Full Length Research Article

Research Journal of Agricultural Sciences
Volume 15; Issue 02 (Mar–Apr 2024); pp 316–320

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Phyto-remediation of Lead (Pb) Assisted with EDTA by Using Amaranth (*Amaranthus hybridus* L.) in Contaminated Soil

Adarsh Bhushan*[1], Dinesh Mani[2] and Pravesh Kumar[3]

[1-3] Sheila Dhar Institute of Soil Science, Department of Chemistry, University of Allahabad, Prayagraj - 211 002, Uttar Pradesh, India

Received: 05 Nov 2023; Revised accepted: 12 Feb 2024; Published online: 04 Mar 2024

Abstract

The pot experiment was conducted to find out the Lead (Pb) remediation potential of Amaranth in contaminated soil with EDTA. The soil samples were tested to different levels of Pb contamination namely control, 300 ppm,300 ppm+EDTA,600 ppm,600 ppm + EDTA, 1200 ppm, 1200ppm+EDTA, 1800ppm, 1800ppm+EDTA per pot respectively with EDTA as a dose of 3.0 mmole EDTA/kg soil. The application of EDTA gives a significant effect on the solubility of Pb in soil and Pb absorption by Amaranth. The result revealed that Pb has been accumulated higher amount in root, shoot and leaves of test crop Amaranth. The TF>1.0 value indicates the hyperaccumulation tendency of Amaranth therefore; it may be a promising plant species for phytoremediation.

Key words: Amaranth, EDTA, Hyperaccumulation, Phyto-remediation

The pollution caused by heavy metals has been reported as critical environmental problem and has profound effects on health of human beings [2]. Soil contamination by heavy metals (HMs) has become a serious threat to the human population, agriculture and food security. Anthropogenic activities are significant sources of metals and metalloids in the environment, for instance through rapid industrialization and urbanization, mining and agricultural practices such as the uncontrolled application of fertilizers and pesticides [31]. There has been an increasing concern with regard to the accumulation of heavy metals in the environment as they pose a threat to both human health and the natural environment [1]. This is due to the fact that unlike many substances, metals are not biodegradable and hence accumulate in the environment [11].

Synthetic chelators, such as EDTA, DTPA and EGTA, form soluble complexes with metals in the soil and can increase the uptake and translocation of heavy metals through the aboveground tissues [5]. Organic and inorganic amendments are used for immobilization of metals in the soils with varying benefits but organic amendments could be better option due to improvement of physical, chemical, biological properties and fertility status of the soil [24]. Organic amendments improve both the physic-chemical status and biological activity of contaminated soil. Repeated applications increase water retention capacity, reduce soil erosion, and affect metal speciation and plant bioavailability [6], [28]. Different organic amendments like manures, composts, biosolid and municipal solid wastes, pressmud, and activated carbon are used for immobilization of metals in the contaminated soils [24], [26].

EDTA is one of the successful and admired chemical regent because it is a powerful, recoverable and comparatively biostable chelator which has ability to remediate soil [13]. The process of EDTA addition is considered as an important aspect controlling the leaching of metals [29]. Romkens *et al*. studied that EDTA having more definite affinity for Cd, increased solubility of metal, but it did not enhance plant metal uptake accordingly and biomass production of shoot and root was depressed. EDTA may also be toxic itself diminishing plant biomass sufficient to curtail its metal mobilizing and transmitting benefits [10], [25].

Phytoremediation of soils contaminated with heavy metals is an emerging technique that intends to extract or immobilize heavy metals and it has great value because it has the advantage of being relatively cheap and an environmentally friendly technology [21-22]. Remediation methods for heavy metal contaminated areas are based on physical (soil replacement, isolation and vitrification), chemical (soil washing and immobilisation of metals) and biological (phytoextraction, phyto stabilisation and phyto evaporation) approaches [15]. Non-biological methods are effective, but they are also expensive and often damage surrounding areas. In contrast to non-biological approaches, biological remediation options, employing plants to clean contaminated soils, appear to be environmentally friendly, low-cost and meet sustainability criteria [9], [19].

Keeping in view the above-mentioned facts, the present investigation was carried out in order to study the effect of different levels of EDTA on growth response of Amaranth, concentration of Lead (Pb) in Amaranth and remedial potential of Amaranth.

MATERIALS AND METHODS

Experimental site

***Correspondence to:** Adarsh Bhushan, E-mail: pateladarsh183@gmail.com; Tel: +91 6307072371

Citation: Bhushan A, Mani D, Kumar P. 2024. Phyto-remediation of lead (Pb) assisted with EDTA by using amaranth (*Amaranthus hybridus* L.) in contaminated soil. *Res. Jr. Agril. Sci*. 15(2): 316-320.

A pot experiment was carried out in order to find out the phytoremediation potential of *Amaranthus hybridus* for lead contaminated soil. The Sheila Dhar Institute Experimental Site is located at Prayagraj in Northern India at 25˚ 57′N latitude, 81˚50′ E longitude and at 120±0.4m altitude. The mean annual temperature range between 22˚C and 9˚C respectively. Soil properties were determined before treating the soil with lead and with or without EDTA. The lead level in the uncontaminated soil sample was found to be 8.1 to 8.5 mg kg^{-1}. Textural triangle was used to determine the class name of the soil. The experimental soil was sandy loam soil. The physico-chemical properties of the soil are shown in (Table 1).

Table 1 Physico-chemical properties of experimental soil

Parameters	Units	Value
Texture		Sandy loam
Clay	(%)	15.25
Silt	(%)	13.22
Sand	(%)	71.53
pH		7.8 ± 0.2
EC	(dS/m) at 25˚C	0.28 ± 0.03
Organic carbon	(%)	0.56 ± 0.15
CEC	[Cmol(p+) / kg soil]	19.6 ± 0.6
Total nitrogen	(%)	0.07 ± 0.02
Total phosphorus	(%)	0.03 ± 0.01
Total lead	mg kg^1	8.25 ± 0.10

± Values indicate Standard Deviation having three replications, EC=Electrical Conductivity, CEC =Cation Exchange Capacity, EDTA= Ethylinediamine tetra acetic acid

Soil collection

Top soils (0-15 cm) were collected from Sheila Dhar Institute of Soil Science, Prayagraj, Uttar Pradesh, India. The soils were thoroughly mixed by a mechanical mixer and passed through 4 mm sieve to remove fibre and non-soil particulate in the sample. The chemical and physical properties of the soils were determined prior to planting.

Experimental plants procurement

The healthy and viable seeds of *A. hybridus* were purchased from local market Chungi (Alopibagh) of Prayagraj. The uniform seeds were collected and used for the pot experiment. The plants height was measured from the soil level to the terminal bud using a meter rule at 7 days interval. Number of leaves was also counted weekly as the plant grew.

Pot experiment

For pot experiment 4 mm sieve used and 2 mm sieve is used for analysis of physico-chemical properties. Some physico- chemical properties are depicted in (Table 1). Firstly, the seeds of Amaranth were germinated on the Whatman No. 42 filter paper in petri dishes, after that the seeds were transferred into earthen pot with untreated soil (control) and treated soil to which lead metal as lead carbonate ($PbCO_3$) was applied with different concentration. Each pot contained 3-5 kg soil. There were 10-20 seeds grown in each pot with three replicates. After 45 days, the plants were grown without Pb metal solution. This would be higher accumulation of metals in plants.

Soil physico-chemical analysis

Soil pH was measured with 1:2.5 soil water ratio using Elico digital pH meter (Model LI127, Elico Ltd., Hyderabad, India) at authors' laboratory. Double distilled water was used for the preparation of all solutions. Organic carbon was determined by chromic acid digestion method, cation exchange capacity (CEC) by neutral 1 N ammonium acetate solution, total nitrogen by digestion mixture (containing sulphuric acid, selenium dioxide and salicylic acid) using micro-Kjeldahl method, Glass Agencies, Ambala, India. Total phosphorus by hot plate digestion with HNO_3 (16M, 71%) and extraction by standard ammonium molybdate solution [8], [17], [20].

Determination of total lead in soil

1.0g of soil sample (passed through 0.5mm sieve) was weighed into crucibles in duplicate. 10ml of conc. H_2SO_4 10ml of conc. $HClO_4$ and 5ml of conc. HNO_3 were added. The mixture was swirled gently and heated at low to medium heat on a hot plate. The heating was continued until the solution dried off and the crucible was allowed to cool. 50ml of distilled-deionized water was added to rinse the crucible gradually and then filtered. The filtrate was then analyzed for lead using AAS.

Plant sample analysis

The crop was harvested separately after 45 days according to soil treatment. The 3 replicates of each treatment were pooled together to give composite sample of each treatment. The plants were then washed carefully in water to eliminate dust, dirt, and they were again washed with deionized water [32]. The leaves, stems and roots of each composite sample were then separated as sub-samples. Each sub-sample was oven-dried at 90°C for 24 hours and ground to a fine powder. One gram of ground plant material was digested with 15 ml of tri-acid mixture containing conc. HNO_3 (16M, 71%), H_2SO_4(18M, 96%) and $HClO_4$ (11M, 71%) in 5:1:2), heated on hot plate at low heat (60 °C) for 30 minutes [17]. The beakers containing the samples were covered with watch glasses and left overnight. The digestion was carried out at a temperature of about 90 °C until about 4ml was left in the beaker. Then, a further 10ml of the mixture of acids was added. This mixture was allowed to evaporate to a volume of about 4ml. After cooling, of solution was filtered to remove small quantities of waxy solids and made up to a final volume (50ml) with distilled water. Lead concentrations were determined using Atomic Absorption Spectrophotometer.

Calculation

Determination of the bio-concentration factor (BCF)

The bio-concentration factor (BCF) of metals was used to determine the quantity of heavy metals that is absorbed by the plant from the soil. This is an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil and is calculated using the formula:

$$BCF = \frac{\text{Metal concentration in plant tissues (whole plant/portal)}}{\text{Initial concentration of metal in substrate (soil)}}$$

Determination of the movement of metals from roots to plants

To evaluate the potential of plants for phytoextraction, the translocation factor (TF) was used. This ratio is an indication of the ability of the plant to translocate metals from the roots to the aerial parts of the plant. It is represented by the ratio:

$$TF = \frac{\text{Metal concentration (Stem + leaves)}}{\text{Metal concentration (roots)}}$$

Metals that are accumulated by plants and largely stored in the roots of plants are indicated by TF values < 1 with values > 1 indicating that the metals are stored in the stems and leaves.

Percentage removal (%) was calculated from the total concentration (TC) of elements initially present in the soil [23].

Data analysis

Statistical analysis was carried out by one-way ANOVA using general linear model and t-Test to evaluate significant differences between means of plant biomass, absorbed heavy metal concentrations in harvested plants at 95% level of confidence [27]. Further statistical validity of the differences among treatment means was estimated using the least significance difference (LSD) method. The diagrams were plotted using the Graph Pad Prism 9.

RESULTS AND DISCUSSION

Dry mater yield

The number of leaves in the control plants was greater than those planted on contaminated soils. The dry matter yield of leaves, stems and roots was highest for control and lowest in the treated plants. For the leaves and stems, the lowest dry matter was found in 1800ppm + EDTA treated plants, while it was found lowest in the 1800ppm + EDTA treatment for the roots. The observations were found that the dry natter yields per pot are related to the concentration of lead in shoots of Amaranth. Dry matter yields decreased with increased lead concentration in shoots of Amaranth which is shown in (Table 2).

Lead concentration

The levels of lead in leaves and stems increased as the level of lead in the roots of Amaranthus. The sufficient amount of lead was detected in the plant roots. The sufficient amount of lead was detected in the plant roots correspond to soluble lead in soil. So, the treatments with EDTA application were found higher amount of lead in their tissues than Pb-contaminated soil without EDTA. Also, the latter had high concentrations of lead in roots when compared with those grown on the soils amended with EDTA.

Table 2 Dry matter yield of *Amaranthus hybridus* in lead contaminated soils with and without EDTA application

Treatment	Dry matter yields (g/pot)			
	Leaves	Stem	Root	Total
Control	6.42 ± 0.39	3.18 ± 0.55	1.38 ± 0.17	10.98
300ppm	5.13 ± 0.50	3.00 ± 0.57	1.11 ± 0.02	9.24
300ppm + EDTA	4.63 ± 0.33	2.86 ± 0.64	1.00 ± 0.28	8.49
600ppm	4.75 ± 0.36	2.47 ± 0.44	0.98 ± 0.28	8.20
600ppm + EDTA	3.97 ± 0.36	2.40 ± 0.46	0.73 ± 0.04	7.10
1200ppm	3.32 ± 0.46	2.23 ± 0.51	0.71 ± 0.06	6.33
1200ppm + EDTA	2.62 ± 0.40	2.12 ± 0.53	0.70 ± 0.20	5.44
1800ppm	2.36 ± 0.47	2.00 ± 0.57	0.66 ± 0.19	5.02
1800ppm + EDTA	1.00 ± 0.28	1.96 ± 0.03	0.63 ± 0.04	3.59

Values are average of three replicates ± S.D (n=3)

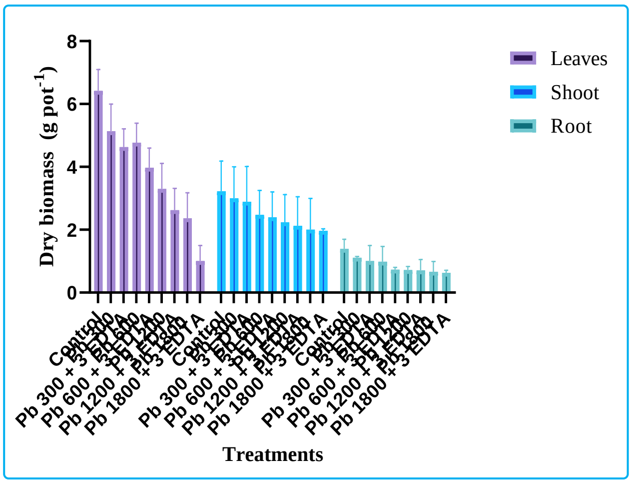

Fig 1 Dry biomass of leaves, root and shoot in *Amaranthus hybridus* under different treatments of Pb and EDTA. Error bar represent the standard deviation of three measurements

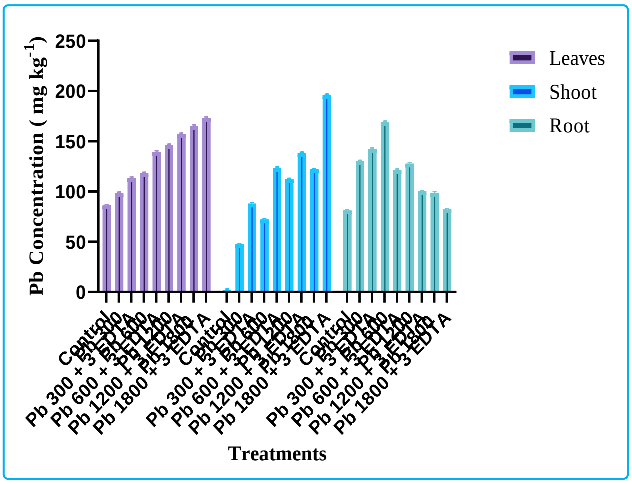

Fig 2 Accumulation of Pb in the leaves, root and shoot of *Amaranthus hybridus* under different treatments of Pb and EDTA. Error bar represents the standard deviation of three measurements

TF and BCF

The application of EDTA resulted in a significant increase in Pb uptake in both the shoots and roots. The most important parameter for phytoremediation is a high uptake of heavy metals into the harvestable biomass of plant. The concentration of lead in soil significantly enhanced the shoot to root ratio of *Amaranthus hybridus*. The percentage of absorbed lead translocated from roots to shoots increased from 52.07% in the control sample to 81.80% in 1800ppm + EDTA amended soils. The distribution of lead in leaves, stems and roots of *Amaranthus hybridus* was affected by EDTA application as there were found increased levels in plants, grown on EDTA amended soils.

The identification of metal hyper-accumulator plants are capable of accumulating high metal levels demonstrate that plant have genetic potential to clean up contaminated soils [18]. Hence bio-concentration factor (BCF) may better characterize hyper-accumulators than concentration ratio (CR) [30]. From this study, the BCF that is based on water soluble lead reflect plant accumulation of lead accurately in the soil rather than on total soil lead, as only a small quantity of total lead in the soil is readily taken up by plant root as reported by Tu *et al*. [30].

Lowest BCF value is found in control whereas the maximum value found in treatment of 300ppm + EDTA (0.67).

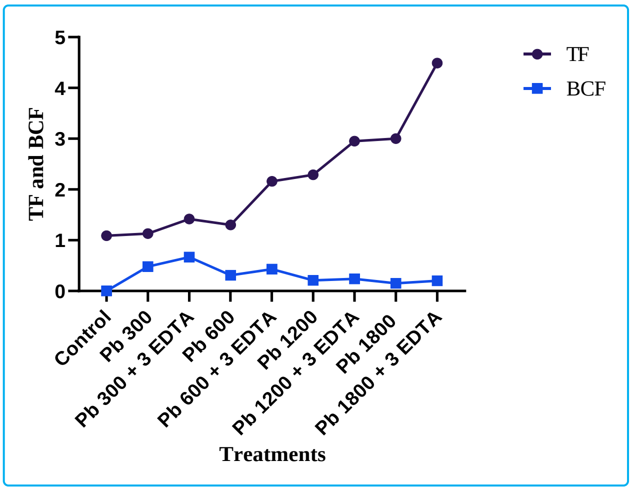

Fig 3 TF and BCF of Pb in *Amaranthus hybridus* under different treatments of Pb and EDTA

There are three categories of plant based on their TF namely accumulator (TF>1), excluder (TF<1), and indicator (TF near 1) [4], [12]. Generally, the TF in all confirmed hyper accumulators are >1 where as it is usually <1 in non – accumulators. More ever, the higher the TF value is, the stronger the phyto-extraction ability. In this study the translocation factor was found >1 for *A. hybridus.* The values of TF in *A. hybridus* were found from 1.09 to 4.49. The treatments with EDTA had higher TF value than Pb-contaminated soil without EDTA.

CONCLUSION

From this study, Amaranth was found the best plant species to carry out phyto-remediation of Pb in contaminated soil due to its hyper-accumulator characteristics. According to the Translocation Factor (TF) the Amaranth plant can be classified as a hyper-accumulator plant to the Pb because the TF value of Amaranth plant in this study found more than 1 (>1). Hence, it is a hyper- accumulator and promising plant for phytoremediation. In case of Pb, availability of suitable soil amendments can be explored that may improve its uptake from soils. The application of EDTA enhanced the remediation efficiency in Pb contaminated soils. However, EDTA may have environmental consequences. Amaranth showed Pb removal potential, as quick and short duration vegetable crops. This provides the opportunity to grow Amaranth many times on a piece of land to clean-up Pb from contaminated soil. However, to take the plant for phyto-remediation works, further studies on lead absorption, concentration and nutrient qualities in the leaves and stems should be carried out in details and monitored regularly.

Conflict of interest

Through this, all authors declare that there is no conflict about the publication of this research paper.

LITERATURE CITED

1. Abioye PO, Abdul Aziz A, Agamuth P. 2010. Enhanced biodegradation of used engine oil in soil amended with organic waste. *Water, Air, and Soil Pollution* 209(1): 173-17.
2. Ali B, Wang B, Ali S, Ghani MA, Hayat MT, Yang C, Xu L, Zhou WJ. 2013. 5-Aminolevulinic acid ameliorates the growth, photosynthetic gas exchange capacity, and ultrastructural changes under cadmium stress in *Brassica napus* L. *Jr. Plant Growth Regulation* 32: 604-614.
3. Ali H, Khan E, Sajad MA. 2013. Phytoremediation of heavy metals—Concepts and applications. *Chemosphere* 91: 869-881.
4. Baker AJM. 1981.Accumulators and excluders: Strategies in the response of plants to heavy metals. *Journal of Plant Nutrition* 3: 643-654.
5. Blaylock MJ, Salt DE, Dushenkov S, Zakharova O, Gussman C, Kapulnik Y, Ensley BD, Raskin I. 1997. Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environ. Sci. Technology* 31: 860-865.
6. Bot A, Benites J. 2005.The importance of soil organic matter. *In*: FAO Soils Bulletin 80. Food and agriculture organization of the United Nations, Rome.
7. Burkil HM. 1985. The useful plant of West Africa. 1985. 2nd Edition. Families A-D Royal Botanic Gardens, Kew. pp 51-52.
8. Chopra SL, Kanwar JS. 1999. *Analytical Agricultural Chemistry*. Kalyani Publication, New Delhi.
9. Cunningham SD, Ow W. 1996. Promises and prospects of phytoremediation. *Plant Physiology* 110: 715-719.
10. Do Nascimento CWA, Amarasiriwardena D, Baoshan X. 2006. Comparison of natural organic acids and synthetic chelates at enhancing phytoextraction of metals from a multimetal contaminated soil. *Environ. Pollution* 140(1): 114-123.
11. Ghanbari-Bonjar A, Lee HC. 2003. Intercropped wheat (*Triticum aestivum*) and bean (*Vicia faba*) as whole-crop forage: Effect of harvest time on forage yield and quality. *Grass and Forage Science*1: 28-36.
12. Ghosh M, Singh SP. 2005. A review on phytoremediation of heavy metals and utilization of it's by products. *Applied Ecology and Environmental Research* 3: 1-1.
13. Hong PKA, Li C, Banerji SK, Regmi T. 1999. Extraction, recovery and biostability of EDTA for remediation of heavy metal-contaminated soil. *Jr. Soil Contamination* 8: 81-103.
14. Kaihura BS, Kullaya IK, Kilasara M, Aune JB, Singh BR, Lal R. 1999. Soil quality effects of accelerated erosion and management systems in three eco-regions of Tanzania. *Soil Tillage Research* 53: 59-70.
15. Khalid S, Shahid M, Niazi NK, Murtaza B, Bibi I, Dumat C. 2017. A comparison of technologies for remediation of heavy metal contaminated soils. *Jr. Geochem. Explorer* 182: 247-268.
16. Khan MA, Khan S, Khan A, Alam M. 2017. Soil contamination with cadmium, consequences and remediation using organic amendments. *Sci. Total Environment* 601: 1591-1605.
17. Kumar C, Mani D. 2010. Enrichment and management of heavy metals in sewage-irrigated soil. Lap LAMBERT Acad. Publishing, Dudweiler (Germany).
18. Lasat MM. 2002. Reviews and analyses – Phyto extraction of Toxic metals; A review of biological mechanisms: A review of biological mechanism. *Journal of Environments Quality* 31: 109-120.

19. Li JT, Baker AJM, Ye ZH, Wang HB, Shu WS. 2012. Phytoextraction of Cd-contaminated soils: Current status and future challenges. *Crit. Rev. Environ. Sci. Technology* 42: 2113-2152.
20. Mani D, Mourya VK, Balak S, Patel NK, Pal N. 2014. Effect of organic matter on the uptake of cadmium by Spinach (*Spinacea oleracea* L.). *Asian Jr. Adv. Basic Sciences* 3(1): 144-150.
21. McGrath SP, Sidoli CMD, Baker AJM, Reeves RD. 1993. The potential for the use of metal accumulating plants for the in-situ de-contamination of metal-polluted soils. *In*: (Eds) Eijsackers HJP, Hamers, T. Integrated Soil and Sediment Research: A Basis for Proper Protection. Kluwer Academic Publishers, Dordrecht. pp 673-677.
22. McGrath SP, Zhao FJ, Lombi E. 2002. Phytoremediation of metals, metalloids, and radionuclides. *Advanced Agronomy* 75: 1-56.
23. Paiva HN, Carvalho JG, Siqueira JO. 2002. Translocation index of nutrients incedro (CedrelafissilisVell.) and ipe-roxo (Tabebuiaimpetiginosa (Mart.) Standl.) seedlings submitted to increasing levels of cadmium, nickel and lead. RevistaArvore 26: 467–473.
24. Park JH, Lamb D, Paneerselvam P, Choppala G, Bolan N, Chung, JW. 2011. Role of organic amendments on enhanced bioremediation of heavy metal (loid) contaminated soils. *Jr. Hazard Mat*. 185(2): 549-574.
25. Romkens P, Bouwman L, Japenga J, Draaisma C. 2002. Potentials and drawbacks of chelate-enhanced phytoremediation of soils. *Environ. Pollution* 116: 109-121.
26. Sabir M, Hanafi MM, Aziz T, Ahmad HR, Rehman MZ, Saifullah, Murtaza G, Hakeem KR. 2013. Comparative effect of activated carbon, pressmud and poultry manure on immobilization and concentration of metals in maize (*Zea mays*) grown on contaminated soil. *Int. Jr. Agric. Biology* 15(3): 559-564.
27. SAS. 2003. SAS Institute, 2003. SAS Version 9.1.2(c). SAS Institute, Inc., Cary, NC. pp 449-453.
28. Shahid M, Sabir M, Ali MA, Ghafoor A. 2014. Effect of organic amendments on phyto availability of nickel and growth of berseem (*Trifolium alexandrinum*) under nickel contaminated soil conditions. *Chem. Speciat. Bioavailab.* 26: 37-42.
29. Sun Y, Zhou Q, Xu Y, Wang L, Liang X. 2011. The role of EDTA on cadmium phytoextraction in a cadmium hyper accumulator *Rorippa globosa. Jr. Environ. Chem. Ecotoxicology* 3(3): 45-51.
30. Tu Cong, Lena Q, Ma, Bhaskar Boondade. 2002. Plant and environment interactions – Arsenic accumulation in the hyper accumulator Chinese Brake and its utilization potential for phyto remediation. *Journal of Environmental Quality* 31: 1671-1675.
31. Wuana RA, Okieimen FE. 2011. Heavy metals in contaminated soils: A review of sources, chemistry, risks and best available strategies for remediation. *Communications in Soil Science and Plant Analysis* 42: 111-122.
32. Yusuf AA, Arowolo T, Bamgbose O. 2002. Cd, Cu and Ni levels in vegetables from industrial and residential areas of Lagos City, Nigeria. *Global Journal of Environmental Sciences* 1: 1- 3.