

EFFECTS OF DIFFERENT CONCENTRATIONS OF CADMIUM ON GROWTH AND MORPHOLOGICAL CHANGES IN BASIL (*OCIMUM BASILICUM* L.)

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Abstract

This study was conducted on four treatments (0, 3, 5 and 10 milligrams per liter of cadmium nitrate solution) and three replications in a completely randomized design. The morphological characteristics including fresh weight, dry weight, stem length, root length, leaf weight ratio, measuring index of the imposed stress, specific leaf area and water availability per unit leaf area, and the amount of cadmium in stems and roots were measured. By increasing cadmium treatment at 10 ppm concentration, the root growth varied between 3.47 and 1.93 cm and the lowest root growth belonged to the treatment at 10 ppm concentration measured 1.93 ± 0.4 cm. The rising stem growth was also indicated. Fresh and dry weight analysis indicates their descending growth. The growth reduction was observed by increasing cadmium treatment at 10 ppm concentration in the study of the trait of weight and specific leaf area. Relative water content (RWC) of the leaves varies between 81 to 89 percent which represents the greatest amount of stress in the treatment at 10 ppm concentration. A notable decline in transport from the root to stem and a significant reduction in plant tolerance index were observed through the analysis of transfer factor and tolerance index in plants. The process of reducing metal transition from the root to stem has become slower by increasing the substance concentration. Basil can absorb and accumulate cadmium and its root is able to accumulate more quantity of the metal than its stem. The morphological signs of cadmium toxicity are remarkable through discolored roots, shortening the distance between nodes, creating an amorphous spots on the leaves, reducing the leaves extent and a slight pallor of the leaves.

Key words: Heavy metals, Cadmium, Toxicity, Morphological characteristics, Basil.

Introduction

Heavy metal pollution in agricultural soils may lead to irregularities in the soil structure, interference in plant growth, and even damage to human health through entering the food chain. Reservoir sediments and mines are used to accumulate heavy metals such that these metals may be stored in sediments and enter the agricultural soils. Bhuiyana *et al.* (2010) examined heavy metal pollution in agricultural soils through several indicators including contamination factor, geoaccumulation index, and pollution load index.

The main soil contaminants are heavy metals, acid precipitation, and organic materials. Heavy metals have been intensively studied in the recent years due to their emission properties in the soil. Spatial variability of heavy metal content in agricultural topsoil may be affected by native soil material and human resources. In other words, these metals may naturally exist in soil but a substantial amount will be added to the soil due to human activities. In fact, human activities can lead to accumulation of heavy metals in the soil. Plants react in two ways in the environments with high concentrations of heavy metals: The first mechanism is avoidance mechanism through which the plants prevent from absorption and transport of metals into their organs. These plants are called non-accumulator. The second mechanism is metals accumulation mechanism through which the plants can have a huge potential for absorption of metals by the roots and transport and store them in the stems. These plants are called hyper accumulator (Baker *et al.*, 2000b). These plants can be

used to remove and cleanup the soils contaminated by heavy metals through phytoremediation process. According to Reeves and Baker (2000), the term "hyper accumulator" was first used by Joffre *et al.* in 1976. It was proved later by Baker *et al.* (2000a) that the percentage of metal accumulated in plant tissue was a criterion for selecting the plant as hyper accumulator of heavy metal. Plants containing more than 100 milligrams of cadmium per kg of dry tissue are considered as cadmium hyper accumulator. Cadmium concentration in most of the plant species is less than 3 mg.kg⁻¹. This concentration may be also reported about 20 mg.kg⁻¹ or greater in cadmium-rich soils. S. Dom'inguez *et al.* (2007) reported that *Echinochloa polystachya* can be named from among the hyper accumulator plant that can, accumulate 157 milligrams cadmium per kg of dry weight.

Some plant species are resistant to a certain amount of heavy metals in soil and are able to absorb and stabilize them in their internal tissues. The toxic effects are not sometimes so apparent in some plants; however, the metal content in plants may endanger the humans or animals health (Arduini *et al.*, 1994). Therefore, study the effect of heavy metals on plants seems necessary to identify resistant plants and using them in phytoremediation, and to develop plant resistant genotypes. For this reason, the effect of different concentrations of cadmium on basil was examined in this research. *Ocimum basilicum* is used as a herb, a spice, and a fresh vegetable. It belongs to Lamiaceae family and is a herbaceous, standing plant with a square, branched stem, an annual herb with relatively high foliage and aromatic compounds. This

plant has oval petiole leaves with an exclusively narrow base and bottom or the sharp ends with smooth edges or short teeth. Basil root is straight and tapered shape. Basil flowers are arranged on the inflorescence in the form of the six-flower cycles along the inflorescence. These small flowers are in various colors including white, light pink, light purple, and sometimes violet. Basil has been introduced as a herbal medicine in most pharmacopoeias.

The total concentration of heavy metals is not a perfect criterion for determining the bioavailability of elements because only part of the concentration of these elements can be absorbed by plants in the soil. The bioavailability of heavy metals depends on various parameters including the chemical form of the element in the soil solution and the soil physicochemical properties such as cation exchange capacity, pH and organic matter content. For example, the solubility and bioavailability of the metal cations increase in the acidic environments. Thus, decreasing the bioavailability of heavy metals is a technique for phytoremediation of the contaminated soil. Therefore, the amount of cadmium residues in soil was measured to study the phytoremediation features of basil.

Contamination of vegetables with heavy metals may be due to irrigation with wastewaters, fertilizers, pesticides, and industrial emissions. Eating more vegetables as a source of vitamins, nutrients and fiber is beneficial to health though these plants may contain toxic substances and result in an excessive risk.

Materials and Methods

This research was conducted at Imam Khomeini Higher Education Center in Karaj located at Km 5 Karaj-Mahdasht Road, with latitude 35°48'N, longitude 51°E and altitude 1250m above sea level.

To this end, some soil was collected from the educational farm of the training center and was mixed with fine sand at a ratio of one-third in order to lighten the soil texture to create favorable conditions for cultivation of basil. Then, before starting the experiment, some of the prepared soil was randomly transferred to the soil science laboratory for analyzing its properties such as texture, structure, pH, electrical conductivity, organic matters, percentage of nitrogen, phosphorus and potassium (NPK), and the amount of cadmium (Table 1). The results revealed that the tested soil was alkaline with an average pH of 8.3 and the electrical conductivity of about 1.57 ds/m; and the soil texture was clay loam with the organic matter content of 0.81%, without any cadmium contamination. This study was conducted in a completely randomized design with 4 treatments (0, 3, 5 and 10 milligrams per liter) and three replications. Each replication was included 10 samples.

To begin the tests, 12 plastic pots with the dimensions of 50×40×20 cm were prepared and an equal of soil was added to them upon ensuring no nuisance seed growth and phytoremediation. 12 g of cadmium nitrate with the purity of 99.9% was, then, prepared using distilled water at concentrations of 3, 5 and 10 ppm. The solutions were added to the pot equally. Distilled water was used as control treatment. After adding the solutions to the soil, 48 hours were taken in order to stabilize the soil. Green basil seeds were obtained from the Research Institute of Forests and Rangelands (RIFR). In order to disinfect seeds, they were, first, washed a few minutes in tap water, and then disinfected with 0.5% sodium hypochlorite for one minute and rinsed with sterilized distilled water.

The sterilized seeds were mixed with some sand after two days upon cadmium stabilization in the soil for uniform cultivation and were, then, spread on the substrate. By employing the Nellessen and Fletcher method (1993), the pots were placed under controlled photoperiod of 16/8 hour (light/dark) and 28/13°C (day/night) temperature and relative humidity of 51%. After 8 days, the planted seeds began to sprout and after two to three weeks cotyledon leaves were developed. The plants were equally watered throughout the growing season with distilled water. Irrigation was made at certain doses proportional to the pot and the soil weight such that no solution can remove from the pot bottom holes. All parameters such as light, humidity and temperature were considered the same for all treatments and the only limiting factor was cadmium concentration. To determine the morphological characteristics and cadmium uptake by the plant, harvesting was made accurately and without any damage to the plants 7 weeks after sowing.

Parameters of length, fresh weight, and dry weight were measured to study the plant growth. To this end, the stem and root length was accurately measured by graph paper. The fresh weight of each plant was measured immediately after removing them from the pots and wiping the dust by Sartorius digital scale model MA40. To measure dry weight, the samples were treated for 72 h at 60°C and re-weighed.

The method of paper copy (Adam *et al.*, 2006), was used to measure the specific leaf area. For this purpose, some plants of each pot were picked up carefully without causing any damage to the plant and their leaves were separated from the stem at petiole. The leaves were, then, laid out on a piece of graph paper and copied. Then, the copy of the target leaves was weighed and calculated through correlations of the leaf area based on their weight to the weight of a square centimeter of the similar paper for each treatment by the following relationships in terms of (m².kg⁻¹).

Table 1. The results of experiments performed on the soil sample.

Cd	Cd	K	P	Texture	Silt	Clay	Sand	OC	N	EC	pH
Total	Absorbable (mg kg ⁻¹)	%							Dsm ⁻¹		
0.5>	0.5>	532	42.2	C.L.	24	37	39	0.81	0.09	1.57	8.3

$$(1) \quad \text{LAR} = \frac{\text{LA}}{\text{TDW}}$$

$$(2) \quad \text{SLA} = \frac{\text{LA}}{\text{TDW}}$$

where, LAR: Leaf Area Ratio; SLA: Special Leaf Area; LA: Leaf Area; TDW: Total Dry Weight; LDW: Leaf Dry Weight.

By Ferrat and Lovat method (1999), to measure the water content of the leaf area per unit, plant leaves were immediately weighed after being collected and their wet weight was measured. The leaves were, then, transferred into the test tubes containing water and were incubated for 24 h at 4°C for complete flooding. After 24 hours, the leaves were reweighed and the weight of saturated leaves was measured. Then, the said leaves were dried in an oven at 75 °C for 48 h and were weighed again. So, their dry weight was measured.

$$(5) \quad \text{Transfer Factor} = \frac{\text{Shoot Cd concentrations (mg kg}^{-1}\text{)}}{\text{Root Cd concentrations (mg kg}^{-1}\text{)}} \times 100$$

To evaluate crop tolerance in the cadmium-contaminated environment, by using Wilkins method

$$(6) \quad \text{Tolerance index} = \frac{\text{Plant growth in metal - containing environment}}{\text{Plant growth in metal - free environment}} \times 100$$

According to Woodies *et al.*, method (1977) Concentration of cadmium ion uptake in roots and stems of plant was measured by atomic absorption spectrometer.

To prepare the sample for plant analysis in order to assess the uptake potential, the fresh plant was, first, rinsed with hydrochloric acid 0.1 mol before drying for 30 seconds due to pollution caused by the dust, and was, then, rinsed with distilled water. The plants were, then, placed in an oven for 48 h at 70°C to become dry. Subsequently, the dried plant matters were grounded to obtain an entirely homogeneous sample of each treatment of roots and stems. To measure the residual cadmium in soil, the soil was transferred to the laboratory upon the entire harvest of plants and two grams of each treatment (soil, root and stem) were weighed with an accuracy of 0.001 g and poured into the Chinese cruise. The oven temperature was gradually reached to 550 °C within two hours. The temperature was kept at the said temperature for approximately 12 hours and, then, the cruises were removed from the oven. The ashes were soaked in a little distilled water after cooling and were covered with a watch glass. 10 ml of 2 mol hydrochloric acid was added to each cruise of the roots and stems, and 10 ml of nitric acid solution was added to the sample ashes. After completion of reactions, the cruises were placed on a water bath and heated to 80°C until the first white fumes were emitted. Then, the cruise contents were poured from the fine Whatman filter paper into a 100 ml volumetric flask and filtered. The cruise and filter paper were washed several times with lukewarm distilled water and the desired volume was achieved. The amount of cadmium of the prepared extracts was finally measured by atomic absorption spectrometer model Shimadzu-AA-670 in the Soil Science Unit of the Laboratory Complex of Agriculture and Natural Resources Campus of Tehran University.

The relative water content of leaves is measured according to formula (3) as follows:

$$(3) \quad \text{RWC}\% = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

where, RWC: Relative Water Content; DW: Dry Weight; FW: Fresh Weight; TW: Turgid Weight.

The following formula was used to calculate the leaf weight ratio:

$$(4) \quad \text{Leaf weigh ratio} = \frac{\text{Leaf dry matter}}{\text{Total dry weight of plant}}$$

In order to evaluate the ability of metal transfer by the plant, with using the Mattina *et al.* method (2003), the transfer factor was calculated according to the formula 5:

(1978), the tolerance index was measured according to the formula 6:

After obtaining the required data, SPSS software and Duncan test were, respectively, used for statistical analysis and the mean comparison. Graphs were also plotted by Excel software.

Results

Measuring the amount of cadmium absorbed by the treated organs of basil suggests that a significant amount of cadmium in the environment is absorbed by the root and some of the absorbed cadmium is transferred to the stem. According to the obtained results, different levels of cadmium concentration have a significant effect on the measured morphological characteristics. By increasing cadmium concentrations, the quantitative amount of all traits except the stem length was significantly decreased comparing to the control treatment.

Cadmium effect on the plant growth parameters including stem and root length and fresh and dry weight: Analysis of data collected from the longitudinal growth of stems and roots using Duncan's test ($p \leq 5$) showed that, by increasing the cadmium concentration, the longitudinal growth of roots comparing to the control plant was decreased at all concentrations while the longitudinal growth of stems was increased. Reduction in fresh and dry weight of the plant at different concentrations of cadmium ion was quite significant. A survey of the subject treatments along the stem indicated that there was no significant difference between the control treatments and 3 ppm. The results also revealed that the drastic reduction in longitudinal growth and weight of the roots in comparison with the stems was more obvious in all treatments (Figs. 1&2).

Cadmium effect on leaf weight: Data analysis of the leaf weight trait indicated that change in leaf weight index reduction comparing to the control treatment with the probability of 0.01% is significant at all cadmium concentrations (Fig. 3).

Cadmium effect on specific leaf area: According to the results, different treatments of cadmium have a significant effect on specific leaf area in a sense that by increasing cadmium concentration up to 10 ppm, the specific leaf area varies up to 15.3 m²/kg (Fig. 4).

Stress effect on the relative water content of leaves: An increase in the cadmium concentration results in a decrease in water content per unit leaf area and, so, a decrease in the relative water content of leaves. RWC in different treatments of 0, 3, 5 and 10 ppm was 89.93%, 88.55%, 85.22%, and 81.38%, respectively, and the difference observed in all various tested treatments was statistically significant (Fig. 5).

Measuring the basil tolerance index in different concentrations of cadmium: Data collected from measuring the tolerance and transmission indices indicates their reduction in the presence of cadmium ions. It shows that basil stores most of the cadmium content in its root. The results demonstrate a significant difference among different treatments of cadmium in measuring tolerance index traits. However, this difference in the confidence level of 0.05% was significant for all the subject treatments in measuring the transmission index (Fig. 6).

Measuring the cadmium concentration in stem and root: Examination of the amount of cadmium absorbed in the treated tissues indicates that a significant amount of cadmium in the environment has been absorbed by the plant organs and some of the absorbed cadmium has been transmitted to the stems (Table 2).

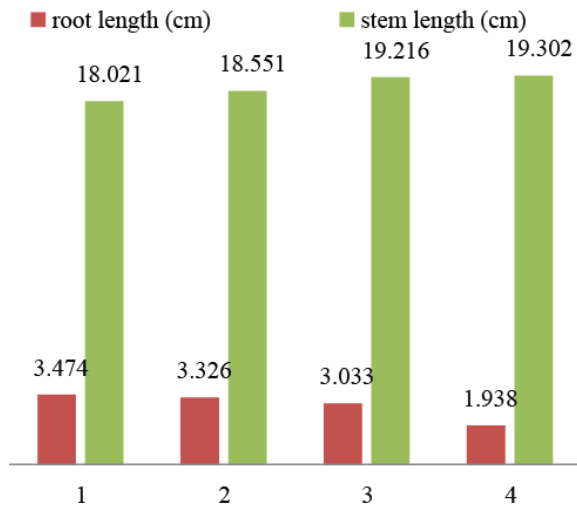


Fig. 1. Comparison of the longitudinal growth of roots and stems in different treatments of cadmium.

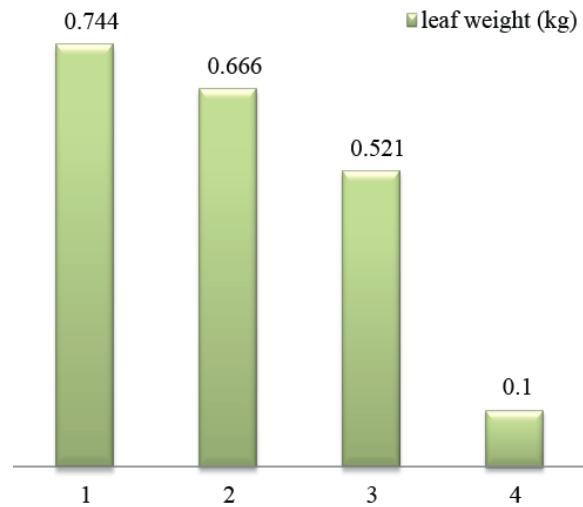


Fig. 3. Evaluation and comparison of the leaf weight in different treatments of cadmium.

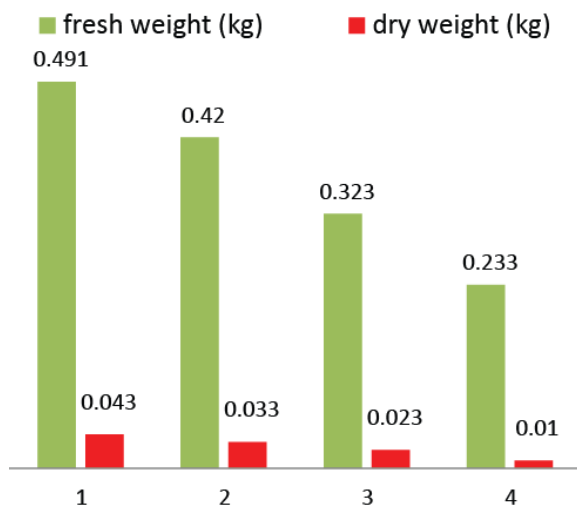


Fig. 2. Comparison of fresh and dry weight in different treatments of cadmium.

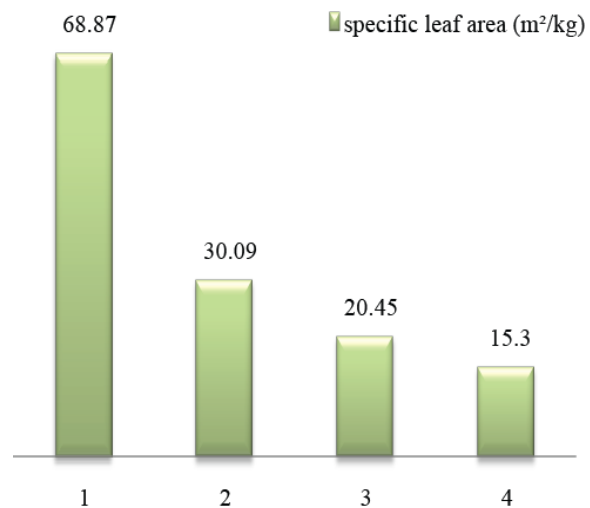


Fig. 4. Evaluation and comparison of the specific leaf area in different treatments of cadmium.

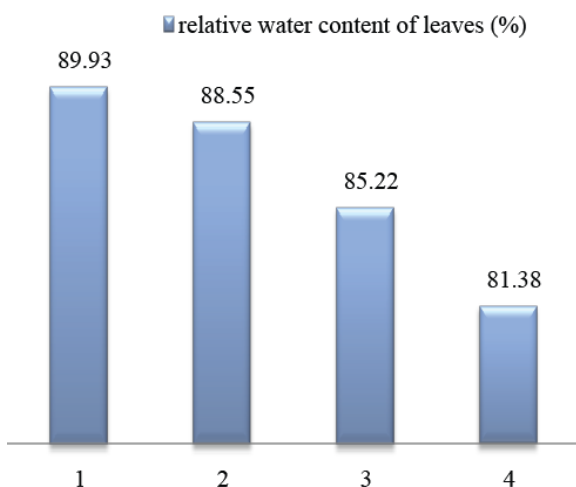


Fig. 5. Comparison of the effect of cadmium stress in different treatments on the leaf water content.

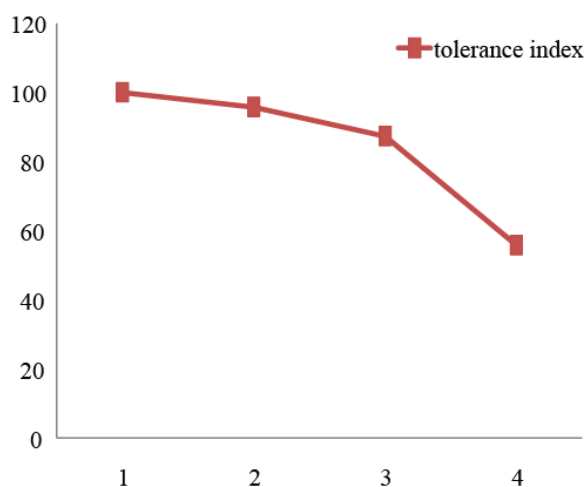


Fig. 6. Determination of tolerance index in different treatments of cadmium.

Table 2. Potential of absorption of cadmium by the organs of basil.

Replication	Root				Stem (aerial organs)			
	3ppm	5ppm	10ppm	control	3ppm	5ppm	10ppm	Control
1	16.4	29	37	0.5>	5.1	7.4	9.4	0.5>
2	16	27	36.3	0.5>	6.5	7.3	8.9	0.5>
3	17.2	29.8	38.6	0.5>	5.7	7.8	9.1	0.5>

The estimated amount of cadmium in the soil after harvesting the plants to assess phytoremediation of basil (Table 3).

Table 3. Basil phytoremediation potential of different treatments.

Replication	Soil			
	3ppm	5ppm	10ppm	Control
1	15.3	17.8	24.8	0.5>
2	14.8	17.9	25.7	0.5>
3	15	18.3	25.4	0.5>

It is to be noted that the measured quantities are based on milligrams per kilogram (mg.kg⁻¹) of the plant dry matter

Discussion

According to the studies conducted by Metwally *et al.* (2003), cadmium is easily absorbed by the plant roots. This metal is toxic for the plant and interferes with many cellular actions by formation of the compound complexes with the secondary groups of organic compounds such as proteins and, thus, prevents from the necessary cellular activities. This accumulation eventually leads to the impaired mineral nutrition of the root cells and significant reduction in the root growth. It can be, generally, said that the longitudinal growth of an organ is directly related to the cell division rate. Cadmium ion stops cell division in the meristemic region and ceases the cells growth in the growth zone. On the other hand, early differentiation and the wooden wall of the cells in the longitudinal cell growth region may be another reason for the decreased growth of the root that is accompanied by root discoloration. Hartley *et al.* (1999) and Papazoglou *et al.* (2005) studied reed and Yang *et al.* (1996) studied rice and barely and reported that the root length was decreased

by increasing Ni concentration. McLaughlin *et al.* (2000) suggested that an increased reduction in the growth of roots rather than stems may be due to the excessive ion accumulation in the roots or the intracellular detoxification of cadmium ion in the stems as suggested by Barcele (1990). As you know, most of the ions in the environment are connected to the root which is the first site of absorption. Lasat *et al.* (1998) and Lasat (2000) reported that some of the ions are absorbed by the root cell walls and cannot be transmitted to the stem. Besides, they may be bonded with other compounds and captured inside the cellular structures such as vacuoles. Therefore, they will be unavailable for being transmitted to the stem. Yet, other specialized mechanisms are available to limit metal transmission. Thus, the ion uptake is high in the roots while the ion transfer to the stems is much limited. The results obtained in this study also demonstrated that the rate of accumulation of cadmium in roots is higher than the stems (Table 2).

Gogorcena *et al.* (2002) and Gussarsson *et al.* (1996) reported that absorption of high amounts of cadmium by the plant reduces and stops the root growth and results in the cork structure of the root, and reduces the electrical conductivity of water in the root. They reported the decreased absorption of essential nutrients such as iron, magnesium, potassium and calcium in their experiments due to the absorption of large quantities of cadmium. Balsberg (1989), Haag-kever *et al.* (1999), and Larsson *et al.* (1998) reported a decline in biomass production due to disruptions in the processes of respiration, photosynthesis, and nitrogen metabolism owing to the toxic concentrations of cadmium. Other researchers such as Bashmakov *et al.* (2005), Peralta *et al.* (2000), Jeliakova *et al.* (2003), and

Gulfraz *et al.* (2003) obtained similar results concerning the prohibition of cadmium in plant growth, biomass loss and disruption of cellular processes. Rion and Alloway (2004) suggested that cadmium and zinc are very similar concerning the ionic radii. Therefore, cadmium can be absorbed by the plant root and mimic the zinc metabolic pathway, but it is considered a toxic element unlike zinc. Toxicity of this element is mainly due to its penchant to thiols (SH) in enzymes and other proteins. Thus, cadmium disrupts the enzymatic activity. Zinc most likely plays a role of cofactor for many enzymes in the biosynthesis of tryptophan amino acid as a precursor for the auxin synthesis or in converting the tryptophan amino acid to indole acetic acid. Auxin triggers cells elongation and, according to the acid growth theory, this hormone increases flexibility of the cells wall and stimulates the growth through transporting proteins to the wall, reducing the acidity in the cells membrane and making changes in cytosolic calcium. Therefore, by increasing the amount of cadmium in plant cells, the zinc ion concentration is reduced and thus the plant organs will stay short due to lack of cell division. Schickler and Caspi (1999) suggested that fresh and dry weight of the plant was significantly decreased by increasing cadmium concentrations and the maximum fresh and dry weight was observed under cadmium-free conditions (control treatment). The toxic concentrations of cadmium result in lower plant water potential through changing the root cells membrane structure and reducing the water absorption levels that has a negative impact on physiological processes such as photosynthesis, transpiration, and respiration and ultimately reduces the crop yield. The previous researchers have also demonstrated that heavy metals such as cadmium decrease the plant fresh and dry weight and ultimately reduce the plant biomass. Reduction in fresh and dry weight of the organs, particularly the roots, that is seen in the figures in this research is owing to disturbance in the general metabolism of cells.

The heavy metal ions remain in the cytosol of cells upon entering the plant until induction of formation of phytochelatin as a result of phytochelatin synthase and this high accumulation of metal in the cytosol inhibits the growth of leaves. Reduction in the leaf area in plants treated with a heavy metal confirms this matter in this research. Rout & Das (2003) reported that the mitochondrial structure is destroyed in the plants exposed to the high concentrations of toxic metals and so their energy-seeking activities are impaired. One of the most important defense systems to control and neutralize free radicals is the induction of synthesis of a number of antioxidant compounds such as flavonoids, anthocyanins, and carotenoids in plants. As suggested by Asada (1984), these compounds react with free radicals and convert them into their stable form by giving electron to these reactive compounds. These antioxidant compounds not only eliminate free radicals but also prevent from production of more free radicals in the plant. According to Tripathi *et al.* (2006), these compounds may facilitate the entry of heavy metals into the vacuoles and collect them from other parts. According to Shahsavan Behboodi & Samadi (2002), some concentrations of heavy metals in different plants may sometimes disrupt this defense mechanism and increase free radicals to the extent that they overcome the

antioxidant system of the plant and lead to lipid peroxidation in the cells membrane. According to Lasat (2000), despite the limitations of metal transfer to the stem, it is necessary to transmit the metal from the root to the stem for phytoremediation. The metal-containing sap movement from root to stem is controlled by two processes: root pressure and leaf transpiration. Cadmium is also able to pass through the root cells membrane and enter into the timber vessel in order to be transmitted to the stem and absorbed into the leaf cells. Accumulation of the metal in plant is determined in accordance with transmission capacity and the ion absorption by the stem. Therefore, the plant potential was evaluated by calculation of the transmission factor in this research and it was determined that transmission of cadmium from the root to the stem was decreased by increasing the cadmium quantity. It is perhaps because of detoxification effect and more metal retention in the root. The decline in transfer of cadmium from roots to stems could be also due to the non-moving of this element in the cell wall or cadmium binding to the organic compounds in the root (phytochelatin).

Lu *et al.* (2012) suggested that vermilion can accumulate large amounts of heavy metals, especially cadmium and manganese, in the stems. This plant can be used as a heavy metals phytoremediator. They used 0, 5, 10, and 50 micro-molar concentrations of cadmium chloride solution and the results of concentration analysis of 1.8-3.6 in the root and 8.1-31.6 in the leaves and stems showed that by increasing the concentrations of cadmium in this plant, the cadmium concentration in roots and leaves was also increased that was in conformity with the results obtained in the present research.

According to Gardea-Torresdey *et al.* (2005), cadmium also reduces the plant growth. As suggested by Ghosh and Singah (2005), this metal inhibits the activity of some enzymes, deposits the essential nutrients and metabolites, and causes cell damage. Amorphous spots on the leaves of basil were shown in this research. These spots appeared gradually by approaching the flowering stage. Tiny newly-sprouted leaves and reduction of the distance between the nodes were observed by increasing the concentrations of cadmium ion that was probably due to the destruction and prevention from zinc ion activity. Arpadjan *et al.* (2008) studied the effect of arsenic, cadmium and lead on some genera of medicinal plants such as Yarrow (*Achillea millefolium*), Chamomile (*Matricaria chamomilla*), Thyme (*Thymus vulgaris*), Oregano (*Origanum vulgare*), Hibiscus (*Hibiscus sabdariffa*), Peppermint (*Mentha piperita*). They suggested that the toxic range of metals varies in medicinal plants from 12 to 25 µg/kg for arsenic, from 15 to 268 µg/kg for cadmium, and from 0.2 to 0.6 µg/kg for lead. They prepared the metal concentrations of 1ppm in nitrate 0.3 w/v from all treatments. Arsenic was found up to 0.4 in all herbal infusions. Injection of cadmium in plants was about 0.7 and lead was only observed between 2-3 in the genus *Hibiscus*. The results of this research revealed that the increased amounts of the toxic cadmium in the soil result in the increased concentration of this element in basil. This relationship may indicate the correlation of concentrations of elements in the plant and soil such that by increasing the quantity of elements in the soil to a certain extent, the concentrations of these elements will also increase in the plant. In this study, the amount of cadmium in the roots and stems grew higher by increasing the cadmium concentration

in the soil. According to the results obtained in this study, basil is also capable to absorb a significant amount of cadmium ions in its stems. One of the major problems in phytoremediation process is that high concentrations of heavy metals may prevent from the plant growth and biomass production. Heavy metals affect the plant growth in different ways and may disturb the physiological and morphological characteristics of the plants.

Street *et al.* (2009) studied the effect of cadmium on the growth and biological activity of the medicinal herb of *Merwillia plumbea* and found that the low concentration of cadmium 2mg.l⁻¹ significantly reduced the fresh weight of leaves, tubers and roots in comparison with the control treatment. Most cadmium was accumulated in the root and scallion that has medicinal uses. Generally, particular attention should be paid to the input sources of emission of this pollutant to the environment due to the toxicity of cadmium heavy metal even at low concentrations. Therefore, considering the toxicity of this metal for the plant and the risk of entering into the food chain, it is necessary to accurately monitor the different stages from production to consumption of agricultural products in order to ensure a healthy product and since the plants root remains in the soil, the root can become a contamination source for water and soil in the subsequent stages in case of being polluted with cadmium and this will lead to numerous problems for humans due to the toxicity of cadmium. Several studies also demonstrate that the plants root is very capable in storage of cadmium. According to Sahmurova *et al.* (2010), identifying the plant species that produce high biomass which can tolerate or concentrate pollutants is of abundant importance for the revival or purification of the sites contaminated with heavy metals through phytoremediation. Ebbs and Khochin (1998) suggested that the increased biomass would improve elimination of the heavy metal and lead to the higher plant efficiency.

By examination of this property in basil in this research, it can be said that although basil accumulates high, toxic concentrations of the metal and is of high potential in storage of this element in its tissues, it is less efficient in removing cadmium from the soil due to the low production of biomass. Thus, it is required to control the resulting environmental pollution considering the mineral resources in the world and the increased exploitation of these resources as well as development of metal smelting industry. Using plants to reduce pollution in mining zones which are mainly located in the remote desert areas will lead to rehabilitation of degraded lands and increased vegetation in these areas. Identification and selection of suitable species in order to be planted in the polluted areas is of great importance due to the high diversity of plant species in Iran and this requires further study of a variety of plant species in different regions and climates.

References

Adam, T., R. Nilesh, C. Sharma, S.V. Sahi, S.R. Singh and K.S. Sajwan. 2006. Effects of lead and chelators on growth, photosynthetic activity and Pb uptake in *Sesbania drummondii* grown in soil. *Environ. Poll.*, 144: 11-18.

- Arduini, I., D.L. Godbold and A. Onnis. 1994. Cadmium and copper change root growth and morphology of *Pinus pinea* and *Pinus pinaster* Ait seedlings. *Tree physiol.*, 15: 411-415.
- Arpadjan, S., G. Celik, S. Taskesen and S. Guser. 2008. Arsenic, Cadmium and Lead in medicinal herbs and their fractionation. *J. Food & Chem. Toxicol.*, 46: 2871-2875.
- Asada, K. 1984. Chloroplast: formation of active oxygen and its scavenging. *Meth. Enzymol.*, 105: 422-429.
- Baker, A., S.P. McGrath, R.D. Reeves and J.A.C. Smith. 2000. *Metal hyper accumulator plants: A review of the ecology and physiology of a biological resource for phytoremediation of metal polluted soils*. USA 7 Lewis publishers, pp. 85-107.
- Baker, A.J.M., S.P. McGrath, R.D. Reeves and J.A.C. Smith. 2000. *Metal hyper accumulator plants: A review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils*. In: *Phytoremediation of contaminated soil and water*. (Eds.): N. Terry and G. Banuelos. USA 7 Lewis publishers, pp. 313-375.
- Balsberg Pahlsson, A.M. 1989. Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. *Water, Air, Soil Poll.*, 47: 287-314.
- Barcele, J. Poschenriederc. 1990. Plant water relation as effected by heavy metal stress: A review. *J. Plant Nuts.*, 13: 1-37.
- Bashmakov, D.I., A.S. Lukatkin and V.V. Revin. 2005. Growth of maize seedlings affected by different concentration of heavy metals. *J. Ekologija*, 3: 22-27.
- Bhuiyana, M.A.H., L. Parvez, M.A. Islam, S.B. Dampare and S. Suzukia. 2010. Heavy metal pollution of coal mine-affected agricultural soils in the northern part of Bangladesh. *J. Hazardous Mat.*, 173: 384-392.
- Ebbs, S.D. and L.V. Khochin. 1998. Phytoextraction of Zinc by Oat (*Avena sativa*), barley (*Hordeum vulgare*) and Indian mustard (*Brassica juncea*). *Environ. Sci. Technol.*, 32: 802-806.
- Ferrat, I.L. and C.J. Lovat. 1999. Relation between relative water content, Nitrogen pools and growth of *Phaseolus vulgaris* L. and *P. acutifolius*, A. Gray during water deficit. *Crop Sci.*, 39: 467-474.
- Gardea-Torresdey, J.L., J.R.P. Videa, G. Rosa and J.G. Parsons. 2005. Phytoremediation of heavy metals and study of the metal coordination by X-Ray absorption spectroscopy. *Coordination Chemis. Rev.*, 249: 1797-1810.
- Ghosh, M. and S.P. Singah. 2005. A comparative study cadmium phyto extraction by accumulator and weed spices. *Environ. Poll.*, 133: 365-371.
- Gogorcena, Y., J.J. Lucena and J. Abadia. 2002. Effects of Cd and Pb in sugar beets plants grown in nutrient solution: Induced Fe deficiency and growth inhibition. *J. Funct. Plant Biol.*, 29: 1453-1464.
- Gulfraz, M., Y. Mussadeq, R. Khanum and T. Ahmad. 2003. Metal contamination in wheat crop (*Triticum aestivum* L.) irrigated with industrial effluent. *Online J. Biol. Sci.*, 3(3): 335-339.
- Gussarsson, M. Asp, H., S. Adalsteinnsson and P. Jensen. 1996. Enhancement of Cadmium effect on growth and nutrient composition of birch (*Betula pendula*) by buthionine sulphoximine (BSO). *J. Exp. Bot.*, 47: 211-215.
- Haag-kever, A., H.J. Schafer, S. Heiss, C. Walter and T. Rausch. 1999. Cadmium expose in *Brassica juncea* cause a decline in transpiration rate and leaf expansion without effect on photosynthesis. *J. Exp. Bot.*, 50: 1827-1835.
- Hartley, J.J., W. Cairney, P. Freestone, P. Woods and A.A. Meharg. 1999. The effects of multiple metal contaminations on ectomycorrhizal Scots pine (*Pinus sylvestris*) seedlings. *Environ. Poll.*, 106: 413-424.

- Jeliazkova, E.A., L.E. Craker and B. Xing. 2003. Seed germination of anise, caraway, and fennel in heavy metal contaminated solutions. *J. Herbs, Spices & Med. Plants*, 10(3): 83-93.
- Larsson, E.H., J.F. Bornman and H. Asp. 1998. Influence of UV-B Radiation and Cadmium on chlorophyll fluorescence *Brassica napus*. *J. Exp. Bot.*, 43: 1031-1039.
- Lasat, M.M. 2000. The use of removal of toxic metals from contaminated soil, U.S. American Association for the Advancement of Science. *Environ. Sci. and Eng. Fellow*. 33p.
- Lasat, M.M., A.J.M. Baker and L.V. Kochian 1998. Altered Zn compartmentation in the root symplasm and stimulated Zn absorption into the leaf as mechanism involved in Zn hyper accumulation in *Thlaspi caerulescens*. *Plant Physiol.*, 118: 875-883.
- Lu, G., P. Kejian, X. Yan, W. Guiping, N. Liyuan, L. Chunlan and S. Zhengu. 2012. Cadmium and manganese accumulation in *Phytolacca americana* L. and the roles of nonprotein thiols and organic acids. *Internat. J. of phytoremediation*, 15(4): 307-319.
- Mattina, M.J.I., W. Lannucci-Berger, C. Musante and J.C. White. 2003. Concurrent plant uptake of heavy metals and persistent organic pollutants from soil. *Environ. Poll.*, 124: 375-378.
- McLaughlin, M.J., M.J. Bell, G.C. Wright and G.D. Cozens. 2000. Uptake and partitioning of cadmium by cultivars of peanut (*Arachis hypogea* L.). *Plant and Soil*, 222: 51-58.
- Metwally, A., I. Finkemeier, M. George and K.J. Dietz. 2003. Salicylic acid alleviates the cadmium toxicity in barley seedlings. *J. Plant Physiol.*, 132: 272-281.
- Nellessen, J.E. and J.S. Fletcher. 1993. Assessment of published literature on the uptake, accumulation, and translocation of heavy metals by vascular plants. *Chemosphere*, 27(9): 1669-1680.
- Papazoglou, E.G., G.A. Karantounias, S.N. Vemmos and D.L. Bouranis. 2005. Photosynthesis and growth responses of giant reed (*Arundo donax* L.) to the heavy metals Cd and Ni. *Environ. Internat.*, 31: 243-249.
- Peralta, J.R., J.L. Gardea-Torresdey and K.J. Tiemann. 2000. Study of the effects of heavy metals on seed germination and plant growth on alfalfa plant (*Medicago sativa*) grown in solid media. *Proceed. of the 2000 Conf. on Hazardous Waste Research*, 135-140.
- Reeves, R.D. and A.J.M. Baker. 2000. *Metal-accumulating plants. In: Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment*, (Eds.): I. Raskin and B.D. Ensley. John Wiley and Sons Inc., New York, pp. 193-229.
- Rion, B. and J. Alloway. 2004. *Fundamental aspect of zinc in soils and plants*. Internat. Zinc Associat. pp. 1-128.
- Rout, G.R. and P. Das. 2003. Effect of metal toxicity on plant growth and metabolism. *Zinc Agronomie*, 23: 3-11.
- S.Dom'inguez, F.A., M.C.G. Chavez, R.C. Gonzalez and R.R. Vazquez. 2007. Accumulation and localization of cadmium in *Echinochloa polystachya* grown within a hydroponic system. *J. Hazardous, Mat.*, 141: 630-636.
- Sahmurova, A., M. Celik and S. Allahverdiyev. 2010. Determination of the accumulator plants in Kucukcekmece Lake (Istanbul). *Afr. J. Biotechnol.*, 6545-6551.
- Schickler, H. and H. Caspi, 1999. Response of the genus *Alyssum*. *Physiol. Plant.*, 105: 39-45.
- Shahsavan Behboodi, B. and L. Samadi. 2002. Morphological study of cadmium-induced changes on root apex of *Allium cepa*. *Iranian Int. J. Sci.*, 3: 11-22.
- Street, R.A., M.J. Kulkarni, W.A. Stirk, C. Southway, H.S. Abdillahi, M. Chinsamy and J. Van Staden. 2009. Effect of cadmium uptake and accumulation on growth and antibacterial activity of *Merwillia plumbea*-An extensively used medicinal plant in South Africa. *South Afr. J. Bot.*, 75: 611-616.
- Tripathi, B.N., S.K. Mehta, A. Amar and J.P. Gaur. 2006. Oxidative stress in *Scenedemus* sp. During short and long-term exposure to Cu and Zn. *Chemosphere*, 62: 538-544.
- Wilkins, D.A., 1978. The measurement of tolerance to edaphic factors by means of root growth. *New Phytologist*, 91: 255-256.
- Woodies, T.C., G.B. Hunter and F.J. Johnson. 1977. Statistical studies of matrix effects on the determination of Cadmium and Lead in fertilizer and material and plant tissue by flame atomic absorption spectrophotometry. *Anal. Chem. Acta*, 90: 127-136.
- Yang, X., V.C. Baligar, D.C. Martens and R.B. Clark. 1996. Plant tolerance to Ni toxicity. I. Influx, transport and accumulation of Ni in four species. *J. Plant Nutr.*, 19: 73-85.

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