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Research article

INDIAN MUSTARD *BRASSICA JUNCEA* L. MEDIATED PHYTOREMEDIATION OF LEAD

Vikas Sarsar[1],Hardeep[2],krishan k Selwal[3],Ranjeet S Tanwar[4],Pankaj K. Tyagi[5],Anami Ahuja[6]*

[1,3]Department of Biotechnology, Deenbandhu Chotu Ram University of Science and Technology, Murthal, Sonipat, India

[2]Department of Environment Studies, Kurukshetra University, Kurukshetra, India

[4]Department of Biotechnology, N.C College of engineering, Israna , India

[5]Department of Biotechnology, Meerut Institute of Engineering and Technology, Meerut, India

[6]Stem Fort Regenerative Medical center, Rohini, New Delhi, India

*Corresponding author: anami.genetics@gmail.com

ABSTRACT: The contamination from heavy metals has risen during the last decade due to increase in Industrialization. This has led to a significant increase in health problems. Many of the known remediation techniques to remove heavy metal from soil are expensive, time consuming and environmentally destructive. Phytoremediation is an emerging technology for removal of heavy metals which is cost effective, and has aesthetic advantages and long term applicability. The present study aims at efficiently utilizing Brassica juncea L. to remove lead (Pb). The result of our study indicate that amount of lead in Indian mustard is increased with the amount of EDTA applied to the soil and maximum accumulation was achieved with 5mmol/kg of EDTA. On further increase in EDTA resulted in leaf necrosis and early shedding of leaves. Therefore EDTA at a concentration of 5mmol/kg was considered optimum for lead accumulation by *Brassica juncea L.*

Key words: Heavy metals, Phytoremediation, EDTA, metals toxicity, Brassica juncea L**.**

INTRODUCTION

Heavy metal contamination of soil and water is one of the most serious environmental problems across the world due to their toxicity to human, animals, plants and microbes (Singh et al., 1997; Meagher, 2000; Chandra et al., 2009). Heavy metals, including lead, are present in soils either as natural components or as the result of human activity. Metal-rich mine tailings, metal smelting, electroplating, gas exhausts, energy and fuel production, downwash from power lines, intensive agriculture, and sludge dumping are the human activities that introduce the largest quantities of lead into soils .Lead is highly toxic and if ingested can accumulate in body organs, including the brain, and result in various degrees of lead poisoning. At high levels of exposure, lead can cause severe damage to the brain and kidneys of adults and children often resulting in death. Lead is estimated to have a soil retention time of about 150–5000 years and is reported to maintain high concentration for as long as 150 years after sludge application to soil. Lead phytoremediation technology can only be feasible if systems can be developed to employ high biomass plants, which are capable of accumulating more than 1% lead in shoots and produce more than 20t of biomass ha $^{-1}$ yr (McGrath et al., 2003).Lead has limited solubility in soil and therefore is not easily available for plant uptake due to the formation of complex with organic matter, sorption on oxides and clays, and precipitation as carbonates, hydroxides and phosphates. Because of its limited bioavailability, an approach to increase its bioavailability is essential for the success of Phytoremediation. A few plants are known to hyper accumulate lead such as *Thlaspi rotundifolium, T. alpestre, Alyssum wulfenianum, Polycarpaea synandra, Armeria martima,* few bryophytes and lichens etc. Quite often, metal poisoning leads to the elevated production of reactive oxygen species (ROS), which can damage macromolecular compounds in cells: proteins,lipids and nucleic acids (Rascio et al.,2011) . The current remediation techniques of heavy metals from contaminated soil or water are expensive, time consuming and environmentally destructive **(**Chhotu et al.,2009).

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An alternative to conventional technologies is phytoremediation, in which specially selected plants with a particular high affinity for heavy metals are used to restore degraded soils (Chhotu et al.,2009; Salt et al.,1998; Pilon &Smits, 2005). Phytoremediation makes use of the ability of green plants to accumulate or degrade contaminants (Pulford et al., 2003). Phytoremediation is a cost effective, environmental friendly, aesthetically pleasing approach most suitable for developing countries (Ghosh & Singh 2005). Phytoremediation can be carried out in a number of ways. In the process known as phytostabilization, plants convert contaminants to less assimilable forms, as a result of which the pollutants are not transported to the upper parts of the plants but remain locked in the rhizosphere. In phytodegradation, contaminants are decomposed within the plant following their uptake by the root system or outside the plant under the influence of plant enzymes secreted into the environment. Plants can also transform contaminants to usually less toxic, volatile forms, a process known as phytovolatalization. In phytostimulation, contaminants decompose in the presence of the micro-organisms present in the rhizosphere. Finally, there is phytoextraction, in which plants accumulate heavy metals in their above-ground organs (Evangelou et al.,2007; Gardea-Torresdey et al 2005; Alkorta et al.,2004; Garbisu et al., 2001;Pilon-Smits et al.,2000). The success of phytoremediation depends mainly on the choice of plant, which must obviously possess the ability to accumulate large amounts of heavy metals. These plants should satisfy the following criteria:

1. The concentration of heavy metals in the shoots should be 50–100 times greater than in 'normal' plants (Jabeen et al., 2009)
2. The bioaccumulation coefficient (the ratio of the concentration of a toxic substance in the tissues of an organism to its concentration in the living environment of that organism) must have a value greater than 1 (McGrath et al., 2003)
3. Metal concentrations in the shoots should be higher than in the roots (Jabeen et al., 2009)
4. Fast growth and high accumulating biomass (Marchiol et al.,2004);
5. Easily grown as an agricultural crop and fully harvestable (Marchiol et al.,2004).

For more than 40 years, chelating agents, such as EDTA are used to enhance the accumulative potential of plants. These compounds substantially intensify the uptake and translocation of metals in plants in that they release metals from the soil and form soluble complexes with them, which are then transported by the xylem and deposited in the leaves. Uptake efficiency depends on the metals affinity for the chelate. Themobility of heavy metals in the soil can also be manipulated by altering its pH: a higher pH > 6.5 significantly reduces the quantity of readily soluble forms of metals in the soil and limits their uptake and accumulation by plants .Another approach to remove heavy metals, radionuclides and orgainc contaminants from contaminated soil and groundwater is electrodic and electro kinetic remediation. This technology employs the application of high–voltage, low level direct current to the polluted soil using electrodes placed in the ground to remove contaminants. Among the plants of the *Brassica* species, the *Brassica juneca* deserve special attention because its relevance to the process of phytoexctration of heavy metals from soil has been confirmed in many experiments. It has been found that *B. juncea* exhibits a high capacity to accumulate Cd- mainly in the shoots, where Cd level was recorded at level of 1450 µg Cd/g dry wt. This is three times more than reported in *Brassica napus* (555 µg/g dry wt) (Nouairi et al.,2006).These plant exhibit a high removal efficiency of other metals such as Pb (28% reduction) and Se (reduced between 13–48%) (salt et al., 1998).However *Brassica juneca* needs to be harvested shortly after the plant becomes mature, which causes problems of disposal of obtained biomass. When these plants are dried, they easily crumble and flake off, greatly reducing the yield obtained, and the rest of the plant residues are a source of secondary emissions of toxic substances. The application of high potential plants from the *Brassicaceae* family for bioaccumulation of heavy metals along with management of plant matter after phytoremediation process, would emerge as one of the most important technologies for cleaning the heavy metals components from the environment.(Marzena Szczygłowska et al., 2011).The objective of our study was to investigate the combination of EDTA and electrodic phytoremediation for the removal of lead contamination from soil.

MATERIALS AND METHODS

Soil collection and treatment

The soil was collected from the garden and air dried and then it was passed through a 1.0 cm sieve. The soil was then autoclaved and was thoroughly mixed with lead chloride ($PbCl_2$), 400mg/kg of soil. The homogenized soil was subsequently transferred to 15 cm. plastic pots. The pH of the homogenized soil was around 5.5.

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Cultivation practices and set up

Indian mustard seeds (*Brassica juncea*) were directly sown in pots with lead containing soil. The plants were grown for 1 month in the pots before phytoremediation experiments involving various factors were initiated. They were watered every 2nd day and fertilized with 1/4th strength MS salts (Murashige and Skoog, 1962) weekly.

Experimental setup

The first set of experiment was setup to study the effect of different concentration of EDTA on lead accumulation. Different concentrations of EDTA (3mmol/kg, 4mmol/kg, and 5mmol/kg) was added to different pots and labeled accordingly. One pot was kept without EDTA that acted as a negative control. The experiments were performed in duplicate. The plants were harvested 10 days following treatment with EDTA.

The second set of experiment was setup to study electrodic phytoremediation. The eletrodic phytoremediation system included electrodes, a power supply, EDTA and plants. Copper wires were used for electrodes. DC power supply was used to supply electric power providing 40v and 1A current.

Sample preparation and analysis

The plants were harvested after 10 days of EDTA treatment and after first week of application of electric current. The plant samples were dried in an oven at about 60^{o}C and were homogenized using motar and pestle. The samples were prepared by ashing method and the ash was extracted by 2.5ml of dilute HCl (20%(v/v)), heated for 10 min and allowed to cool. The extract was filtered through Whatmann No. 1 filter paper and diluted with distilled H_2O to make the final volume upto 100ml. Lead concentrations were determined by UV-VIS spectrophotometer. Lead standards (10 ppm) were used to prepare standard curve and lead concentration in samples was determined.

RESULTS AND DISCUSSION

In the present study, the combination of EDTA-enhanced phytoremediation with electrodics for lead in Indian mustard was investigated. The addition of EDTA was shown to significantly increase the accumulation of lead in B*rassica juncea*. However, the use of electric potential with EDTA caused increased phytoremediation to many folds. The result of these experiments demonstrates the feasibility of phytoremediation in relation to the high concentration of lead in the soil. The plants were harvested after 10 days of EDTA treatment and the results were analysed. . It was found that the amount of lead accumulated increased with the application of electric potential and increasing EDTA concentration clearly indicating the role of electrodic remediation in removing heavy metals from contaminated soil. However, at high EDTA concentration it proved to be necrotic for the plants resulting in burning effect. (Table 1, 2, 3).

Table 1:- Represents the Lead concentrations that were determined by UV-VIS spectrophotometer.

S.No.	Sample	Lead Concentration (µg/ml)	Absorbance (235 nm)
1.	Standard	10.00	0.021
2.	Sample a	7.35	0.390
3.	Sample b	7.75	0.507
4.	Sample c	7.90	0.546
5.	Sample d	9.15	0.620
6.	Sample e	9.70	0.780
7.	Sample f	10.95	1.170
8.	Sample g	10.60	1.365

In Table 1:-
Standard: represents Lead standard sample at 10 ppm
Sample a: represents plant grown on soil containing 400mg/kg of Pb
Sample b: represents plant grown on soil containing 400mg/kg of Pb +3mmol/kg of EDTA
Sample c: represents plant grown on soil containing 400mg/kg of Pb +4mmol/kg of EDTA
Sample d: represents plant grown on soil containing 400mg/kg of Pb +5mmol/kg of EDTA
Sample e: represents plant grown on soil containing 400mg/kg of Pb +3mmol/kg of EDTA+ electric potential of 40v
Sample f: represents plant grown on soil containing 400mg/kg of Pb +4mmol/kg of EDTA+ electric potential of 40v
Sample g: plant grown on soil containing 400mg/kg of Pb +5mmol/kg of EDTA+ electric potential of 40v

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Table 2:- Represent the effect of EDTA concentration on amount of Pb accumulated by 0.2g of dried plant sample

S.No.	EDTA Concentration (mmol/kg)	LEAD Concentration (mg/kg)
1.	0	67
2.	3	87
3.	4	95
4.	5	107

Table 3:- Represents the combined effect of application of electric potential and different EDTA concentration on amount of Pb accumulated by 0.2g of dried plant sample

S.NO.	EDTA CONCENTRATION* (mmol/kg)	LEAD CONCENTRATION (mg/kg)
1.	3	135
2.	4	187
3.	5	230

*Electric potential of 40V for 15 min were supplied to each concentration EDTA.

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REFERENCES

A.A. Belimov, N. Hontzeas, V.I. Safronova, S.V. Demchinskaya, G. Piluzza, S. Bullitta, B.R. Glick (2005). Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (Brassica juncea L. Czern.). Soil Biology & Biochemistry .37:241–250.

Alkorta, I.; Hernandez-Allica, J.; Becerril, J.M.; Amezaga, 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. Rev. Environ. Sci. Biotech., 3:71–90.

Chandra, R., R.N. Bhargava, S. Yadav and D. Mohan (2009). Accumulation and distribution of toxic metals in Wheat (Triticum aestivum L.) and Indian mustard (Brassica compestries L.) irrigated with distillery and tannery effluent. J. Haz. Mat.

Chhotu, J.D, Fulekar, M.H.(2009).Phytoremediation of heavy metals: Recent techniques. Afr. J.Biotechnol., 8:921–928.

Evangelou, M.; Mathias-Ebel, M.; Andreas-Schaeffer, A.(2007). Chelate assisted phytoextraction of heavy metals from soil. Effect, mechanism, toxicity, and fate of chelating agents. Chemosphere .68:989–1003.

Garbisu, C.; Alkorta, I.(2001). Phytoextraction: A cost-effective plant-based technology for the removal of metals from the environment. Bioresour. Technol.,77:229–236.

Gardea-Torresdey, J.; Peralta-Videa, J.; Parsons, J.G.(2005). Phytoremediation of heavy metals and sudy of the metal coordination by X-ray absorption spectroscopy. Coord. Rev.Chem., 249:1797–1810.

Ghosh , Singh(2005). A Review On Phytoremediation Of Heavy Metals And Utilization Of Its By Products. Applied Ecology And Environmental Research.3(1): 1-18.

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

Jabeen, R.; Ahmad, A.; Iqbal, M.(2009). Phytoremediation of heavy metals: Physiological and molecular mechanisms. Bot. Rev.,75:339–364.

Kuldeep Bauddh, Rana P. Singh,(2011).Differential toxicity of cadmium to mustard (Brassica juncia L.) genotypes under higher metal levels. J. Environ. Biol.,32:355-362 (2011)

M. Turan, A. Esringü(2007). Phytoremediation based on canola (Brassica napus L.) and Indian mustard (Brassica juncea L.) planted on spiked soil by aliquot amount of Cd, Cu, Pb, and Zn. PLANT SOIL ENVIRON., 53, (1):7–157

Marchiol, L.; Assolari, S.; Sacco, P.; Zerbi, G.(2004).Phytoextraction of heavy metals by Canola (Brassica napus) and radish (Raphanus sativus) grown on multicontaminated soil.Environ. Pollut.,132: 21–27

Marzena Szczygłowska , Anna Piekarska, Piotr Konieczka and Jacek Namiesnik (2011). Use of Brassica Plants in the Phytoremediation and Biofumigation Processes. Int. J. Mol. Sci., 12:7760-7771

McGrath, S.P.; Zhao, F.J.(2003). Phytoextraction of metals and metalloids from contaminated soils.Curr. Opin. Biotechnol.,14: 277–282.

Meagher, R.B.(2000). Phytoremediation of toxic elemental and organic pollutants .C. Op. Plant Biol., 3:153-162

Nouairi, I.; Wided Ben Ammar, W.; Youssef, N.; Douja Ben Miled Daoud, D.B.; Habib Ghorbal, M.;Zarrouk, M.(2006). Comparative study of cadmium effects on membrane lipid composition of Brassica juncea and Brassica napus leaves. Plant Sci.,170:511–519.

Pilon-Smits, E.(2005). Phytoremediation. Annu. Rev. Plant Biol., 56:15–39.

Pilon-Smits, E.; Pilon, M.(2000). Breeding mercury-breathing plants for environmental cleanup. Trends Plant. Sci.,5: 235–236.

Pulford, I.D.; Watson, C(2003). Phytoremediation of heavy metal-contaminated land by trees. A review.Environ. Int., 29:529–540.

Rascio, N.; Navari-Izzo, F.(2011). Heavy metal hyperaccumulating plants: How and why do they do it?And what makes them so interesting? Plant Sci.,180:169–181

S.P., Zhao, F.J.(2003). Phytoextraction of metals and metalloids from contaminated soils.Curr. Opin. Biotechnol.,14:277–282.

Salt, D.E.; Smith, R.D.; Raskin, I.(1998). Phytoremediation. Annu. Rev. Plant Physiol. Plant Mol. Biol., 49:643–668.

Singh R.P., G. Dhania, A. Sharma and P.K. Jaiwal(2007).Biotechnological approach to improve phytoremediation efficiency for environmental contaminants. In: Environmental bioremediation technologies. (Eds.: Singh S.N., R.D. Tripathi). Springer. pp. 223-258

Singh, R.P., S. Dabas, A. Choudhary and R. Maheshwari(1997). Effect of lead on nitrate reductase activity and alleviation of lead toxicity by inorganic salts and 6-benzylaminopurine. Biol. Plant., 40: 339-404.