Journal of**A**pplied **B**iotechnology **R**eports

Original Article

Investigating the effect of Dimercaprol on phytoremediation of lead by *Zea mays*, *Helianthus annuus*, *Sinapis arvensis*

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Abstract

Application of plants to remove heavy metals from soil (phytoremediation) is expanding due to its cost-effectiveness as compared to conventional methods and it has shown a great potential. Since contaminants such as Pb have a limited bioavailability in the soil, methods to facilitate their translocation to the shoots and roots of plants are required for efficient phytoremediation. The present pots experiment were to investigate the effect of Dimercaprol chelator with different concentration (0, 1.5, 3 mmol Dimercaprol chelator kg-1) and also different ranges of Pb (100, 200 mg Pb kg-1) and a control group, on the amount of Pb accumulation, by corn (*Zea mays*), sunflower (*Helianthus annuus*) and mustard (*Sinapis arvensis*). The results showed that the amount of Pb accumulation increased as the Pb concentration was increased. Also the results of this experiment showed that the addition of Dimercaprol chelator is most likely to increase the bioavailability of Pb and consequently the accumulation of this heavy metal in the shoots. The highest accumulation of Pb was noticed with the highest does of Dimercaprol chelator (3 mmol Dimercaprol chelator kg^{-1}) and Pb (200 mg Pb kg-1) in the shoots of *Helianthus annuus*.

Keywords: Phytoremediation, Lead, Dimercaprol, Corn (*Zea mays*), sunflower (*Helianthus annuus*), Mustard (*Sinapis arvensis*)

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Submission Date: 4/3/2014
Accepted Date: 5/14/2014

Phytoremediation is a cleansing technique for soils, which uses the ability of metal accumulator plants to extract metal from polluted soil with their roots and to concentrate these metals in above-ground plant parts and the metal-accumulating plant material, can be securely harvested and removed from the site without the extensive excavation [1-8].

Lead (Pb) is potential pollutants that readily accumulate in soils and sediments. Apart from natural disasters such as volcano, lead contamination of the environment has resulted from industrial activities such as mining and smelting processes, agricultural activities such as application of insecticide and municipal sewage and sludge, paint, batteries, and other material [9]. Eventually, Pb transfers to the food chain and accumulates in the body of humans and endangers their health [10].

The Pb-contaminated soils are difficult to remediate with natural phytoremediation that employs hyperaccumulators, because natural hyperaccumulator plants species generally exhibit slow growth and low biomass making the remediation process effective over a long time [11].

In order to overcome such drawbacks, the use of high- biomass producing crop plants such as corn (*Zea mays*), sunflower (*Helianthus annuus*) and mustard (*Sinapis arvensis*) with a chemically enhanced method has been proposed as a viable strategy to eliminate metals from soils in a reasonable time frame [12-15].

In order to enhance the accessibility of Pb in soil solution and its translocation from root to shoot, application of some chelating agents such as ethylene diaminetetraacetic acid (EDTA), diethylenetrinitrilopentaacetic acid (DTPA), nitrilotriacetic acid (NTA), ethylenediaminedisuccinate (EDDS) and Dimercaprol have been proposed by various workers [12, 16-21]. Dimercaprol or British anti-Lewisite (BAL), which was developed as an antidote for lewisite, is used medically in treatment of arsenic, mercury, gold, lead, antimony, and other toxic metal poisoning. BAL is an efficient chelator [21-23] and its probable to have a good potential to increase the concentration of various metal specially lead in above ground plant tissues. The aim of present experiment was to study the effects of different concentration of Pb and BAL on phytoextraction potentiality of lead in artificial contaminated soil, to increase transfer of Pb from contaminated soil to above ground parts in corn (*Zea mays*), sunflower (*Helianthus annuus*) and mustard (*Sinapis arvensis*).

Efforts have been made to understand the mechanisms by which cells react when exposed to exogenous metals. Proteomics is an important tool for elucidating the role of various proteins in stress tolerance and adaptation. Proteomics provides direct information of the dynamic protein expression in tissues or whole cells, thus making global analysis possible. Owing to the significant accomplishments of genomics and bioinformatics, systematic analysis of all expressed cellular components has become a reality in the post-genomic era, and attempts to grasp a comprehensive picture of biology have become possible [10]. Two-dimensional polyacrylamide gel electrophoresis (2D PAGE) in combination with mass spectrometry is currently the most widely used technology for comparative bacterial proteomics analysis [11].

In this study RSM is used to determine the optimum pH, iron concentration, temperature, and nitrogen concentration, on the growth and activity of indigenous bacterium that has been isolated from Koshk Zn/Pb sulfide mine, central of Iran. In addition the ability of this bacterium for extracting zinc and lead has also been studied. We have also investigated the response of the strain to external exposure of the exogenous metals Pb^{2+} and Zn^{2+} and characterized the differential expression profile of protein expression by 2D PAGE and mass spectrometry.

Materials and Methods

Pot experiment was established with corn (*Zea mays*), sunflower (*Helianthus annuus*) and mustard (*Sinapis arvensis*). In the Botanical Garden of the Department of applied Biotechnology, Baghiatallah University, Tehran, Iran. The seeds were collected from Iranian Research Institute of Plant Protection, Tehran, Iran.

The soil used in this experiment was peat moss (Italy). Some physical and chemical properties of soil are given in Table 1.

Table 1. Physical and chemical properties of soil

Parameter	Value
Organic Carbon (gkg^{-1})	52.2 %
Organic Nitrogen (gkg^{-1})	1 %
Organic Materials (gkg^{-1})	99.95%
Maximum Humidity	40-50%
Soil pH	4.8-5.3
EC (ms/cm)	<0.2
CEC (Cmol(+)/Kg)	8.28
Available P (gkg^{-1})	0.43
Exchangable K (gkg^{-1})	0.68
Soil Ash	1-5 %
Natural Peat Moss	H1- H4
Lead Concentration	Near to 0 ppm

Each pot was supplied with a different concentration of Pb and BAL with 3 times repetition; they were dissolved in deionized water. BAL used in this experiment was 2 ml ampoules containing 50 mg/ml (100 mg/ampoule) 5% dimercaprol dissolved in almond oil and 10% benzyl benzoate. There were two different concentrations of lead as $Pb(NO_3)_2$ (100, 200 mg kg^{-1}) and three concentration of BAL (1.5, 3 mmolkg-1) and a control which was not treated with BAL. $Pb(NO_3)_2$ (Merk, Germany) and BAL (Serb Laboratories, France) were added to the soil by a spraying method.

The FC (field capacity) of the soil was determined and the Water content of the soil was adjusted to 70% of field capacity [24]. The pots were incubated to ensure soil equilibrium for one month under natural light at a minimum temperature of 10–11°C and maximum of 25–30°C and a relative humidity of about 30–40%. During incubation the soil's humidity was kept constant at the level of the field capacity.

The seeds were grown in 80L × 40W × 20 cm Hsized pots, each filled with 4 kg soil. The seeds were pre-soaked in 0.5% hypochlorite for 1 minute, and then 70% alcohol for 1 minute and finally they were thoroughly washed with deionized water and sown in the pots. Fertilizers were added to the pots after six weeks of germination. The plants were harvested after sixteen weeks. Plants were harvested by cutting the shoots from the soil surface and washed with deionized water until they were free of soil and other unwanted substances. Plant shoots were dried for 48 hours at 70°C and were ground. 1 gram of each Sub- sample of ground plant material was weighted and incinerated by furnace for 5 hours at 470 °C.

Pb was determined after dry digestion of dried and ground sub-samples in Hypochlorous acid (1 N) (Merk, Germany) [25]. The digested solution were filtered through the Whatman filter paper 40, and then diluted with deionized water to a volume of 50ml in flask. Samples of Pb extracts were analyzed by atomic absorption spectrophotometer.

Statistical Analysis

Analysis of variance (one-way and two-way ANOVA) was performed to determine which means differed significantly ($\alpha=0.05$). Statistically significant differences ($p<0.05$) are reported in the text and shown in the figure and tables.

Results

Effects of Lead on Growth

For *Zea mays* there were no disruption seen in growth and the plant continued to rise till the end of the germination period. Similarly for *Helianthus annuus* germination was preceded till week four. But after that the growth range was slightly reduced to the chemical fertilizers were added and peaked the amount of growth. Generally it seems that lead toxicity more or less affected the pace of germination in *Helianthus annuus*. Also the lead adversely affected the growth of *Sinapis arvensis*. Only after adding fertilizers a huge amount of plants were appeared in *Sinapis arvensis*.

The lead accumulation in plants without BAL

All plants showed maximum accumulation of Pb in shoots at 200 mg Pb kg^{-1} soil treatment (53.35, 78.87 and 39.15 respectively for *Zea mays*, *Helianthus annuus* and *Sinapis arvensis*) as compared to treated groups at 100 mg Pb kg^{-1} soil and untreated control groups. Results showed that the increasing of Pb concentration in soil from 100 mg Pb kg^{-1} to 200 mg Pb kg^{-1}, significantly ($p<0.05$) increased the Pb accumulation in shoots in all three plants (Fig. 1).

The effect of chelating agent BAL on lead accumulation

Zea mays

The results of present pot experiment for *Zea mays* for 100 mg Pb kg^{-1} treatments showed that the concentration of 1.5 mmol BAL kg^{-1} did not make a significant change in lead accumulation. However comparing the 3 mmol BAL kg^{-1} treatments with the 1.5 mmol BAL kg^{-1} and control groups indicated a significant ($p<0.05$) increasing in Pb uptake with the highest accumulation of 60.32 mg Pb kg^{-1} for the mentioned treatments (100 mg Pb kg^{-1}). Moreover results for 200 mg Pb kg^{-1} treatments showed that all concentrations of BAL (1.5 and 3 mmol BAL kg^{-1}) significantly ($p<0.05$) increased Pb accumulation, with the most concentration of Pb for BAL 3 mmol BAL kg^{-1} (81.85 mg Pb kg^{-1}) and the least for the control (without BAL treated plants) group (53.35 mg Pb kg^{-1}) (Fig. 1).

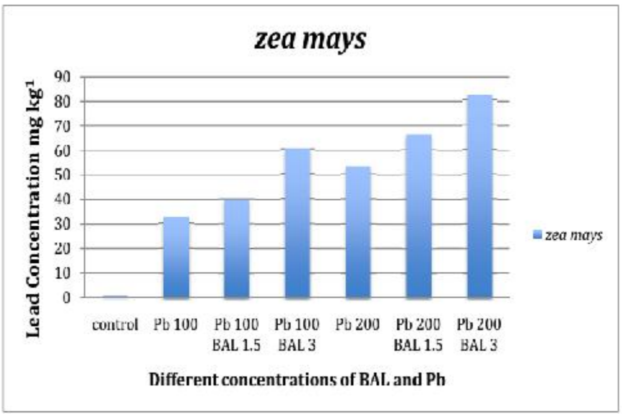

Figure 1. Effect of different concentrations of BAL and Pb on Lead accumulation in *Zea mayes* shoots

Helianthus annuus

Investigating the results of *Helianthus annuus* pots for 100 mg Pb kg^{-1} treatments, represented that there was a significant ($p<0.05$) increase for BAL 1.5 mmol BAL kg^{-1} in compare with the control group (without BAL treated plants), with the highest amount of accumulation (53.72 mg Pb kg^{-1}). While no significant ($p>0.05$) change was seen between BAL 1.5 and BAL 3 after the application of BAL 3 mmol BAL kg^{-1}. Furthermore the results for 200 mg Pb kg^{-1} treatments demonstrated that, all the concentrations of BAL (1.5 and 3 mmol BAL kg^{-1}) significantly ($p<0.05$) increased Pb accumulation in shoots of *Helianthus annuus* as compared to control group. While it was shown that, application of BAL 3 comparing to BAL 1.5 have made a significant ($p<0.05$) increase in the uptake of Pb (Fig. 2).

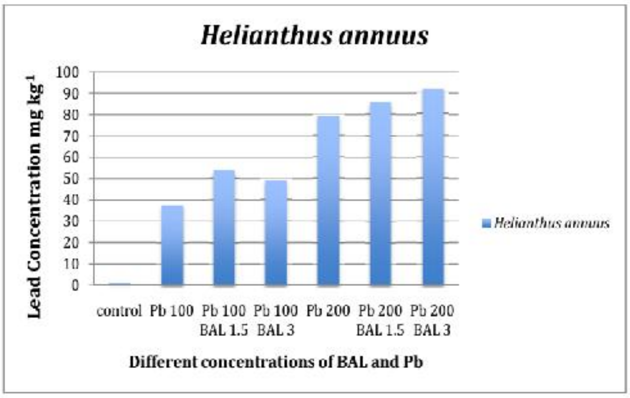

Figure 2. Effect of different concentrations of BAL and Pb on lead accumulation in *H. annuus* shoots

Sinapis arvensis

Considering the results of 100 mg Pb kg^{-1} treatments, for the regarded plant, indicates that however there was a slight increase in the accumulation of Pb for BAL 1.5 mmol BAL kg^{-1} comparing to the control group (without BAL treated plants), but it was not significant ($P>0.05$). Nonetheless the application of BAL 3 mmol BAL kg^{-1} significantly ($P<0.05$) increased the accumulation of lead in shoots of *Sinapis arvensis* in compare to BAL 1.5 and the control group. Moreover the pot experiments for 200 mg Pb kg^{-1} showed that, while treatments of BAL 1.5 and BAL 3, significantly ($P<0.05$) increased Pb accumulation in compare with the control group, there was not any significant ($P>0.05$) change in between of them (Fig. 3).

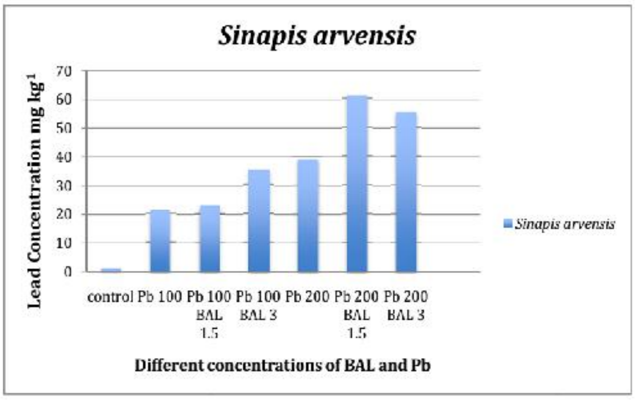

Figure 3. Effect of different concentrations of BAL and Pb on lead accumulation in *S. arvensis* shoots

The results of present investigation shows that in over 75% of cases BAL treated pots increased the accumulation of lead in the shoots of regarded plants, which this confirms the earlier findings that chelators are effective in increasing the bioavailability of Pb for roots and consequently its successful transfer to above ground parts (stem and shoots) of mentioned plants.

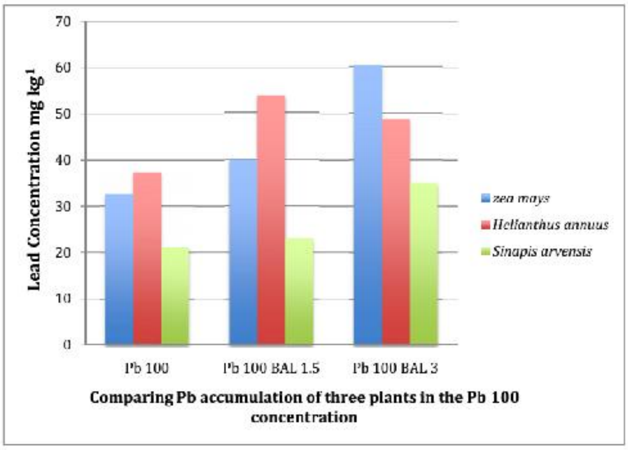

Figure 4. Comparing Pb accumulation of three plants in the pb 100 ppm

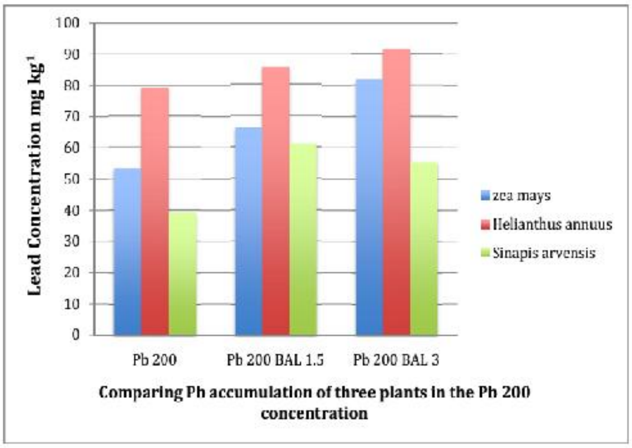

Figure 5. Comparing Pb accumulation of three plants in the pb 200 ppm

Discussion

In this experiment it was shown that *Zea mays* had a good capacity to tolerate the wide range of Pb toxicity [26]. However the toleration of *Helianthus annuus* was more or less similar to *Zea mays*, but Pb had a slight effect on the growth range. Also in *Sinapis arvensis*, Pb induced a toxic effect on the germination factor. Considering this matter, it seems *Zea mays* had the most sustainability for Pb toxicity and is an ideal candidate for phytoremediation. Various workers reported similar results earlier [7, 27, 28]. The reasons why *Helianthus annuus* and *Sinapis arvensis* showed lower ranges of toleration, in addition to primary stress of Pb toxicity, might be as a result of low water potential, biomass reduction, interrupt nutrient uptake and secondary stress such as oxidative stress [29]. Furthermore Pb can also disturb microtubule structure in meristematic cells [30]. The results of Pb accumulation, demonstrated that all three plants were capable to uptake Pb [5, 12, 31] (Fig. 4). It was shown that the amount of Pb accumulation increased as the Pb concentration was increased (Fig. 5). Also the results of this experiment showed that the addition of BAL is most likely to increase the bioavailability of Pb from soil to roots and from roots to shoots, and in 75% of cases of the present pot experiment, this concept was confirmed. Investigating the results showed that *Helianthus annuus* is meant to have the highest range of Pb accumulation in its shoots in comparison with the other two plants, in most cases and also generally the most accumulation was seen in this plant for 3 mmol BAL kg^{-1} (200 mg Pb kg^{-1} treatment). Therefore, although it's lower toleration compared to *Zea mays*, it is also an ideal candidate for phytoremediation [32, 33, 34]. In *Zea mays* the 1.5 mmol BAL kg^{-1} and 3 mmol BAL kg^{-1} group (in all Pb concentrations), significantly ($p<0.05$) increased Pb accumulation comparing the control (EDTA non-treated plants) and 1.5 mmol BAL kg^{-1} groups respectively. Also this significant increasing was seen in 1.5 mmol BAL kg^{-1}, 3 mmol BAL kg^{-1} (100 mg Pb kg^{-1} treatment) and 3 mmol BAL kg^{-1} (200 mg Pb kg^{-1} treatment) comparing regarded controls, for *Helianthus annuus* plant. Similar results were also seen for 3 mmol BAL kg^{-1} (100 mgPbkg-1 treatment) and 1.5 mmol BAL kg^{-1}, 3 mmol BAL kg^{-1} (200 mg Pb kg^{-1} treatment) comparing regarded controls, for *Sinapis arvensis*. Also the 1.5 mmol BAL kg^{-1} (100 mg Pb kg^{-1} treatment) group of this plant showed the least accumulation, comparing all BAL treated cases. Results from this experiment are in agreement with the view that Dimercaprol, like other chelators such as EDTA, is potentially an efficient chelating agent to increase Pb accumulation in the regarding plant shoots.

Conclusion

The results of present pot experiment showed that these three plants could more or less tolerate a wide range of Pb concentrations, and accumulate high concentrations of Pb in their aboveground parts (shoots). And also BAL is potential to dissolve the metal in the soil and also enhance the accumulation of Pb in shoots of regarding plants with the 3 mmol BAL kg^{-1} treat. The phytoremediation ability of these plants could be amplified by genetic engineering techniques in further investigations. Since using these chelators, which are available in today's market, are not affordable for bigger project, it's better to find an alternative for these kinds of chelators.

Acknowledgement

The authors would like to thank Ms. Sama Kamalpoor for her valuable and constructive suggestions during the planning and development of this research work. And also the Iranian Research Institute of Plant Protection for providing the seeds.

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