



Effect of Pb toxicity on growth and antioxidant status in *Trigonella foenum graceum*

Suganthi.V¹, Mansoor Mohamed.M.P¹ and Rajalakshmi .K²

¹Department of Biochemistry, Dr.N.G.P Arts & Science College, Coimbatore-48.

²Department of Botany, NGM College, Pollachi.

ARTICLE INFO

Article history:

Received: 19 February 2013;

Received in revised form:

28 March 2013;

Accepted: 6 April 2013;

Keywords

Phytoaccumulation,
Phytotoxicity,
Germination.

ABSTRACT

In the present study, the lead phytoaccumulation and its effects on the growth and antioxidant status were determined in *Trigonella foenum graceum*. The phytotoxicity was studied in the germinating seeds under the exposure to increasing concentrations of lead acetate (25, 50, 100, 200 µM). Although there was a decrease in the germination percentage of the seeds to increasing concentrations, normal growth was observed after germination. The plant was capable of tolerating various concentrations of lead revealed by its vigorous growth after the 16th day of treatment. Pb caused significant reductions in the chlorophyll content, protein content and induced changes in the antioxidant enzymes. Pb decreased the activity of SOD and Catalase while the activity of GPx and LPO were induced significantly after Pb exposure. The plant expressed higher tolerance to Pb which is mainly attributed by its normal growth and physiology.

2013 Elixir All rights reserved.

Introduction

Heavy metals are important environmental pollutants and many of them are toxic even at very low concentrations. Lead (Pb) is one of the major heavy metals of the antiquity and has gained considerable importance as a potent environmental pollutant. Lead (Pb) was noted as primary contaminant due to the ability to disperse across environmental multimedia. (Cunningham, S.D. and W.R. Berti, 1993.) (USEPA, 1993). Significant increases in the Pb content of cultivated soils have been observed near industrial areas. Pb tends to accumulate in the surface ground layer and its concentration decreases with soil depth. It is easily taken up by plants from the soil and is accumulated in different organs. Pb is considered a general protoplasmic poison, which is cumulative, slow acting and subtle. Soils contaminated with Pb causes sharp decreases in crop productivity thereby posing a serious problem for agriculture (Freitas *et al.*, 2008).

The major factor limiting the potential for Pb Phytoextraction is low metal bioavailability for plant uptake. To overcome this limitation, synthetic chelators have been proposed to be added to the soil to increase the amount of available Pb (United States Environmental Protection Agency 2000a, b). Once introduced into the soil matrix, Pb is very difficult to remove. The capacity of the soil to adsorb Pb increases with increasing pH, cation exchange capacity, organic carbon content, soil/ water Eh (redox potential) and phosphate levels (USEPA 1992). Pb hyperaccumulation is particularly rare and restricted only to several species grown in soil or solution (Xiong, 1998; Baker *et al.*, 2000; Sahi *et al.*, 2002; Tamura *et al.*, 2005), since Pb prefers to complex with natural organic matter, absorb on clays and oxides, and precipitate as carbonates, hydroxides and phosphates, which limits its solubility in soil (Epstein *et al.*, 1999). The application of certain chelating agents, such as ethylenediaminetetraacetic acid (EDTA), to soils is a way to increase Pb solubility and thus the

translocation of Pb from the soils into the plant shoots. This method provides a possibility for an efficient Pb Phytoextraction, although it simultaneously increases the risk of leaching Pb into groundwater (Huang and Cunningham, 1996; Huang *et al.*, 1997; Blaylock *et al.*, 1997; Epstein *et al.*, 1999; Wu *et al.*, 2004).

Strong binding of Pb to the carboxyl groups of carbohydrate in cell walls leads to its diminished transport via apoplast. An electron microscopic study of root tips from tolerant plants reveals the presence of Pb in the cell wall as well as the cytoplasm. Within the cell, the major part of Pb is sequestered in the vacuole in the form of complexes. This may represent another mechanism of Pb detoxification in plants. Pinocytosis is observed in leaf cells of many plants treated with Pb salt solution. Through pinocytotic vesicles, Pb particles could be discharged into the vacuole (Wierzbica and Antosiewicz, 1993). Accumulation of excess total amino acid in response to Pb can be regarded as an important adaptive response of plants to avoid Pb toxicity (Thanbhom *et al.*, 2006).

Lead is widely known to be a non-essential element for plants, and can cause adverse effect on the plant's photosynthesis, chlorophyll synthesis and antioxidant enzymes, resulting in various symptoms of phytotoxicity, such as chlorosis, reduction of biomass, inhibition of root elongation and finally death (Milone *et al.*, 2003). Plant species have developed two basic strategies to resist the toxicity of heavy metals: avoidance and accumulation (Baker, 1987). Most heavy metal-tolerant species have the capability of preventing heavy metal accumulation in their shoots and therefore are called 'excluders', while others can take up heavy metals, translocate them into the shoots, and sequester them in non-metabolic-active tissues and organs in less harmful forms (Kupper *et al.*, 2007).

Trigonella foenum graceum belongs to the family fabaceae which has been proved to be have different hyperaccumulators. The crop is of significant commercial and nutrient value. In the

study, we investigated responses of the plant to varied supply levels of Pb including the Pb concentration in plant tissues, antioxidative enzymes, and chlorophyll content in pot culture. The objectives of the present study were to identify the capability investigate the effects of Pb on normal growth and physiology and the mechanisms involved in the detoxification of Pb by *Trigonella foenum-graceum*.

1. Materials and methods

Trigonella foenum-graceum seeds were obtained from Tamilnadu Agricultural University, Coimbatore. The seeds were surface sterilized using 0.1% sodium hypochlorite solution and 0.1% mercuric chloride for 10min and rinsed with double distilled water. Seeds were sown in earth pots (30 cm × 25 cm, diameter and depth) containing red soil and farmyard manure in 3:1 proportion. The seeds were randomly placed in the pots. Each pot contained 20 seedlings and made triplicates for the control as well as for different concentrations of lead. They were kept under natural photo radiation with a photoperiod of 12 hours at 200µmol/m²/s and at a temperature of 25 ± 2°C and 40 ± 5% relative humidity. The seeds were initially treated with four different concentrations of lead (lead acetate solutions 0 (control), 25, 50, 100 and 200 µmol/L) in separate pots, till the maximum growth of the crop was observed and the observations were recorded (triplicates). The solutions used for the treatments were 30 ml for each pot and four different chosen concentrations. Totally the solutions were added 8 times during the experimental period. The soil pH was 7.58. The seeds were treated with four different Pb concentrations once every three days with the above solutions. The duration of exposure to the heavy metal was increased to 23 days and the growth was observed.

1.1 Seed germination test

Different concentrations of lead acetate solutions were exposed to the germinating seeds. The number of germinated seeds on the day three and seven after initiation were germination energy and germination percentage respectively. After three days, the shoot length was measured from culms base to the tip of the longest leaf.

Germination index (GI) and vitality index (VI) were calculated using the following equation.

$$GI = \Sigma (G_t/D_t)$$

$$VI = \Sigma (G_t/D_t) \times S$$

G_t – germination rate at day

D_t – day t

S – Shoot length

The germination index was calculated according to the method of Ana *et al.*, (2004).

1.2 Estimation of plant growth and biochemical parameters

The plant growth was observed and recorded once every three days. The plant growth parameters including the plant height, the morphology of the leaf and leaf size were measured using an mm scale. The control group was grown normally without the addition of lead acetate. The duration of exposure to the heavy metal was increased to 23 days and the growth was observed.

1.3 Analysis of chlorophyll-a, chlorophyll-b, total chlorophyll, protein

The leaves were washed with distilled water and then grinded using mortar and pestle for physiological and biochemical studies. Chlorophyll content in the leaves (1000 mg) of treated and control plants were extracted in 80% chilled acetone and estimated by the method of Arnon (Arnon, 1949)

and determined the absorbance of the solution at 645, 663, and 652 nm against the solvent (80%) blank, using spectrophotometer (UV-Vis double beam spectrophotometer 118, Systronics, India). The Chlorophyll content was expressed as mg/g fw (fresh weight).

The Protein content in the leaves (1000 mg) were extracted in with buffers used for enzymes assay, grind well the samples with a pestle and mortar in 5–10 ml of buffer and centrifuge, the supernatant for protein was measured at 660 nm by Lowry's method (Lowry *et al.*, 1951) using bovine serum albumin (BSA) as the standard protein. The total Protein content was expressed as mg/g fw.

1.4 Effect of Pb on antioxidant status

1.4.1 Enzymic antioxidant system

The plant material (1000mg) was homogenized in 100mM chilled potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% polyvinyl pyrrolidone (w/v) at 4°C. The homogenate was squeezed through four layers of cheese cloth and the extract obtained was centrifuged at 10000 X for 15 mins at 4°C. The supernatant was used to measure the activities of antioxidant enzymes.

The Catalase activity was assayed according to the method of Sinha (Sinha, 1972). The assay mixture contained 0.5 ml of H₂O₂, 1.0 ml of 0.01 M phosphate buffer, pH 7.0 and 0.4 ml of water along with 1.0 ml of the newly prepared leaf homogenate. 0.2 ml of the enzyme was added to initiate the reaction. 2.0 ml of the dichromate/acetic acid reagent was added after 0, 30, 60, 90 seconds of incubation. The colour developed was read at 610 nm. The activity of Catalase was expressed as µmol H₂O₂ oxidised/mg of protein/min.

The activity of Superoxide dismutase was ascertained using the nitrite method of Das *et al.*, (2000). About 100 mg leaves from freshly uprooted plants were extracted using chilled mortar and pestle in 5 ml of 50 mmol/L K-phosphate buffer (pH 7.4) containing 1% (v/v) triton X-100, 10 mM hydroxylamine hydrochloride and 50 µM EDTA. One unit of the enzyme activity was defined as the amount of SOD capable of inhibiting 50% of nitrite formation under the assay conditions. The activity of SOD was expressed as units/ µg protein min.

The activity of Lipid peroxidase was determined as 2-thiobarbituric acid reactive substances (TBARS) by using the standard methods of Okhawa *et al.* (1979) with minor modifications. For the measurement of the level of lipid peroxidation in plant leaves, the extraction was done in 5 ml of 0.02 M Tris HCl Buffer (pH 7.5). 3 ml of the reaction mixture contained thoroughly mixed TBA-TCA-HCl reagent. The absorbance of chromophore was read at 535 nm. The results are expressed as units of MDA/ g of tissue.

Glutathione Peroxidase was assayed according to the method of Rotruck *et al.*, (1973). 4.0 ml of the reaction mixture contained 0.4 M sodium phosphate buffer (pH 7.0), 10 mM sodium azide, 2.5 mM hydrogen peroxide and 4 mM reduced glutathione and a suitable aliquote of enzyme. The increase in the absorbance was read at 412 nm. The activity of GPx was expressed as µg glutathione utilized/min/mg protein.

2.0 Statistical analysis

All the measurements were replicated three times. Average values and the standard deviations (S.D) were calculated by the Microsoft Office Excel 2007 for all the data in this paper. To confirm the variability of the data obtained and validity of results, all the data were subjected for the statistical significance with the package SPSS 11.5 using one way Analysis of Variance

(ANOVA) and the significance of difference between sample means were calculated. The significant difference was set between treatments as $p < 0.05$ or $p < 0.01$.

Results and Discussion

The percentage of germination may reflect the reaction rate of plant seeds to their living environment. Germination index (GI) and vitality index (VI) are two important parameters that reflect the seed quality. From table 1 it can be

Seen that Germination Index and Vitality Index decreased with the increase of Pb concentrations.

Digital in the brackets represent the percentage compared to the control (%).

a-Comparison between the control and 25 μ M

b-Comparison between 25 μ M and 50 μ M

c-Comparison between 50 μ M and 100 μ M

d-Comparison between 100 μ M and 200 μ M

e-Comparison between control and 200 μ M

* -Indicates significant difference at $p < 0.01$.

Physiological and biochemical effects

Effect of lead on the plant growth

In this study, the experimental evidence shows that *T.foenum graecum* was well tolerant to the lead in the soil and grew healthy. The plant showed increased growth response after an exposure of 16 days, the growth response being the increase in the plant height and increase in the leaf area as compared to the control group (Fig. 1). The accumulation of metals in various parts of higher plants is often accompanied by an induction of variety of cellular changes, some of which directly contribute to metal tolerance capacity of plants (Devi and Prasad, 1998; Hall, 2002).

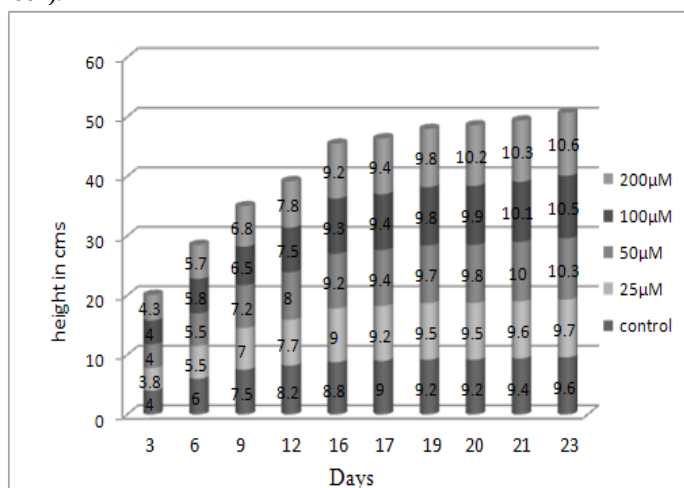


Fig.1: The growth pattern and height of *T.foenum graecum* under different concentrations of lead.

Effect on photosynthetic pigments

Photosynthetic surface area and leaf chlorophyll contents are the main key factors determining the dry matter production in plants. An increase in chlorophyll-a and total chlorophyll content was recorded in leaves of *T.foenum graecum* at 50 μ M but chlorophyll b showed decreased concentrations. The highest concentrations of chlorophyll a and (a + b) were observed at 100 μ M Pb, which differed significantly from those at 200 μ M Pb ($P < 0.05$). However, the concentration of chlorophyll b was not statistically different among all Pb treatments ($P > 0.05$). (Fig. 2).

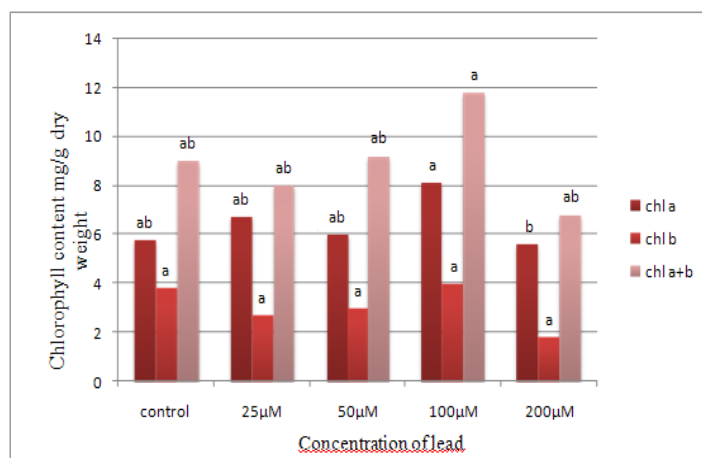


Fig. 2. Effects of Pb on the concentrations of chlorophyll a, b and (a + b) of *T.foenum graecum*. Different letters in the same type of column are significantly different at $P < 0.05$ level. Bars represents ($N = 3$).

Effect on protein content

The protein content in the plant leaves showed significantly increased concentrations in 200 μ M lead acetate (Fig.3). From the experimental evidence it was observed that as the concentration of lead increases the protein content also increases. A steady increase in the total protein content was observed in all the plants grown under lead treatment with different concentrations of lead acetate. Compared to the control, 25 μ M treated plant had 88% less protein content. Other concentrations of protein are 99%, 103%, 109% protein content for 50 μ M, 100 μ M and 200 μ M treated plant respectively. Lead induced increment in the total protein content was observed in other ecotypes during treatment with increasing concentrations of lead (Ejazul Islam et al., 2008).

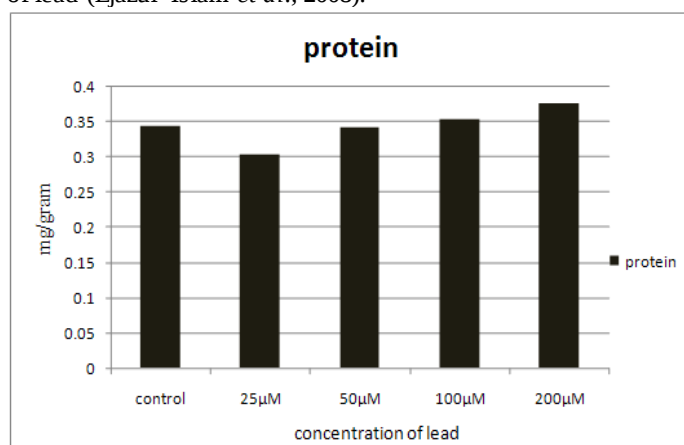


Fig. 3. Effect of lead on the protein content

Enzymic Antioxidants

The values are expressed in mean $\pm$ SD ($n = 3$).

The means are followed by a common letter

1- Inhibition of 50% nitrite formation/min/mg protein

2- 1 μ M of H_2O_2 decomposed/min/mg protein

3- 1 μ M of glutathione consumed/min/mg protein

4- nanomoles of MDA/g tissue.

* - the values are significant at 1 % level of significance

- the values are non significant at 5% level of significance

From the table, it is evident that the activity of Superoxide dismutase and Catalase significantly decreased among the groups. Glutathione peroxidase showed an increase in activity as compared to the control.

Table 1: Changes of germination index and vitality index at different lead concentrations

Parameter	Control	25µM	50 µM	100 µM	200 µM
Germination index	12.56±0.51 (100%)a*	11.08±0.45 (88.2%)b*	10.76±0.46 (85.67%)c*	10.23±0.54 (81.45%)d*	9.45±0.49 (75.24%)e*
Vitality index	50.80±0.23 (100%)a*	48.21±0.28 (94.90%)b*	41.01±0.21 (80.73%)c*	38.65±0.32 (76.08%)d*	37.33±0.29 (73.48%)e*

Concentration	SOD ¹	CAT ²	GPx ³	LPO ⁴
Control	4.81±0.05a*	2.80±0.34a#	0.05±0.002a*	0.62±0.05a#
25µg	3.05±0.14b*	2.52±0.19b#	0.19±0.002b*	1.03±0.10b#
50µg	2.04±0.06c*	1.48±0.19c#	0.22±0.004c*	1.10±0.09c#
100µg	1.16±0.05d*	1.92±0.12d#	0.37±0.002d*	1.13±0.05d#
200µg	0.16±0.01e*	1.90±0.24e#	0.46±0.014e*	1.32±0.08e#

The activity of lipid peroxidation also significantly increased as the concentration of lead increased. The activity of SOD and Catalase was found to be declining in *Elsholtzia argyi* during lead treatment (Ejazul Islam *et al.*, 2008).

Catalase is an iron containing enzyme catalyzing the dismutation of H₂O₂ into O₂ and H₂O. It is a major antioxidant enzyme in curtailing the peroxidative damage in a biological system. The enzyme is found in all aerobic eukaryotes and is important in the removal of H₂O₂ generated in the peroxisomes by oxidases (Redinbaugh *et al.*, 1988) and also in glyoxylate cycle and purine catabolism. Various isoforms are also reported in cytosol and mitochondria. Stress conditions such as salinity, heat, shock or cold causes the depletion of Catalase activity). This may have significance in the plants ability to tolerate oxidative components under these environmental stresses.

It has been well established that glutathione peroxidase, a selenium enzyme, plays a major role in the regulation of concentration of H₂O₂ and wide variety of organic peroxides (Sarita sinha *et al.*, 2005).

Effect of lead on activity of Catalase

The activity of Catalase had diminished activity in 200µM treated plant compared to the control. The activity of Catalase was found to be 90%, 70%, 69%, 68% respectively for the increasing concentrations of 25, 50, 100 and 200µM respectively. Gorinova *et al.*, 2007 found that at lower heavy metal concentrations, the activity of antioxidant enzymes increased, whereas at higher concentrations, CAT activity decreased in plants.

Effect of lead on activity of Glutathione Peroxidase

From the experimental evidences the activity of glutathione peroxidase was found to be increasing in different treatments of lead. The increased activity was found to be in 200µM treated plant. GPx, an oxidative stress inducible enzyme plays a significant role in the peroxyl scavenging mechanism and in maintaining functional integration of the cell membranes. The rise in the activity of GPx could be due to its induction to counter the effect of increased oxidative stress. Marc Dazy *et al.*, (2008) reported that similar findings showing the increase in the GPx activity has been observed in the metal treated plants. The increase in the activity of GPx levels could be the consequence of either the microenvironment or the tissue specific gene expression in the treated plants.

Effect of lead on activity of lipid Peroxidase

The activity of lipid peroxidation increased significantly in 100µM as compared to the control. The highest activity was found to be in 100µM whereas in 200µM the activity decreased. Compared to the control 25µM had increased activity. The role of peroxidase as a stress enzyme in plants has been widely

accepted. Increased peroxidase activity with Lead treatment can be correlated with the release of peroxidase localized in the cell walls

Conclusion

Lead is one of the toxic heavy metal and exerts adverse effects on morphology, growth and photosynthetic processes of plants and causes inhibition of enzyme activities, water imbalance, alterations in membrane permeability and disturbs mineral nutrition. Some plants have developed special metal detoxification mechanisms, which allow them to grow in the soils having elevated levels of Lead and other heavy metals (Singh *et al.*, 1997; Sharma *et al.*, 2005).

The data presented in the study have demonstrated that the lead treatment in *Trigonella foenum graecum* impairs the chlorophyll, protein and the antioxidant defense system. This suggested that the exposure of the plant to lead resulted in ROS release in the plant cells leading to the induction of various physiological changes.

The absence of toxicity symptoms, comparatively higher net growth and better tolerance to lead are mainly attributed to the maintenance of its leaf growth, induction of antioxidants and the integrity of the cell organelles.

Certain levels of lead induced a stimulatory effect on biomass production and chlorophyll concentrations. These indicated that this species had interesting mechanisms for lead detoxification. The discovery of this phenomenon thus provides for a new material to study the underlying mechanisms of lead detoxification. Further study is needed at the cellular and molecular level for better understanding of the adaptive mechanisms in response to the stress due to excess lead.

It was concluded that *Trigonella foenum graecum* could tolerate and accumulate high concentrations of lead and it could be regarded as a potential accumulator with respect to the growth pattern observed under increasing concentrations of lead in the soil. The plant in view of its fast and indeterminate growth and high accumulation potential seems to be a suitable candidate to decontaminate landfills moderately contaminated with lead. Longer field experiments are required to verify the real tolerance to lead of *Trigonella foenum graecum*.

References

- Alkorta, I., Hernánde-Allica, J., Becerril, J. M., Amezcaga, I., Albizu, I., & Garbisu, C. (2004). Recent findings on the Phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead, and arsenic. Reviews in Environmental Science and Bio/Technology, 3, 71–90.
- Amon, D.I., 1949. Copper enzymes in isolated chloroplast, polyphenol Oxidase in Beta vulgaris. Plant Physiol. 24, 1–15.

- Baker, A.J.M., 1987. Metal tolerance. *New Phytol.* 106, 93–111.
- Baker, A.J.M., McGrath, S.P., Reeves, R.D., Smith, J.A.C., 2000. Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for Phytoremediation of metal-polluted soils. In: Terry, N., Bañuelos, G. (Eds.), *Phytoremediation of Contaminated Soil and Water*. Lewis Publishers, pp. 86–107.
- Berti, W. R., & Cunningham, S. D. (2000). Phytostabilization of metals. In I. Raskin & B. D. Ensley (Eds.), *Phytoremediation of toxic metals: Using plants to clean-up the environment* (pp. 71–88). New York: Wiley.
- Blaylock, M.J., Salt, D.E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., Ensley, B.D., Raskin, I., 1997. Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environ. Sci. Technol.* 31, 860–865.
- Cunningham, S. D., & Ow, D. W. (1996). Promises and prospects of phytoremediation. *Plant Physiology*, 110(3), 715–719.
- Devi S R, Prasad M N V, 1998. Copper toxicity in *Ceratophyllum demersum* L. (Coontail), a free floating macrophyte: response of antioxidant enzymes and antioxidants. *Plant Sci*, 138: 157–165.
- Ejazul Islam , Dan Liu , Tingqiang Li , Xiaoe Yang, Xiaofen Jin , Qaisar Mahmoo, Shengke Tian , Junying Li, Effect of Pb toxicity on leaf growth, physiology and ultrastructure in the two ecotypes of *Elsholtzia argyi*, *Journal of Hazardous Materials* 154 (2008) 914–926.
- Epstein, A.L., Gussman, C.D., Blaylock, M.J., Yermiyahu, U., Huang, J.W., Kapulnik, Y., Orser, C.S., 1999. EDTA and Pb-EDTA accumulation in *Brassica juncea* grown in Pb-amended soil. *Plant Soil* 208, 87–94.
- Freitas, O.M.M, Mastins R.J.E., Delerue C.M., Boaventura. R.A.R, Boaventura, 2008. Removal of Cd(II) Zn(II) and Pb from aqueous solution by brown marine macroalgae kinetic modeling. *J. Hazard. Mater.* 153(2008) 493–450.
- Gorinova, N., Nedkovska M., Tosoroska E, Simova-stoliva, L, Stoyanova, Z., Georgieva, K., Demirevska- Kepova, K., Atanassova. A., and Herzig R. (2007). Improved phytoaccumulation of cadmium by genetically modified tobacco plants. physiological and biochemical response of the transformation to cadmium toxicity, *Environmental pollution* 145(2007) 161–170.
- Huang, J.W., Chen, J.J., Berti, W.R., Cunningham, S.D., 1997. Phytoremediation of lead contaminated soils: role of synthetic chelates in lead phytoextraction. *Environ. Sci. Technol.* 31, 800–805.
- Huang, J.W., Cunningham, S.D., 1996. Lead phytoextraction: species variation in lead uptake and translocation. *New Phytol.* 134 (1), 75–84.
- Kumar, P. B. A. N., Dushenkov, V., Motto, H., & Raskin, I. (1995). Phytoextraction: The use of plants to remove heavy metals from soils. *Environmental Science and Technology*, 29(5), 1232–1238.
- Kupper, H., Parameswaran, A., Leitenmaier, B., Trtilek, M., Setlik, I., 2007. Cadmium-induced inhibition of photosynthesis and long-term acclimation to cadmium stress in the hyperaccumulator *Thlaspi caerulescens*. *New Phytol.* 175, 655–674.
- Lowry, O.H., Rosebrought, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193, 265–275.
- Marc.Dazy, Eric Beracud, Sylvie Cotellet, Eric meus, Jean-Francois Masfaraud and Jean-Francois Ferard (2008). Antioxidant enzyme activity as affected by trivalent and hexavalent chromium species in *Fontinalis antipyretica*.
- Milone, M.T., Sgherri, C., Clijsters, H., Navari-Izzo, F., 2003. Antioxidative responses of wheat treated with realistic concentration of cadmium. *Environ. Exp. Bot.* 50, 265–276.
- Sharma, P, R.S. Dubey, Lead toxicity in plants, *Braz. J. Plant Physiol.* 17 (2005) 35–52. (2005) 35–52.
- Prasad, M. N. V, & Freitas, H. (2003). Metal hyperaccumulation in plants – Biodiversity prospecting for Phytoremediation technology. *Electronic Journal of Biotechnology*, 6, 275–321.
- R.P. Singh, R.D. Tripathi, S.K. Sinha, R. Maheshwari, H.S. Srivastava, Response of higher plants to lead contaminated environment, *Chemosphere* 34 (1997) 2467–2493.
- Sahi, S.V., Bryant, N.L., Sharma, N.C., Singh, S.R., 2002. Characterization of a lead hyperaccumulator shrub, *Sesbania drummondii*. *Environ. Sci. Technol.* 36, 4676–4680.
- Salt, D. E., Blaylock, M., Kumar, P. B. A. N., Dushenkov, V., Ensley, B. D., Chet, L., et al. (1995). Phyto-remediation: a novel strategy for the removal of toxic metals from the environment using plants. *Biogeochemistry*, 13, 468–474.
- Sarita sinha, Rohit saxena, shroddha sinh, (2005). Chromium induced lipid peroxidation in the plants of *Pistia stratiotes*. Role of antioxidants and antioxidant enzymes, *Chemosphere*, 58, 595–604.
- Shanker A K, Carlos Cervantes, Herminia Loza-Tavera et al., 2005. Chromium toxicity in plants [J]. *Environ International*, 31: 739–753.
- Tamura, H., Honda, M., Sato, T., Kamachi, H., 2005. Pb hyperaccumulation and tolerance in common buckwheat (*Fagopyrum esculentum* Moench). *J. Plant Res.* 118, 355–359.
- Thanabhorn, S., Jaijoy, K., Thamarase, S., Ingkanian, K., Panthong, A. (2006). Acute and subacute toxicity study of ethanol extract from *Ionicera japonica* thumb. *J. Ethnopharmacol.* 370–373.
- USEPA, 1996. Test methods for evaluating Soils and wastes (SW-846): Acid digestion of sediments, Sludges and soils (Method 3050). US Environmental Protection Agency.
- Wierzbicka, K. (1999). The effect of lead in the cell cycle in the root meristems of *Allium cepa* L. *Protoplasma* 207, 286–294.
- Wu, L.H., Luo, Y.M., Xing, X.R., Christie, P., 2004. EDTA-enhanced Phytoremediation of heavy metal contaminated soil with Indian mustard and associated potential leaching risk. *Agric. Ecosyst. Environ.* 102, 307–318.
- Yang X, E, Li T. Q, Jin X. F, He Z. L, Stoffella P. J, Q. H. Hu, Root responses and metal accumulation in two contrasting ecotypes of *Sedum alfredii* Hance under lead and zinc toxic stress, *J. Environ. Sci. Heal. A* 40 (2005) 1081–1096.
- Xiong, Z.T., 1998. Lead uptake and effects on seed germination and plant growth in a Pb hyperaccumulator *Brassica pekinensis* Rupr. *Bull. Environ. Contam. Toxicol.* 60, 285–291.