Volume 56(1):43-49, 2012
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

Phytoremediation potential of *Raphanus sativus* L. for lead contaminated soil

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ABSTRACT Phytoremediation is an emerging technology that employs the use of higher plants for the clean up contaminated environment. Phytoextraction, the use of plants to extract toxic metals from contaminated soils, has emerged as a cost-effective, environment-friendly clean up alternative. Pot culture experiments using radish (*Raphanus sativus* L.) was performed to investigate lead (Pb) phytotoxic effects on antioxidant enzymes and other early warning biomarkers of soil Pb exposure. The study included an assessment of heavy metal accumulation in root, shoot and leaf, effect of lead stress on growth parameter (root length, root and shoot dry weight), photosynthetic pigment content, bioaccumulation coefficient (BAC) and the activity of anti-oxidant enzymes. Results demonstrated that efficient Pb uptake was observed by the roots in contaminated plants. Root growth was higher in control plants, as compared to the contaminated. Lead exposure also influenced biochemical and physiological parameters. Administration of excess of lead was followed by an increase of Pb accumulation in leaves, and associated symptoms of toxicity. Typical symptoms of Pb toxicity developed 30 days after the beginning of treatment. Chlorophyll concentration was decreased in response to heavy metal toxicity. Activity of anti-oxidative enzymes e.g. peroxidase and catalase were increased in response to oxidative stress. Atomic absorption spectrophotometer (AAS) was used for analysis of heavy metal in soil and plant samples. The results of this research showed that radish are hyperaccumulator plants that can concentrate heavy metals in their different parts, thus they can be used for remediation of polluted area. Study also showed that potential of metal accumulator plants for extraction of metal from soil occur up to a certain level of concentration, after that when the concentration of metal increased the phytoextraction rate of metal or bioaccumulation coefficient (BAC) were decreased. **Acta Biol Szeged 56(1):43-49 (2012)**

KEY WORDS

oxidative stress
peroxidase
phytoremediation
radish

The continuous application of large amounts of fertilizers and other soil amendments to agricultural land has raised concern regarding the possible accumulation of elevated levels of their trace element constituents and potential harm to the environment (Colbourn and Thornton 1978; Raven and Leoppert 1997). Furthermore, increasing amounts of urban and industrial wastes (Parry et al. 1981; Gibson and Farmer 1983) which may contain significant quantities of heavy metals are being disposed on the agricultural lands (Raven and Leoppert, 1997). Severe heavy metal contamination in soil may cause a variety of problems, including the reduction of yield and metal toxicity of plant, animals and humans. The decontamination of these soils by engineering methods is high costing project (Baker et al. 1991; Salt et al. 1995). Over the last 15 years there has been an increasing interest in developing a plant based technology to remediate heavy metal contaminated soils (Chaney 1983; Cunningham and Berti 1993; Raskin et al. 1994). Phytoextraction is the use of plants to remove heavy metals from contaminated soils. The concept of using plants to clean up contaminated environments is not new. About 300 years ago, plants were proposed for use in the treatment of waste water.

At the end of the 19th century, *Thlaspi caerulescens* and *Viola calaminaria* were the first plant species documented to accumulate high levels of metals in leaves (Hartman 1975). The idea of using plants to extract metals from contaminated soil was reintroduced and developed by Utsunamyia (1980) and Chaney (1983), and the first field trial on Zn and Cd phyto extraction was conducted by Baker et al. (1991). Since plant cultivation and harvesting are relatively inexpensive processes as compared to traditional engineering practices that rely on intensive soil manipulation. Phytoextraction may provide an attractive alternative for the cleanup of heavy metal-contaminated soils. The goal of heavy metal phyto extraction is to reduce metal levels in the soil up to the acceptable levels within a reasonable time frame (Raskin et al. 1994; Nanda-Kumar et al. 1995; Huang et al. 1997). The process of phyto extraction generally requires the translocation of heavy metals to the easily harvestable shoots. A few plant species are able to survive and reproduce on soils heavily contaminated with

Accepted May 31, 2012
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Zn, Cu, Pb, Cd, Ni, Cr and As. Such species are divided into two main groups. The first group called pseudo metallophytes that grow on both contaminated and non-contaminated soils, and second group called as absolute metallophytes, that grow only on metal- contaminated and naturally metal rich soil (Baker 1987).

Depending on plant species, metal tolerance may result from two basic strategies: metal exclusion and metal accumulation (Baker 1981; Baker and Walker 1990). The exclusion strategy, comprising avoidance of metal uptake and restriction of metal transport to the shoots (De Vos et al. 1991), is usually used by pseudo-metallophytes. The accumulation strategy caused high uptake of metal and storage in vacuoles to prevent metal toxicity. The extreme level of metal tolerance in vascular plants is called hyper-accumulation. According to Brooks et al. (1977), metal hyper accumulation is a rare phenomenon that occurs in some plants called hyper accumulators. However hyper-accumulators are often described as slow growing and low biomass plants (Dushenkov et al. 1995; Nanda-Kumar et al. 1995; Ebbs et al. 1997; Rouhi 1997). The potential of some crop plants from brassicaceae for phyto-remediation has been extensively studied (Brown et al. 1995b; Dushenkov et al. 1995; Huang and Cunningham 1996; Ebbs and Kochian 1997; Ebbs et al. 1997) and it was demonstrated that some efficient shoot accumulators of the genus Brassica contained up to 3.5% on a dry weight basis of heavy metals (Nanda-Kumar et al. 1995).

Heavy metal such as Mn, Cu, Fe, Zn and Ni are essential mineral nutrients for higher plants.

Normal concentration of these heavy metals play a crucial role in plant growth and development but generally these heavy metals can caused oxidative stress, eliciting enzymatic and non-enzymatic antioxidative reaction responses and lipid per-oxidation in plants.

Nitrate reductase, the key enzyme of nitrate assimilation, is subjected to inhibition by heavy metal treatment. Inhibitory effect of heavy metal may be due to a) blocking the supply of reducing equivalents of nitrate reduction, b) formation of mercurial derivatives of –SH of nitrate reductase c) synthesis of phytochelatins (Sharma and Subhadra 2010).

The present research aimed to study the accumulation of lead in roots, shoot and leaves of radish plant. This study also examined the growth performance and physiological response in respect to activity of important enzymes like catalase (CAT) and nitrate reductase. We identified changes in CAT and nitrate reductase activity and measured chlorophyll level, to determine the potential efficiency of these varieties to remediate the metal from the contaminated soil in which they were cultivated.

Material 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. 2006). In addition, the use of Raphanus sativus has many interests: fast growth, large biomass, sensitivity to heavy metals.

Culture 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 of 4 treatments with 6 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 500 mg / l

6 pots watered with distilled water containing lead acetate at a concentration of 750 mg/ l.

The pots treated with lead acetate and control pots were watered with 300 ml of lead solution and 300 ml of distilled water respectively for a month.

Plant harvest and analysis

Plant samples were gently removed from the pots 30 days after for the measurement of various growth parameters and biochemical analysis. Shoot and roots were separated and length was measured. Plant shoots and roots were washed with distilled water for 20 minutes and then divided into two bundles of shoot and roots. They were blotted dry on filter paper and dried at 70°C for 2 days to determine plant dry weight (Bohm 1979). The biochemical constituent's viz., total chlorophyll (a+b) was extracted with 80% acetone and quantified according to Amon's (1949). Antioxidant enzymes Catalase (CAT) activity was assayed by Chandlee and Scandalios (1984) and Kumar and Khan (1982), respectively. We homogenized 0.5 g of frozen plant material in a prechilled pestle and mortar with 5 ml of ice cold 50 mM sodium phosphate buffers (pH 7.5). The extract was centrifuged at 4 °C for 20 min at 12,500 rpm. The supernatant was used for enzyme assay. The assay mixture contained 2.6 ml of 50mM potassium phosphate buffer (pH 7.0), 400µl of 15 mM H_2O_2 and 40µl of enzyme extract. The decomposition of H_2O_2 was followed by a decline in absorption at 240 nm.

Lead analysis

The leaves and roots were washed thoroughly in deionized water to remove soil particles. They were then oven-dried at 70°C for 48 hours and their dry weights recorded. Subsamples

Table 1. Lead concentration in Radish plant (*Raphanus sativus* L.); Data are mean ± SD.

Lead concentration	Pb concentration (µg /g dry weight) Root	Stem	Leaf
Control	0	0	0
250 mg/l	266±23,1*	199±16,1*	178,19,2*
500 mg/ l	292±17,6**	232±25,1**	200±29,2**
750 mg/ L	332±30,1*	282±25,1*	229±16,5*

(*P<0, 05; **P<0, 01)

of dried plant tissue were digested in a mixture of nitric acid and perchloric acid (Merck) and the lead concentration in the sample was determined by AAS (Atomic Absorption Spectrophotometry, Varian Spectra AA220).

The calculation of bioaccumulation coefficient and statistical analysis

The following formula was used for calculation of Bioaccumulation coefficient (BAC) = Element concentration in plant part (µg metal g dry weight-1 of plant part) / Element concentration in soil (µg metal g dry weight-1 of soil) (Bini et al. 1995). The experiments were repeated thrice and the statistical analysis was done using randomized block design.

Nitrate reductase activity

The activity of nitrate reductase in the treated material was estimated in vivo by Srivastava (1974) with slight modifications. About 200 mg of leaf material from various treatment radish were incubated with 10 ml of incubation medium consisting of 0.1M sodium phosphate buffer (pH 7.2), 0.2M KNO_3 and 25% isopropanol in dark vial of 20 ml capacity. The whole set was incubated in dark for 30 min at 30°C. Nitrite released in the incubation mixture due to enzyme activity was measured by color development by the formation of diazo compound with sulfanilamide and nitrate coupled with NED to give a red dye. The absorbance was read at 540 nm after 20 min by using UV-spectrophotometer.

Statistical analyis

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

The Pb concentration in root, shoot and leaves after exposure to different solution of lead are shown in Table 1. Efficient Pb uptake was observed in the roots of contaminated plants compared with the control. Accumulation metal element in

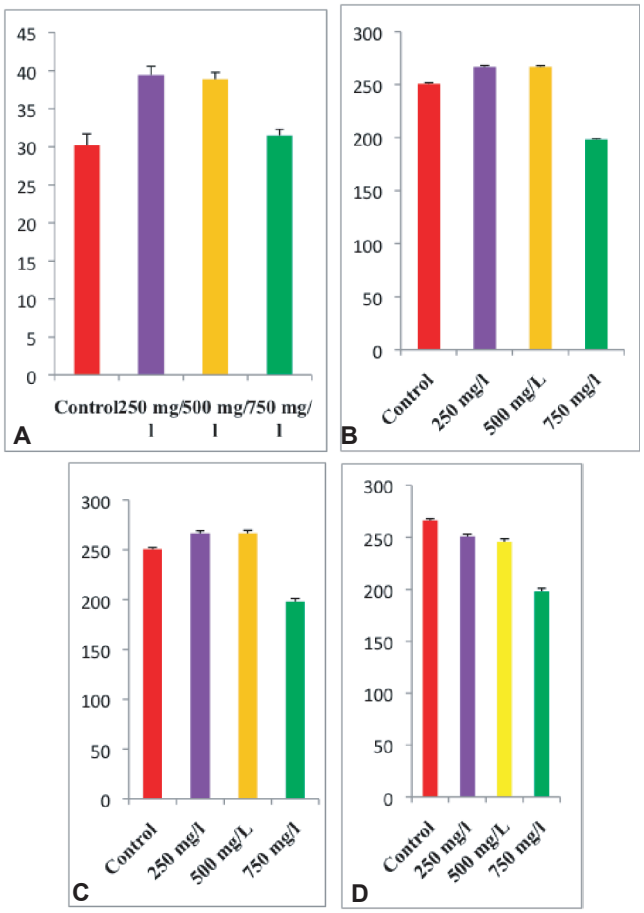

Figure 1. A, B) shoot and root dry weight (mg/plant),(C) root length (cm/plant) and (D) chlorophyll concentration (mg/g fresh weight) in radish (*Raphanus sativus* L.) plant in response to elevated levels of lead in the growth medium (mg/l). Data are mean ± SD (*P<0, 05; **P<0, 01).

the roots was, much higher than in shoot and leaves.

Root growth increased by increasing the lead ion concentration up to low level (250 mg/l). Root and shoot dry weight radish plant had decreased in response to high concentration of Pb ions in the growth medium (Fig. 1A, B).

Root length of radish contaminated plant is not affected under the toxicity of all concentration of lead metal, showed susceptibility to elevated levels of lead metal (Fig. 1C). Lead exposure influenced several biochemical and physiological parameters. Administration of excess amount of lead was followed by an increase of Pb ions and its associated symptoms of toxicity in leaves.

Chlorophyll concentration was decreased in response to heavy metal toxicity. Highest reduction was observed plants contaminated with 750 mg/l of lead. Necrotic lesions were seen on the leaves of plants treated with 750 mg/l. Chlorosis symptoms appeared, reflecting a decrease in chlorophyll a

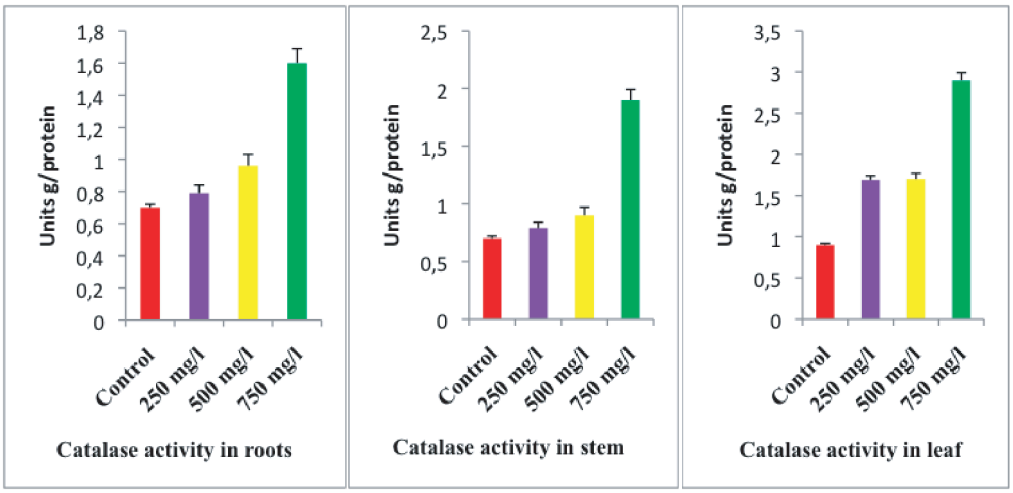

Figure 2. Catalase activity (Units g/protein) in root, stem and leaf radish (*Raphanus sativus* L.) (*P<0, 05; **P<0, 01).

and b, confirming that excess lead is damaging to the photosynthetic apparatus (Fig. 1D).

CAT activity increases by the increasing the Pb ions concentration in root, shoot and leaf tissues contaminated plants compared with control. The highest CAT enzyme activity was observed after the administration of 750 mg/l in root tissue of radish (Fig. 2).

The bioaccumulation coefficient/ phytoextraction rate of lead, which was defined as the ratios between µg of metal/g dry weight of shoot, root or leaf and µg of metal/g dry weight of soil was calculated in different parts of radish plant (Table 1). The maximum bioaccumulation coefficient was observed in radish plant in the concentration of 750 mg/l. In roots, however, a high accumulation rate was observed in all the three concentration. It was observed that the potential of radish plants for extraction of metal from soil occur up to a certain level of concentration, after that when the concentration of metal increased, bioaccumulation rate decreased in radish plants.

The effect of Pb on NRA was dependent on the concentration of the metal. A decrease in the activity of nitrate reductase was noticed with increase in metal concentration. Tables 3, represent the statistical analysis of the results found in *Raphanus sativus*. A significant decrease in the NR activity following metal treatment has been observed in free leaf.

Discussion

Heavy metals are conventially defined as elements with metallic properties (ductility, conductivity, stability as cations etc.) and an atomic number _ 20. The most common heavy metal contaminants are Cd, Cr, Cu, Hg, Pb and Zn. Plants grown in metal enriched substrata take up metal ions in varying degrees. 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 (Brooks et al. 1977). Soil remediation is needed to eliminate risk to humans or the environment from toxic metals. Several studies dealing with metal hyperaccumulating plants, and they have concluded that phytoextraction of metals was a feasible remediation technology for the decontamination of metal polluted soils (Chaney 1983; Mc Grath and Zhao 2003; Brown et al. 1994; Brown et al. 1995a, b; Salt et al. 1995). Recent studies looking at the feasibility of phytoextraction, demonstrated that both metal hyperaccumulation and good biomass yields are required to make the process efficient (Nanda-Kumar et al. 1995; Blaylock et al. 1997; Huang et al. 1997). The chemistry of metal interaction with soil matrix is again the important criterion to the phytoremediation concept. Chaney (1997) reported that soils with high cation exchange capacity (CECs) could absorb large amounts of heavy metals than soil with low CEC. Accumulation of lead was higher in root tissue of radish contaminated plants, perhaps the result of a tolerance mechanism developed by the plant in order to reduce heavy metal stress. 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 (Jones and Clement 1972; Khan and Frankland 1983). 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 orthat the toxicity was associated with ionic imbalance involving other other essential trace metals. Therefore, limitation of transfer

Table 2. Bioaccumulation Coefficient (BCA) of lead metal in different parts of radish plant (*Raphanus sativus* L.).

Lead concentration	Root	Stem	Leaf
Control	0	0	0
250 mg/ l	1,40*	1,12*	0,97*
500 mg/l	1,43*	1,16*	0,99*
750 mg/l	1,48**	1,26**	0,87*

(*P<0, 05; **P<0, 01).

Table 3. Effect of lead on NR activity in leaves segments.

Lead concentration	NR activity (µmol NO_2 /hr/g fresh weight)
Control	7,20±0,05
250 mg/l	6,45±0,02*
500 mg/l	3,03±0,13**
750 mg/l	2,36±0,15**

(*P<0, 05; **P<0, 01).

of lead to stem and leaves explained lead tolerance in plants (Ozounidou 1994). In our study, lead treatment caused a decrease in chlorophyll in leaves of moderately and high metal accumulator plants. Van Assche and Clijsters (1990) and Luna et al. (1994) reported that lead stopped the formation of chlorophyll and caused destruction of chlorophyll. The effect of heavy metals on photosynthetic pigments may be due to the heavy metals entering the frond chloroplast with a resulting over-accumulation locally causing oxidative stress and subsequent damage through the peroxidation of the chloroplast membranes (Clemens et al. 20002). Also, heavy metals can directly destroy the structure and function of chloroplast by binding to the –SH groups of enzymes and affect chlorophyll biosynthesis (Baker 1981). Heavy metals inhibit the uptake and transportation of other metal elements such as Mn, Zn and Fe by antagonistic effects and cause the fronds to lose the capacity of pigment synthesis (Cobbett 2000). Heavy metals may also activated the pigment enzyme and accelerate the decomposition of pigment (Helmy Latif 2010).

Cells can be protected from reactive oxygen species by the combined action of enzymatic antioxidant systems like catalase (CAT) and non-enzymatic antioxidant like ascorbate, glutathione and phenolic compounds. In our experiment catalase enzyme activity was increased under metal stress condition. Catalase is one of the major systems for the enzymatic removal of H_2O_2 and the peroxidative damage of cell walls is controlled by the potency of the antioxidative peroxidase enzyme system (Sreenvasulu et al. 1999; Velikova et al. 2000).

Previous studies have found a positive relationship between increased CAT enzyme activity and amounts of heavy metals such as, Pb, Cu and Zn in plant tissue (Girotti 1985; Mazhoudi et al. 1997; Mocquot et al. 1996). These enzymes remove superoxide radicals, which are harmful to cell membranes. Peroxidase activity and photosynthetic pigments are sensitive indicators of heavy metal stress and can be used to anticipate events on the organism level (Wu et al. 2003; Mac Farlane and Burchett 2001.)

Most of the studies on candidate species are mainly based on the interpretation of the analysis of metal concentrations in their plant parts (Nanda-Kumar et al. 1995; Huang and Cunningham 1996; Huang et al. 1997). Therefore, the content value of metal plant or organ seems to be a better estimate for heavy metal extraction efficiency in a given species, and reflects the extent of metals which could be removed by an individual plant. Thus on the basis of bioaccumulation coefficient (BAC) analysis plant can be considered in three groups by their capability of heavy metal uptake and sensitivity to high metal pollution (Bini et al. 1995). With the above mentioned criterions, it was observed that radish plant should be considered as high accumulator plants for Pb. 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 (Van Assche and Clijsters 1990; Madhu et al. 2008).

Inhibition in nitrate reductase activity by metal stress has been reported in sunflower (*Helianthus annuus*), pearl millet (*Pennisetum typhoides*) leaves, as well (Venketramana et al. 1978), maize (Sinha et al. 1994), mungbean and radish (Pandey et al. 2007) and in higher plant (Smarelli et al., 1983). In the present study, intact and excised leaves of treated with Pb showed a markedly different pattern in enzyme activity. The lower concentration of lead considerably increased NRA activity. The possibility of direct influence of pb on enzyme synthesis cannot ruled out. To this base and with considering the metal accumulation capacity, we suggest that radish can be used effectively for phytoremediation processing. This is a novel report about their ability to clean up the contaminated soil by the accumulation of Pb element in plant parts. The benefit of this technology is the potential for low cost remediation. This is accordance with finding of Kabata-Pandias (2000), who stated that Chenopodiaceous is one of the families that are good hyper accumulator of heavy metals. It can be concluded on the basis of the experimental findings of the present study that *Raphanus sativus* is a good accumulator of heavy metal.

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