*Journal of Chemical Health Risks*

Journal of Chemical Health Risks (2016) 6(1), 69–78

ORIGINAL ARTICLE

# Tolerance and Accumulation of Heavy Metals by *Descurainia sophia* L.

**Hoda Karamooz**, **Akbar Safipour Afshar*, Fatemeh Saeid Nematpour**

Department of Biology, Neyshabur Branch, Islamic Azad University, Neyshabur, Iran

*(Received: 22 September 2015 Accepted: 28 November 2015)*

**KEYWORDS**

Phytoremediation;

Accumulator's Species;

Excluders Species;

Soil Pollution;

Cadmium;

Nickel;

*Descurainia sophia* L.

**ABSTRACT:** Today, biosphere pollution has accelerated strongly with start of industrial revolution by toxicity of heavy metals. One of existing pollution is soil pollution. Unfortunately, soil pollution by metals is as intensive environmental stress for plant hence for human. Plants, which are able to store heavy metals in their organs, can be used for phytoremediation of polluted soils and utilization of these plants is effective for phytoremediation as a cheap and economic method. In this research, the absorption rate of Cd (II), Ni (II) by *Descurainia sophia* was considered in hydroponic conditions. Plants were grown in Hoagland media containing different concentrations of Cd (II), Ni (II). An experiment in a completely randomized design with three replications was conducted. Two weeks after treatment of plants the sample were gathered and metal concentration was measured by atomic absorption spectroscopy. Besides, the content of chlorophyll and proline was measured. The results showed the chlorophyll content in high concentrations of the metals (Cd (II), Ni (II)) was decreased in plants that were sign of pigment degradation in presence of heavy metals. Similarly, the proline content in plants was increased under stress which was sign of damage of heavy metal stress on plant and activation of defensive mechanisms in this condition. The effects of toxic concentration of nickel and cadmium on metal accumulation in these plants showed that roots were able to absorb more than shoots, which is sign of elements connection to root cell wall.

## INTRODUCTION

In current time the environment is excessively polluted by various toxic metals, which create a danger for all living beings. These toxic metals including heavy metals have a relatively high density and are environmentally unfriendly. The sources of heavy metals in soil include mining, combustion of fossil fuels, agrochemicals and sewage sludge applications [1, 2].

* Corresponding author: asafshar@iau-neyshabur.ac.ir (A. Safipour Afshar).

One of the fundamental micronutrient for higher plants is Nickel. Its performance is like a co-factor of enzymes and is useful for animals in trace quantities but in higher concentrations have adverse effects in plant growth. Accumulation of nickel in plants decreases the rate of metabolic activities and cause water and nutrient deficiency [3].

Cadmium (Cd) ranks the highest in terms of damage to plant growth and human health. Moreover, its uptake and accumulation in plants poses a serious health threat to humans via the food chain. Pollution of agricultural soils by Cd induces many stress symptoms in plants, such as reduction of root growth, disruption in mineral nutrition and carbohydrate metabolism, and may thus clearly reduce yield [4].

Heavy metals have significant effects on chlorophyll and protein content in plants. These metals impede with synthesis of chlorophyll by inducing deficiency of $Mg^{2+}$ ions [5]. The effect of different concentrations of lead and cadmium on seed germination and seedling growth of *Leucaena leucocephala* was done by Shafiq et al. [6].

Proline accumulates in plants as a response of environmental stresses and is also believed to have osmoprotective role [7]. Amassing of proline in plant cells in stress condition is result of an increase in proline biosynthesis or proline utilization [8].

Recently, the use of plants in metal removal from contaminated soils or waters called phytoremediation. Numerous methods improved to remediate contaminated soils, such as solidification remediation technology, vitrification remediation technology, and electrokinetic remediation and so on. However, these technologies have many weak points which include degeneration of soil quality, secondary pollution and high cost. For the cleaning of large areas of contaminated soil and to defeat these disadvantages, phytoremediation is believed as a promising method. Plants can remediate soils in different processes, these include: phytoextraction, phytofiltration, phytostabilisation and phytovolatisation [9-11]. Among the plants that had been studied, members of Brassicaceae family are well-known accumulators [12, 13]. Accordingly, *Descurainia sophia* were suggested for phytoremediation technology. *Descurainia sophia* belongs to Brassicaceae family and is an annual plant which can be found in most parts of Iran.

The aim of this study was to investigate Cd (II), Ni (II) absorption rate against concentration increase of these metals in *D. sophia* plant and to determine whether *D. sophia* could be presented as a Cd (II), Ni (II) accumulator.

## MATERIALS AND METHODS

### *Plant Cultivation*

Seeds were germinated in glass Petri dishes. After 30 days, uniform seedlings were selected and transplanted to vessel containing 1.5 L of modified Hoagland's solution at pH 6.5. After 7 days, $CdCl_2$ was added at the concentrations of 0, 40, 70 and 100 $10^{-6}$ mol $L^{-1}$ and $NiSO_4$ was added at the concentrations of 0, 40, 70 and 100 $10^{-6}$ mol $L^{-1}$ also mix Cd and Ni to vessel containing of modified Hoagland's solution. The hydroponic medium was changed once a week. Each vessel contained five plants which represented one replicate. There were three replicates (fifteen plants for each treatment). Temperature for grow was approximately 24 °C in the day and 20 °C at night and a photoperiod of 16 h the day and 8 h at night.

### *Chlorophyll and proline measurements*

The total chlorophyll contents (mg $g^{-1}$ fresh leaf), were analyzed by following Arnon method. Leaf samples were homogenized in 80% acetone and optical densities were measured at 652 nm with Spectrophotometer ($T80^+$ PG Instruments) [14].

Proline was extracted from the leaves and estimated by the methods of Bates et al. [15]. Homogenates of the leaf samples were prepared in 3% sulphosalicylic acid. Pink colour was developed by a reaction with glacial acid and ninhydrin. The colour was separated in toluene layer and intensity of the colour was measured at 546 nm spectrophotometrically.

### *Analytical Method*

At the end of the experiment, after 3 weeks, plants were harvested, and the root and shoot tissues were separated and oven-dried for 2 days at 70 °C. Subsequently, dry weights for each sample were obtained and then 0.5 gram of milled plant tissue (shoot) and less than 0.5 g of root were digested in 20 ml of concentrated nitric acid for two hours at 90 °C in a water bath. Metal concentrations were measured by Flame Atomic Absorption Spectroscopy (AA240 Varian Inc.).

### *Translocation factor*

To evaluate the potential of *D. sophia* for phytoextraction, the translocation factor (TF) was calculated. This ratio is an indication of the ability of the plant to translocate metals from the roots to the aerial parts of the plant [16]. It is represented by the ratio:

$$\text{Translocation factor (TF)} = \frac{\text{Metal concentration in aerial parts}}{\text{Metal concentration in roots}}$$

### *Bio-accumulation Factor (BAF)*

Bio-accumulation Factor can be employed to quantify toxic element accumulation efficiency in plants by comparing the concentration in biota and an external medium (e.g. soil) [17, 18].

$$\text{Bioacculation factor (BAF)} = \frac{\text{Metal concentration in aerial parts}}{\text{Metal concentration in soil}}$$

## STATISTICAL ANALYSIS

Finally, the collected data were statically analyzed for variance using SAS 9.2 software (Chicago, IL, USA). The mean values were compared by applying Duncan's multiple range tests at 5% probability level.

## RESULTS AND DISCUSSION

### *Effect of heavy metals on chlorophyll*

The application of Cd and Ni at concentrations of 0, 40, 70 and 100 $10^{-6}$ $molL^{-1}$ and a mixture of two heavy metals caused a significant decrease ($P$>0.05) in chlorophyll contents (Table 1).

Various abiotic stresses decrease the chlorophyll content in plants [4, 19] as pigment content alterations linked to evidence of plant illness and photosynthesis [20].

Many studies report chlorophyll degradation by metals in higher plants [4]. The decrement in chlorophyll content in plants exposed to $Cd^{2+}$ and $Ni^{2+}$ stress is supposed to be due to:

(a) Inhibition of important enzymes, such as δ-aminolevulinic acid dehydratase (ALA dehydratase) and protochlorophyllide reductase associated with chlorophyll biosynthesis [4, 9, and 18].

(b) Peroxidation of chloroplast, pigments and membrane lipids by oxygen radicles as result of oxidative stress [21, 12]. Besides, these ions can directly destroy the structure and function of chloroplast by binding with-SH group of enzymes [21, 23, and 24].

(c) Heavy metal ions prevent uptake and transportation of other metal elements such as Mn, Zn and Fe by opposing effects and therefore cause the leaves lose the capacity of synthesis of chlorophyll [22, 21].

Our data of decrease in chlorophyll content confirmed with the results of Siedlecka and Krupa who also found a reduction in chlorophyll content with heavy metal stress in *Zea mays* and *Acer rubrum* [25].

Preeti Pandey Pant has stated decreased chlorophyll in *Shorea robusta* and Fikriye Kirbag Zengin et al. have reported chlorophyll reduction in *Phaseolus vulgaris* L. under the impact of heavy metals (Cd) [21, 26].

### *Effect of heavy metals on proline*

Table 1, shows the influence of Cd and Ni on proline content. The results show that the two heavy metal induced proline accumulation.

For decades, plant biologists have studied the synthesis of free proline in several species exposed to oxidative stress. They found that lots of plants in response to oxidative stress accumulate proline in cells and tissues. Proline has been suggested to act as an appropriate osmolyte and to be a sink of carbon and nitrogen [27]. Proline accumulation in plants following water stress is a well-established fact [28, 29]. Cd & Ni induced changes in the cell water balance. Water deficit changes the regulation of pyrroline-5-carboxylate (an enzyme involved in Proline synthesis), resulting in high Proline accumulation [26, 30]. Proline through stability of enzymes to denaturation, and improving of mRNA translation increases the stress tolerance of plants [20, 31]. Moreover, amino acid proline has been suggested to chelate Cd ion in cells and create a non-toxic Cd-proline compound [32]. Heavy metal induced proline accumulation has been reported in lemongrass (*Cymbopogon flexuous* Stapf.) [28] Gram (*Cicer arietinum* L.) [31]. Pandey and Sharma demonstrated that Co, Ni and Cd induced an increase of Proline concentration in cabbage leaves, and suggested an association with the changed water status of the treated plants [33].

**Table 1**. Effect of heavy metals (Cd-Ni & Cd+Ni) on Chlorophyll, Proline in *D. sophia* L. at 12 days of treatment.

| | **Control** | **Ni40** | **Ni70** | **Ni100** | **Cd40** | **Cd70** | **Cd100** |
|---|---|---|---|---|---|---|---|
| **Proline** | 1.468±2.04<br>Max=3.14<br>Min=0.80 | 8.042±10.66<br>Max=20.36<br>Min=1.85 | 11.38±1.17<br>Max=12.38<br>Min=10.08 | 29.13±4.56<br>Max=34.04<br>Min=25.02 | 2.04±5.65<br>Max=8.36<br>Min=-2.55 | 13.93±16.72<br>Max=32.87<br>Min=1.17 | 16.68±8.44<br>Max=26.29<br>Min=10.46 |
| **Chlorophyll** | 6.04±1.92<br>Max=7.84<br>Min=4.02 | 4.97±1.10<br>Max=6.18<br>Min=4.02 | 3.49±0.60<br>Max=4.04<br>Min=2.85 | 2.77±1.17<br>Max=3.96<br>Min=1.61 | 3.78±0.14<br>Max=3.94<br>Min=3.66 | 3.16±1.25<br>Max=4.15<br>Min=1.76 | 2.49±1.29<br>Max=3.48<br>Min=1.03 |

| | **Cd40+Ni70** | **Cd70+Ni40** | **Cd70+Ni70** | **Cd70+Ni100** | **Cd100+Ni70** |
|---|---|---|---|---|---|
| **Proline** | 3.17±7.78<br>Max=12.1<br>Min=1.36 | 18.29±9.21<br>Max=27.14<br>Min=8.76 | 47.48±31.65<br>Max=81.70<br>Min=19.25 | 28.72±9.70<br>Max=39.44<br>Min=20.55 | 10.4±3.65<br>Max=14.55<br>Min=7.65 |
| **Chlorophyll** | 2.63±1.70<br>Max=4.6<br>Min=1.62 | 2.45±0.44<br>Max=2.93<br>Min=2.07 | 2.31±0.70<br>Max=2.86<br>Min=1.52 | 1.88±0.10<br>Max=2<br>Min=1.8 | 1.65±1.18<br>Max=2.5<br>Min=0.3 |

***Heavy Metal Content in Root & Shoot***

*Descuriania sophia* was exposed to various nickel and cadmium ($Ni^{2+}$ & $Cd^{2+}$) concentrations (0, 40, 70, 100 $10^{-6}$ $molL^{-1}$ and mix Cd and Ni) accumulated high content of nickel and cadmium at higher concentrations (Fig. 1). Cd and Ni accumulation in the *D. sophia* were significantly ($P \leq 0.05$) influenced by the treatments. Regardless of the different movability of metal ions in plants, the metal concentration is usually greater in roots than in the shoots [34]. In our studies the maximum nickel and cadmium content was observed in the root than the shoot, which showed low transport of nickel and cadmium towards aerial parts of plants. Uptake in root and transportation in shoot may be based with low translocation factor which indicated great potential for phytostabilization of heavy metals in root. Other works confirmed more accumulation of heavy metal in root than the shoot in radish and spinach [20].

Vajpayee et al. noted high concentration of heavy metal (Cr) in root of *Vallisneria spiralis* L. than the shoot [35]. Our findings indicate that various levels of heavy metals influence uptake and translocation of nickel and cadmium.

Furthermore, the root cells wall are first target for metals ion in soil solution. The integration of the metal ions in to the cell wall has been noted in several papers reviewed by Ernst et al. [36]. Of the total amount of ions connected with the root; only a portion is uptake by cells. A remarkable metal ion fraction is physically linked into the negatively charged sites ($COO^{-}$) of the root cell walls. The cell wall bound fraction cannot be translocated to the shoots and, therefore, cannot be removed by harvesting shoot biomass (phytoextraction). Thus, it is conceivable that a plant representing important metal accumulation into the root, to exhibit a restricted capacity for phytoextraction [36].

Attachment to the cell wall is not the exclusive plant mechanism valid for metal immobilization into roots and following inhibition of ion translocation to the shoot. Metals can also be aggregate and separated in cellular organelles (e.g. vacuole) becoming inaccessible for translocation to the shoot [37]. Moreover, some plants, named excluders, have specific mechanisms to reduce metal uptake into roots. However, the idea of metal exclusion is not well known [38].

Wojcik et al. found higher metal accumulation in roots than in shoots of hydroponically grown *Thlaspi caerulescens* [39].

Our studies show a higher accumulation of Cd than Ni by treated plants. There are many reports that the presence of one metal in solution affected the absorption of another metal. Cd effect on plant parameters was more significant than that of Ni. It seems Cd ions uses the same transmembrane transporter of nutrients, such as K, Ca, Mg, Fe, Mn, Cu, Zn and Ni and thereby restrict uptake of these nutrients [40, 41].

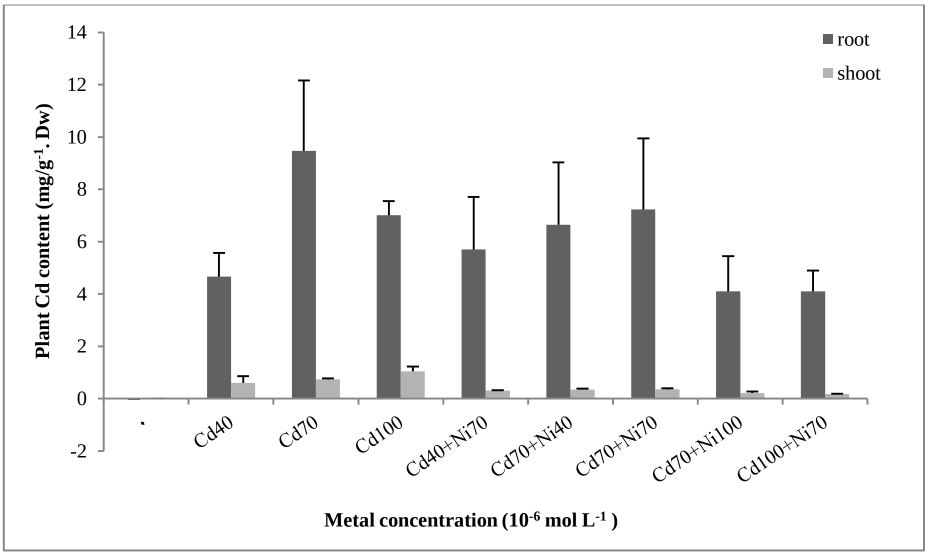


**Figure 1**. Cd & Ni concentrations of *D. sophia* L. after 2 week of growth in the Hoagland nutrient solution

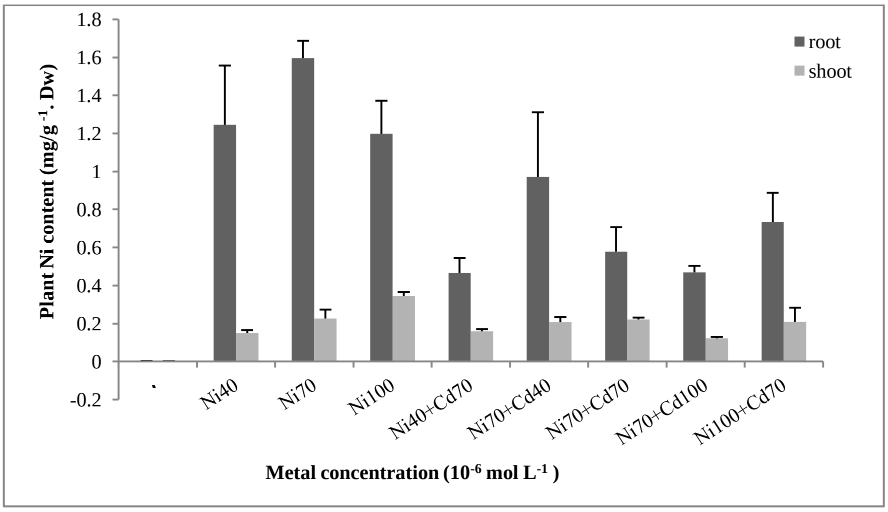


**Figure 1.** Cd & Ni concentrations of D. sophia L. after 2 week of growth in the Hoagland nutrient solution

***Translocation factors and Bio-accumulation factor***

The Translocation Factor (TF) of the 9 different concentrations for each metal is recorded in Fig. 2. Our result showed a TF<1 suggesting that Cd and Ni could not be effectively translocated from the roots to the shoots. In the case of Ni accumulation at all the concentration, the metal was translocated to the shoot than to control but Cd result indicated that the metal was stored mainly in the roots. Therefore, the translocation factor of S/R for Ni is more than Cd. Bioaccumulation factors (BAF) of heavy metal are presented in Fig. 3. Plant species under investigation had BAF<1, that shows *D. sophia* L. can be considered excluder of these metals.

The translocation factors (TF) usually showed the transition of metal from soil to root and shoot, displaying the capability to uptake the metals from the soil [42].

Therefore, translocation factors (TF) can be used to estimate a plant potential for phytoremediation purpose. However, plants with a high translocation factor have the potential for phytoextraction [43].

Plants grouped to three type i.e. accumulator, excluder and indicator. Translocation factors for these three category plants are >1, <<1 and near 1, respectively [4, 17].

Thus, *D. sophia* in this study was an excluder for Cd and Ni. They mainly restrict metal in their roots. The plant may change its cell membrane permeability, alter metal binding capacity of cell walls, or exclude more chelating substances [44]. High accumulation of heavy metals in roots and low translocation in shoots may indicate appropriateness of a plant species for phytostabilization [45- 47].

The BAF represents the contaminant concentration in plants comparing with the environment concentration (in soil) [48]. BAF values are mostly based on metals particularity, environmental efficacy, disposal route and species-specific features. For selection a plant to phytoremediation of the contaminated soil, the BAF have to be higher than 1 [49].

Result of the present study highlighted that this plant had relatively low BAF and TF<1. The elevated concentration of Cd and Ni in roots of plant under investigation and low translocation into the shoot indicated their propriety for phytostabilization of these elements.

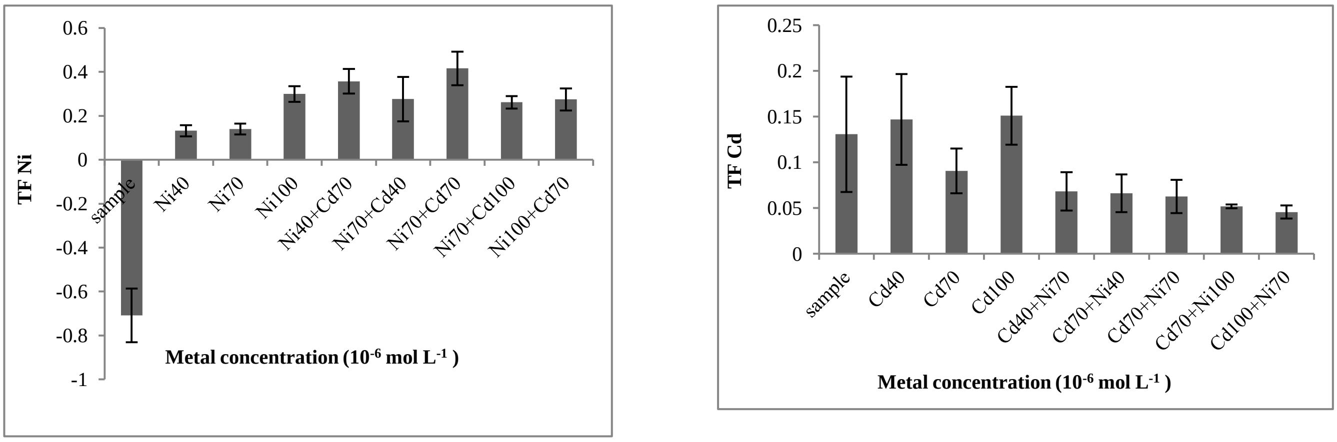


**Figure 2**. Translocation factors (TF) values of Ni and Cd in *D. sophia*

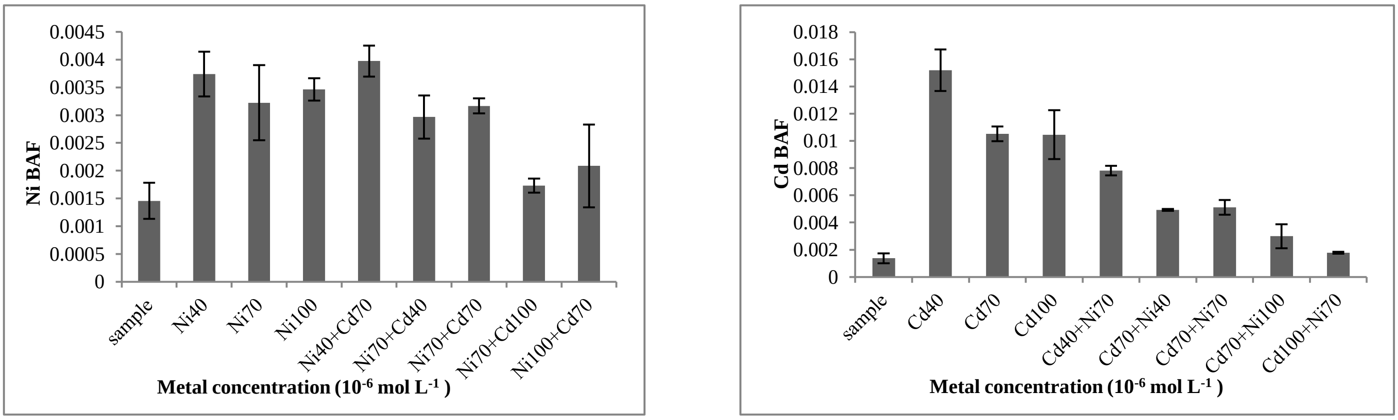


**Figure 3.** Bioaccumulation Factor (BAF) values of Ni and Cd in *Descurinia sophia*

**ACKNOELEDGMENTS**

This work was supported by Islamic Azad University, Neyshabur Branch, Neyshabur, Iran. The authors declare that there is no conflict of interests.

**REFERENCES**

1. Nagajyoti P.C., Lee K.D., Sreekanth T.V.M., 2010. Heavy metals, occurrence and toxicity for plants: a review. Environ Chem Lett. 8, 199–216.

2. Sangman Lee., Jae S., Moon T., Petros D., Goldsbrough B., Korban S., 2003. Overexpression of Arabidopsis Phytochelatin Synthase Paradoxically Leads to Hypersensitivity to Cadmium Stress. Plant Physiol. 131, 656–663.

3. Singh K., Pandey S.N., 2011. Effect of nickel-stresses on uptake, pigments and antioxidative responses of water lettuce, *Pistia stratiotes* L. J Environ Biol. 32, 391-394.

4. John R., Ahmad P., Gadgil K., Sharma S., 2008. Effect of cadmium and lead on growth, biochemical parameters and uptake in *Lemna polyrrhiza* L. Plant Soil Environ. 54(6), 262–270.

5. Van A., Clijsters H., 1990. Effect of metals on enzyme activity in plants. Plant Cell Environ. 13, 195–206.

6. Shafiq M., Zafar I.M., Athar M., 2008. Effect of lead and cadmium on germination and seedling growth of Leucaena leucocephala. J Appl Sci Environ Manage. 12(2), 61-66.

7. Alia P., Saradhi P.P., 1991. Proline accumulation under heavy metal stress. J Plant Physiol. 138, 554–558.

8. Charest C., Phan C.T., 1990. Cold acclimation of wheat (*Triticum aestivum*) properties of enzymes involved in proline metabolism. Physiologia Plantarum. 80, 159–168.

9. Lone M.I., He Z., Stoffella P.J., Yang X., 2008. Phytoremediation of heavy metal polluted soils and water: progress and perspectives. J Zhejiang Univ Sci B. 9, 210-220.

10. Mary C., Teresa J., Van Keulen H., Wei R., 2008. Hydroponic phytoremediation of Cd, Cr, Ni, As, and Fe: Can *Helianthus annuus* hyper accumulate multiple heavy metals? January M.C., et al. Chemosphere. 70, 531–537.

11. Shuha W., Zhou Q., Mathews S., 2008. A newly found cadmium accumulator *Taraxacum mongolicum*. J Hazard Mater. 159, 544–547.

12. Romero Puertas M.C., Rodriguez Serrano M., Corpas F.J., Gomez M., delRiao L.A., Sandalio L.M., 2004. Cadmium-induced subcellular accumulation of $O^{2-}$ and $H_2O_2$ in pea leaves. Plant Cell Environ. 27, 1122-1134.

13. Simon T., Eberhard A., 2000. Effect of Ni and As on Radish tuber cultivated on artificially polluted soils. Eur J Soil Biol. 36, 73-80.

14. Arnon D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol. 24, 1.

15. Bates L.S., Waldren R.P., Teare I.D., 1973. Rapid determination of free proline for water stress studies. Plant Soil. 39, 205-207.

16. Marchiol L., Assolari S., Sacco P., Zerbi G., 2004. Phytoextraction of heavy metals by canola (*Brassica napus*) and radish (*Raphanus sativus*) grown on multi contaminated soil. Environ Poll. 132, 21-27.

17. Baker A.J.M., 1981. Accumulators and excluders-Strategies in the response of plants to heavy metals. J Plant Nutri. 3(1-4), 643-654.

18. Ma L.Q., Komar K.M., Tu C., Zhang W., Cai Y., Kenelly E.D., 2001. A Fern that hyper-accumulates arsenic. Nature. 409, 579-582.

19. Ahmad P., Sharma S., Srivastava P.S., 2007. *In vitro* selection of $NaHCO_3$ tolerant cultivars of *Morus alba* (Local and Sujanpuri) in response to morphological and biochemical parameters. Hort Sci (Prague). 34, 114–122.

20. Pandey Pant P., Tripathi A.K., Dwivedi V., 2011. Effect of Heavy Metals on Some Biochemical Parameters of Sal (*Shorea robusta*) Seedling at Nursery Level, Doon Valley, India. J Agri Sci. 2(1), 45-51.

21. Kirbag Zengin F., Kirbag S., 2007. Effects of copper on chlorophyll, proline, protein and abscisic acid level of sunflower (*Helianthus annuus* L.) seedlings. J of Environ Biol. 28(3), 561-566.

22. Gardea-Torresdey J.L., Peralta-Videa J.R., Montes M., de la Rose G., Corral-Diaz B., 2004. Bioaccumulation of cadmium, chromium and copper by *Convolvulus arvensis* L.: Impact on plant growth and uptake of nutritional elements. Bioresource Technol. 92, 229-235.

23. Liu J., Xiong Z., Li T., Huang H., 2004. Bioaccumulation and ecophysiological responses to copper stress in two populations of *Rumex dentatus* L. from Cu contaminated and non-contaminated sites. Environ Exp Bot. 52, 43-51.

24. Shainberg O., Rubin B., Rabinowitch H.D., Tel-Or E., 2001. Loading beans with sub lethal levels of copper enhances conditioning to oxidative stress. J Plant Physiol. 158, 1415-1421.

25. Siedlecka A., Krupa Z., 1996. Interaction between cadmium and iron and its effects on photosynthetic capacity of primary leaves of *Phaseolus vulgaris*. Plant Physiol Biochem. 34, 833–841.

26. Ali G., Srivastava P.S., Iqbal M., 2001. Responses of *Bacopa monniera* cultures to cadmium toxicity. Bull Environ Contamination Toxicol. 66, 342–349.

27. Verbruggen N., Hermans C., 2008. Proline accumulation in plants: a review. Amino Acids. 35, 753–759.

28. Handique G.K., Handique A.K., 2009. Proline accumulation in lemongrass (Cymbopogon flexuosus Stapf.) due to heavy metal stress. J Environ Biol. 30(2), 299-302.

29. Yamada M., Morishita H., Urano K., Shiozaki N., Yamaguchi K., Shinozaki K., Yoshiba Y., 2005. Effects of free proline accumulation in petunia under drought stress. J Exp Bot. 56, 1975-1981.

30. Nikolic N., Kojic D., Pilipovic A., Pajevic S., Krstic B., Milan Borisev M., Orlovic S., 2008. Responses of hybrid poplar to cadmium stress: Photosynthetic characteristics, cadmium and proline accumulation, and antioxid enzyme activity. Acta Biological Cracoviensia Series Botanica. 50(2), 95–103.

31. Shafi Tantrey M., Agnihotri R.K., 2010. Chlorophyll and Proline Content of Gram (*Cicer arietinum* L.) under Cadmium and Mercury Treatments. Res J Agri Sci. 1(2), 119-122.

32. Sharma S.S., Schat H., Vooijs R., 1998. Phytochemist. 46, 1531-1535.

33. Pandey N., Sharma C.P., 2002. Effect of heavy metals $Co^{2+}$, $Ni^{2+}$ and $Cd^{2+}$ on growth and metabolism of cabbage. Plant Sci. 163(4), 753-758.

34. Ramos I., Esteban E., Lucena J.J., Garate A., 2002. Cadmium uptake and subcellular distribution in plants of lactuce sp. Ca-Mn interaction. J plant Sci. 162, 761-767.

35. Vajpayee P., Rai U.N., Ali M.B., Tripathi R.D., Yadav V., Sinha S.N., 2001. Chromium induced physiological changes in *Vallisneria spiralis* L. and its role in phytormediation of tannery effluent, Bull Environ Contam Toxicol. 67, 246-256.

36. Ernst W.H.O., Verkleji J.A.C., Schat H., 1992. Metal tolerance in plants. Acta Bot Neerl. 41, 229-248.

37. Lasat M.M., Baker A.J.M., Kochian L.V., 1998. Altered Zn compartmentation in the root symplasm and stimulated Zn absorption into the leaf as mechanisms involved in Zn hyperaccumulation in *Thlaspi caerulescens*. Plant Physiol. 118, 875-883.

38. Peterson P.J., Adaptation to toxic metals. In: Metals and Micronutrients: Uptake and Utilization by Plants, eds DA Robb, WS Pierpoint, Academic Press, London, 1983. Pp 51-69.

39. Wojcik M., Vangronsveld J., Tukiendorf A., 2005. Cadmium tolerance in *Thlaspi caerulescens*. I. Growth parameters, metal accumulation and phytochelatin synthesis in response to cadmium. Environ Exp Bot. 53, 151–161.

40. Rivetta A., Negrini N., Cocucci M., 1997. Involvement of $Ca^{2+}$ calmodulin in $Cd^{2+}$ toxicity during the early the early phases of radish (*Raphanus sativus* L.) seed germination. Plant, cell and Environment. 20, 600-608.

41. Barcelo j., Poschenrieder Ch., 1990. Plant water relations as affected by heavy metal stress: A review. J plant Nutr. 13, 1-37.

42. Ghosh M., Singh S.P., 2005. A review on phytoremediation of heavy metals and utilization of it's by products. Appl Ecolo Environ Res. 3, 1-18.

43. Yoon J., Cao X., Zhou Q., Ma L., 2006. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci Total Environ. 368, 456-464.

44. Lasat M.M., Pence N.S., Garvin D.F., Ebbs S.D., Kochian L.V., 2000. Molecular physiology of zinc transport in the Zn hyperaccumulator *Thlaspi caerulescens*. J Expt Botany. 51, 71-79.

45. Archer M.J.G., Caiwell R.A., 2004. Response of six Australian plants species to heavy metal contamination at an abandoned mine site. Water Air Soil Pollut. 157, 257-267.

46. Malik R.N., Husein S.Z., Nazir I., 2010. Heavy metal contamination and accumulation in soil and wild plants species from industrial area of Islamabad Pakistan. Pak J Bot. 42(1), 291-301.

47. Shu W.S., Xia H.P., Zhang Z.Q., Wong M.H., 2000. Use of vetiver and other three grasses for re-vegetation of Pb/Zn mine tailings at Lechang, Guangdong province: field experiment. Int J Phytoremediation. 4(1), 47-57.

48. Scragg A. Environmental Biotechnology. Oxford University Press, New York. 2005

49. Samuel N., 2005. Luoma and Philip S. Rainbow, Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. J Env Sci Tech. 39(7), 1921-193