To Study the Effect of Garlic Extract as a Chelating Agent against the Synthetically Available EDTA during Phyto-Extraction by Hyper Accumulator Species

Sidi. Shahenaz[1], Dr.Tank.S.K[2]

1. Research scholar, Dept. of Biosciences, VNSGU, Surat
2. Professor, Dept. of Biosciences, VNSGU, Surat

Email: sjadeja9@gmail.com (email of corresponding author)

Abstract: 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). Furthermore, increased leaching of Pb during EDTA-assisted phytoextraction hasmainly been attributed to the solubility of the Pb–EDTA complexes. It is further favored by the low sorption of Pb–EDTA complexes on soil particles (Benyahya and Garnier, 1999; Chen et al., 2003; Wu et al., 2003a) compared to free ions. Release of soil-bound Pb could increase with an increase in EDTA concentration. Therefore, high levels of soluble Pb in soil solution resulting from application of EDTA may lead to contamination of ground water .The adverse effects of EDTA are not only related to the enhanced solubility of the metals but also to their low biodegradability. EDTA therefore remains in soil for extended periods of time after treatment (Thomas et al., 1998; Nörtemann, 1999; Bucheli-Witschel and Egli, 2001; Meers et al., 2005b).Due to its limited lead bioavailability, an approach to increase its bioavailability is essential to the success of phytoremediation. Hence the potential of natural chelator: garlic extract was investigated. Therefore, Chelate – enhanced phytoextraction was performed using *Helianthus annus, Brassica juncea, spinacea oleracea and Amaranthus. virids* by applying the chelators (EDTA and garlic extract) as solution to the soil about 1 week before harvesting.

Key words: Garlic, Chelator, Lead, Soil

Introduction:

Lead is naturally present in soils. It is a trace constituent of common rock-forming and readily weatherable minerals such as Kfeldspar, plagioclase and mica, and a major constituent of various sulphide, sulphate, oxide, carbonate and silicate minerals (e.g., galena PbS, anglesite PbSO4, minium Pb3O4, cerussite PbCO3, alamosite PbSiO3) locally found in Pb ores and their weathering products (Reimann and de Caritat, 1998). In uncontaminated soils, Pb concentrations are generally below 50 mg kg_1 (Reimann and de Caritat, 1998). Mining, industrial, and agricultural activities have lead to an accelerated release of various metals including Pb into the environment, creating potential hazards to ecosystems and human health (Lantzy and Mackenzie, 1979; Nriagu, 1979; Mushak, 1993).

Conventional clean-up technologies are costly and feasible only for small but heavily polluted sites where rapid and complete decontamination is required (Blaylock et al., 1997; Cooper et al., 1999; Shen et al., 2002). The increasing demand for uncontaminated agricultural land fueled by population growth has increased the need for an effective clean-up of moderately contaminated soils as well. Phytoremediation, especially phytoextraction, has received increasing attention as a promising, cost-effective alternative to conventional engineering-based remediation methods (Salt et al., 1998). A plant is said to be ahyperaccumulating species if it accumulates metals in aboveground biomass above a given concentration, for example,100 mg kg_1 for Cd on a dry weight (DW) basis, 1000 mg kg_1 for As, Co, Cu, Pb or Ni, or more than 10000 mg kg_1 for Mn or Zn DW (Brooks, 1998; Baker et al., 2000; Ma et al., 2001; McGrath et al., 2002; Schmidt, 2003).

Phytoextraction approach involves the use of high yielding plant species. These species lack inherent ability to take up large concentrations of metals, but can accumulate elevated amounts when cultivated on soils that have been chemically treated with soil amendments to enhance metal phytoavailability and plant uptake (Meers et al., 2005b). The success of phytoextraction is strongly determined by the amount of biomass, the concentration of heavy metals in plant tissues, and the bioavailable fraction of heavy metals in the rooting medium (McGrath, 1998; Grcˇman et al., 2001). Pb is a widespread metal pollutant in soils (Mushak, 1993). However, it is usually poorly bioavailable (Miller, 1996; Raskin et al., 1997) due to the formation of insoluble precipitates (McBride, 1994; Blaylocket al., 1997; Ruby et al., 1999; Adriano, 2001; Shen et al., 2002). Pb contamination is usually accumulated in the uppermost horizons of soil profiles (Anderson, 1977; Wang et al., 1995; Abreu et al., 1998;

Johnson and Petras, 1998; Sanchez-Camazano et al., 1998). Only a very minor fraction of the total soil Pb is in soil solution (Davies, 1995; Maiz et al., 2000).

Accelerated, uncontrolled release and leaching of metals from sparingly soluble metal compounds is likely to increase groundwater pollution (Grcˇman et al., 2001; Römkens et al., 2002; Madrid et al., 2003; Wenzel et al., 2003). Careful management of soils and the appropriate selection of plants and irrigation strategies are of paramount importance (Chen et al., 2004), while the focus might need to shift towards the use of more degradable alternatives, thus effectively reducing the risks implied with this technology (Meers et al., 2004).

A study by Cui et al. (2004) showed that the combined application of EDTA and elemental sulfur significantly enhanced solubilization of heavy metals (Pb and Zn). Similarly, citric acid in conjunction with EDTA has been shown to enhance the solubilization of Pb and significantly increase the rate of metal translocation from root to shoot in Fetuca arundianacea Scherb grown on Pb-contaminated soil (Begonia et al., 2005). With thus in mind garlic extract which is an excellent source of sulphur and effective metal chelator in huan body was tried upon for its chelation ability.

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 Helianthus annus, Brassica juncea, spinacea oleracea and Amaranthus. virids by applying the chelators (EDTA and garlic extract) as solution to the soil about 1 week before harvesting (Wu et al.,2004)

Methodology

Plants were grown on the soils spiked with 5, 50, 250 ppm lead stock. They were watered regularly and a week before harvest the chelators were added to 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 ground into fine powder using mortar and pestle and used for heavy metal analysis. 0.5 grams powder of the plant sample\ was weighed into a 100 ml conical flask. 30 ml of concentrated nitric acid was added and the contents were digested on a hot plate at 80°C for 2 hrs. After cooling the volume was made up to 100 ml with double distilled water and filtered through Whatman No.1 filter paper (Pratt, 1965) and then fed into the ICP: AES.

Metal uptake by plants involves a series of processes such as metal desorption from soil particles, transport of soluble metals to root surfaces via diffusion or mass flow; metal uptake by roots and metal translocation from roots to shoot. Chelators enhance desorption of heavy metals from the soil matrix to the soil solution (Means et al., 1978; Stanhope et al., 2000; Nowack, 2002; Nascimento et al., 2006), facilitate metal transport into the xylem (Huang and Cunningham 1996; Blaylock et al., 1997; Huang et al., 1997) and increase metal translocation from roots to shoots (Barber and Lee, 1974; Hamon et al., 1995; Vassil et al., 1998; Gleba et al., 1999).

Observations:

The plants were harvested after chelator application. The photographs of the 5 ppm set have been shown in the figure 1 given below.

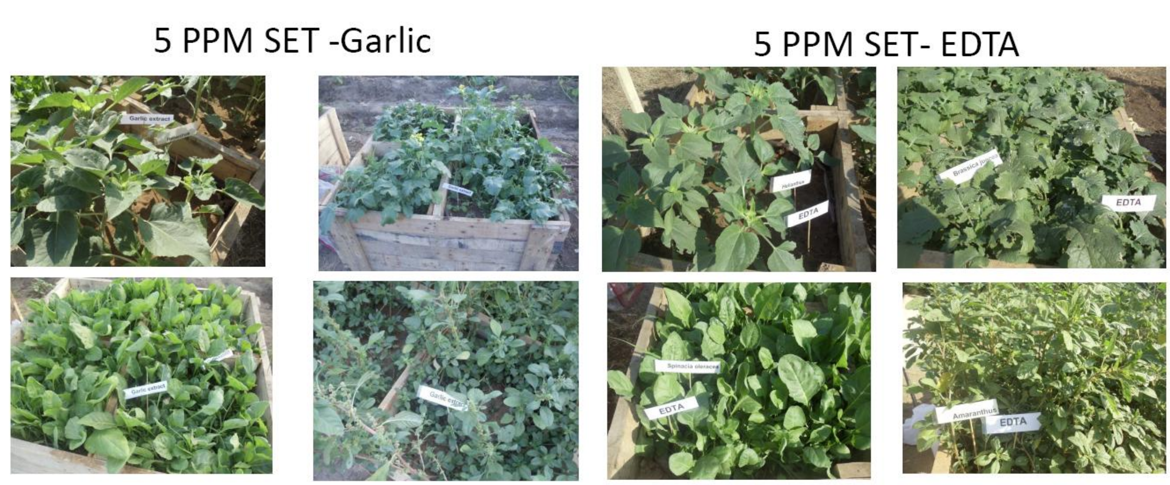

Figure 1: Plants exposed to 5 ppm lead nitrate and treated with EDTA and garlic extract one week before harvesting

The plant digests were analyzed for lead using ICP AES at SAIF, IIT Bombay. The results obtained were as mentioned in table 1

Table 1: Bioconcentration factror (BF) , Translocation factor (TF) and Total metal concentration in root and shoot of plants exposed to EDTA and garlic extract.

Helianthus annus						
Soil conc	Treatment	Root (mg/kg)	Shoot (mg/kg)	Total uptake (mg/kg)	BF	TF
5	EDTA	8.4832032	3.8605521	12.3437554	11.5716	0.4550818
50	EDTA	21.916319	62.544931	84.4612512	6.75690	2.8538063
250	EDTA	288.91736	272.92191	561.839275	8.98942	0.9446366
5	Garlic extract	3.2793209	1.7411491	5.02047015	4.01637	0.5309480
50	Garlic extract	86.863166	20.791168	107.654334	8.61234	0.2393554
250	Garlic extract	692.07660	82.022471	774.099077	12.3855	0.1185164

Brassica juncea						
Soil conc	Treatment	Root (mg/kg)	Shoot (mg/kg)	Total uptake (mg/kg)	BF	TF
5	EDTA	36.8393647	13.1168831	49.9562479	39.9649	0.35605617
50	EDTA	215.521904	150.045956	365.56786	29.2454	0.69619817
250	EDTA	1302.44962	837.190742	2139.64037	34.2342	0.64278167
5	Garlic extract	4.34201737	27.1912502	31.5332676	25.2266	6.26235408
50	Garlic extract	123.271434	92.8526514	216.124086	17.2899	0.75323737
250	Garlic extract	423.119065	370.658442	793.777507	12.7004	0.87601451

Spincea oleracea						
Soil conc	Treatment	Root (mg/kg)	Shoot (mg/kg)	Total uptake (mg/kg)	BF	TF
5	EDTA	9.81639702	5.43794912	15.2543461	12.2035	0.55396589
50	EDTA	151.898734	70.3638755	222.26261	17.7810	0.46322885
250	EDTA	96.3798778	16.3881748	112.768053	1.80429	0.17003731
5	Garlic extract	8.59106529	3.23991576	11.8309811	9.46478	0.37712619
50	Garlic extract	151.213592	39.0063642	190.219956	15.2176	0.25795541
250	Garlic extract	313.683458	72.7309562	386.414415	6.18263	0.23186099

Amaranthus viridus

Soil Conc	Treatment	Root (mg/kg)	Shoot (mg/kg)	Total uptake (mg/kg)	BF	TF
5	EDTA	3.36	2.96296296	6.32296296	5.05837	0.88183422
50	EDTA	43.2260737	116.41648	159.642554	12.7714	2.69320043
250	EDTA	36.5988909	107.515658	144.114549	2.30583	2.93767529
5	Garlic extract	3.4529062	4.22751729	7.68042349	6.14434	1.22433598
50	Garlic extract	4.49486301	28.2437885	32.7386515	2.61909	6.28357047
250	Garlic extract	19.7245999	43.7940141	63.518614	1.01630	2.22027388

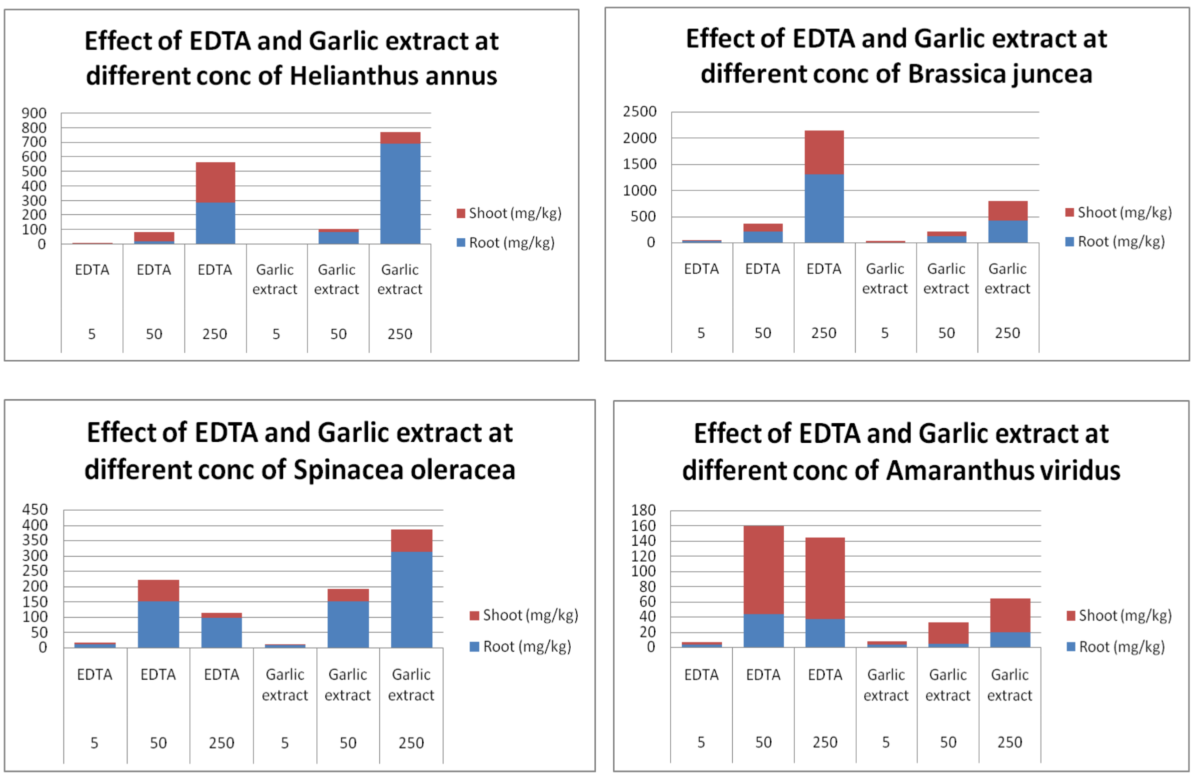

Fig 2 : Graphical representation of effect of EDTA and garlic extract as chelators

Conclusion:

In case of Helianthus, BF was higher in 50 and 250 ppm treatments due to Garlic extract and TF was highest in 50 ppm treatment due to EDTA. (Refer Figure 2)

In case of Brassica, BF was higher in case of plants treated with EDTA but garlic proved to be promising for increased translocation at 5ppm. (Refer Figure 2)

In case of Spinacea, highest BF was seen in plant treated with 50 ppm and EDTA. Garlic extract proved to be effect ive at 250 ppm conc. (Refer Figure 2) Morel et al. (1986) reported that the majority of Pb taken up by roots is bound to carboxyl groups of mucilage uronic acids. According to Jarvis and Leung (2002), Pb retention in roots is based on the binding of Pb to ion exchange sites on the root cell walls and extracellular precipitation, mainly in the form of Pb-carbonates. Once absorbed by roots, Pb is rather immobile, showing very limited translocation into above-ground foliage (Malone et al., 1974; Zimdahl and Koeppe, 1977; Reeves and Brooks, 1983; Rudakova et al., 1988; Kumar et al., 1995; Zheljazkov and Nielsen, 1996; Brennan and Shelley, 1999; Salt and Kramer, 2000; Kabata-Pendias, 2001; Wilde et al., 2005).

In case of Amaranthus, highest BF was higher at 50 ppm and 250 ppm with EDTA and highest Translocation of metal was seen at 50 ppm treated ith garlic extract. (Refer Figure 2)

Overall garlic extract was proved to most effective in Brassica juncea and Spinacea oleracea. The effect of both the chelators was most prominent in Brassica juncea with highest BF of 40.

Metal uptake by plants involves a series of processes such as metal desorption from soil particles, transport of soluble metals to root surfaces via diffusion or mass flow; metal uptake by roots and metal translocation from roots to shoot. Chelators enhance desorption of heavy metals from the soil matrix to the soil solution (Means et al., 1978; Stanhope et al., 2000; Nowack, 2002; Nascimento et al., 2006), facilitate metal transport into the xylem (Huang and Cunningham 1996; Blaylock et al., 1997; Huang et al., 1997) and increase metal translocation from roots to shoots (Barber and Lee, 1974; Hamon et al., 1995; Vassil et al., 1998; Gleba et al., 1999).

The above experiments suggests that further research on different concentrations of garlic extract can be studied upon for further application as it’s a greener alternative to EDTA for phytoextraction

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