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Potential of *Ricinus Communis* L. and *Brassica Juncea* (L.) Czern. under natural and induced Pb Phytoextraction

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Abstract:

Put "*metal into the petal*" of plants which literally means bioavailability and translocation of the heavy metal to the plants is an important factor in phytoextraction. A green house experiments was conducted to study potential of *Ricinus communis* and *Brassica juncea* in metal accumulation at different concentration of Pb and also the effect of the application of two different chelators i.e., EDTA and Citric acid, on the Pb phytoextraction was studied in an artificially contaminated soil. *Ricinus communis* and *Brassica juncea* accumulated high concentrations of Pb in its shoots but the biomass, root and shoot length of *Brassica juncea* was highly affected due to increased Pb accumulation. *Ricinus communis* showed high tolerance to Pb since the biomass, root and shoot length of the plant was not affected. Chelators had a potential role in enhancing Pb content in both the plants. The results analysed for metal extraction using chelates revealed that EDTA treated pots increased Pb concentration in *Ricinus communis* and *Brassica juncea* compared to citric acid treated pots and untreated pots (control). Both the plants showed high phytoextraction levels but the growth of *Brassica juncea* was impaired at high levels of Pb and can be used in marginally polluted soils.

Keywords: Chelators, Citric acid, EDTA, High biomass plants, Pb uptake, Phytoextraction

1.0 Introduction:

Heavy metal pollution is a world wide problem (Chehregani et al., 2009) and considered as one of the major toxic environmental pollution causing global disaster. Major portions of soils have became unfit for cultivation due to heavy metals from mining, smelting, electroplating, energy and fuel production, Sludge dumping, Industrial emissions and applications of agrochemicals and fertilizers (Giachetti and Sebastiani, 2006). This toxic pollutant are generally non-biodegradable and they persist as long-term in aquatic and terrestrial ecosystems which is potentially harmful to all biota and represents one of the most pressing threats to water and soil resources and to human health (Jiang et al., 2010). Therefore, remediation of heavy metal contaminated soil is needed. Due to some disadvantages associated with physical and chemical remediation methods such as destruction of soil structure, secondary pollution and huge costs. Since, the ultimate goal of any soil remediation process should be not only to remove the pollutant(s) from the soil but also to restore the soil health as well (Hernández-Allica et al., 2006). Phytoremediation can provide an effective technique for removing heavy metals and can be introduced into remediation field (Wei et al., 2006) actually, with some added advantages such as low cost environment friendly technology (Chehregani et al., 2009) which clean – up metal polluted soils (Arshad et al., 2008).

Metal hyperaccumulation by plants is of great interest in recent years because of its potential application for phytoremediation of heavy metal contaminated soils (Tang et al., 2009). Phytoremediation is a use of green plants, soil amendment and agronomic techniques to remove pollutants from the environment or to render them harmless (Salt et al., 1998) and for successful phytoremediation, hyperaccumulators and accumulators has to be screened in heavy metal contaminated soils (Zhang et al., 2010). Phytoextraction of heavy metal contaminated soils is widely considered as a promising remediation technology in the future.

Most of the research focused on the use of high biomass plants which exhibits high tolerance to environment contaminated with heavy metals. But, chelators which facilitates metal uptake were also used for high translocation efficiency in the plants. For the first time wallance (1974) suggested that metal – EDTA complexes formed in the soil can increase solubility and phytoavailability in metals. A number of chelators

such as EDTA (ethylenediamine tetraacetic acid), CDTA (trans – 1,2 diaminocyclohexane – N , N , N^1 , N^1 – tetraacetic acid), EGTA [ethylene glycol – bis (β -aminoethylether), N , N , N^1 , N – tetraacetic acid] and EDDHA [ethylene diamine – di (0 – hydroxyphenyl acetic acid)] (Shen et al., 2002), HEDTA (diethylenetrinitrilo penta acetic acid (Blaylock et al., 1997), NTA (nitrilotriacetate) (Grčman et al., 2003) and EDDS (S,S – ehylenediaminedisuccinic acid) (Epelde et al., 2008) which desorbs heavy metals from soil, thus facilitating their plant absorption and increase translocation of metal from roots to shoots (Shen et al., 2002). So far, EDTA is most commonly used on chelate – induced phytoextraction, because of its high efficiency in extracting many metals (Epelde et al., 2008).

Two strategies of phytoextraction methods have been adopted in our research work. First, natural phytoextraction, here *Ricinus communis* and *Brassica juncea* were grown in extremely polluted environment and its metal accumulation ratio was assessed at different levels of Pb. Secondly, The effect of chelators on metal extraction, through the utilization of high biomass *Ricinus communis* and *Brassica juncea* by enhancing the metal bioavailability was studied, refered as induced phytoextraction.

2.0 Materials and Methods:

2.1 Green house experiments:

Two green house experiments were conducted to find out the Pb accumulation patterns in *Ricinus communis* and *Brassica juncea*. First, to find out the performance of the plants at different concentrations of Pb and second, to assess the role of chelators in Pb induction in both the plants.

2.2 Experimental soil:

Two different soils were used in this study. Soil samples were collected from the top 20 cm profile of the agricultural lands from Nellithurai and Umapalayam near Mettupalayam, Coimbatore, Tamil Nadu, India. Soils were air dried and passed through 2 mm sieve and used for cultivation of plants. The physical and chemical properties of the soils were assessed prior to experiment studies and are presented in Table 1.

2.3 Experimental design and treatments:

The experiment was carried out in green house with mud pots (Diameter = 35 cm, height = 35 cm) lined with plastic bags filled with 15 kg of soil and the pots were arranged in a completely randomized factorial design with three replicates. The soil was weighed and the field capacity was calculated. Soil portions equivalent to 15 kg of dried soil were individually contaminated (by mechanical mixing in plastic bags) with five treatments Viz., T1 (Control, no external Pb added), T2, T3, T4 and T5 (200, 400, 600 and 800 mg kg^{-1} of Pb as $Pb(NO_3)_2$ dissolved in deionised water). Control and polluted soil were equilibrated for two weeks. Two types of soil and plants with five treatments and three replicates, giving a total of 60 pots.

For experiment II, T1 (Control, no external Pb added) and T2 (500 mg kg^{-1} of Pb as $Pb(NO_3)_2$ dissolved in deionised water). Before one week of harvest, the following chelate treatments were applied: (i) a single dose of 10 mmol EDTA per kg soil. (ii) a single dose of 10 mmol Citric acid per kg soil. Two types of soil and plants with six treatments and three replicates, giving a total of 72 pots.

2.4 Plant cultivation:

Seeds were sown at a rate of 3 pot $^{-1}$ for *Ricinus communis* and 10 pot $^{-1}$ for *Brassica juncea* and thinned to one seedling in each pot 2 week after germination. Deionised water was added on alternative days throughout the experiment to keep the water content near to field capacity.

2.5 Harvest:

Plants were harvested after 90 days (after flowering stage) for *Brassica juncea* and 180 days (upto maturity stage) for *Ricinus communis*.For Second experiment, before one week of harvest, 10 mmol of EDTA and citric acid per kg soil was added.

2.6 Analysis of soil and plant:

Soil pH (Model - Hanna HI 98107, USA) and EC (Model - Hanna HI 98304, USA) was measured in a prepared 1:2.5 (w/w) ratio soil : water suspension (Jackson, 1973). Organic carbon present in organic matter of the soil was oxidized by chromic acid in the presence of Conc. H_2SO_4. Potassium dichromate on reaction with H_2SO_4 provides nascent oxygen which combines with carbon and forms CO_2. The H_2SO_4 enables easy digestion of organic matter by rendering heat of dilution. Only a certain quantity of chromic acid is used for oxidation. The excess chromic acid left unused by the organic matter is determined by back titration with 0.5 N ferrous sulphate using diphenylamine indicator (Walkley and Black, 1934).

Exactly 0.5 g of soil was weighed and transferred to 100 ml conical flask. Then, 15 ml of aqua regia

(Con. HCl: Con. HNO_3 at 3:1) was added and the contents were digested in hot plate at 200°C. After cooling volume was made up to 100 ml with double distilled water and filtered through Whatman No.1 filter paper and then fed into the Atomic Absorption Spectrophotometer (Model - Perkin Elmer AAnalyst 200, USA) and analyzed for total Pb concentrations in the soil. Harvested plants were washed with running tap water to remove adhering soil particles, then rinsed twice with deionised water, blotted with tissue paper and their fresh weight, root length, shoot length and total length was recorded. Plant materials was dried in an oven at 70°C for 48 h. Root and shoots were separated using stainless steel scissors and milled in a metal – free Willey mill and used for heavy metal analysis. 0.5 gram of the plant sample was weighed into a 100 ml conical flask. 15 ml of triacid mixture (Con. HNO_3, Con. H_2SO_4, Con. $HClO_4$ at 9:2:1) was added and the contents were digested on a hot plate at 200°C until white fumes appear. After cooling the volume was made up to 50 ml with double distilled water and filtered through Whatman No.1 filter paper (Pratt, 1965) and then fed into the Atomic Absorption Spectrophotometer (Model - Perkin Elmer AAnalyst 200, USA) and analyzed for Pb.

Two parameters were calculated to evaluate plant's phytoextraction efficiency. The translocation factor ($TF = C_{shoots}/C_{roots}$) (Marchiol et al., 2004) and bioaccumulation factor ($BF = C_{shoots}/C_{soils}$) (Evangelou et al., 2007).

***2.7 Statistical analysis*:**
Data were statistically analysed for one-way ANOVA to find out the significant differences between the treatments.

3.0 Results and Discussion:

As represented in Table 1, the characteristics of soil 1 & 2 selected for pot culture experiments appeared to be similar. The concentrations of Pb in both the soil was high under natural conditions (221.8 and 298.8 mg Pb kg^{-1} in soil 1 and 2, respectively). At the end of all the experiments, soil Pb concentrations studied after plant harvest was decreased which is mainly due to increase in plant Pb uptake (Table 2, 4, 6 & 8) and there is also a slight increase or decrease in the soil pH value which is mainly due to release of root exudates by plants during metals uptake (Kim et al., 2010).

There was no significant reduction in biomass, root and shoot length of the *Ricinus communis* under different levels (0 to 800 mg kg^{-1}) of Pb (Table 3) indicating high tolerance of *Ricinus communis* to Pb. As presented in Table 2, Pb accumulation in the shoot of *Ricinus communis* in T_{400} (soil 1) was 212.5 mg kg^{-1} and in the roots was up to 139 mg kg^{-1} in T_{200} (Soil 1) which was the highest value recorded between different treatments of Pb. The judging criteria for a Pb – hyperaccumulator should be > 1000 mg kg^{-1} in its shoots (Baker et al., 1994). Since, soil Pb content was only upto 800 mg kg^{-1} and the plant was able to accumulate 190 mg kg^{-1} of Pb in its shoots which was highest compared to the root within short duration. The translocation factor was also > 2, a judging criterion for a hyperaccumulator (Ma et al., 2001). *Ricinus communis* can be considered as a hyperaccumulator.

As indicated in Table 5, increase in Pb concentrations in the soil significantly reduced the biomass, root and shoot length of the *Brassica juncea* but significantly increased the Pb content in the shoot (649.6 mg kg^{-1}) and root (206.5 mg kg^{-1}). These results were consistent with those of previous studies (Lai and Chen, 2004; Chen et al., 2004). The translocation factor was also > 3 (Table 4) which is a judging criteria for a plant to be a accumulator (Rotkittikhum et al., 2006). The reason in reduction of biomass, root and shoot length of the plant might be due to high concentration of Pb in the shoot and root of the plant. Pb accumulation by *Brassica juncea* was comparatively higher than *Ricinus communis* but biomass and growth pattern of *Brassica juncea* was highly affected due to high degree of Pb accumulation in the plant parts. *Brassica juncea* can also be considered as hyperaccumulator due to high Pb content in its shoots (649.6 mg kg^{-1}) at 800 mg kg^{-1} of Pb in the soil with in short duration (Ma et al., 2001) but Kumar et al. (1995) indicated that experiments using soils artificially spiked with heavy metals may result in high performance of phytoextraction effects because of the high availability of heavy metals in artificially spiked soils.

Pb solubility in soil and availability for plant uptake is limited due to its complexation with organic matter, sorption on oxides and clays, and precipitation as hydroxide, carbonates and phosphates (Bride, 1994). Due to its limited bioavailability, an approach to increase its bioavailability is essential to the success of phytoremediation. Therefore, Chelate – enhanced phytoextraction was performed using *Ricinus communis* and *Brassica juncea* by applying the chelators (EDTA and Citric acid) as solution to the soil about 1 week before harvesting (Wu et al.,

2004). Regarding shoot Pb concentrations of both plants, EDTA appeared much more efficient than citric acid (Table 6 & 8) and control. Although shoot Pb concentrations observed as a result of EDTA addition did not reach 1000 mg kg^{-1} threshold proposed by Baker et al. (1994) for a plant to consider them as hyperaccumulator.

Table 1: Physico-chemical properties of the soil selected for pot culture experiments

S.No	Soil	pH	EC (mS cm^{-1})	OC (%)	Total Pb content (mg kg^{-1})
1	Soil 1	6.6	0.15	1.26	221.8
2	Soil 2	7.5	0.20	1.02	298.8

Table 2: Phytoextraction potential of *Ricinus communis* L. **under different levels of Pb pollution in an artificially contaminated soil.**

Soil	Treatments	Soil			Pb concentration in plants (mg kg^{-1})			
		pH	EC (mS cm^{-1})	Pb (mg kg^{-1})	Root	Shoot	BF	TF
Soil 1	T_{Ctrl}	6.73 ± 0.2c	0.14 ± 0.0 a	42.5 ± 2.05 f	61.3 ± 2.08 c	78 ± 3 f	0.35 ± 0.01 c	1.27 ± 0.01 de
	T_{200}	6.66 ± 0.05c	0.07 ± 0.0 bc	55.9 ± 2.00 cd	139 ± 25.1 a	179 ± 5.14 abc	0.89 ± 0.02 a	1.31 ± 0.21 de
	T_{400}	6.86 ± 0.05c	0.09 ± 0.0 b	62.4 ± 2.20 ab	114 ± 10.43 ab	212.5 ± 9.74 a	0.53 ± 0.02 b	1.86 ± 0.08 ab
	T_{600}	7.30 ± 0.1b	0.07 ± 0.0 bc	65.8 ± 0.91 a	115 ± 3.53 ab	166.8 ± 3.25 bc	0.27 ± 0.0 cd	1.43 ± 0.01 cd
	T_{800}	7.26 ± 0.3b	0.07 ± 0.01 bc	58.1 ± 1.67 bc	92.4 ± 7.32 bc	190 ± 5.61 ab	0.23 ± 0.0 d	2.06 ± 0.10 a
Soil 2	T_{Ctrl}	7.76 ± 0.15a	0.08 ± 0.01 bc	41.6 ± 1.50 f	78.5 ± 3.23 bc	87 ± 1ef	0.29 ± 0.0 cd	1.10 ± 0.04 de
	T_{200}	7.80 ± 0.1a	0.07 ± 0.0 bc	48.2 ± 1.05 e	94.1 ± 17.18 bc	107 ± 3.23 def	0.53 ± 0.01 b	1.16 ± 0.19 de
	T_{400}	7.73 ± 0.05a	0.06 ± 0.01c	59.8 ± 2.13 bc	89.4 ± 25.23 bc	105.9 ± 21.5 def	0.26 ± 0.05 d	1.20 ± 0.10 de
	T_{600}	7.83 ± 0.05a	0.08 ± 0.0 bc	51.9 ± 1.76 de	85.6 ± 21.73 bc	141.6 ± 44.1 cd	0.23 ± 0.07 d	1.65 ± 0.21 bc
	T_{800}	7.60 ± 0.1ab	0.08 ± 0.0 bc	42.3 ± 2.32 f	115.9 ± 1.8ab	123 ± 7.88 de	0.15 ± 0.0 e	1.06 ± 0.05 e

T_{Ctrl} means the control without Pb addition; T_{200}, T_{400}, T_{600} & T_{800} means 200, 400, 600 & 800 mg kg^{-1} of Pb added to the soil. BF – Bioaccumulation Factor, Concentration ratio of shoots to soil; TF – Translocation factor, Concentration ratio of shoots to roots. Mean ± SD. Data in the same column followed by the same letter are not significantly different, whereas with different letters data are significantly different ($p < 0.05$).

Table 3: Growth and biomass of *Ricinus communis* L. **at different concentrations of Pb**

Soil	Treatments	Biomass (g / Pot)	Root length (cm)	Shoot length (cm)	Total length (cm)
Soil 1	T_{Ctrl}	109 ± 3.2 b	21 ± 4 cd	100.3 ± 0.57 cd	121.3 ± 4.04 cd
	T_{200}	121 ± 1.0 a	29.3 ± 1.5 ab	98 ± 1.0 d	127.3 ± 2.51 abc
	T_{400}	118 ± 1.0 a	18.3 ± 1.5 cd	98 ± 1.7 d	116.3 ± 3.05 d
	T_{600}	120 ± 1.0 a	23 ± 2 bcd	110 ± 1.5 a	133.3 ± 2.08 a
	T_{800}	121 ± 1.5 a	23 ± 1 bcd	101 ± 2.08 cd	124.3 ± 1.52 abcd
Soil 2	T_{Ctrl}	120 ± 1.0 a	23 ± 4.3 bcd	99.6 ± 1.52 d	122.6 ± 5.77 cd
	T_{200}	117 ± 1.5 a	31 ± 2 a	98.6 ± 0.57 d	129.6 ± 1.52 abc
	T_{400}	120 ± 1.5 a	17.6 ± 0.5 d	105.6 ± 5.03 abc	123.3 ± 4.50 bcd
	T_{600}	120 ± 0.57 a	24.6 ± 2.5 bc	107.6 ± 1.52 ab	132.3 ± 4.04 ab
	T_{800}	121 ± 1.5 a	24.3 ± 1.5 bcd	103 ± 3.0 bcd	127.3 ± 1.52 abc

T_{Ctrl} means the control without Pb addition; T_{200}, T_{400}, T_{600} & T_{800} means 200, 400, 600 & 800 mg kg^{-1} of Pb added to the soil. Mean ± SD. Data in the same column followed by the same letter are not significantly different, whereas with different letters data are significantly different ($p < 0.05$).

Table 4: Phytoextraction potential of *Brassica juncea* **(**L.) Czern.**under different levels of Pb pollution in an artificially contaminated soil.**

Soil	Treatments	Soil			Pb concentration in plants ((mg kg^{-1})			
		pH	EC (mS cm^{-1})	Pb (mg kg^{-1})	Root	Shoot	BF	TF
Soil 1	T_{Ctrl}	6.80 ± 0.10 c	0.04 ± 0.0 c	72.26 ± 2.41 f	51 ± 1 h	57.3 ± 2.51 g	0.25 ± 0.01 fg	1.12 ± 0.07 de
	T_{200}	6.66 ± 0.15 c	0.07 ± 0.0 ab	83.6 ± 1.44 e	105 ± 5 e	85.6 ± 4.04 f	0.42 ± 0.02 c	0.81 ± 0.005 ef
	T_{400}	6.80 ± 0.10 c	0.06 ± 0.0 bc	107.4 ± 2.33 c	167 ± 13.4 d	120.6 ± 10.5 e	0.3 ± 0.02 ef	0.72 ± 0.01fg
	T_{600}	6.83 ± 0.05 c	0.06 ± 0.0 bc	141.4 ± 1.5 b	235.6 ± 4.04 b	166.6 ± 4.72 d	0.27 ± 0.0 fg	0.7 ± 0.02 fg
	T_{800}	6.86 ± 0.05 c	0.05 ± 0.0 c	153.5 ± 3 a	464.3 ± 8.73 a	191.3 ± 9.01 d	0.23 ± 0.01 fg	0.41 ± 0.01 g
Soil 2	T_{Ctrl}	7.60 ± 0.10 b	0.05 ± 0.0 c	44.6 ± 1.52 g	80.6 ± 3.78 fg	69.3 ± 3.78 fg	0.23 ± 0.01 g	0.86 ± 0.04 ef
	T_{200}	7.83 ± 0.05 ab	0.07 ± 0.0 ab	47.9 ± 1.37 g	57.2 ± 0.88 h	70.3 ± 9.39 fg	0.35 ± 0.04 de	1.22 ± 0.16 d
	T_{400}	7.86 ± 0.05 a	0.08 ± 0.0 a	71.93 ± 2.1 f	66.3 ± 4.16 gh	275 ± 5 b	0.68 ± 0.01 b	4.15 ± 0.24 a
	T_{600}	7.90 ± 0.0 a	0.08 ± 0.0 a	95.2 ± 1.7 d	86 ± 5.56 f	235 ± 5 c	0.39 ± 0.0 cd	2.74 ± 0.20 c
	T_{800}	7.76 ± 0.11 ab	0.08 ± 0.01 ab	103 ± 3 c	206.5 ± 1.85 c	649.6 ± 23.4 a	0.81 ± 0.02 a	3.14 ± 0.14 b

T_{Ctrl} means the control without Pb addition; T_{200}, T_{400}, T_{600} & T_{800} means 200, 400, 600 & 800 mg kg^{-1} of Pb added to the soil. BF – Bioaccumulation Factor, Concentration ratio of shoots to soil; TF – Translocation factor, Concentration ratio of shoots to roots. Mean ± SD. Data in the same column followed by the same letter are not significantly different, whereas with different letters data are significantly different ($p < 0.05$).

Table 5: Growth and biomass of *Brassica juncea* **(**L.) Czern. **at different concentrations of Pb**

Soil	Treatments	Biomass (g / Pot)	Root length (cm)	Shoot length (cm)	Total length (cm)
Soil 1	T_{Ctrl}	75 ± 1 a	12.3 ± 1.52 bc	104.3 ± 4.93 bc	116.6 ± 6.42 bc
	T_{200}	67.3 ± 2.08 b	14 ± 1.73 abc	107.3 ± 2.08 ab	121.3 ± 1.52 b
	T_{400}	54.3 ± 2.08 d	9 ± 1 c	98 ± 1 cd	107 ± 2 d
	T_{600}	42 ± 1 e	12.6 ± 2.08 bc	85 ± 1 e	97.6 ± 1.15 e
	T_{800}	31 ± 1 f	13.3 ± 1.52 abc	78 ± 2.64 f	91.3 ± 1.15 ef
Soil 2	T_{Ctrl}	74 ± 1 a	18.6 ± 3.21 a	111.3 ± 1.52 a	130 ± 3.6 a
	T_{200}	62 ± 1 c	16 ± 1 ab	106.6 ± 2.30 ab	122.6 ± 1.52 ab
	T_{400}	60 ± 1 c	12.6 ± 2.08 bc	97.6 ± 0.57 d	110.3 ± 1.52 cd
	T_{600}	44.6 ± 0.57 e	12.6 ± 2.08 bc	85 ± 1 e	97.6 ± 1.15 e
	T_{800}	31.6 ± 1.52 f	15.6 ± 1.15 ab	70.3 ± 1.52 g	86 ± 1 f

T_{Ctrl} means the control without Pb addition; T_{200}, T_{400}, T_{600} & T_{800} means 200, 400, 600 & 800 mg kg^{-1} of Pb added to the soil. Mean ± SD. Data in the same column followed by the same letter are not significantly different, whereas with different letters data are significantly different ($p < 0.05$).

Table 6: Effect of EDTA and citric acid as enhancers of Pb in *Ricinus communis* L. in an artificially contaminated soil.

Soil	Treatments	Soil			Pb concentration in plants (mg kg^{-1})			
		pH	EC (mS cm^{-1})	Pb (mg kg^{-1})	Root	Shoot	BF	TF
Soil 1	$T1_{Ctrl}$	6.86 ± 0.05 a	0.08 ± 0.0 a	42.3 ± 2.5 ef	59.6 ± 2.0 f	73.3 ± 2.0 g	0.33 ± 0.00 e	1.23 ± 0.07 de
	$T1_{EDTA}$	6.9 ± 0.1 a	0.08 ± 0.0 a	29.3 ± 1.1 g	32.0 ± 3.0 g	136.0 ± 5.5 cd	0.61 ± 0.02 a	4.28 ± 0.54 a
	$T1_{Citric\ acid}$	7.1 ± 0.1 a	0.08 ± 0.0 a	35.6 ± 1.1 fg	44.3 ± 2.0 g	109.6 ± 6.8 f	0.49 ± 0.03 b	2.47 ± 0.23 c
	$T2_{Ctrl}$	6.86 ± 0.05 a	0.08 ± 0.0 a	91.7 ± 4.0 a	105.1 ± 4.7 d	112.3 ± 2.5 ef	0.22 ± 0.00 h	1.06 ± 0.02 e
	$T2_{EDTA}$	6.4 ± 0.1b	0.08 ± 0.0 a	54.3 ± 2.0 cd	132.0 ± 10.5 b	171.6 ± 5.0 b	0.34 ± 0.01 de	1.30 ± 0.09 de
	$T2_{Citric\ acid}$	7.1 ± 0.1 a	0.08 ± 0.0 a	46.6 ± 1.5 de	116.3 ± 1.5 cd	144.6 ± 6.4 c	0.28 ± 0.01 fg	1.24 ± 0.06 de
Soil 2	$T1_{Ctrl}$	6.86 ± 0.05 a	0.08 ± 0.0 a	42.3 ± 2.0 ef	82.3 ± 2.5 e	98.3 ± 2.0 f	0.32 ± 0.0 ef	1.19 ± 0.01 de
	$T1_{EDTA}$	6.9 ± 0.1 a	0.08 ± 0.0 a	33.0 ± 7.0 fg	58.3 ± 3.5 f	179.0 ± 7.2 b	0.59 ± 0.02 a	3.08 ± 0.30 b
	$T1_{Citric\ acid}$	7.1 ± 0.1 a	0.08 ± 0.0 a	31.0 ± 1.0 g	70.6 ± 1.5 ef	125.6 ± 5.0 de	0.42 ± 0.01 c	1.77 ± 0.08 d
	$T2_{Ctrl}$	6.86 ± 0.05 a	0.08 ± 0.0 a	95.3 ± 4.7 a	123.0 ± 2.0 bc	132.0 ± 1.0 cd	0.26 ± 0.0 gh	1.07 ± 0.01 e
	$T2_{EDTA}$	6.4 ± 0.1 b	0.08 ± 0.0 a	75.6 ± 4.7 b	146.6 ± 5.1 a	194.6 ± 3.5 a	0.38 ± 0.0 cd	1.32 ± 0.02 de
	$T2_{Citric\ acid}$	7.1 ± 0.1 a	0.08 ± 0.0 a	64.3 ± 3.2 c	124.6 ± 6.4 bc	169.0 ± 5.2 b	0.33 ± 0.01 e	1.35 ± 0.02 de

T1 means control without Pb addition; T2 means 500 mg kg^{-1} of Pb added to the soil. T1 & $T2_{Ctrl}$ means control with out chelators; T1 & $T2_{EDTA}$ means EDTA added; T1 & T2 $_{Citric\ acid}$ means Citric acid added. BF – Bioaccumulation Factor, Concentration ratio of shoots to soil; TF – Translocation factor, Concentration ratio of shoots to roots. Mean ± SD. Data in the same column followed by the same letter are not significantly different, whereas with different letters data are significantly different ($p < 0.05$).

Table 7: Effect of EDTA and citric acid on growth and biomass of *Ricinus communis* L.

Soil	Treatments	Biomass (g / Pot)	Root length (cm)	Shoot length (cm)	Total length (cm)
Soil 1	$T1_{Ctrl}$	113.3 ± 1.5 ef	18.6 ± 3.2 b	102.0 ± 1.0 d	120.6 ±3.5 c
	$T1_{EDTA}$	125.3 ± 4.5 cd	58.3 ± 0.5 a	163.3 ± 1.5 bc	221.6 ±1.1 ab
	$T1_{Citric\ acid}$	142.3 ± 2.5 a	63.0 ± 2.0 a	168.3 ± 4.7 abc	231.3 ±6.6 ab
	$T2_{Ctrl}$	106.3 ± 7.2 f	19.6 ± 4.0 b	100.3 ± 2.0 d	120.0 ±6.0 c
	$T2_{EDTA}$	126.6 ± 4.7 cd	59.3 ± 1.5 a	161.0 ± 2.0 c	220.3 ±3.5 b
	$T2_{Citric\ acid}$	142.6 ± 2.0 a	63.0 ± 2.0 a	171.0 ± 1.0 ab	234.0 ±2.6 ab
Soil 2	$T1_{Ctrl}$	115.3 ± 0.5 ef	27.6 ± 1.5 b	105.6 ± 1.1 d	133.3 ±2.5 c
	$T1_{EDTA}$	127.0 ± 1.0 c	61.0 ± 2.6 a	162.6 ± 2.5 bc	223.6 ±2.0 ab
	$T1_{Citric\ acid}$	143.3 ± 1.5 a	64.3 ± 3.0 a	169.3 ± 7.3 abc	233.6 ±10.4 ab
	$T2_{Ctrl}$	117.3 ± 1.1 de	19.6 ± 6.8 b	102.3 ± 2.5 d	122.0 ±8.6 c
	$T2_{EDTA}$	128.0 ± 2.0 bc	60.3 ± 2.0 a	164.3 ± 3.2 abc	224.6 ±5.0 ab
	$T2_{Citric\ acid}$	137.0 ± 2.0 ab	64.3 ± 3.0 a	173.3 ± 4.9 a	237.6 ±6.5 a

T1 means control without Pb addition; T2 means 500 mg kg^{-1} of Pb added to the soil. T1 & $T2_{Ctrl}$ means control with out chelators; T1 & $T2_{EDTA}$ means EDTA added; T1 & T2 $_{Citric\ acid}$ means Citric acid added. Mean ± SD. Data in the same column followed by the same letter are not significantly different, whereas with different letters data are significantly different ($p < 0.05$).

Table 8: Effect of EDTA and citric acid as enhancers of Pb in *Brassica juncea* (L.) Czern. in an artificially contaminated soil.

Soil	Treatments	Soil			Pb concentration in plants (mg kg^{-1})			
		pH	EC (mS cm^{-1})	Pb (mg kg^{-1})	Root	Shoot	BF	TF
Soil 1	$T1_{Ctrl}$	6.8 ± 0.1 ab	0.08 ± 0.0 a	79.3 ± 1.5 cd	52.0 ± 2.6 h	59.0 ± 3.0 hi	0.26 ± 0.01 e	1.13 ± 0.11 ab
	$T1_{EDTA}$	6.9 ± 0.5a	0.08 ± 0.0 a	46.3 ± 5.1 e	78.6 ± 4.0 g	90.3 ± 2.5 f	0.40 ± 0.01 bc	1.15 ± 0.08 ab
	$T1_{Citric\ acid}$	7.1 ± 0.1 a	0.08 ± 0.0 a	38.3 ± 3.0 e	124.6 ± 3.7 e	50.3 ± 4.5 i	0.22 ± 0.02 f	0.40 ± 0.03 d
	$T2_{Ctrl}$	6.7 ± 0.0 ab	0.08 ± 0.0 a	145.3 ± 5.8 a	170.0 ± 7.8 d	73.3 ± 7.0 g	0.14 ± 0.01 g	0.43 ± 0.02 d
	$T2_{EDTA}$	6.2 ± 0.0 a	0.08 ± 0.0 a	125.0 ± 3.0 b	192.3 ± 4.9 c	214.3 ± 1.5 b	0.42 ± 0.0 b	1.11 ± 0.03 ab
	$T2_{Citric\ acid}$	7.0 ± 0.1 a	0.08 ± 0.0 a	119.3 ± 1.5 b	172.0 ± 1.0 d	183.3 ± 1.5 c	0.36 ± 0.0 d	1.06 ± 0.0 b
Soil 2	$T1_{Ctrl}$	6.7 ± 0.1 ab	0.08 ± 0.0 a	48.3 ± 5.5 e	79.6 ± 2.0 g	69.6 ± 5.0 gh	0.23 ± 0.01 ef	0.87 ± 0.06 c
	$T1_{EDTA}$	6.9 ± 0.2 a	0.08 ± 0.0 a	43.6 ± 4.1 e	98.3 ± 2.5 f	100.3 ± 4.1 ef	0.33 ± 0.01 d	1.02 ± 0.01 bc
	$T1_{Citric\ acid}$	7.1 ± 0.1 a	0.08 ± 0.0 a	42.6 ± 2.0 e	108.3 ± 4.7 f	111.0 ± 5.2 e	0.37 ± 0.01 cd	1.02 ± 0.01 bc
	$T2_{Ctrl}$	6.7 ± 0.0 ab	0.08 ± 0.0 a	84.0 ± 3.6 c	250.0 ± 5.5 a	133.3 ± 1.5 d	0.26 ± 0.0 e	0.53 ± 0.01 d
	$T2_{EDTA}$	6.4 ± 0.1 bc	0.08 ± 0.0 a	71.0 ± 3.6 d	205.6 ± 5.0 b	251.0 ± 7.8 a	0.50 ± 0.01 a	1.22 ± 0.0 a
	$T2_{Citric\ acid}$	7.1 ± 0.1 a	0.08 ± 0.0 a	73.0 ± 2.0 d	197.6 ± 1.5 bc	204.0 ± 2.6 b	0.40 ± 0.0 bc	1.03 ± 0.0 b

T1 means control without Pb addition; T2 means 500 mg kg^{-1} of Pb added to the soil. T1 & $T2_{Ctrl}$ means control with out chelators; T1 & $T2_{EDTA}$ means EDTA added; T1 & T2 $_{Citric\ acid}$ means Citric acid added. BF – Bioaccumulation Factor, Concentration ratio of shoots to soil; TF – Translocation factor, Concentration ratio of shoots to roots. Mean ± SD. Data in the same column followed by the same letter are not significantly different, whereas with different letters data are significantly different ($p < 0.05$).

Table 9: Effect of EDTA and citric acid on growth and biomass of *Brassica juncea* (L.) Czern.

Soil	Treatments	Biomass (g / Pot)	Root length (cm)	Shoot length (cm)	Total length (cm)
Soil 1	$T1_{Ctrl}$	78.0 ± 2.6 ab	11.0 ± 1.0 c	104.0 ± 5.2 bcd	115.0 ± 5.5 b
	$T1_{EDTA}$	64.6 ± 1.5 de	15.3 ± 1.5 b	95.0 ± 4.3 cde	110.3 ± 5.6 b
	$T1_{Citric\ acid}$	85.0 ± 1.0 a	17.0 ± 1.0 ab	113.0 ± 2.0 ab	130.0 ± 1.7 a
	$T2_{Ctrl}$	66.0 ± 1.0 cde	8.0 ± 1.0 c	97.0 ± 1.0 cde	105.0 ± 2.0 b
	$T2_{EDTA}$	40.3 ± 2.0 f	17.6 ± 1.5 ab	91.6 ± 5.6 e	109.3 ± 4.1 b
	$T2_{Citric\ acid}$	69.3 ± 2.5 cd	15.6 ± 1.5 ab	113.0 ± 3.6 ab	128.6 ± 2.5 a
Soil 2	$T1_{Ctrl}$	72.6 ± 2.0 bc	8.0 ± 1.0 c	105.6 ± 6.8 abc	113.6 ± 6.6 b
	$T1_{EDTA}$	61.3 ± 2.0 e	19.3 ± 1.5 a	95.3 ± 3.7 cde	114.6 ± 2.3 b
	$T1_{Citric\ acid}$	82.0 ± 1.0 a	17.0 ± 1.0 ab	116.0 ± 1.0 a	133.0 ± 0.1 a
	$T2_{Ctrl}$	64.6 ± 1.5 de	7.3 ± 1.5 c	97.0 ± 1.0 cde	104.3 ± 1.1 b
	$T2_{EDTA}$	38.3 ± 5.5 f	17.3 ± 2.08ab	94.0 ± 3.6 de	111.3 ± 3.2 b
	$T2_{Citric\ acid}$	63.0 ± 2.6 de	16.0 ± 1.0 ab	115.6 ± 3.5 a	131.6 ± 4.5 a

T1 means control without Pb addition; T2 means 500 mg kg^{-1} of Pb added to the soil. T1 & $T2_{Ctrl}$ means control with out chelators; T1 & $T2_{EDTA}$ means EDTA added; T1 & T2 $_{Citric\ acid}$ means Citric acid added. Mean ± SD. Data in the same column followed by the same letter are not significantly different, whereas with different letters data are significantly different ($p < 0.05$).

In the present experiment, as expected, phytoextraction by *Ricinus communis* and *Brassica juncea* was relatively very high (194.6 and 251.0 mg kg^{-1}, respectively in the 500 mg kg^{-1} of Pb – polluted soil) compared to the controls. The application of EDTA solution significantly increased concentration of Pb in soil which also led to increased Pb uptake by *Ricinus communis* and *Brassica juncea* (Table 6 & 8). Thus, adding EDTA solution can significantly increase the bioavailability of the contaminated soil and concentration of Pb accumulation in plants (Lai and Chen, 2004). In agreement with our results, Epelde et al. (2008a) proved that Pb translocation from roots to shoots of cardoon plants was higher in the presence of EDTA. It can be concluded that high translocation of Pb in the shoot of the plant by EDTA was due to the stronger chemical affinity of EDTA for Pb (Luo et al., 2005). Since, EDTA and Pb were taken up together by the plant, and then Pb was translocated in the plant as the Pb – EDTA complex (Epstein et al., 1999). Then, these complexes rapidly enter the roots through endodermis and the Casparian stripes which is finally transported to the shoots of the plants (Bell et al., 1991). The transport of these metal complexes inside the plant cells are connected with internal transport proteins which play an important role in homeostasis of heavy metals. First the metal enters through two phospholipids layers and then into the cytoplasm of the cell. Here, Pb ions are chelated by compounds containing the – SH groups (i.e., phytochelatins, glutathione, organic acids and other ligands) and enters into the vacuole of the cells (Piechalak et al., 2003) where metal detoxification occurs by sequestration (Irtelli and Navari-Izzo, 2006). Blaylock et al. (1997) reported that EDTA enhanced Pb upto 15000 mg kg^{-1} in *Brassica juncea* shoots which was 6.6 % of Pb extracted in one phytoextraction cycle out of the total Pb in the soil. Citric acid treated pots also showed significant increase in Pb accumulation in *Ricinus communis* and *Brassica juncea* comparatively higher than control but lower than EDTA treated pots (Table 6 & 8). The results were in accordance with Huang et al. (1998) but in contrast with Wu et al. (2004) reported that citric acid has no effect on uptake of Pb.

The biomass, root and shoot length decreased significantly in *Brassica juncea* (Table 9) but there was no significant reduction in growth parameters of *Ricinus communis* (Table 7) with increase in Pb accumulation by plants. Biomass of the plant is very important factor in successful soil remediation because 1% of dry biomass would be required to reduce 500 mg Pb kg^{-1}over 20 – 25 years (Luo et al., 2005). There was no visible symptom of Pb toxicity in *Ricinus communis* during germination and growth period. However, leaves at the bottom of the plant became yellow and fell down. Leaf – fall may be due to metal detoxification mechanism in the leaves (Ernst et al., 1992; Neumann et al., 1995).

4.0 Conclusion:

Phytoextraction is dependent upon plants capacity to accumulate the heavy metal and independent upon the concentration of the metal(s) or pollutant(s). Even, EDTA had high efficiency in extracting Pb by both the plants. Its time to optimize the best method of application the chelate – assisted phtoextraction technique can be tested in the field. Since, there is a potential risk of leaching of metals into ground water. Accumulation of heavy metals by plants using chelators is close dependant and fixing the choice and time of application of soil amendment important for effective remediation. Further research is needed to determine the effectiveness of *Ricinus communis* and *Brassica juncea* in field conditions and on a multicontaminated soil.

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