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COMPARATIVE STUDY OF NATURAL PHYTOEXTRACTION AND INDUCED PHYTOEXTRACTION OF LEAD USING MUSTARD PLANT (*BRASSICA JUNCEA ARAWALI*)

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Abstract: The objective of the present study was to assess the extraction efficiency of two different synthetic chelants [Ethelyne diamine tetra acetic acid (EDTA) and Salicylic acid (SA)] for desorbing Pb from Pb contaminated soil. Pot experiments were conducted in randomized block designing. First group of plants were treated with different Pb concentrations like 0, 100, 200, 400 and 800 mg/L. Second group of plants were treated with same Pb concentrations along with Chelant EDTA of 2.4 mM and third group of plants were also treated with same Pb concentrations along with Chelant salicylic acid (2.4 mM). EDTA and SA were applied after 6 weeks of growth i.e., at rosette stage. Different physical and biochemical parameters were studied. The concentrations of Pb in plant samples were determined by Atomic Absorption Spectrophotometer. It was found that Pb accumulation in mustard plant was significantly more enhanced by EDTA than that of SA. Chelants were found to improve Pb uptake by plants. In conclusion, chelate-assisted phytoextraction showed better results than continuous phytoextraction. *B. juncea arawali* is a suitable candidate for chelate-assisted phytoextraction of Pb.

Keywords: Phytoextraction, *Brassica juncea arawali*, Lead, EDTA, Salicylic acid

INTRODUCTION

Soil contamination due to heavy metals is a major environmental problem facing the current world. Although a small portion of heavy metals in soils is derived from natural processes (e.g., bedrock weathering), a much higher amount originates from anthropogenic sources such as the mining and smelting industry, use of mineral fertilizers and pesticides, sewage sludge application and in the case of Pb, from the former use of leaded gasoline [1]. Traditional remediation methods are generally costly (soil excavation and dumping) or require long-term monitoring/maintenance (stabilization) and harmful to soil properties. Hence scientists and environmentalists put emphasis on phytoextraction as an alternative remediation method which is cost-effective and environmentally friendly phytoremediation mechanism.

Phytoextraction is the accumulation of contaminants/pollutants in the above ground parts of the plant from the contaminated environment. The success of this process depends up on biomass production and metal concentration in plan shoots [2]. Research on phytoextraction has been started using hyper accumulators like *Thalspi caerulescens*. Hyper accumulators are plants which grow on highly contaminated soils and accumulate high concentrations of pollutants in their tissues [3, 4]. For example, *T. caerulescens* accumulated about 33600 mg/kg Zn in shoots in hydroponics condition, *Ipomea alpine* and *Haumaniastrum katangense* could accumulate about 12300 mg/kg Cu and 19800 mg/kg Zn in their leaves, respectively [5]. This process of phytoextraction using hyper accumulators is called as continuous phytoextraction. However, many of hyper accumulators are slow growing and produce small amounts of biomass. Thus, they cannot remove large quantities of heavy metals per unit of land area in a given period of time [6]. Some plant species are capable to accumulate and tolerate moderate to high levels of heavy metals in their tissues as well as produce a relatively large biomass. For example, some varieties of corn (*Zea mays L.*), barley (*Hordium vulgaris L.*) and ryegrass (*Lolium perenne L.*) have demonstrated significant heavy metal tolerance [7].

Phytoextraction mechanism has its own limitations i.e., low mobility and bioavailability of some heavy metals (especially Pb) in polluted soils [1]. An increase of heavy metal mobility can be achieved by adding synthetic chelants which are capable of solubilisation and complexion of heavy metals into the soil solution as well as promote heavy metal translocation from roots to the harvestable parts of the plant [8, 9]. This mechanism is known as Induced phytoextraction.

Ethylene Di-amine Tetra acetic Acid (EDTA) is the most common chelant for heavy metal phytoextraction especially for Pb phytoextraction [9, 10]. However, EDTA can possibly leach down the soil profile and therefore pose an important environmental risk for ground water quality [11]. We have taken salicylic acid (SA) as chelant to do comparative study of EDTA and SA in enhancement of heavy metal accumulation.

Objective:

The objective of the present study was to assess the extraction efficiency of two different synthetic chelants [Ethelyne diamine tetraacetic acid (EDTA) and Salicylic acid (SA)] for desorbing Pb from Pb contaminated soil.

MATERIALS AND METHODS

Under field conditions a pot experiment was conducted to study the comparative effect of EDTA as well as SA on Pb accumulation by mustard plants (*Brassica juncea arawali*).

The experimental site:

Experiments were conducted at the Micromodel experimental site of the Indian Institute of Technology, Delhi. It is situated at 77.09°E longitude and 20.45°N latitude, and 28 m altitude above sea level. The mean maximum and minimum temperature during the study period were 18-43°C and 3-15°C, respectively. The field soil used for experiment was sandy loam with organic carbon 0.72 %, available N 272 kg/ha, available P 9.0 kg/ha, available K 200.7 kg/ha and pH 7.5.

Chelant treatments:

The seeds of *Brassica juncea arawali* were procured from the National seeds Corporation Ltd., Beej Bhawan, Pusa, New Delhi. Treatments were designed as shown in Table.1.

Table.1: Designing of chelant treatments

Treatment No.	Treatment Name	Treatment No.	Treatment Name	Treatment No.	Treatment Name
T1	Control	T6	Control EDTA	T11	Control SA
T2	100 ppm Pb	T7	100 ppm Pb + EDTA	T12	100 ppm Pb + SA
T3	200 ppm Pb	T8	200 ppm Pb + EDTA	T13	200 ppm Pb + SA
T4	400 ppm Pb	T9	400 ppm Pb + EDTA	T14	400 ppm Pb + SA
T5	800 ppm Pb	T10	800 ppm Pb + EDTA	T15	800 ppm Pb + SA

About 20 seeds were sown in 11x11 cm pots containing unsterilized field soil, farm yard manure (organic carbon 12.2 %, total N 0.55 %, total P 0.75 %, total K 2.30 % and pH 7.2) and sand in a 2:2:1 ratio. In chemical treatment, Pb, EDTA and SA were added as per the designed treatment. The pots were arranged in a complete randomized block design with three replication of 15 treatments in 5 rows, with a total of 225 pots. Watering was done regularly to maintain optimal moisture level (water-holding capacity 0.44 m^2/gm soil). Seed germination started after the seventh day of sowing and after 30 days of sowing the plants were thinned to 3 plants per pot.

Seed germination, plant growth and production study:

To study the germination percentage in different treatments, seedlings were counted up to 30 days of sowing. The first harvesting was done after 30 days of sowing and subsequent harvestings were also done at 8 days after chelant application, at flowering stage and at maturation stage. After taking data on plant height, number of tillers per pot, number of leaves per plant per pot and fresh weight, samples were dried at 60°C to constant weight to determine total dry weight. The dried samples were used for the estimation of lead content.

Lead analysis:

The dried plant samples were heated in a muffle furnace at 500°C for 6 hours. The ash of each sample was dissolved in 5 ml of 20% HCl to dissolve the residue. Samples were heated on a hot plate to boiling. Required amount of HCl (20%) was added to avoid sample drying. The resulting solutions were filtered and diluted to 50ml with deionized water in volumetric flasks. The Pb content of these plant samples were determined by using flame atomic absorption spectrophotometer (ECIL AAS4129) with the following settings: wavelength 217 nm, lamp current 5 mA, slit 1 nm, fuel-acetylene and oxidant air. Pb concentration in plant samples was calculated using the following equation:

$$\text{Pb concentration} = \frac{\text{Reading of Pb in sample} * \text{Total volume of the sample}}{\text{Dry weight of the sample}}$$

Chemical analysis:

The organic C, N, P and K were estimated by the methods of Walkley and Black, Micro-kjeldahl, Olsen and Flame photometer, respectively, in soil and farm yard manure as descried by Rowell [12]. Various biochemical parameters like chlorophyll, total soluble sugar, soluble protein and proline content were estimated by the methods of Arnon, Anthrone, Bradford and Ninhydrin, respectively, as described by Thimmaiah [13].

Statistical analysis:

The experiment was conducted as a factorial randomized block design with each treatment replicated thrice. Statistical analysis of the data was done following analysis of variance (ANOVA); when the ANOVA was significant the means were separated using least significant difference at P≤ 0.05 level of significance.

RESULTS AND DISUSSION

Effect of treatments on seed germination and plant survival:

Seed germination and percentage survival of Indian mustard reduced with increasing concentration of Pb (Table.2). Addition of SA enhanced germination and survival of *B. juncea*, while EDTA played negative role. The highest values of RSG (131%) and GI (1.08) were seen in 100 ppm Pb+SA treatment after 10 days while lowest were observed in 800 ppm of Pb+EDTA treatment. These results are consistent with Xiong [14] who demonstrated a concentration-dependent inhibition of the seed germination and observed that seed germination parameter decreased with the increasing Pb concentration in the solution in *Brassica pekinensis* Rupr.

The results on the effect of different Pb treatments on plant survival are given in Table.3. The maximum plant survival was 85% in control SA treatment and the minimum (60%) in 800 ppm Pb+EDTA treatment. In general, the results demonstrated a concentration-dependent inhibition of the plant survival percentage. The positive effect of SA for attenuating metal stress in plants can be explained by three reasons: (i) SA may prevent cumulative damage development in response to heavy metals; (ii) SA may alleviate the oxidative damages caused by metals; and (iii) pretreatment with SA may exert a protective effect on the membrane stability [15].

Table.2: Effect of Pb treatments on seed germination of *B. juncea*

Treatment	Seed germination		Relative seed germination (RSG in %)		GI
	10 days	25 days	10 days	25 days	
Control (T1)	3.3±2.5^{a,b}	17±2^{a,b}	-	-	-
100 ppm Pb (T2)	4±3.6^{a,b}	18±1.7^{a,b}	121	105.8	1.05
200 ppm Pb (T3)	3.6±3.5^{a,b}	16.3±1.1^{a,b}	111	103.92	1.00
400 ppm Pb (T4)	2±1^{a,b}	16.3±0.57^{a,b}	60.6	96.07	0.93
800 ppm Pb (T5)	1±0^{a}	16.3±1.5^{a,b}	30.3	88.23	0.82
0 ppm Pb+EDTA (T6)	2±1^{a,b}	15.3±4.5^{a,b}	60.6	90.19	0.84
100 ppm Pb+EDTA (T7)	3.6±3.05^{a,b}	17±2^{a,b}	111	100	1.00
200 ppm Pb+EDTA (T8)	3.6±2.5^{a,b}	16±2.6^{a,b}	111	94.11	0.99
400 ppm Pb+EDTA (T9)	2.3±0.57^{a,b}	15.6±2.5^{a,b}	70.7	92.15	0.76
800 ppm Pb+EDTA (T10)	1±1^{a}	15.6±0.57^{a,b}	30.3	92.15	0.75
0 ppm Pb+SA (T11)	2.3±1.5^{a,b}	17±1^{a,b}	70.7	100	1.00
100 ppm Pb+SA (T12)	4.3±2.5^{b}	18.6±1.1^{b}	131	109.8	1.08
200 ppm Pb+SA (T13)	3.3±0.57^{a,b}	17.6±0.57^{a,b}	101	96.07	0.94
400 ppm Pb+SA (T14)	3±3^{a,b}	16±1^{a,b}	90.9	94.11	0.91
800 ppm Pb+SA (T15)	1.3±0.57^{a,b}	15±1^{a}	40.4	88.23	0.88

Values represent mean ± standard deviation (n=3). Means followed by the same letter within a column do not differ significantly according to DMRT at P=0.05., GI = Germination Index

Table.3: Effect of different Pb treatments on plant survival

Pb Treatment	Survival (%)	Pb Treatment with EDTA	Survival (%)	Pb Treatment with SA	Survival (%)
Control	70^{a}	0 ppm Pb+EDTA	65^{a}	0 ppm Pb+SA	85^{b}
100 ppm Pb	80^{a,b}	100 ppm Pb+EDTA	70^{a}	100 ppm Pb+SA	80^{a,b}
200 ppm Pb	75^{a}	200 ppm Pb+EDTA	65^{a}	200 ppm Pb+SA	75^{a}
400 ppm Pb	70^{a}	400 ppm Pb+EDTA	65^{a}	400 ppm Pb+SA	70^{a}
800 ppm Pb	65^{a}	800 ppm Pb+EDTA	60^{a}	800 ppm Pb+SA	65^{a}

Values represent mean ± standard deviation (n=3). Means followed by the same letter within a column do not differ significantly according to DMRT at P=0.05.

Effect of treatments on plant growth:

Plant growth parameters like numbers of branches and leaves, root length and shoot length increased with time in all treatments and decreased with increased concentrations of Pb. These findings (Table.4 and Table.5) are corroborated by Opeolu et al. [16]. In all the cases SA influenced growth parameters while EDTA was found to play negative role. Probably, EDTA presence affects negatively the balance of minerals, e.g. Zn, Cu, Fe and Ca, which leads to disturbances in cell metabolism and destabilizes biological membranes. An additional danger is connected with the formation of EDTA chelates with ions of metals necessary in functioning of plants, which may lead to disturbances in basic metabolic mechanisms [17].

Effect of Pb on root length of *B. juncea* is shown in Figure 1. It is clear from the figure that root length was affected with increased concentrations of Pb, however, there was direct relationship between root length and time. Decline in root length ranged from 38% (with 100 ppm Pb) to 69% (with 800 ppm Pb) after 120 days. The present study indicated that Pb stress reduced the growth of roots in Indian mustard plants. These results are in agreement with those reported by others [18-20].

Table.4: Effect of Pb treatments on number of branches of *B. juncea*

Treatment	No. of branches with days of treatment			
	30 days	60 days	90 days	120 days
Control (T1)	4.7±1.5^{a,b}	6.0±2.6^{a,b}	7.0±3.1^{a,b}	10.0±2.0^{c,d}
100 ppm Pb (T2)	3.7±2.0^{a,b}	4.3±2.1^{a,b}	6.0±2.0^{a,b}	8.0±2.5^{b,c,d}
200 ppm Pb (T3)	3.3±1.5^{a,b}	3.6±2.9^{a}	5.0±3.1^{a,b}	6.3±1.5^{a,b,c}
400 ppm Pb (T4)	2.7±2.1^{a}	3.3±3.0^{a}	4.2±2.1^{a,b}	5.3±1.5^{a,b}
800 ppm Pb (T5)	2.7±1.2^{a}	3.0±1.5^{a}	4.0±1.0^{a}	4.7±1.5^{a,b}
0 ppm Pb+EDTA (T6)	4.0±1.5^{a,b}	4.2±1.5^{a,b}	4.3±2.0^{a}	5.3±2.5^{a,b}
100 ppm Pb+EDTA (T7)	3.7±1.0^{a,b}	4.0±1.0^{a}	5.0±1.5^{a,b}	6.7±2.1^{a,b,c}
200 ppm Pb+EDTA (T8)	3.3±1.5^{a,b}	3.5±1.5^{a}	4.6±1.0^{a,b}	6.3±1.5^{a,b,c}
400 ppm Pb+EDTA (T9)	2.7±1.5^{a}	3.0±1.0^{a}	3.5±1.0^{a,b}	6.0±1.0^{a,b}
800 ppm Pb+EDTA (T10)	2.3±1.5^{a}	3.0±1.0^{a}	3.3±1.5^{a}	4.4±2.1^{a}
0 ppm Pb+SA (T11)	6.3±1.5^{b}	7.0±1.0^{b}	8.0±1.0^{b}	12.0±3.0^{d}
100 ppm Pb+SA (T12)	4.0±2.0^{a,b}	4.7±2.0^{a,b}	6.3±1.5^{a,b}	8.7±1.5^{b,c,d}
200 ppm Pb+SA (T13)	3.7±2.3^{a,b}	4.3±1.0^{a,b}	5.2±1.7^{a,b}	7.3±1.5^{a,b,c}
400 ppm Pb+SA (T14)	3.3±2.6^{a,b}	3.8±2.0^{a}	4.7±1.5^{a,b}	7.0±2.0^{a,b,c}
800 ppm Pb+SA (T15)	2.7±1.5^{a}	3.5±1.5^{a}	4.3±2.0^{a}	6.3±1.5^{a,b,c}

Values represent mean ± standard deviation (n=3). Means followed by the same letter within a column do not differ significantly according to DMRT at P=0.05.

Table.5: Effect of Pb treatments on number of leaves of *B. juncea*

Treatment	No. of leaves with days of treatment			
	30 days	60 days	90 days	120 days
Control (T1)	5.3±1.5^{b,c}	5.7±1.5^{a,b}	8.3±1.5^{b,c}	20.3±1.5^{d}
100 ppm Pb (T2)	6.0±1.2^{b,c}	8.0±1.7^{b,c}	9.3±1.0^{c,d}	25.0±1.0^{e}
200 ppm Pb (T3)	4.7±1.5^{b}	7.3±1.5^{b,c}	8.0±2.0^{b,c}	20.0±1.5^{d}
400 ppm Pb (T4)	4.0±1.7^{a,b}	6.3±1.5^{a,b}	7.3±1.5^{b,c}	11.3±1.5^{b}
800 ppm Pb (T5)	3.3±1.5^{a,b}	5.7±1.2^{a,b}	6.7±2.1^{a,b}	9.3±1.5^{a,b}
0 ppm Pb+EDTA (T6)	5.3±1.5^{b,c}	5.7±1.2^{a,b}	8.0±1.0^{b,c}	11.3±1.5^{b}
100 ppm Pb+EDTA (T7)	5.3±1.5^{b,c}	7.7±1.2^{b,c}	8.0±2.0^{b,c}	23.0±2.0^{d,e}
200 ppm Pb+EDTA (T8)	4.3±1.5^{a,b}	7.0±1.7^{b}	7.3±1.5^{b,c}	14.7±1.5^{c}
400 ppm Pb+EDTA (T9)	3.3±1.5^{a,b}	6.0±1.5^{a,b}	7.0±1.0^{b}	10.3±2.0^{b}
800 ppm Pb+EDTA (T10)	2.3±1.5^{a}	5.0±1.0^{a}	5.3±1.5^{a}	6.0±2.5^{a}
0 ppm Pb+SA (T11)	7.7±1.5^{c}	9.3±1.5^{c}	9.5±1.5^{c,d}	24.7±2.5^{e}
100 ppm Pb+SA (T12)	6.3±1.5^{b,c}	9.0±1.0^{b,c}	11.0±1.0^{d}	30.0±2.0^{f}
200 ppm Pb+SA (T13)	5.3±1.5^{b,c}	8.7±1.5^{b,c}	9.0±1.0^{c,d}	22.0±1.0^{d,e}
400 ppm Pb+SA (T14)	4.7±1.0^{b}	8.0±1.0^{b,c}	8.7±2.1^{c}	15.0±1.5^{c}
800 ppm Pb+SA (T15)	4.3±1.5^{a,b}	7.3±1.5^{b,c}	7.4±1.5^{b,c}	10.3±1.5^{b}

Values represent mean ± standard deviation (n=3). Means followed by the same letter within a column do not differ significantly according to DMRT at P=0.05.

SA showed better results than EDTA in all treatments. Positive role of SA on root length is supported by El-Tayeb et al. [15, 21]. Popova et al. [15] reported that SA alleviated the negative effect of Cd on growth of pea plants. Similar to our results, reduction of root elongation by EDTA was also observed by Wang et al. [22] and Chen et al. [23]. The decline in shoot length in *B. juncea* was observed with the increasing concentrations of Pb (Figure 2). Shoot length declined from 12% (with 100 ppm Pb) to 55% (with 800 ppm Pb) after 120 days. Shoot length increased with the increase in time in all treatments. Results showed that shoot growth was better in SA treatments compared to EDTA. Shoot length was significantly affected by all the different metal concentrations studied. Our results pertaining to the effect of higher concentration of Pb on plant height (*Solanum melongena*) are corroborated by Yilmaz et al. [24].

Our results on positive effect of SA on different plant growth parameters are supported by others. El-Tayeb et al. [21] showed that exogenous application of SA increased the growth of roots, stems and leaves of both the control and Cu-stressed sunflower plants. Similarly, the negative effect of EDTA is supported by Sinhal et al. [25] who showed that Zn, Cu, Pb and Cd in combination with 30 mg/l concentration of EDTA and citric acid caused significant reduction in growth of marigold in terms of plant height, fresh weight, total chlorophyll, carbohydrate and protein contents.

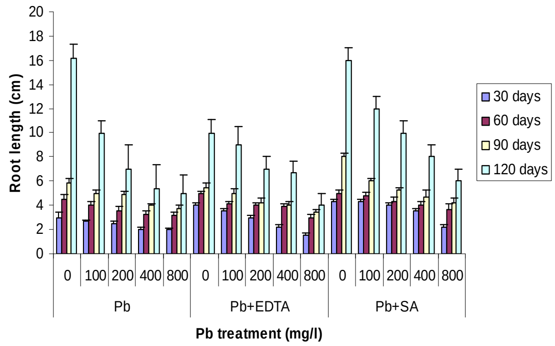

Figure.1: Effect of Pb treatments on root length of *B. juncea*

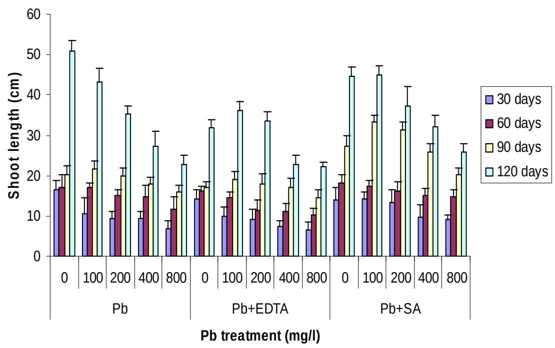

Figure.2: Effect of Pb treatments on shoot length of *B. juncea*

Plant weight significantly increased with the increase in exposure time in all treatments (Figure 3). At higher levels of Pb treatment (800 ppm), fresh weight declined 20% of control for the same time period. Plant showed maximum dry weight in control SA treatment (Figure 4). Dry weight was significantly lower in EDTA treated plants.

EDTA decreased significantly plant dry weight, whereas SA stimulated plant dry weight compared to control. Plant dry weight was significantly higher in all SA treatments compared to control, however the time of application of chelant on plant dry weight is very important.

In our results, Pb reduced fresh weight of Indian mustard plants and the reason may be that they were exposed to very high concentration of Pb and at very early stage of their development. Similar to our results, the adverse effect of EDTA on the growth of Indian mustard was also reported by Van Engelen et al. [26].
In our results, SA stimulated yields of *B. juncea* and this is in agreement with Gunes et al. [27] who reported that exogenous levels of SA increased dry yield of maize significantly both in saline and non-saline conditions. Increased dry matter of metal stressed plants in response to SA may be related to the induction of antioxidant response and protective role of membranes that increase the tolerance of plant to damage.

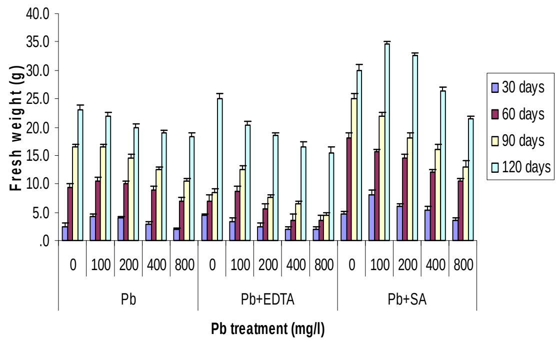

Figure.3: Effect of Pb treatments on fresh weight of *B. juncea*

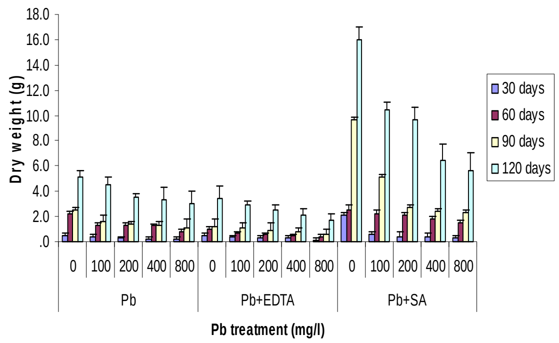

Figure.4: Effect of Pb treatments on dry weight of *B. juncea*

Effect of treatments on biochemical parameters:

All biochemical parameters showed declination with increasing Pb concentrations. A higher accumulation of chlorophyll, soluble sugars, soluble proteins and proline occurred in Indian mustard plants treated with SA. Addition of EDTA enhanced chlorophyll content, soluble sugar and soluble protein but reduced proline content in all treatments. The ratio of chlorophyll a/b shows more sensitivity as stress indicator than total chlorophyll content [28]. The changes in chlorophyll a/b ratio indicate that there are differential changes in the photosynthetic pigment stoichiometry. The ratio of chlorophyll a/b increased slightly with increasing Pb treatments (Table.6) which is consistent with the results of Zengin & Munzuroglu [29] and it may be linked to the reduction in light harvesting chlorophyll proteins (LHCPs). The ratio of chl a/b dropped with the addition of EDTA and SA. This could be an indication of some inhibition of growth by EDTA and SA.

Table.6: Effect of treatments on Chl. a/b ratio of *B. juncea*

Treatment	Chl. a/b	Treatment	Chl. a/b	Treatment	Chl. a/b
Control	1.53^{a}	0 ppm Pb+EDTA	1.46^{a}	0 ppm Pb+SA	1.48^{a}
100 ppm Pb	$2.29^{b,c}$	100 ppm Pb+EDTA	2.10^{b}	100 ppm Pb+SA	1.27^{a}
200 ppm Pb	$2.50^{c,d}$	200 ppm Pb+EDTA	2.13^{b}	200 ppm Pb+SA	1.24^{a}
400 ppm Pb	$2.60^{c,d}$	400 ppm Pb+EDTA	$2.29^{b,c}$	400 ppm Pb+SA	1.29^{a}
800 ppm Pb	2.71^{d}	800 ppm Pb+EDTA	2.03^{b}	800 ppm Pb+SA	1.36^{a}

Values represent mean ± standard deviation (n=3). Means followed by the same letter within a column do not differ significantly according to DMRT at P=0.05.

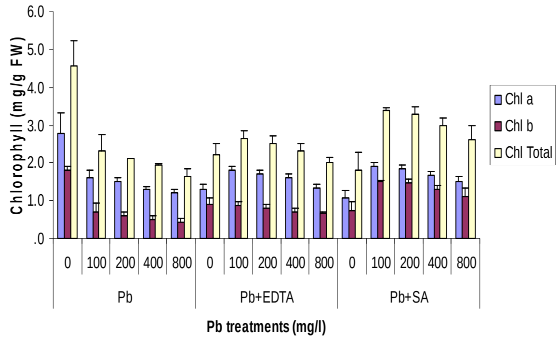

Figure.5: Effect of Pb treatments on chlorophyll content of *B. juncea*

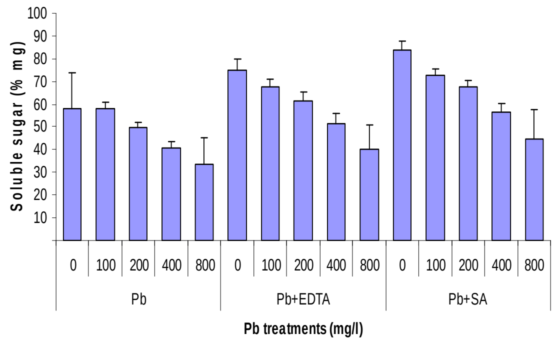

Figure.6: Effect of Pb treatments on total soluble sugar content of *B. juncea*

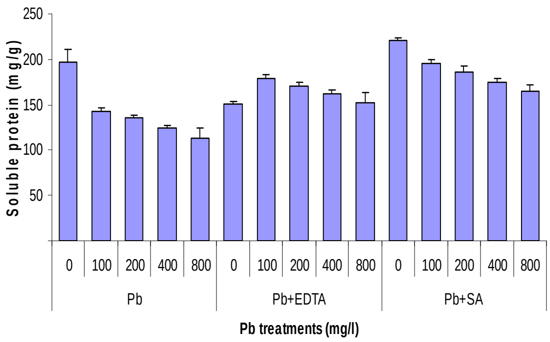

Figure.7: Effect of Pb treatments on soluble protein content of *B. juncea*

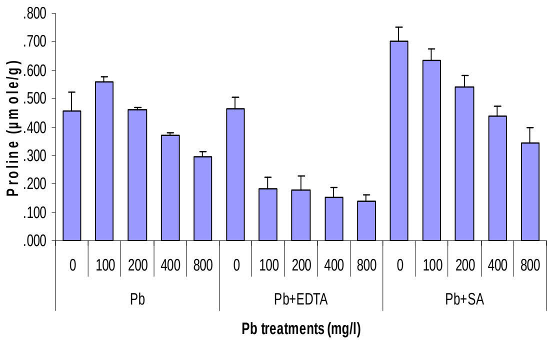

Figure.8: Effect of Pb treatments on proline content of *B. juncea*

Effect of treatments on Pb accumulation:

The relative increases in the extraction efficiencies of *B. juncea* after EDTA treatment, as compared to the control (without chelant) were 45, 56, 68 and 58% higher Pb accumulation rates for the applied Pb doses of 100, 200, 400 and 800 ppm, respectively after 120 days (Figure 9). Pb accumulation was significantly different ($P<0.05$) with treatments and time and it increased with increase in Pb concentration and time. EDTA was found to be more effective in metal uptake than SA. The difference in their efficiency may be due to the difference in their stability constants with the metal [30]. At 800 ppm Pb treatment, Indian mustard plants achieved hyper accumulator status (1462±59 mg/kg) while with EDTA it was 1150±50 to 3479±71 mg/kg for the applied Pb doses of 100-800 ppm. The Pb metal accumulation order was Pb+EDTA > Pb+SA > Pb.

The content of heavy metals in the soil of individual pot was analyzed after harvesting the plant biomass. The amounts of Pb remaining in the pots with different treatments are given in Figure 10. Statistically significant differences were found among all treatments. There was an increase in heavy metal contents in the control treatment, in which soils were polluted with different concentrations of Pb. Soil contents of Pb decreased due to enhanced metal uptake by plants in chelants treated soil. EDTA was found to be more efficient. This supremacy of EDTA over SA may be due to higher stability constant of metal-EDTA complexes than metal-SA complexes.

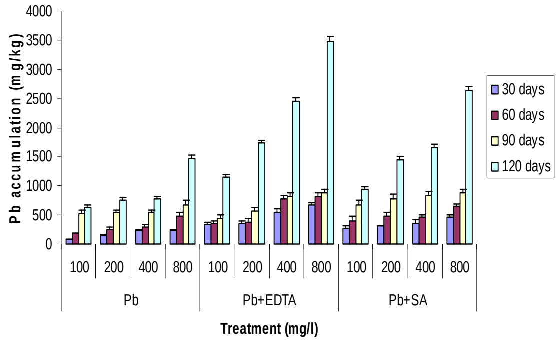

Figure.9: Effect of chelants on Pb accumulation by *B. juncea* in Pb treatments

The results of this study indicated that EDTA enhanced the removal of Pb from contaminated soil and also the accumulation of Pb in plants and these results are consistent with those of previous studies [31-34].

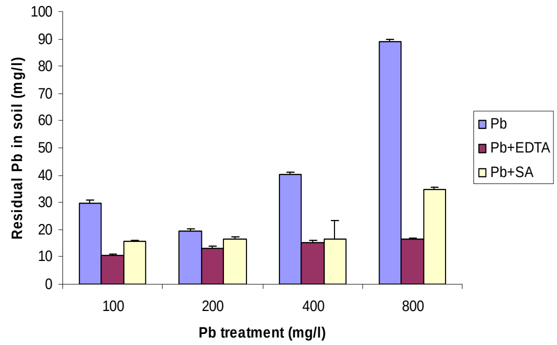

Figure.10: Effect of chelants on residual Pb remained in Pb treated soils

CONCLUSION

Overall, it could be concluded that chelate-assisted phytoextraction showed better results than continuous phytoextraction. *B. juncea arawali* is a suitable candidate for chelate-assisted phytoextraction of Pb. EDTA proved to be more efficient chelant than SA for removal of Pb from contaminated soil.

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