) was calculated by the formula (1 x/y) × 100, where y was the average value detected in the control and x was one in each samples treated [7]. Three replicates were set up for each treatment and the experiments were carried out in duplicate for comparison.

Determination of Biochemical Parameters

Plant Growth Condition

To determine the biochemical parameters, the seeds of two ecotypes were sterilized as previously mentioned and then placed in Petri dishes (90-mm diameter) on filter paper (Whatman # 42) under dark condition, to simulate the soil conditions at 20 °C. The uniform germinated seeds were transferred to the pots (diameter 9.5 cm and height 14 cm) containing sands at sizes 1-3 mm (40%) and perlite (60%). The pots were incubated in a growth chamber at a temperature of 20±1 °C and a 16h day and 8h night photoperiod. The plants were fertilized by Hoagland solution once a day. After 21 days seedlings were treated with 0 (as control), 300, 450, 600, 750 and 1050 µM of Cd under the above mentioned laboratory condition for 7 days. Each treatment was replicated three times for statistical purposes and the experiments were carried out in duplicate for comparison.

Determination of Chlorophyll

The chlorophyll content was determined by the method of Hiscox and Israelstam [8]. Fresh leaves (100 mg) were kept in the extraction reagent, dimethylsulphooxide (DMSO). The tubes were kept in oven at 65 °C for 40 min. 1 mLof sample was mixed with 2 mL DMSO and vortexed. Absorbance was determined photometrically at 480, 510, 645, 663 nm (Analytik Jena, Germany) using DMSO as a blank.

Protein Estimation

Proteins were estimated by the method of Bradford [9]. 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. 0.5mL of freshly prepared trichloroacetic acid (TCA) was added and centrifuged at 8000 × g for 15 min. The debris was dissolved in 1 mL of 0.1 N NaOH and 5

mL Bradford reagent was added. Absorbance was recorded photometrically at 595 nm (Analytik Jena, Germany) and bovine serum albumin was used as standard curve drawing.

Estimation of Proline Content

Proline concentration was determined using the method of Bates *et al*. [10]. Fresh leaves (300 mg) were homogenized in 10 mL of aqueous sulphosalicylic acid (3%). The homogenate was centrifuged at 9000 × g for 15 min. A two mL of the supernatant was mixed with an equal volume of acetic acid and ninhydrin and incubated for 1 h at 100 °C. The reaction was terminated on ice bath and extracted with 4 mL of toluene. The extract was vortexed for 20 s and the chromatophore-containing toluene was aspirated from the aqueous phase and absorbance determined photometrically at 520 nm (Analytik Jena, Germany) using toluene as a blank.

Statistical Analysis

The experiments were carried out based on complete randomized block design with three replications. The statistical analyses were carried out using the SAS version 9. Changes in growth and biochemical parameters were tested statistically by performing one-way analysis of variance (ANOVA). The treatment means compared using Duncan's multiple-range test (DMRT) taking $p < 0.01$ as significant (Table 1).

Table 1 Analysis of variation of the effect of $CdCl_2$ on measured traits (Isfahan ecotype (up), Khorasan ecotype (bellow)

Source of Variation	Degree of Freedom	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Protein	Proline	Germination	Root Growth
Cadmium Chloride	5	0.5389**	0.1341**	1.2129**	337.203**	4030.860**	3970.844**	16.323**
Error	12	0.0029	0.00021	0.0025	0.0905	0.4483	1.117	0.0104
CV%	-	5.024	3.102	3.2968	1.3061	0.4492	3.1382	5.775

Source of Variation	Degree of Freedom	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Protein	Proline	Germination	Root Growth
Cadmium Chloride	5	0.5602**	0.1292**	1.2245**	323.639**	4304.658**	4789.333**	9.5035**
Error	12	0.00019	0.00017	0.00032	0.0884	0.3033	0.7688	0.0104
CV%		1.349	2.985	1.212	1.403	0.379	2.391	6.373

Significant at 0.01 probability level**

Table 2 Effect of different concentrations of Cd on seed germination percentage of tow ecotypes after 7 days exposure

Cd concentration (µM)	Isfahan ecotype (%)	Khorasan ecotype (%)
0	93.10±1.42a	98.66±2.01a
300	62.66±2.58b	75.33±2.57b
450	20.66±1.95c	19.66±0.98c
600	19.00±2.91c	12.66±0.98d
750	6.70±0.16d	7.00±0.86e
1050	0.00e	6.70±0.16e

Values are means (n=3)± SE, means followed by the same letter(s) are not significantly different for $p < 0.01$ according to the Duncan's test.

Table 3 Effect of different concentrations of Cd on root length of seedlings of two ecotypes after 7 days exposure

Cd concentration (µM)	Root in Isfahan ecotype		Root in Khorasan ecotype	
	Root length (cm)	Inhibitory rate (%)	Root length (cm)	Inhibitory rate (%)
0	5.84 ± 0.98a	0.00	4.66 ± 0.26a	0.00
300	3.27 ± 0.27 b	44.00	2.38 ± 0.50b	48.92
450	0.96 ± 0.20c	83.56	1.90 ± 0.29c	59.22
60	0.36 ± 0.04d	93.83	0.30 ± 0.08d	93.56
75	0.17 ± 0.06de	97.00	0.22 ± 0.06d	95.28
105	0.00 ± 0e	100	0.14 ± 0.01d	97.00

Values are means (n = 3) ± SE, means followed by the same letter(s) are not significantly different for $p < 0.01$ according to the Duncan's test.

Table 4 Effect of different concentrations of Cd on chlorophyll content (mg g^{fw-1}) of two ecotypes after 7 days exposure.

Cd concentration (µM)	Isfahan ecotype			Khorasan ecotype		
	a	b	total	a	b	total
0	1.496 ±0.026a	0.686±0.02a	2.183±0.043a	1.490±0.016a	0.680± 0.016a	2.170±0.016a
300	1.396±0.026ab	0.630±0.016b	2.023±0.023b	1.363±0.022b	0.623± 0.042b	1.986±0.029b
450	1.316±0.195b	0.580±0.033c	1.896±0.162b	1.243± 0.019c	0.520± 0.016c	1.763±0.035c
600	1.083±0.178c	0.450±0.029d	1.533±0.17c	1.036±0.01d	0.420±0.016d	1.456±0.026d
750	0.800±0.033d	0.343±0.026e	1.133±0.041d	0.723± 0.042e	0.316± 0.010e	1.040±0.045e
1050	0.380±0.016e	0.120±0.016f	0.500±0.033e	0.343± 0.026f	0.120±0.016f	0.463±0.033f

Values are means (n = 3) ± SE, means followed by the same letter(s) are not significantly different for $p < 0.01$ according to the Duncan's test**.**

Results

Effect of Cd on Seed Germination and Root Growth

The results showed that Cd adversely affected the seed germination in both ecotypes (Tables 2 and 3). The percentage of seed germination inhibition after Cd exposure at 7 days was 30% and 23% for Isfahan and Khorasan ecotypes respectively, when the concentration of cadmium was 300 µM ($p<0.01$). When the concentration of Cd in the growing media was 1050 µM, the inhibitory rate of seed germination was up to 100 and 91.9% for Isfahan and Khorasan ecotypes respectively. In other words, in this concentration complete inhibition was occurred in the seed germination of Isfahan ecotype and seed germination of Khorasan ecotype with reduction more than 90% compared with control strongly affected. The root growth of cumin seedlings can be assessed using the observation and measurement of variation in root length in response to environmental pollution. The results demonstrated that root length inhibited substantially compared with control seedlings with the increase of Cd concentration 300, 450, 600, 750, and 1050 µM ($p<0.01$) following 7 days of treatment. The percentage of root-growth inhibition at 7 days after cadmium exposure was 43.6% and 48.7% for Isfahan and Khorasan ecotypes respectively, when the concentration of Cd was 300 µM in the media ($p< 0.01$). The higher concentration of Cd, the more serious the inhibitory effect on root length. When the concentration of Cd was 1050 µM, the inhibitory rate of root growth was 100% and 97% for Isfahan and Khorasan ecotype respectively.

Table 5 Effect of different concentrations of Cd on protein content (mg g^{fw-1}) of two ecotypes after 7 days exposure.

Cd concentration (µM)	Isfahan ecotype	Khorasan ecotype
0	38.40±1.291a	36.86±1.454a
300	28.06±0.752b	26.10±1.010b
450	25.13±1.494c	22.40±1.291c
600	23.26±0.908d	21.06±0.752d
750	16.06±1.01e	14.46±0.888e
1050	7.3±00.716f	6.26±0.902f

Values are means (n = 3) ± SE, means followed by the same letter(s) are not significantly different for $p < 0.01$ according to the Duncan's test.

Table 6 Effect of different concentrations of Cd on proline content (µg g^{fw-1}) of two ecotypes after 7 days exposure.

Cd concentration (µM)	Isfahan ecotype	Khorasan ecotype
0	116.26±2.310e	120.46±1.809e
300	161.86±3.275c	158.83±2.739c
450	184.36±2.103b	180.20±1.641b
600	189.26±3.335a	187.40±1.895a
750	144.36±1.454d	135.23±1.770d
1050	98.13±1.155f	88.00±1.710f

Effect of Cd on Biochemical Characters

In this study, exposure to Cd stress significantly decreased chlorophyll content (Chl a, Chl b and total Chl) in comparison to control seedlings (Table 4). The chlorophyll content was decreased significantly with increase in metal concentration. Parallel decrease in Ch1 a, Chl b and total Chl due to Cd stress were observed in both ecotypes. The decrease in Chl a, Chl b and total Chl contents of Isfahan ecotype was 74.6%, 82.5%, and 77% in a higher level of Cd treatment (1050 µM) respectively, compared with control (100%). However, in Khorasan ecotype the reduction in Chl a, Chl b, and total Chl contents was 77.45%,

82.35% and 78.98% respectively in 1050 µM Cd concentration compared with control. The reduction in Chl b content was higher than Chl a in both ecotypes. Additionally, the reduction in photosynthetic pigments in Khorasan ecotype was greater than in Isfahan ecotype.

The results pertaining to the effect of Cd on proline content are presented in table 6. Maximum proline accumulation was observed with 600 µM Cd in both ecotypes but the 1050 µM Cd declined the proline to about 15.6% and 26.94% of the control respectively in Isfahan and Khorasan ecotype.

In both ecotypes exposures to Cd stress significantly declined ($p<0.01$) protein content (Table 5). 450 µM Cd declined protein content to 34.5% and 1050 µM decreased it to 80.9% of the control in Isfahan ecotype. However, in Khorasan ecotype the reduction in protein content was 39.23% and 83% respectively at 450 µM and 1050 µM Cd concentration compared to control.

Values are means (n=3) ± SE, means followed by the same letter(s) are not significantly different for $p<0.01$ according to the Duncan's test.

Discussion

Plant growth and development under stress conditions are generally negatively affected. One of these stress conditions that affect plants is heavy metals. Recently, heavy metals have become a hot topic of research for many researchers around the world, mostly due to their detrimental effects on many organisms including plants. Much research has been conducted on the effect of Cd on crops and other agricultural plants. However, little information is available on the toxicity of Cd on medicinal plants. Therefore, the experiments were performed to evaluate the inhibitory effect of Cd contamination on seed germination, root growth and some biochemical responses of cumin seedlings at all designed concentrations.

The results of this study showed that Cd levels affected all of the studied parameters in *C. cyminum* by different magnitudes. In general, it was closely observed that increasing concentrations of cadmium obviously decreased the germination percentage of seeds and root growth in two ecotypes compared with control. The results demonstrate clearly a dose dependent response of seed germination and root growth of cumin and confirmed that Cd is indeed a toxic agent for plants as described in literature. The spectrum and dose-response relationships of inhibitory effects on seed germination and root growth in Cumin are largely similar to those in *Andrographis paniculata* [11], Arabidopsis [12], wheat and cucumber [13], *Cichorium pumilum* [14], *Catharanthus roseus* [15] and cowpea [16]. The decrease in the germination percentage of cumin seeds may be related to the negative effects of cadmium on water uptake and water movement [16]. Shafiq *et al*. [17] indicated that seed germination reduction of *Leucaena leucocephala* due to heavy metal stress could be attributed to higher levels of seeds stored nutrient breakdown and/or change in permeability characteristics of the cell membrane.

The inhibitory action of excess of cadmium in root length might be due to a reduction in cell division, toxic effect of heavy metals on photosynthesis, respiration and protein synthesis. These obviously contributed to the retardation of normal growth [16].

In the present study with increase in concentration of Cd, the seed germination and root growth indices were reduced in both ecotypes. However, with increasing Cd more than 450 µM, the adverse effects were more obvious. In Isfahan ecotype, the seed germination and root growth completely stopped in 1050 µM cadmium.

Chlorophyll a and b are essential for photosynthesis and they are very sensitive to environmental stresses such as heavy metals [18]. Several reports show chlorophyll biosynthesis inhibition by metals in higher plants [19-22]. In the present study, exposure to Cd stress significantly ($p<0.01$) decreased chlorophyll content (Chl a, Chl b and total Chl) concentrations of both ecotypes in comparison to control seedlings. Decrease in chlorophyll content may be due to reduce synthesis of chlorophyll due to inhibition of enzymes activity such as δ-aminolevulinic acid dehydratase (ALA-dehydratase) [23] and protochlorophyllide reductase [24], replacement of Mg with heavy metals in chlorophyll structure [25], decrease in the source of essential metals that involved in chlorophyll synthesis such as Fe^{2+} and Zn^{2+} [24, 26], destruction of chloroplast membrane by lipid peroxidation due to increase in peroxidase activity and lack of antioxidants such as carotenoids [27], decrease in density, size and the synthesis of chlorophyll and inhibition in the activity of some enzymes of Calvin cycle [28, 29]. The loss of chlorophyll content can consequently lead to disruption of photosynthetic machinery [29].

Abiotic stress may inhibit the synthesis of some proteins and promote others [30] with a general

trend of decline in the overall content. The total soluble protein levels in the seedlings of both ecotypes decreased substantially ($p < 0.01$) compared with the control plantlets along with the increase of Cd concentration following 7 days treatments. Our studies coincide with John *et al*. [1] who also reported a decrease in soluble protein content under cadmium and lead stress in *Lemna polyrrhiza*. Similar results obtained by Anayat*et al*. [31] who reported that treatment with mercury and cadmium caused reduction in the soluble protein level in fennel (*Foeniculum vulgare*).

It is thought that decrease in total soluble protein content under heavy metals stress may be due to increase in protease activity [32], various structural and functional modifications by the denaturation and fragmentation of proteins [33], DNA-protein cross-links [34], interaction with thiol residues of proteins and replacement of them with heavy metals in metalloproteins [35]. It has been reported that cadmium is able to decrease protein content by inhibiting the uptake of Mg and K and promote posttranslational modifications [36, 37], decrease in synthesis or increase in protein degradation [38] and the prevention of Rubisco activity [39].

Proline, an amino acid, is well known to get accumulated in a wide variety of organisms ranging from bacteria to higher plants on exposure to abiotic stresses. In present study, proline showed increase at lower concentrations of Cd in both ecotypes but at higher concentrations (750 and 1050 µM) it showed decrease. In response to heavy metal stress, plants accumulate a large quantity of proline. Increase in proline content may be either due to *de novo* synthesis or decreased degradation or both [40]. Ozdener and Guray Kutbay [41] reported that proline levels increased in the leaves of *Verbascum wiedemannianum* with Cd stress. Dinakar *et al*. [42] also reported that Cd treated *Arachis hypogaea* tissues showed a significant increase in proline compared to control. Al Khateeb and Al-Qwasemeh [43] reported that proline content increased under Cd in *Solanum nigrum* and *Solanum lycopersicum*. It was suggested that this amino acid acts as an osmolyte by antioxidative, osmoprotection properties and metal chelator [44], takes part in reconstruction of chlorophyll [45], regulation of cytosolic acidity [46], tolerance to stress by osmoregulation and stabilization of protein synthesis [47], stabilize the macromolecules and organelles [1], the protection of enzymes from denaturation [46] and also serve as source of nitrogen and energy in recovery growth [1].

The present results allow us to conclude that the cumin plants adversely affected by cadmium toxicity. Decrease in seed germination percentage, root growth, chlorophyll and protein may be considered as circumstantial evidence for the toxicity of cadmium. The present study demonstrated that under cadmium stress, *Cuminum cyminum* underwent biochemical changes to survive under high concentrations of this metal. Increase in metal chelate components (proline) proves this fact. It can be concluded that Isfahan ecotype was superior to Khorasan ecotype in most of the measured parameters and it can be suggested that Isfahan ecotype is more tolerant to Cd stress than Khorasan ecotype.

References

1. John R, Ahmad P, Gadgil K, Sharma S. Effect of cadmium and lead on growth, biochemical parameters and uptake in *Lemna polyrrhiza* L. Plant Soil Environ. 2008;54:262-270.
2. Shamsi IH, Wei K, Zhang GP, Jilani G, Hassan MJ. Interactive effects of cadmium and aluminum on growth and antioxidative enzymes in soybean. Biol Plantarum. 2008;52:165-169
3. Sharma P, Dubey RS. Cadmium Uptake and Its Toxicity in Higher Plants. In: Khan NA, Samiullah S (eds.) Cadmium Toxicity and Tolerance in Plants, Narosa publishing House, 2006, pp. 63-86.
4. Schutzendubel A, Schwanz P, Teichmann T, Langenfeld-Heyser GK, Godbold DL, Polle A. Cadmium-induced changes in antioxidative systems, hydrogen peroxide content, and differentiation in scots pine roots. Plant Physiol. 2001;127:887-898.
5. Amirthalingam T, Velusamy G, Pandian R. Cadmium - induced changes in mitotic index and genotoxicity on *Vigna unguiculata* (Linn.) Walp. J Environ Chem Ecotoxicol. 2013;5:57-62.
6. Einafshar S, Poorazarang H, Farhoosh, R, Seiedi M. Antioxidant activity of the essential oil and methanolic extract of cumin seed (*Cuminum cyminum*). Eur J Lipid Sci Tech. 2011;114:168–174.
7. Liu W, Li P, Qi X, Zhou Q, Zheng L, Sun TH, Yang YS. DNA changes in barely (*Hordeum vulgare*) seedlings induced by cadmium pollution using RAPD. Chemosphere. 2005;61**:**158-167.
8. Hiscox JD, Israeltam GF. A method for extraction of chlorophyll from leaf tissue without maceration. Canadian J Bot. 1979;59:1332-34.
9. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the protein dyes binding. Annu Rev Biochem. 1976;72:248-254.

10. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant Soil. 1973;39:205-207.
11. Bhattacharya S, Puri S, Jamwal A, Sharma S. Studies on seed germination and seedling growth in kalmegh (*Andrographis paniculata*) under abiotic stress conditions. Int J Sci Environ Tech. 2012;1:197-204.
12. Liu W, Sun L, Zhong M, Zhou M, Zhou Q, Gong Z, Li P, Tai P. Cadmium-induced DNA damage and mutations in Arabidopsis plantlet shoots identified by DNA fingerprinting. Chemosphere. 2012;89:1048–1055.
13. Munzuroglu O, Geckil H. Effects of metals on seed germination, root elongation, and coleoptile and hypocotyls growth in *Triticum aestivum* and *Cucumis sativus*. Arch Environ ConTox. 2002;43:203-213.
14. Al Khateeb W. Cadmium-induced changes in germination, seedlings growth, and DNA fingerprinting of *in vitro* grown *Cichorium pumilum* Jacq. Int J Biol. 2014;6:65-74.
15. Pandey S, Gupta K, Mukherjee AK. Impact of cadmium and lead on *Catharanthus roseus* – A phytoremediation study. J Environ Biol. 2007;28:655-662.
16. Vijayaragavan M, Prabhahar C, Sureshkumar J, Natarajan A, Vijayarengan P, Sharavanan S. Toxic effect of cadmium on seed germination, growth and biochemical contents of cowpea (*Vigna unguiculata* l.) plants. Int Multidiscip Res J. 2011;1:01-06.
17. Shafiq M, Iqbal MZ, Athar M. Effect of lead and cadmium on germination and seedling growth of *Leucaena leucocephala*. J Appl Sci Environ Manag. 2008;12:61-66.
18. Ekmekçi Y, Tanyolaç D, Ayhan B. Effects of cadmium on antioxidant enzyme and photosynthetic activities in leaves of two maize cultivars. Plant Physiol. 2008;165:600-611.
19. Hayat S, Ali B, Aiman S, Ahmad HA. Brassinosteroid enhanced the level of antioxidants under cadmium stress in *Brassica juncea*. Environ Exp Bot. 2007;60:33-41.
20. Gill SS, Khana NA, Tutejab N. Cadmium at high dose perturbs growth, photosynthesis and nitrogen metabolism while at low dose it up regulates sulfur assimilation and antioxidant machinery in garden cress *(Lepidium sativum* L.). Plant Sci. 2012;182:112-120.
21. Pesko M, Kralova K, Masarovicova E. Responses of *Hypericum perforatum* plants to supply of cadmium compounds containing different forms of selenium. Ecol Chem Eng. 2010;17(3):279-287.
22. Gupta P, Khatoon S, Tandon PK, Rai V. Effect of cadmium on growth, bacoside A, and Bacopaside I of *Bacopa monnieri* (L.), a memory enhancing herb. The Scientific World Journal. 2014;Article ID 824586, 6 pages.
23. Padmaja K, Prasad DDK, Prasad ARK. Inhibition of chlorophyll synthesis in *Phaseolus vulgaris* L. seedlings by cadmium acetate. Photosynthetica. 1990;24:399-405.
24. Van Assche F, Clijsters H. Effects of metals on enzyme activity in plants. Plant Cell Environ. 1990;13:195-206.
25. Küpper H, Küpper F, Spiller M. In situ detection of heavy metal substitution chlorophylls in water plants. Photosynth Res. 1998;58:123-133.
26. Küpper H, Küpper F, Spiller M. Environmental relevance of heavy metal substituted chlorophylls using the example of water plants. J Exp Bot. 1996;47:259-266.
27. Prasad MNV, Strzalka K. Impact of Heavy Metals on Photosynthesis. In: Prasad MNV, Hagemeyer J (eds.) Heavy Metal Stress in Plants: from Molecules to Ecosystems, Springer-Verlag Publication, 1999, pp. 177-198.
28. Baryla A, Carrier P, Franck F, Coulomb C, Sahut C, Havaux M. Leaf chlorosis in oilseed rape plants (*Brassica napus*) grown on cadmium-polluted soil: causes and consequences for photosynthesis and growth. Planta. 2001;212:696-709.
29. Benavides MP, Gallego SM, Tomaro ML. Cadmium toxicity in plants. Braz J Plant Physiol. 2005;17:21-34.
30. Ericson MC, Alfinito AE. Proteins produced during salt stress in tobacco cell cultures. Plant Physiol. 1984;74:506-509.
31. Anayat M, Agnihotri RK, Vamli R, Kumar S, Sharma R. Role of Cd and Hg on biochemical contents of fennel and its reduction by exogenous treatment of nitrogen. Int J Sci Res Pub. 2014;4(3):1-6.
32. Palma JM, Sandalio LM, Javier Corpas 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.
33. John P, Ahmad P, Gadgil K, Sharma S. Heavy metal toxicity: Effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea* L. Int J Plant Prod. 2009;3:65-76.
34. Atesi I, Suzen HS, Aydin A, Karakaya A. The oxidative DNA base damage in tests of rats after intraperitoneal cadmium injection. Biometals. 2004;17:371-377.
35. Pál M, Horváth E, Janda T, Páldi E, Szalai G. Physiological changes and defense mechanisms induced by cadmium stress in maize. J Plant Nutr Soil Sci. 2006;169:239-246.
36. Gardea-Torresdey JL, Peralta-Videa JR, Montes M, de la Rosa G, Corral-Diaz B. Bioaccumulation of cadmium, chromium and copper by *Convolvulus arvensis* L.: impact on plant growth and uptake of nutritional elements. Bioresource Technol. 2004; 92:229-235.
37. Romero-Puertas MC, Corpas FJ, Rodríguez-Serrano M, Gómez M, del Río LA, Sandalio LM. Differential expression and regulation of antioxidative enzymes by cadmium in pea plants. Plant Physiol. 2007;164:1346-1357.
38. Monteiro MS, Santos C, Soares A, Mann RM. Assessment of biomarkers of cadmium stress in lettuce. Ecotox Environ Safe. 2009;72:811-818.
39. Muthuchelian K, Bertamini M, Nedunchezhian N. Triacontanol can protect *Erythrina variegata* from cadmium toxicity. Plant Physiol. 2001;158:1487-1490.

40. Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A. Role of proline under changing environments. Plant Signal Behav. 2012;7(11):1456-1466.
41. Ozdener Y, Guray Kutbay H. Physiological and biochemical responses of the leaves of *Verbascum wiedemannianum*to cadmium. Pakistan J Bot. 2011;43(3):1521-1525.
42. Dinakar N, Nagajyothi PC, Suresh S, Udaykiran Y, Damodharam T. Phytotoxicity of cadmium on protein, proline and antioxidant enzyme activities in growing *Arachis hypogaea* L. seedlings. J Enviro Sci. 2008;20:199-206.
43. Al Khateeb W, Al-Qwasemeh H. Cadmium, copper and zinc toxicity effects on growth, proline content and genetic stability of *Solanum nigrum* L., a crop wild relative for tomato; comparative study. Physiol Mol Biol Plants. 2014;20(1):31-39.
44. Farago ME, Mullen WA. Plants which accumulate metals. Part IV. A possible copper-proline complex from the roots of *Armeria maritima*. Inorg Chim Acta. 1979;32:L93-L94.
45. Carpena RO, Vazquez S, Esteban E, Fernandez-Pascual M, Rosario de Felipe M, Zornoza P. Cadmium–stress in white lupin: effects on nodule structure and functioning. Plant Physiol Bioch. 2003;161:911-919.
46. Gajewska E, Skłodowska M. Differential biochemical responses of wheat shoots and roots to nickel stress: antioxidative reactions and proline accumulation. Plant Growth Regul. 2008;54:179-188.
47. Kuznetsov VV, Shevyakova NI. Stress responses of tobacco cells to high temperature and salinity. Proline accumulation and phosphorylation of polypeptides. Physiol Plantarum. 1997;100:320-326.