ÉVALUATION DE L'EFFET TOXIQUE DU PLOMB SUR CERTAINS PARAMETRES DE CROISSANCE DU RADIS " RAPHANUS SATIVUS L".

EVALUATION OF THE TOXIC EFFECT OF LEAD ON SOME PARAMETERS OF GROWTH RADISH PLANT "*Raphanus sativus L.*"

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ABSTRACT : The lead pollution has become a serious problem threatening our ecosystems with detrimental effects on crop production and biodiversity, so much research is currently focused on new environmentally friendly methods such as phytoremediation .The work undertaken registers with a view to study the physiological, biochemical behavior of the plant radish (*Raphanus sativus L.*). In this context, the dose (250, 500 and 1000 mg / l) of lead acetate were applied to radish (*Raphanus sativus L.*) relative to a control (absence of the Pb), for a period of two months. The results show apparent changes in various parameters studied in stressed plants compared to controls. Macroscopic (inhibition of germination rate, decreased biomass and size reduction), physiological (disruption of water status, decreased chlorophyll pigments and carotenoids content) were observed. Histological changes at the level of the rods (deformation of the walls of the medullary parenchyma cells), and roots (occlusion of xylem vessels) were observed.

However, a strong correlation between soil Pb and plant Pb was noticed. Accordingly, lead uptake by *Raphanus sativus L*. is influenced by its bioavailability in soil.

Key words: Lead, Phytoremediation, *Raphanus sativus*, *Soil Pollution.*

RESUME : La pollution par le plomb est devenue un véritable problème menaçant nos écosystèmes, avec des effets préjudiciables sur la production végétale et sur la biodiversité, c'est pourquoi actuellement de nombreuses recherches sont axées sur de nouvelles méthodes plus écologiques comme la phytoremediation. Le travail entrepris s'enregistre dans la perspective d'étudier le comportement physio-biochimique de la plante du radis (*Raphanus sativus L.)* en réponse à des apports excessifs de Plomb dans le milieu de croissance et l'évaluation de sa tolérance à ce polluant, pour l'utiliser dans la phytoremédiation des sols contaminés. Les résultats obtenus montrent des modifications apparentes dans les différents paramètres étudiés chez les plantes stressées par rapport aux témoins. Dans ce cadre, des doses de (250, 500 et 1000 mg/l) d'acétates de plomb sont appliquées sur des graines de radis (*Raphanus sativus L*.) par rapport à un témoin (absence du Pb pendant deux mois. Des modifications histologiques, au niveau des tiges (déformation des parois des cellules du parenchyme médullaire), et des racines (occlusion des vaisseaux de xylème) ont été observées. Des changements histologiques au niveau des tiges (déformation des parois des cellules du parenchyme médullaire), et les racines (occlusion des vaisseaux du xylème) ont été observés. Cependant, une forte corrélation entre Pb du sol et des plantes Pb a été remarquée. En conséquence, l'absorption de plomb par *Raphanus sativus* est influencée par sa biodisponibilité dans le sol.

Mots clés : Plomb, phytoremédiation , *Raphanus sativus* , Pollution des sols.

INTRODUCTION

Contamination of the biosphere by heavy metals has increased sharply at the beginning of the 20th century, posing major environmental and human health problems worldwide (Singanan *et al.*, 2005). Industrial operation such as smelting, mining, metal forging, manufacturing of alkaline storage batteries, combustion of fossil fuel, utilization of fertilizers and pesticides and disposal of waste cause phenomenal increase in the extent of heavy metals in the environment (Alloway, 1995; Mcllveen & Negusanti, 1994). Lead toxicity may be the cause of a number of biological and physiological processes in plants. The remediation technologies of heavily heavy metal contaminated soils are generally extremely complicated and expensive. Phytoremediation is an alternative emerging technology utilizing plants to reduce, remove, degrade or immobilize xenobiotics from contaminated environment (Sharma & Subhadra, 2010). The advantages of phytoremediation are relatively simple, eco-friendly and cost effective compared to other conventional strategies (Burd *et al.*, 2000; Glick, 2003; McGrath *et al.,* 1998). Phytoremediation is becoming an important tool for decontaminating soil, water, and air by detoxifying, extracting, hyperaccumulating, and/or sequestering contaminants, especially at low levels where, using current methods, costs exceed effectiveness. Phytoremediation is the direct use of living green plants for in situ, or in place, removal, degradation, or containment of contaminants in soils. Phytoremediation can be defined as "the efficient use of plants to remove, detoxify or immobilise environmental contaminants in a growth matrix (soil, water or sediments) through the natural biological, chemical or physical activities and processes of the plants". Phytoremediation involves growing plants in a contaminated matrix, for a required growth period, to remove contaminants from the matrix, or facilitate immobilization (binding/containment) or degradation (detoxification) of the pollutants. The ideal plant for use in phytoextraction should have the following traits: (i) ability to accumulate the metal(s) intended to be extracted, preferably in the aboveground parts; (ii) tolerance to high metal concentrations in soils; (iii) fast growth and high accumulating biomass; (iv) easily grown as an agricultural crop and fully harvestable (Khan & Frankland, 1983; Klang-Westin & Perttu, 2002). The experiment reported in this paper was undertaken to assess the capability of radish to phytoextract lead from polluted soils and to obtain the efficiency of radish for phytoextraction purpose. Consequently, an extensive experiment was conducted to find out if radish can be introduced as a Pb-hyperaccumulative plant.

MATERIALS AND METHODS

Biological material

Radish (*Raphanus sativus*), family Cruciferae, has been chosen because of its use as a model plant for laboratory toxicology studies of various pollutants (Forbes *et al.,* 1997). In addition, the use of *Raphanus* *sativus* has many interests: fast growth, large biomas, and sensitivity to heavy metals.

Condition and sampling

Raphanus sativus seeds were washed with bleach to 1% for 5 minutes to remove any fungal contamination. After several rinses in distilled water the seeds are germinated in pots containing soil to a depth of 1 cm under normal conditions at a rate of 1 to seeds per pot.

Experimental desigN

The experiment consists to 4 treatments with 5 replicates for each treatment:

- 6 pots watered with distilled water (control)
- 6 pots watered with distilled water containing lead acetate at a concentration of 250 mg/l.
- 6 pots watered with distilled water containing lead acetate at a concentration of 750 mg/l.
- 6 pots watered with distilled water containing lead acetate at a concentration of 1000 mg/l.

The pot treated with lead acetate and control pots were watered with 300 ml of distilled water respectively for two months

Experimental device

PLANT HARVEST AND ANALYSIS

PARAMETERS STUDIED

Measurements on the morphological parameters of plants (number of leaves, height of the aerial parts) were made each week of exposure to lead. In addition, measurements of fresh weight, plant height and the study of physiological, biochemical and histological parameters were made at the end of the experiment.

MORPHOLOGICAL PARAMETERS

Throughout the period of the experiment, visual observation (discoloration, discoloration, pigmentation, withering ...) and a measure of growth of the aerial part of plants (number of leaves, length) are performed each week.

Biometric parameters

After sowing we performed the counting of the seeds germinated in each pot, we made the calculated using the following formula:

Germination rate (%) = germinated seeds / seed sowing x 100

The number of leaves, and fresh weight were measured.

PHYSIOLOGICAL PARAMETERS

Physiological parameters (relative water content, chlorophyll and carotenoid) are only made on the leaves of radish.

THE RELATIVE WATER CONTENT (RWC)

The relative water content is determined according to the method of (Scippa *et al.*, 2004), the sheet is cut to the base of the blade and immediately weighed to give fresh weight (PF) the sheet was then dipped into a test tube containing distilled water, the assembly is placed in the dark and at a temperature of 4 ° C for 24 hours. The recovered leaves are gently wiped with a filter paper to remove surface water, reweighed to give the maximum weight after rehydration (full turgor weight (PPT)). The dry weight (DW) was determined after passage of the sheets in the oven set at 80 ° C for 48 hours.

The relative water content RWC is calculated using the following formula:
RWC (%) = [(PF - PS) / (PPT - PS)] x 100extracted with 80 % acetone
The biochemical constituent's total chlorophyll (a+b) was extracted with 80% acetone and quantified according to (Arnon, 1949).

ANATOMICAL METHODS

Cross-sections of stems and roots were taken by hand. Sections were cleared in sodium hypochlorite and stained by carmine-vest (1% w/v in 50% ethanol) and methyl green (1% w/v, aqueous) and mounted in gelatin. Then well-stained sections were photographed with an optical microscope (ZEISS 37081) and all the measurements and observations were performed10 times on different slides were performed by measurement software with 5 repeats at each part.

STATISTICAL ANALYSIS

The data are average ± SD of three duplicate experiments. Employing the t-test the significant differences from the control were statistically evaluated at p=0, 05 and 0, 01.

RESULTS

Effect of lead on the morphological parameters

During the experiment, the macroscopic observation reveals some abnormalities in poisoned plants:

• During the second and third week we noticed a yellowing and brown spots on the leaves of plants treated with lead acetate (250, 500 and 1000 mg / l) (Fig. 1).

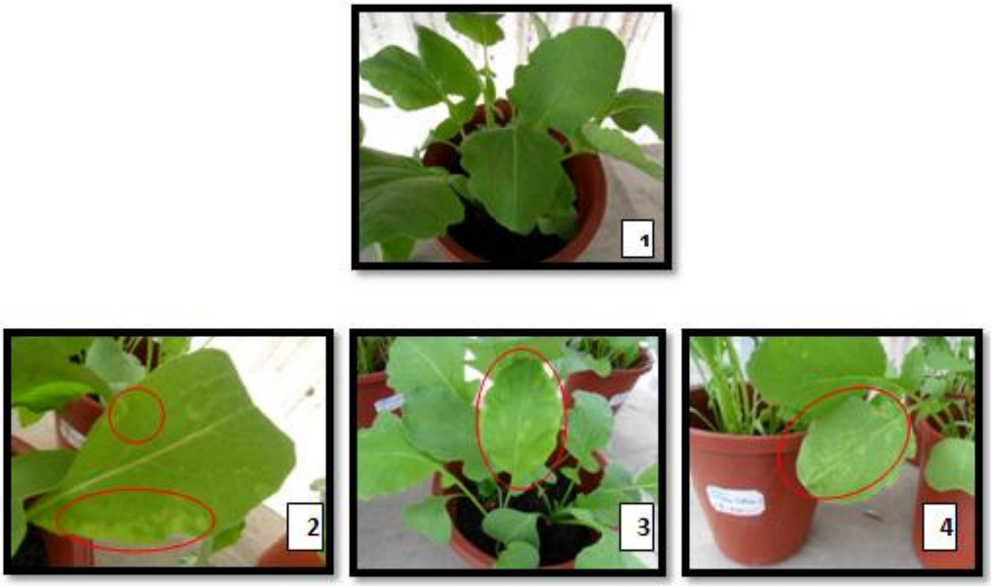

Fig.1. Yellow spots on the leaves of plants intoxicated (**1:** control, **2:** Pb 250mg / **l, 3:** Pb 500mg / l, **4:** Pb 1000mg / l).

In the weeks that followed (4, 5, 6, 7 and 8), we observed that the poisoned plants seem more fragile than control plants, and there were breaks in the stems of plants subjected to different concentrations Pb (250, 500 and 1000 mg / l), other signs are noticed (wilting, yellowing, brown spots) (Fig. 2).

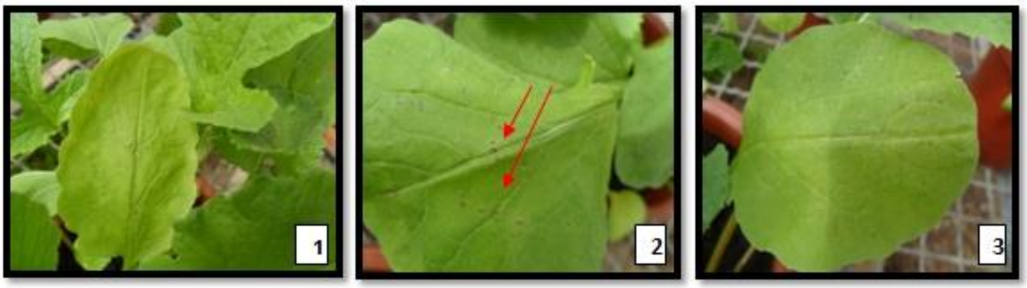

Fig. 2. Yellowing and brown spots on the leaves of stressed plants (**1** and **2**: 500mg / l, **3**: 1000 mg / l).

EFFECT OF LEAD ON BIOMETRICS PARAMETERS

Effect of lead on the germination rate of seeds

Exposure radish seeds to lead during the germination period, showed a non-significant decrease in germination rate among groups of stressed plants, 93.75%, 93.75% and 75% at concentrations of lead 250, 500 and 1000 mg / l, respectively, compared to the control with the germination rate is 100% (Fig. 3).

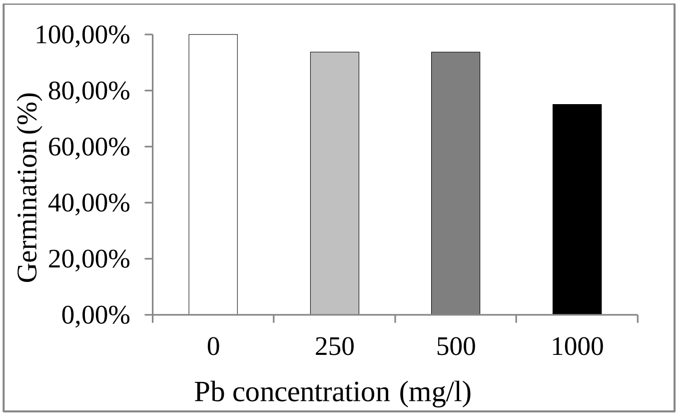

Fig. 3. Effect of lead (0, 250, 500, 1000 mg / l) on the rate of germination of radish (*Raphanus sativus L.*).

EFFECT OF LEAD ON THE NUMBER OF LEAVES

The results showed that the number of leaves is higher (9 leaves per plant at the seventh week), compared to intoxicated plants (8, 8, 7 sheets at the seventh week) for concentrations of lead, 250, 500 and 1000 mg / l respectively. But this difference is not significant (P > 0.05) (Fig. 4).

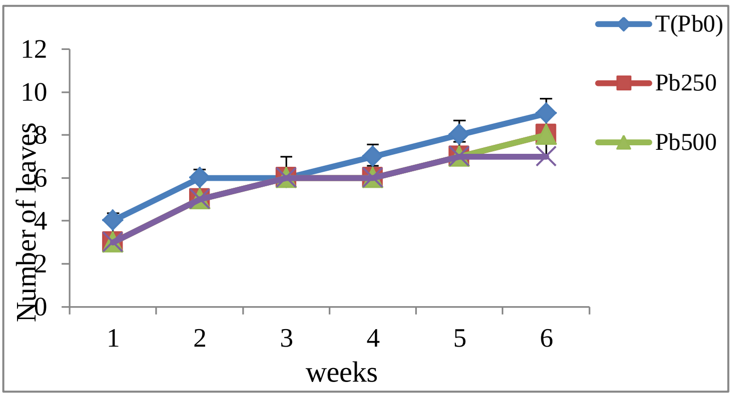

Fig. 4. Effect of lead (0, 250, 500, 1000 mg / l) on the number of f radish leaves (*Raphanus sativus L.*), Values are expressed as mean (± SD, n = 5).

EFFECT OF LEAD ON FRESH WEIGHT (BIOMASS)

Fresh weight intoxicated plants was not significantly reduced ($p > 0.05$) at 42.19 ± 1.94 for Pb concentration 250 mg/l, and significantly ($p < 0.05$), 37.80 ± 2.95, 36.56 ± 2.03g at concentration of lead 500, 1000 mg /l, respectively compared to the control which is 55.38 ± 1.44 g (Fig. 5).

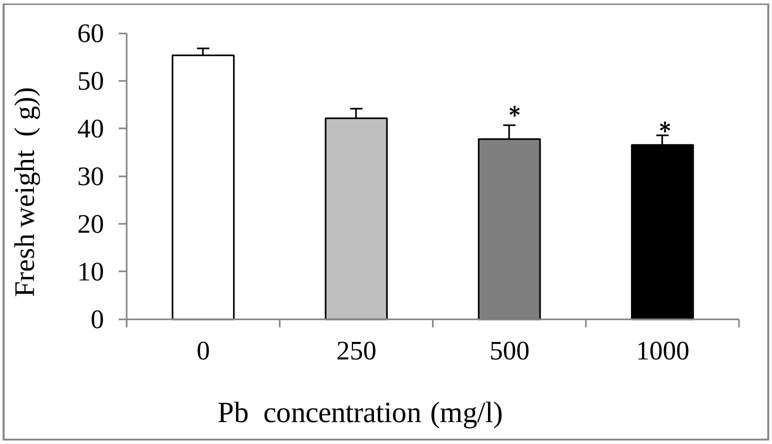

Fig. 5. Effect of lead (0, 250, 500, 1000 mg / l) on the fresh weight (biomass) (g). Values are expressed as mean (± SD, n = 5) * significant difference ($p< 0.05$).

EFFECT OF LEAD ON THE SIZE (LENGTH) OF PLANTS

The Figure 6 showed no significant decrease in plant size poisoned at 250mg/l and 500mg/l (37.2 ± 3.20 cm; 39.94±1.96 cm) respectively.

Contrary, at de 1000mg/l, the results showed a significant (($p < 0.05$) increase in plant size (44.29±1.80cm) compared with the control (Fig.6).

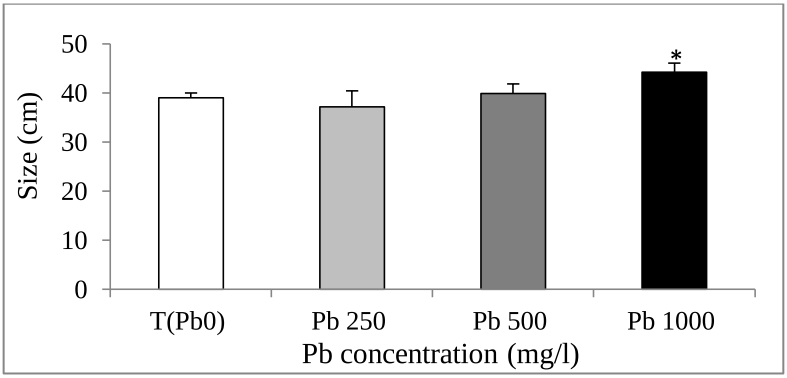

Fig.6. Effect of lead (0, 250, 500, 1000 mg / l) on plant size (cm). Values are expressed as mean (± SD, n = 5) * significant difference (p< 0.05).

EFFECT OF LEAD ON PHYSIOLOGICAL PARAMETERS

Effect of lead on the relative water content (rwc)

Fig. 7 showed a no significant decrease (P > 0.05) in the relative water content of leaves (86.15% ± 0.48, 82.18 ± 0.55% and 81. ± 29 0.48%) at Pb concentration (250, 500 and 1000 mg / l) respectively compared to the control (85.82 ± 0.61%).

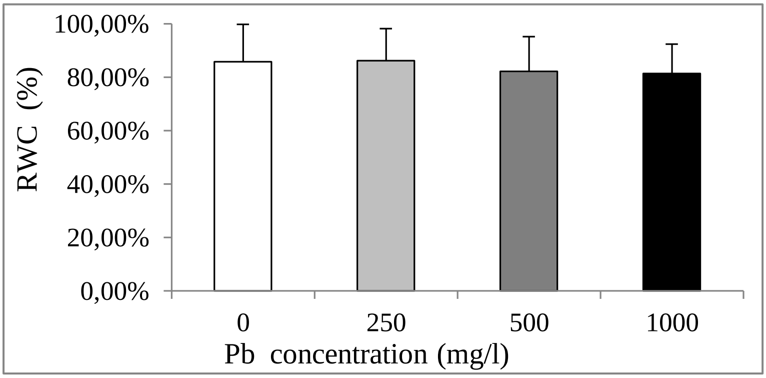

Fig.7. Effect of lead (0, 250, 500, 1000 mg / l) on plant RWC (%). Values are expressed as mean (± SD, n = 5) * significant difference.

EFFECT OF LEAD IN LEAF CHLOROPHYLL PIGMENTS

Chlorophyll a (ch)

The results of Fig. 8 show a decline in total chlorophyll (CH) as and when the metal stress (Pb concentration) was increasing. We observed no significant decrease ($p > 0.05$) in total chlorophyll in poisoned plants at 250mg / l (39 ± 1.08 mg / g). Furthermore, this decrease in total chlorophyll is very significant in poisoned plants at 500 mg/l and 1000 mg/l ($p < 0.01$).

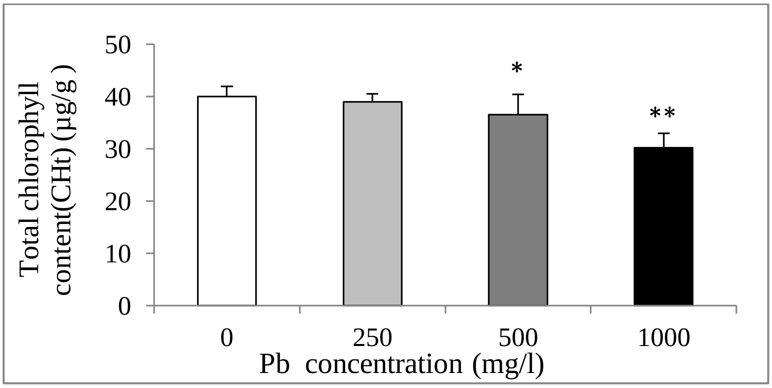

Fig. 8. Effect of lead (0, 250, 500, 1000 mg / l) in the total chlorophyll content of plants. Values are expressed as mean (± SD, n = 5). * Significant difference ($p < 0.05$), ** very significant difference ($p < 0.01$).

HISTOLOGICAL STUDY

Histological examination was performed on cross sections of different parts of the plant (leaf, stem, and root). Microscopic observations of the different cuts were made using a microscope with two optical magnifications x 100 and x 400. Different changes were observed in stressed plants at the roots and stems. At the leaf level we have not noticed any differences between control and poisoned plants.

LEAVES

The microscopic observations (x100) showed similar structures for histological sections of control leaves (Fig. 9) with those subjected to various lead concentrations.

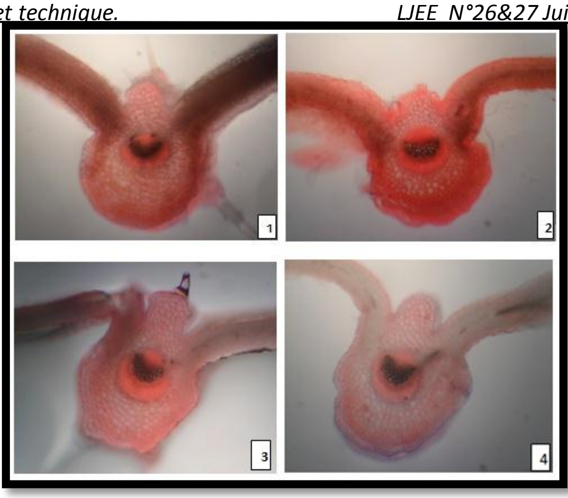

Fig. 9. Histological sections of the leaves of radish plants (*Raphanus sativus L.*) in different concentrations of lead (1: control, 2: 250, 3: 500, 4: 1000 mg / l) (X 100).

STEMS

Histological analysis of radish stems (*Raphanus sativus L.*) showed a deformation of the cell walls of the medullary parenchyma in plants treated by lead compared to control. This phenomenon is more pronounced in plants poisoned in 1000 mg / l (Fig. 10).

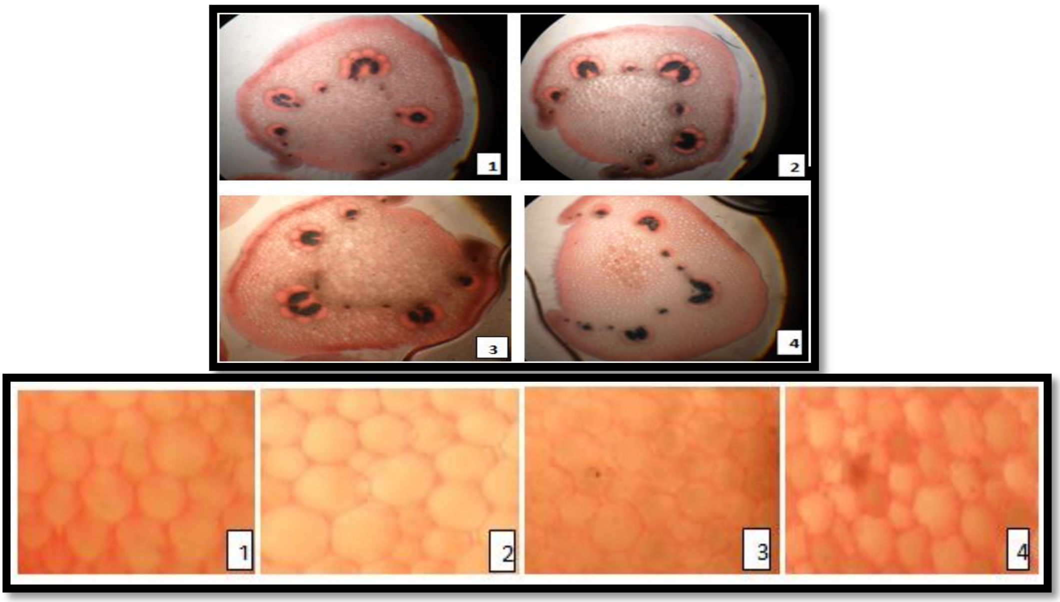

Fig.10. Histological structure of radish stems (*Raphanus sativus L.*) under different concentrations of lead (**1**: control, **2**: 250, **3**: 500 and **4**: 1 000 mg / l) (X100 and X 400) (cell changes medullary parenchyma).

ROOTS

Histological study showed a decrease or reduction of quantity and size of lignified xylem cells compared to control plants. This decrease is very significant at high doses (500 and 1000 mg / l) (Fig. 11).

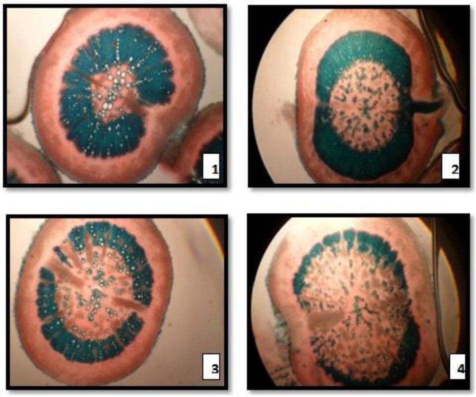

Fig.11. Histological structure of radish roots (*Raphanus sativus L.*) in different concentrations of lead (1: control, 2: 250 3: 500 4: 1000 mg / l) (X100).

DISCUSSIONS

A raising concern regarding human health risks and environmental consequences associated with heavy metal pollution have created a need for efficient and effective remediation strategies. Phytoremediation has recently attracted a great attention as a highly promising strategy because it is relatively inexpensive, environmentally-friendly, and more aesthetically pleasing compared to the other remediation technologies (Chomchalow, 2011; Roongtanakiat & Chairoj, 1962). Therefore, the present study explored the capability and compared the efficiency of heavy metal uptake, translocation and accumulation of *Raphanus sativus*. The experiments were intentionally conducted under axenic condition to avoid un-estimated effects of other environmental factors that might affect the metal uptake efficiency. Uptake is largely influenced by the availability of metals, which is in turn determined by both external (soil associated) and internal (plant associated) factors. In only a limited number of plant species a heritable tolerance or

resistance occurs, which enables these plants to grow on metal contaminated soils. (Sharma & Subhadra, 2010).
Soil remediation is needed to eliminate risk to humans or the environment from toxic metals. Several studies dealing with metal hyper accumulating plants, and they have concluded that phyto extraction of metals was a feasible remediation technology for the decontamination of metal polluted soils (Cheraghi *et al.*, 2011). The chemistry of metal interaction with soil matrix is again the important criterion to the phytoremediation concept. Soil pH affects not only metal bioavailability, but also it affects the metal uptake by the roots (Brown *et al.*, 1994a). Sharma & Dubey, 2005 reported that the solubility of heavy metals is generally greater as pH decreases within the pH range of normal agricultural soils. The high pH values of soils could have accounted for a low transfer of metals from soil to plants. In our study, lead treatment caused a decrease in chlorophyll in leaves of moderately and high metal accumulator plants. Barrs & Weatherley, 1962; Luna *et al.* 2000 reported that Pb stopped the formation of chlorophyll and caused destruction of chlorophyll. The decrease of chlorophyll may be due to blocking of enzymes acting in chlorophyll synthesis or to degradation of chlorophyll. Study also showed that radish accumulated lead mainly in roots and shoots. Inhibition in dry weight and length of root and shoot of *Raphanus sativus* has been observed; these effects of lead on growth and biomass accumulation are possibly a consequence of effect on metabolic processes of plant (Madhu *et al.,* 2008). The absence of severe toxicity symptoms in plants grown in Pb-contaminated soils could be partly due to the lower absolute toxicity of Pb and partly due to the restricted transport to the shoot. It is generally understood that roots act as a barrier to the movement of toxic heavy metal through the soil-plant system (Kershaw & Webber, 1986). The high levels of contaminant metals in the plant tissue reached phytotoxic concentration. It is not however certain whether the toxicity symptoms produced in the plant were solely due to the excessive levels of Pb in the plant tissue or that the toxicity was associated with ionic imbalance involving other essential trace metals. In this research we focused the effect of lead in plant tissues and anatomical and structural alterations in *R. sativus* plants under Pb treatments (Kuiper, 1978; Vollenweider *et al.,* 2006). Heavy metal treatments deal to appearance the symptoms similar to water stress including increase leaf thickness, palisade mesophyll and size of epidermal cells, increase the number of stomata, reduction of the stomata opening and increase the size to guard cells (Rai *et al.*, 2010). The reason of decreasing the stomata opening seem is increased cell wall thickness and reduction of turgor pressure. The decrease in the size of stomata aperture in

the leaves is in line with the hypothesis that metals induce water stress (Singh, 2004). Closed stomata of the leaf result in a slower rate of diffusion due to greater diffusion gradient of water vapors (Bondada & Oosterhuis, 2000). The various effects of water deficit seen on stomata structure are clearly mechanisms to enable plants to survive in stress conditions (Comstock, 2000). The main reason behind the stomata closure under metal stress may be a strategy to prevent the water loss through transpiration as the translocation of water and solutes get disturbed in the presence of metals. The effect of metals on stomata opening was thought to be due to either metal-induced inhibition of an energy system or the alterations of K+ fluxes through membranes (Bondada & Oosterhuis, 2000). Pb physically block the uptake of water and water stress led to substantial losses in dry weight, leaf area, root dry weight and length. (Azmat *et al.,* 2009). The structure and morphology of epicuticular waxes is a indicator of plant health and regulate the resistance to pollution stress. Changes in leaf wet ability, rate of transpiration, and loss of solutes from leaf cells are some of the effects that result from disruption of the epicuticular wax layer. (Azmat *et al.,* 2009; Heidari & Sarani, 2011).

CONCLUSION

The ability of brassicas to bioaccumulate heavy metals can be used to reduce the level of contaminants in the soil (phytoremediation), and thus to clean up and prepare soils for cultivation. Phytoremediation is a new and promising approach to remove contaminants in the environment. But using plants alone for remediation is confronted with many limitations.

REFERENCES

Alloway BJ. (1995) *Heavy metals in soils*. Blackie Academic & Professionals. p.368.

Arnon DI. (1949) *Copper enzyme polyphenoloxides in isolated chloroplast in Beta vulgaris*. Plant Physiology 2, 1-15.

Azmat R, Haider S, Nasreen H, Aziz F, Riaz M. (2009). *A Viable alternative mechanism in adapting the plants to heavy metal environment*. Pak J Bot 41(6), 2729-2738.

Barrs HD, Weatherley PE. (1962) *A re-examination of the relative turgidity technique for estimating water deficits in leaves*. Aust J Biol Sci 24, 519-570.

Bondada BR, Oosterhuis DM. (2000) *Comparative epidermal ultrastructure of cotton (Gossypium hirsutum L.) leaf, bract and capsule wall*. Ann Bot 86, 1143– 52.

Brown SL, Chaney RL, Angle JS, Baker AJM. (1994a) Phytoremediation *potential of Thlaspi caerulescens and bladder campion for zinc- and cadmium contaminated soil*. J Environ Qual 23,1151–7.

Burd GL, Dixon DG, Glick BR. (2000) *Plant growth-promoting bacteria that decrease heavy metal toxicity in plants*. Can J Microbiol 46, 237-245.

Cheraghi M, Lorestani B, Khorasani N, Yousefi N, Karami M. (2011) *Findings on the phytoextraction and phytostabilization of soils contaminated with heavy metals, Biological*. Trace Element Research 144(1-3), 1133-1141.

Chomchalow N. (2011) *Research, Development and Applications in Thailand* AU JT 14(4), 268-274.

Comstock JP. (2002) *Hydraulic and chemical signaling in the control of stomatal conductance and transpiration*. Journal of Experimental Botany 53, 195-200.

Forbes PJ, Black KE, Hooker JE. (1997) *Temperature-induced alteration to root longevity in Lolium perenne*. Plant Soil 190, 87–90.

Glick BR. (2003) *Phytoremediation: Synergistic use of plants and bacteria to clean up the environmen*t. Biotechnology Advance 21, 383-393.

Heidari M, Sarani S. (2011) *Effects of lead and cadmium on seed germination, seedling growth and antioxidant enzymes activities of mustard (Sinapis arvensis L.)*. ARPN Journal of Agricultural and Biological Science 6, 44-47.

Kershaw KA, Webber M R. (1986) *Seasonal changes in the Chlorophyll content and the quantum efficiency of the moss Brachythecium rutabulum*, J Bryol 14, 151-158.

Khan DH, Frankland B. (1983) *Effects of cadmium and lead on radish plants with particular reference to movement of metals through soil prople and plant*. Plant and Soil 70, 335-345.

Klang-Westin E, Perttu E. (2002) *Effects of nutrient supply and soil cadmium concentration on cadmium removal by willow.* Biomass and Bioenergy 23, 415-426.

Kuiper PJC. (1978) *Mechanism of to physical and chemical factors in plants*. In Freysn, A H. J. andWoldendorp, J. W. (eds). Structure and Functioning of Plant populations. Co., NY, p. 215-235.

Luna CM, Casano LM, Trippi VS. (2000) *Inhibition of wheat nitrate reductase activity by zinc*. Biologia. Plantarum 43(2), 257-262.

Madhu G, Rakesh SS, Sengar KG, Kalpana S, Reshu S. (2008) *Effect of lead on seed germination, seedling growth, chlorophyll content and nitrate reductase activity in Mung bean (Vigna radiata)*. Res J Phyto*chem* 2(2), 61-68.

McGrath SP, Shen ZG, Zhao FJ. (1998) *Heavy metal uptake and chemical changes in the rhizosphere of Thlaspi caerulescens and Thlaspi ochroleucum grown in contaminated soils*. Plant and Soil 188, 153–159.

Mcllveen WD, Negusanti JJ. (1994) *Nickel in the terrestrial environment*. Sci. Total. Environ 148, 109-139.

Rai A, Kulshreshtha K, Srivastava PK, Mohanty CS. (2010) *Leaf surface structure alterations due to particulate pollution in some common plants*. Environmentalist 30, 18–23.

Roongtanakiat N, Chairoj P. (1962) *Vetiver grass for remedying soil contaminated with heavy metals.* Paper no. 1962. In: Proceedings of the 17th World Congress of Soil Science, 14–21 August 2002, Bangkok, Thailand.

Scippa G, Michele DI, Onelli M, Patrignani E, Chiatante G, Bray E. (2004) *The histone-like protein H1-S and the response of tomato leaves to water defici*t. J Exp Bot 55, 99-109.

Sharma JOT, Subhadra AV. (2010) *The effect of mercury on nitrate reductase activity in leaf segments (Phaseolus vulgaris) its chelation by phyto -chelating synthesis*. Life Science and Medicine Research 13, 1-8.

Sharma P, Dubey RS. (1994a) *Lead toxicity in Plants*. Braz J Plant Physio. 17, 35-52.

Singanan M, Abebaw A, Singanan V. (2005) *Removal of lead ions from industrial wastewater by using biomaterials-A novel method*. Bull Chem Soc Ethiop 19(2), 289-294.

Singh S. (2004) *Scanning electron microscopic studies and growth response of the plants of Helianthus annuus L. grown on tannery sludge amended so*il. Environment. International 30, 389– 395.

Soares AM, Gomes MP, Marques T, Oliveira M, Nogueira G, Castro EM. (2005) *Ecophysiological and anatomical changes due to uptake and accumulation of heavy metal in Brachiaria decumbens*. Sci Agric 68, 566-573.

Vollenweider S, Cosio C, Keller C. (2006) *Localization and effects of cadmium of cadium toleranwillow (Salix viminalis) I. Macrolocalization and phytotoxic effects of cadmium*. Environmental and Experimental Botany 58, 64-74.

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