JIMMA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF CHEMISTRY

A RESEARCH THESIS ON

PHYTOEXTRACTION OF LEAD AND CHROMIUM BY LEAFY VEGETABLES (LETTUCE, KALE AND SWISS CHARD)

Sep. 2011

Jimma, Ethiopia

PHYTOEXTRACTION OF LEAD AND CHROMIUM BY LEAFY VEGETABLES (LETTUCE, KALE AND SWISS CHARD)

BY

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A THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES JIMMA UNIVERSITY IN PARTIAL FULFILLMENT OFTHE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

School of Graduate Studies

Department of Chemistry

Phyto extraction of Lead and Chromium by leafy vegetables (lettuce, kale and Swiss chard)

By:

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A thesis Submitted to School of Graduate Studies of Jimma University with Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry *(Analytical Chemistry)*

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Acknowledgment

I would like to acknowledge my advisor Dr. Dejene Ayele for his advice and cooperation until the completion of this research. I would like to acknowledge Prof. Selemon Demeke, Mr. Wasihun Hassen and Mr. Abyot Hundie from animal science department; Mr. Abebe Nigusie and Mr. Bayu Dume from natural resource management department; and Mr. Nassir Abamecha from horticulture department; all from Jimma University college of Agriculture and veterinary medicine for their technical and material support.

I would like also to acknowledge chemistry department. In addition to this, I would like to address my acknowledgement to Universal Higher Diagnostic Laboratory owner Mr. Debebe Asrat for his material support. Lastly I would like to acknowledge my laboratory assistances Mr. Abdisa and Mr. Adam Biftu for their technical support.

Table of Content

List of tables

List of figures

Abbreviations and acronyms

BAC	Bioaccumulation coefficient
CEC	Cation exchange capacity
CLEA	Contaminated land exposure assessment
CS	Control soil
CS_1	Control soil 1
CS_2	Control soil 2
Dwt	Dry weight
EC	Electrical conductivity
EDTA	Ethylenediaminetetraaceticacid
EU EPA	European Union environmental protection administration
FAAS	Flame atomic absorption spectrometer
HEDTA	Hydroxyethylethylene-diaminetriacetic acid
LMWOAs	Low molecular weight organic acids
NTA	Nitrilotriacetate
OM	Organic matter
PES	Polluted and crude spinach extract treated soil
PES_1	Polluted and crude spinach extract treated soil 1
PES_2	Polluted and crude spinach extract treated soil 2
PS	Polluted soil
PS_1	Polluted soil 1
PS_2	Polluted soil 2
TF	Translocation factor

Abstract

Phytoextraction of metal from soil is a plant-based technique of removing metal pollutants from the soil. This research was carried out to assess the accumulation of Cr and Pb in leafy vegetables (lettuce, kale and Swiss Chard) in their harvestable part and root from artificially polluted soil with Pb and Cr. Soil physicochemical properties; pH, organic matter (OM), cation exchange capacity (CEC), Electrical conductivity(EC) and texture of the Soil sample under investigation was determined. S_2 was more acidic and relatively great OM (5.48, 7.78) respectively than S_1. Shoot part and root of Plant sample collected from green house grown in the control, contaminated and polluted with spinach extract soil was taken and dried in an oven at 70^0c for 48 hrs. The grinded plant sample was digested by concentrated HNO_3 and $HClO_4$ (2:1) mixture.

The digest was analyzed by FAAS. Results of the study indicate that Pb accumulation in shoot was on average greater (318.36 to 1235.20 mg/kg) in S_2 than S_1 (169.66 to 404.80 mg/kg). TF value of Swiss chard was generally higher in S_2 than S_1. Translocation of Pb from root to shoot was enhanced by the addition of spinach extract in kale and Swiss chard. TF and shoot accumulation of kale was enhanced by 36.5% & 33.3% respectively. Similarly Swiss chard TF and shoot accumulation was enhanced 8.3% &10.6% respectively with spinach extract. Chromium extraction by the three plants showed that translocation of Cr from root to shoot was limited. All TFs were less < 1 and these plants were able to accumulate less than 1000 mg/kg Cr except lettuce (1196.67 mg/kg) grown in PES_2. Lettuce without spinach extract amendment; kale with spinach extract; and Swiss chard with spinach extract and without spinach extract amendment could be used in Pb extraction in slightly acidic soil. In addition to this, kale with spinach extract and without spinach extract; and Swiss chard with spinach extracts could be used in Pb extraction in moderately acidic soils. Extraction of Cr was suitable by lettuces with spinach extract amendment in moderately acidic soil.

1. INTRODUCTION

Industrialization has led to the release of enormous quantities of toxic compounds into the environment. Industrial activities such as chemical works, garages and service stations, metal fabrication shops, paper mill, tanneries, textile plants, waste disposal sites and intensive agriculture are particularly guilty of polluting the environment either with organic or elemental pollutants [1].

There are numerous options for the remediation of contaminated sites. Commonly used engineering techniques include excavation and land filling, chemical treatment and verification. These methods are extremely expensive. This financial burden probably plays a role in slowing down global efforts to eradicate pollution, particularly in developing countries where these techniques are clearly not affordable. Due to the acute toxicity of Heavy metal contaminants, there is an urgent need to develop low-cost, effective, and sustainable methods to remove or to detoxify from the environment. One of these methods is a plant based approach known as phytoremediation [1, 2].

Phytoremediation is a method of environmental treatment that makes use of the ability of some plant species to stabilize, degrade uptake pollutants or accumulate certain elements, including heavy metals, in amounts exceeding the nutrition requirements of plants [2]. It is a broad term that comprises several techniques used for water and soil decontamination. The term phytoremediation (“phyto” meaning plant, and the Latin suffix “remedium” meaning to clean or restore) refers to a diverse collection of plant-based technologies that use either naturally occurring, or genetically engineered plants to clean contaminated environments [3].

The increase in industries that can pollute soil around by releasing toxic metals as byproduct requires an environmental friendly technique to remediate the polluted site. This is a plant based remediation technique. Therefore, the main purpose of undertaking this research is to evaluate the lead and chromium extraction abilities of kale, lettuce and Swiss chard from soils having different physico-chemical properties, and assess the influence of

crude spinach extract on the enhancement of lead and chromium uptake by the plants. This is based on the recommendation give by Fiseha who found that lettuce and Swiss chard had high toxic metal and metalloids accumulating ability from a research conducted in Addis Ababa in toxicological aspect of the metals [4].

1.1 Statement of the problem

Industrial development with improper waste disposal causes soil pollution. For the remediation of moderately polluted soil, plants are most friendly applicable without disturbing natural soil profile and low cost. Leafy vegetables can accumulate heavy metal in their harvestable part. Accumulation potential varies from species to species, soil condition and bioavailability of metals in soil. The uptake of metals like lead and chromium by plants is limited due to low bioavailability in soil. This research was aimed at assessing plants for their accumulation potential of Pb and Cr in their tissues and the effect of crude spinach extract on the enhancement of uptake of the metals under the same growth condition.

1.2 Objective of the study

1.2.1 General objective

- To assess the suitability of leafy vegetables (lettuce, kale and Swiss chard) for the phytoextraction of lead and chromium from two different soil types and the effect of crude spinach extract on the enhancement of metal uptake and translocation.

1.2.2 Specific objective

- To grow the vegetables: Lettuce, Kale and Swiss chard on Cr and Pb polluted soils.
- To treat polluted soil with crude spinach extract.
- To determine the concentrations of Cr and Pb in the shoots and roots of the vegetables.
- To determine the translocation factor for each of the vegetables.

1.3 Significance

Soil phytoextraction by plants is cost effective. It is ten times less than other conventional methods of treatment in cost. The method is applicable in a large area which is moderately polluted by toxic heavy metal disposal. It is sun light driven process. For the effective application of phytoextraction to clean polluted soils screening locally available plants which can accumulate high concentrations of pollutants is mandatory. In addition to this, the addition of synthetic chelators like EDTA and NTA into soils to enhance phytoextraction has been found to be successful. However, such practices have been found to be costly and also risky for underground water contamination due to high mobilization of metal contaminants. The chelators also have the disadvantage of low degradability. Unlike synthetic ligands, natural organic acids like oxalic acid and citric acid are easily biodegradable. The use of crude extracts of plants that contain significant concentrations of organic acids which could complex and make metals available to phytoextractant plants have the advantage of solving the problems associated with synthetic chelators. Therefore, the outcome of this research has a huge environmental significance in that it seeks finding out plants which are applicable for the remediation of polluted soils which is currently becoming a serious problem in our country. Moreover, a successful enhancement of the phytoextraction of a given plant by the application of crude plant extract has also a significant environmental implication in that it reduces cost and introduces environmentally friendly chemical components into the soil, which are easily degradable.

2. REVIEW OF LITERATURE

2.1 Phytoremediation techniques

Phytoremediation technique includes:

Phytotransformation (phytodegradation**)**- Is a technique in which organic pollutants are converted by internal or secreted enzymes into compound with reduced toxicity [1].

Phytostabilization (space uniformity**)** - the use of certain plant species to immobilize contaminants in soil, through absorption and accumulation by roots, adsorption onto roots or precipitation within the root zone and physical stabilization of soils [2].

Rhizodegradation - involves the enzymatic breakdown of organic pollutants, but through microbial enzymatic activity. These breakdown products are either volatilized or incorporated into the microorganisms and soil matrix of the rhizosphere. It is also called plant assisted technique [5].

Phytofiltration (rhizofiltration**)** - is the use of plant roots or seedlings (blastofiltration) to absorb or adsorb pollutants, mainly metals, from aqueous waste streams [2].

Phytoextraction (phytoaccumulation**)** - is a remediation technology that removes metals from contaminated soil by plant absorption and translocation to harvestable plant parts and it has attracted attention for its low cost of implementation. 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 [6].

Phytoextraction processes extract both metallic and organic constituents from soil by direct uptake into plants and translocation to aboveground biomass using metal- (hyper) accumulating plants. Hyperaccumulators are:

- plant species capable of accumulating a concentration 100 times higher then in normal

plants for each metal of interest, i.e. more than 10 mg kg^{-1} of Hg; 100 mg kg^{-1} of Cd; 1000 mg kg^{-1} of Co, Cr, Cu, Pb and Ni; and 10,000 mg kg^{-1} of Zn and Mn etc.;

- Bioconcentration factor > 1 (concentration of the element in the plant > concentration in the soil); and
- Translocation factor > 1 (element concentration in the over ground part of the Plant > than in roots) [7].

Phytoextraction has commonly two strategies. These are natural Phytoextraction and enduced Phytoextraction. In natural Phytoextraction, certain plants have been identified which have natural potential to uptake heavy metals. At least 45 families have been identified to have hyperaccumulating plants; some of the families are ***Brassicaceae***, ***Fabaceae***, ***Euphorbiaceae***, ***Asteraceae***, ***Lamiaceae***, and ***Scrophulariaceae***. *Brassica juncea*, commonly called Indian mustard, has been found to have a good ability to transport lead from the roots to the shoots. Induced Phytoextraction is assisted by the addition of chelating agents like Ethylenediaminetetraaceticacid (EDTA), Nitrilotriacetate (NTA) and Hydroxyethylethylene-diaminetriacetic acid (HEDTA) which enhances the uptake of metals from soil. [1, 8-11].

2.2 Metals in soil

Metals exist in soil in five different pools: fraction 1) soluble *i.e.* metals in the soil solution (as free metal ions and metal complexes); fraction 2) exchangeable *i.e.* metals adsorbed on ion-exchange sites and on inorganic soil constituents; fraction 3) organic *i.e.* metals bound with the organic matter; fraction 4) insoluble *i.e.* metals precipitated mainly as oxides, carbonates and hydroxides; and fraction 5) residual *i.e.* metals incorporated in the silicate minerals. Anthropogenic contamination affects the metal content of fractions 1- 4, whereas the fraction 5 reflects the background *i.e.* indigenous metal concentration [7, 12].

Only metals in the soluble fraction (fraction 1) and in some components of the exchangeable fraction (fraction 2) are readily available for plant uptake; those of fraction 3 and 4 can be released by different soil amendments; whereas metals in the fraction 5 are potentially non-available. In general, the metal concentration in plants is correlated with the soil metal concentration in the soluble fraction, making this fraction as the most important indicator of the metal phytoavailability [7].

Lead

Lead occurs most commonly with an oxidation state of 0 or +II. Pb (II) is the more common and reactive form of lead forming oxide and hydroxide. Lead released to groundwater, surface water and land is usually in the form of elemental lead, lead oxides and hydroxides, and lead-metal oxyanion complexes from metal smelting industries, lead battery manufacturing, pigment and chemical manufacturing [13].

Once introduced into the soil matrix, Pb is very difficult to remove. The capacity of the soil to adsorb Pb increases with increasing pH, cation exchange capacity, organic carbon content and phosphate levels. In any case, one major factor limiting the potential for Pb Phytoextraction is low metal bioavailability for plant uptake. To overcome this limitation, synthetic Chelators have been proposed to be added to the soil to increase the amount of available Pb [14].

Chromium

Chromium is a steel-gray, lustrous, hard, brittle metal of Group VIB of the transition series. It occurs in nature in bound forms like $FeCr_2O_4$.that constitute 0.1- 0.3 mg kg^{-1} of the Earth's crust. Chromium mobility depends on sorption characteristics of the soil, including clay content, iron oxide content and the amount of organic matter present. Chromium has several oxidation states ranging from Cr (−II) to Cr (+VI). The trivalent and hexavalent states are the most stable, although Cr with valences of I, II, IV and V have also been shown to exist in a number of compounds. Major sources of Cr contamination include

releases from electroplating processes and the disposal of chromium containing wastes [12, 15, 16].

Hexavalent Cr (Cr (VI) is highly toxic carcinogen due to their high solubility in water, rapid permeability through biological membranes and subsequent interaction with intracellular proteins and nucleic acids, and may cause death to animals and humans if ingested in large doses. Chromium is not considered to be essential for plant growth and development. Some studies have indicated that at low concentrations (1μM), Cr stimulates plant growth [16, 17].

Heavy metals in soil may be solubilized by decreasing the soil pH. At around neutral soil pH, most of heavy metals in soil are strongly bound to soil and not available to plants; particularly Pb and Cr are inherently immobile. Cations readily absorbed in clay than in sandy soil [7, 18, 19].

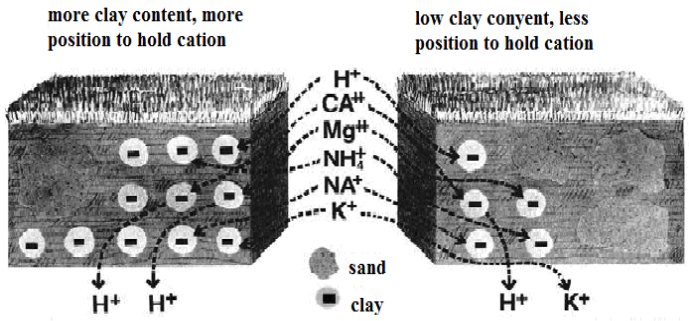


Fig. 1 Cation exchange site of soil

Soil acidity could be increased by hydrolysis of Aluminum present in soil solution.

$$Al^{3+}\ (soln.) + H2O \rightarrow Al\ (OH)^{2+} + H^{+}$$

$$Al\ (OH)^{2+} + H_2O \rightarrow Al\ (OH)_2^{+} + H^{+}$$

$$Al\ (OH)_2^{+} + H_2O \rightarrow Al\ (OH)_3 + H^{+}$$

Phytoavailability of metals in soil is the first step for successful phytoextraction. A major proportion of metals in soil exists as the bound fraction and needs to be mobilized into the

soil solution to make available for plant uptake. Although, this can be achieved artificially through soil amendments (chelant-induced phytoextraction), natural hyperaccumulators have the inherent capability to overcome this constraint by reducing the soil-bound metals by specific plasma membrane bound metal reductases; through root exudation of organic ligands like phytosiderophores and low-molecular weight organic acids (LMWOAs) which form metal complexes; and by acidifying the rhizosphere through the activity of proton pump and/or exudation of LMWOAs [7].

Plant uptake of metals varies with the types of Chelators present in solution at the same free metal activity. Furthermore, given the same chelate, total metal concentration in solution affects metal uptake by plants. The possible reactions of complexed metals at the soil-root interface and the potential uptake by plants of metal-organic complexes are depicted below [20].

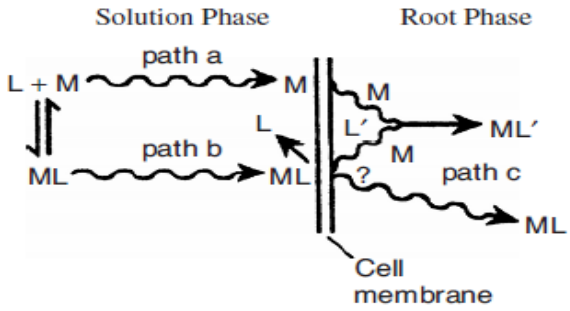


Fig. 2 Conceptual model for potential uptake of metal ions (M) by plants complexed with organic ligands (L)

The use of plants for environmental restoration is an emerging technology and plants capable of accumulating high levels of metals are grown in contaminated soils. Studies carried out in multi metal polluted soil showed that *E. cheiradenia*, *R. lutea, S. excelsa*, *S. orientalis, C.oblonga* and *C. virgata* are considered as *accumulators* [21]. Phytoremediation efficiency of most metal Hyperaccumulator is limited by their slow growth rate and low biomass. For example, Thlaspi caerulescens, a Cd and Zn

Hyperaccumulator, successfully removed 43% Cd and 7% Zn from an industrially contaminated soil, but it took 391 days [3].

The plant species *Leptospermum scoparium* (Myrtaceae) is an accumulator of Cr. it showed up to 20,000 mg Cr kg^{-1} in the foliage ash when grown on serpentine soils. Few Cr hyperaccumulator species have been identified to date. *Brassica juncea* has been found to be an excellent accumulator plant for Cr in soils. Other metals accumulated by it are Cd, Ni, Zn and Cu [22].

Chromium toxicity was evident in form of reduction of shoot and root length and total biomass of non-edible plants. Experimental findings showed that, *Phragmytes karka* plant was able to grow above 20 mg Cr kg^{-1} soil. Others like *Ipomoea carnea*, *Dhatura innoxia*, *Cassia tora and Lantana camara*, with two accumulator plants (*Brassica juncea* and *Brassica campestris*) were not able to tolerate the toxicity. At 50 mg kg^{-1} Cr, plants were able to germinate and grow up to 10 days, this clearly shows that conc. of 50 mg Cr kg^{-1} soil is toxic to plants. Though the uptake of *Phragmytes karka* was low, it was more effective at translocating Cr from soil to plant shoot. The order of Cr extraction was *I. carnea* > *D. innoxia* > *C. tora* > *P. karka* > *B. juncea* > *L. camara* > *B. campestris* [23].

Research on the phytoextraction of Cr from contaminated soils and sediments has been scarce. Very few plant species such as *Sutera fodina*, *Dicoma niccolifera* and *Leptospermum scoparium* have been reported to accumulate Cr to high concentrations in their tissues. In an attempt to use promising aquatic plant species for the phytoextraction of Cr from contaminated tannery sludge, a study has been conducted on the ability of three plant species (*Scirpus lacustris*, *Phragmites karka* and *Bacopamonnieri*) to absorb, translocate and concentrate Cr in their tissues. Most of the Cr removed by these species was accumulated in their roots with very low Cr being concentrated in their shoots. In order for the phytoextraction process to be effective substantial amounts of the Cr removed from the root medium must be translocated to the harvestable plant parts so that it can be completely removed from the contaminated site. [15]

Pot experiment carried out to assess the capacity of *C. Odorata* and *V. zizanioides* to remove Pb and Cd from artificially contaminated soil with 100 mg kg^{-1} dwt showed that both plants have high accumulation in their root than other parts. But *C. Odorata* performs better in Cd accumulation than *V. zizanioides* in their above ground biomass [21].

A study has been carried out in China to identify hyperaccumulator of Zn and Cd in polluted soil. Concentrations of Cd and Zn in leaves and stems increased with increasing Cd and Zn supply levels. The distributions of the metals in different plant parts decreased in the order: stem> leaf >root for Zn and leaf>stem>root for Cd. These results indicate that *S. alfredii* has an extraordinary ability to tolerate Cd/Zn toxicities [10].

Recently a research has been conducted in Nigeria on the effect of texture, pH and soil metal concentration on plant growth and Cd and Zn uptake. Control plant show high grow, soil with lower pH and sandy texture absorb more than the one with high pH and sandy loam at the same soil metal concentration. There was an increase in metal uptake with increase soil metal concentration [24].

Translocation factor (TF) and bioaccumulation coefficient (BAC) values >1 had been used to evaluate the potential of plant species for Phytoextraction. Research results indicated that accumulation of Pb, Cu, Zn, Ni, Co and Cr in none of the shoots of 16 plant species studied was more than 1000 mg/kg, which is one measure of accumulators for these metals. However, based on BACs and TFs, values, which were >1; plant species were identified which have the potential for phytoextraction [11]

Brassica juncea, commonly called Indian mustard, has been found to have a good ability to transport lead from the roots to the shoots. The Phytoextraction coefficient (factor) for ***Brassica juncea*** is 1.7 and it has been found that a lead concentration of 500 mg/l is not phototoxic to ***Brassica*** species. Some calculations indicate that Brassica juncea is capable of removing 11550 kg of lead per acre [9].

A green house study has been to examine the accumulation of Pb in different parts of rice plant *Prathum Thani* grown in soil polluted with different level of Pb. The result showed that maximum Pb accumulation in root than stem and leaf. Pb concentration in root increases with increase of soil concentration. Lead concentration in stems and leaves was in the same order of magnitude as found in roots, suggesting that lead can be readily translocated to rice shoot [22].

Lead and cadmium uptake from soil by fruit vegetable; chilies (capsicum annum) and long beans (Vigna sinensis) has been studied in Malaysia. It has been obtained that Pb concentration was greater than Cd in both vegetable plants. Heavy metal content in long beans(Vigna sinensis) and chilies(Capsicum annum) studied were low and below the maximum level allowed by the Malaysian food act (for pb 2.00 mg $kg^{-1}$and Cd 1.00 mg kg^{-1}) [25]

Laboratory research has shown that different vegetable crops varied in their ability to accumulate Cr in their tissues. Highest Cr concentrations were detected in members of the Brassicaceae family (i.e., cauliflower, kale, cabbage), which are known to be S-loving plants indeed, studies conducted have clearly show on Brassica species (e.g., Indian mustard) shown that an unusual ability to take up heavy metals such as Pb, Cr, Cd, Ni, Zn and Cu from root substrates and concentrate these metals in their tissues [15].

Even though the tendency to retain Cr in the roots seems to be common to all plant species studied thus far by various workers, there are quantitative differences among plant species in this regard. Leafy vegetables that tend to accumulate Fe (e.g., spinach, turnip leaves) appeared to be the most effective in translocating Cr to the plant top. The leafy vegetables that do not accumulate relatively high concentrations of Fe in their leaves (e.g., lettuce, cabbage) are substantially less effective in translocating Cr to their leaves. [15, 26]

A research carried out on garden soil treated with different level of Cr solution showed clear trend of increase in the amount of chromium accumulation in plants with increase in the quantity of Cr added to soil was observed. A significant and positive correlation between Cr accumulated and Cr Added to the soil confirmed it. Similar pattern of chromium uptake was observed in amaranthus plants. Maximum chromium uptake (47.79 ±2.75 μg/g DW in shoot and 110.29±10.33 μg/g DW in root) was observed in amaranthus plants grown on 100 μg Cr /g in soil [26].

Field experiment on crops has been carried out in Poland to determine lead accumulation and distribution in the plants' organs to select species suited for Phytoremediation. *Pumpkins* accumulated most lead in the leaves (12.81 mg kg^{-1} d. wt,). The least amount was found in the stem and fruits. Red beet accumulates high amounts of lead in leaves (8.71 mg kg^{-1} d. wt,). The roots contained 3.6 less lead than the leaves. In the case of white cabbage, the most lead contaminated organs were the leaves of the rosette (11.60 mg kg^{-1} d. wt of Pb) [27]

Ethylenediaminetetraaceticacid (EDTA) has been the most investigated organic amendment in phytoextraction and has been successfully used to enhance the phytoextraction of Pb and other heavy metals. EDTA was better in releasing soil-bound Pb compared to citric acid. The ability of EDTA to enhance the release of Pb from insoluble or sparingly soluble compounds compared to other chelating agents has been attributed to its higher binding capacity for Pb [7, 28-30].

Low molecular weight organic acids (LMWOAs), e.g. citric acid, oxalic acid, gallic acid and acetic acid. Chelants can be particularly useful in mobilizing heavy metals at high soil pH as the stability of metal-organic complex increases with increasing pH. The efficiency of metal solubilization by chelating agents depends on the stability constants of the metal-chelate complex and follows the order EDTA (and related compounds) > NTA > citric acid > oxalic acid > acetic acid. Besides mobilizing metals in soil, chelants also facilitate metal translocation from root to shoot. [7]

EDTA and DTPA though mobilized more Cr and Ni than LMWOAs (citric and oxalic acids) but reduced the biomass of *B. juncea* due to metal phytotoxicity. The chelant phytotoxicity may often limit the phytoextraction potential that relies not only on the high metal concentration in shoots but also on the high biomass production. EDTA and EDTA-heavy metal complexes are toxic to the soil micro flora as well as to plants causing drastic growth reduction in several plant species [7, 18]. The use of EDTA and other synthetic Chelators in induced phytoextraction has a risk of ground water contamination due to its leaching effect [18].

The LMWOAs are of particular importance in mobilizing soil metals due to their dual function; soil acidification, and forming complexes with heavy metals. However, the metal complexing capacity of LMWOAs rather than soil acidification has been considered more important in metal mobilization and uptake by plants [7].

Leafy vegetable plants like lettuce (*Lactuca sativa),* Swiss chard (*Beta vulgaris*) and kale or Abyssinia kale (*Brassica oleracea acephala)* are commonly native around Jimma. These vegetables were the plant taken for our study. The species have fast growth rate and large above ground biomass compared to underground part.

3. MATERIALS AND METHOD

3.1 Soil Sampling and Selection of vegetables

Two separate composite surface soil (30 cm depth) samples were selected purposely from higher-3 Mendera Kochi kebelle (S_1) and Jimma University College of Agriculture and Veterinary medicine (JUCAVM) farm site (S_2). The soil samples were transported to JUCAVM green house. Seeds of the vegetables (lettuce, kale and Swiss chard) were obtained from JUCAVM Post Harvest Management (PHM) resource center.

3.2 Chemicals and reagents

Analytical grade chemicals; $K_2Cr_2O_7$ (neo-Lab), $Pb(NO_3)_2$ (FINKEM, India), $FeSO_4.7H_2O$ (Uni-chem, China), (Sigma-Aldrich, Germany), NaCl (Sigma-Aldrich, Germany), HgI_2 (Uni-chem, China), methyl red (HIMEDIA, India), KI (Alpha laboratory reagent), 98% H_2SO_4 NaOH (neo-Lab), 85%H_3PO_4 (Riedel-de Haen), $CH_3CO_2NH_4$ (C.D.H), 96% CH_3CH_2OH (Lab-MERK), 65% HNO_3 (Riedel-de Haen), 70% $HClO_4$ (Riedel-de Haen), have been used

3.3 Sample preparation and analysis

3.3.1 Soil physicochemical properties

Soil pH

An air dried 50 g soil sample was ground by using mortar and pastel and placed in 100 ml glass beaker. Fifty ml of distilled water was added and mixed well. Suspension of the mixture was stirred with glass rode for 15 sec and allowed to stand for 30 min. After that, the combined electrode was immersed in the suspension and the pH reading was taken after 30 seconds [31].

Organic matter (OM)

Determination of organic matter involves reduction of potassium dichromate ($K_2Cr_2O_7$) by organic carbon compounds and subsequent determination of the unreduced dichromate by redox titration with ferrous ammonium sulfate.

$$2Cr_2O_7^{2-} + 4C + 16H^+ \rightarrow 4Cr^{3+} + 3CO_2 + 8H_2O$$

An air dried 0.5 g of S_1 or 0.25 g of S_2 was placed in each of two separate 500 ml beakers. Ten ml 1N potassium dichromate and 20 ml concentrated H_2SO_4 were added into each of the beakers. The mixtures were swirled gently and allowed to stand for 30 min. Then 200 ml of distilled water and 10 ml of H_3PO_4 were added consecutively into the suspensions. Ten drops of barium diphenylamine phosphate indicator (0.16%) was added into each of the mixtures. Finally the mixtures were titrated with 0.5 N ferrous sulfate heptahydrated until the color changed from violet-blue to light green.

$$6Fe^{2-} + Cr_2O_7^{2-} + 14H^+ \rightarrow 2Cr^{3+} + 6Fe^{3+} + 7H_2O$$

A blank was prepared and titrated in the same manner [32].

Percentage organic matter in the soil was calculated as follows

$$\% \text{ oxidizable organic carbon (w/w)} = \frac{[V_{blnk} - V_{sample}] \times 0.3 \times N}{W}$$

$$\text{where, } N = \frac{(V_{K_2Cr_2O_7} \times N_{K_2Cr_2O_7})}{V_{FeSO_4.7H_2O}} \quad \text{(blank titration)}$$

% Total organic carbon -TOC (% w/w) = 1.3 x % oxidizable organic carbon

% organic matter (w/w) = % total organic carbon/0.58 = 1.724 x %TOC

where: V_{sample} = volume of ferrous ammonium sulfate solution required to titrate the sample.

V_{blank} = volume of ferrous ammonium sulfate solution required to titrate the blank.

Wt = weight of air dried soil (g)

$$0.3 = \frac{12}{4 \times 1000} \times 100$$

$\frac{12}{4 \times 1000}$ = milliequivalent weight of C in grams.

Determination of Cation Exchange Capacity (CEC)

Determination of CEC was conducted by using the ammonium acetate method. Five gram of an oven dried soil sample was soaked with 100 ml of 1M ammonium acetate (pH 7.0) solution for 24 hrs. The suspension was then filtered and the residue was washed with 50 ml 1 M ammonium acetate. The sample was further washed three times with 25 ml 1 M ammonium acetate each time. After this, the sample was washed three times with 25 ml 96% ethanol each time, in order to remove excess ammonium acetate. The absence of yellow precipitate was checked by the Nessler's reagent on the ethanol filtrate. The soil was saturated with Na^+ ions by washing it five times successively with 20 ml of sodium chloride (10%) and a total of 100 ml filtrate was collected. Blank was prepared in similar procedure. The leachate was transferred into a Kjeldhal flask. Fifteen ml of 0.20 N H_2SO_4 was poured in to a 250 ml Erlenmeyer flask. Then 10 ml of 1 N NaOH was added to the Kjeldhal flask and connected immediately to the distillation apparatus. Finally the distillate was titrated with 0.1 N NaOH using methyl red indicators (purple- yellow) and the cation exchange capacity was calculated by using the following equation [33].

$$\text{CEC (meq/100g soil)} = \frac{(s-b) \times N \times 100}{W}$$

Where; s = volume of NaOH for sample titration

b = volume of NaOH for blank titration

W = sample weight (g)

N = Normality of titrant (NaOH)

Soil Texture

Particle size analysis was carried out by using the Bouyoucos hydrometer ASTM No152H method. Fifty gram soil sample was dispersed in to 1000 ml solution which is 4.0% (w/v) in sodium hexametaphosphate and 1.0% (w/v) in sodium carbonate. The resulting suspension was then mixed thoroughly by using a high-speed mechanical soil mixer and filled to 500 ml with distilled water. The solution was stirred for 5 min, and then transferred to a 1.0 liter graduated cylinder. The mixture was then made to 1.0 L with distilled water. The suspension was mixed well and temperature reading was taken. The graduated cylinder with its contents was allowed to stand on a flat surface so that the soil particles of different sizes settle to the bottom of the cylinder. Finally the rate of fall of suspended particles was related to size: sand settling faster than silt and silt faster than clay.

The Hydrometer was carefully lowered in to the suspension until it floats without oscillating. The first hydrometer reading was taken at after 40 seconds which measures the silt and clay percent (< 50 microns) in the suspension. The cylinder was allowed to stand for additional two hrs and a second temperature and hydrometer reading was taken. This reading gives the clay percent (<2 microns) in the suspension [34].

Results were corrected to 20 ^{0}C. Temperature readings above and below 20 ^{0}C corrections were added and subtracted from the hydrometer reading respectively. Correction for the compensation of dispersing agent was made by subtracting 2 from the hydrometer reading.

$$\% \text{ Sand} = 100 - [(d_1 + Tc_1 - 2) \times 100/50]$$

$$\% \text{ Clay} = (d_2 + Tc_2 - 2) \times 100/50]$$

$$\% \text{ Silt} = 100 - (\% \text{ sand} + \% \text{ clay})$$

where, d_1 = first hydrometer reading, Tc_1 = first temperature correction

d_2 = second hydrometer reading , Tc_2 = second temperature correction

100/50 = conversion of sample weight to 100 %

Electrical conductivity (EC)

Soil salinity refers to the concentration of soluble salts in the soil. It is normally measured by extracting the soil sample with water. EC of the soil sample under investigation was measured in 1:5 soils: water suspension. Conductivity cell was firs calibrated by 0.01 M KCl to read 1413 µs/m at 25 ^{0}C. Ten grams of air dry soil was measured to a 100 ml beaker. Fifty milliliters of deionized water was added to the beaker containing the sample and stirred using glass rode for one minute. The solution was allowed to stand for one hr. the conductivity cell was inserted to the suspension and reading was taken [32].

3.3.2 Green house Experiment

A 2.5 kg of each soil sample was placed in to each 36 pots, 18 pots in one soil sample. Pots were arranged in such a way that the first six of both soil samples as first raw. The second six as second raw and the third six pots as third raw. Based on EU EPA level of lead in agricultural soil to be taken it as polluted (50 - 300 mg/kg soil). United State Environmental Protection Agency (USEPA) has state maximum contamination levels for lead metal concentration in soil to be 420 mg/kg. The contaminated land exposure assessment (CLEA) model 2002 has stated soil guide line values of different metals in residential soil (with and without vegetable growing). Maximum limit of Chromium was given to be 130 - 150 mg/kg soil. Four pots of the six in each soil were polluted by solution lead nitrate and potassium di chromate in 300 and 100 mg/kg to make the soil toxic. Two pots in each six pot are controls. The soil was watered with tap water and left to equilibrate in green house. After the equilibration time, seed of the plants were sowed (lettuce on the first raw, kale on the second and Swiss chard on the third raw).

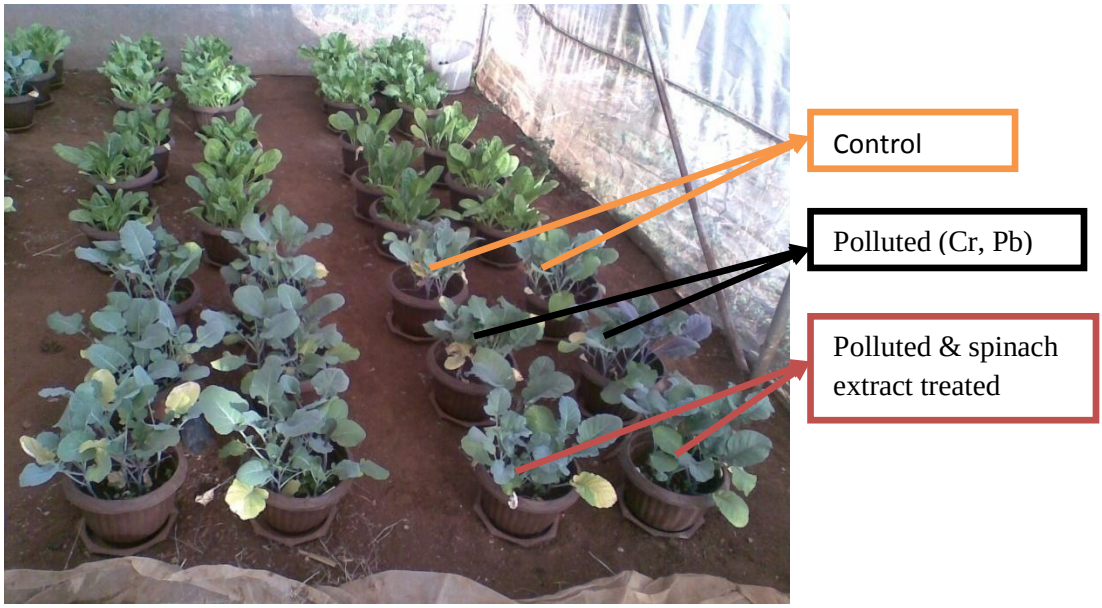


Fig. 3 Green house Experiment

Many other green, leafy vegetables contain organic acid like oxalic acid. Spinach contains organic acids mainly oxalic acid in its leaf [35]. After 25 days of germination, four of the polluted pots in each row were treated with 200 ml crude extract spinach in two batches one day gap to evaluate the effect of the extract content on the metal uptake. Plant sample was collected from green house 53 days after germination.

3.3.3 Soil sample analysis

A small portion of the homogenized and air-dried soil sample was ground using mortar and pestle. An aliquot, 1.0 g, of the ground soil was accurately weighed and transferred into a 100 ml beaker. Twelve milliliter mixture of 66.67 % conc. HNO_3 and 33.33 % conc. $HClO_4$ (v/v) was added into the beaker and the mixture was heated on a hot plate until the end of white fume evolution from the beaker. The resulting digest was allowed to cool at room temperature and filtered through a Whatman No 42 filter paper. The filtrate was then diluted to 50 ml and analyzed for its Pb and Cr content by FFAAS. [36].

3.3.4 Plant available Pb and Cr in soil

For the extraction of the water soluble (plant available) fraction of Pb or Cr in the soil samples of various treatments, 0.2 g of soil sample was transferred into an Erlenmeyer flask and 50 ml of deionized water was added. The resulting suspension was shaken for 1 hr at a rate of 200 rpm on a shaker and filtered. The extract was analyzed for Pb and Cr content by FAAS [37].

3.3.5 Plant sample preparation and analysis

The above ground (shoot) and underground (root) parts of the vegetables were collected on the 8^{th} week of plantation. The collected samples were dried in an oven at 70 ^{0}C for 48 hours. The dried samples were ground using mortar and pestle to less than 2 mm size. A portion, 0.5 g, of each of the ground shoot and root samples was transferred into an Erlenmeyer flask and added 12 ml of a mixture of concentrated HNO_3 and $HClO_4$, in which the volume ratio of HNO_3 to $HClO_4$ is 2:1. The flask with its contents was put on a hot plate and heated until the end of white fume evolution from the beaker. The resulting digest was then filtered with Whatman No 42 filter paper. The filtrate was diluted to 50 ml with deionized water. The diluted extract was analyzed by FAAS for its chromium and lead contents [23, 36, 38].

3.3.6 Method validity test

Method detection limit

The minimum concentration of the analyte (Cr and Pb) that can be measured and reported with 99 % confidence that the analyte concentration is greater than zero was determined from seven replicate analyses of samples in a given matrix containing the analyte. The standard deviation calculated was used for determining the concentration of each metal [39]. The method detection limit was calculated as;

$$MDL = t \times SD$$

where, t = critical t value at 99% and n -1 degree of freedom.

SD = standard deviation

Recovery Test

The efficiency of the sample preparation method was evaluated by carrying out percent recovery test for each of the metals. One milliliter 100 ppm solution of each of Pb and Cr was spiked into each of 0.5 g portions of the ground root and shoot samples and 1.0 g portions of the soil samples and digested in the same way as their respective non-spiked samples. The extract was diluted to a final volume of 100 ml ideally to contain 1 ppm of each metal ion. Each sample was analyzed and percent recovery was calculated [40].

$$\text{Recovery (\%)} = \frac{\text{concentration measured}}{\text{Concentration spiked}} \times 100$$

Repeatability

Precision between successive measurements of the adopted method was determined from a replicate analysis of sample containing an analyte in the range of the working concentration at a time. Repeatability of the measurements was expressed by relative standard deviation (RSD) [39].

$$\text{RSD} = \frac{\text{SD}}{\text{Mean}} \times 100$$

Reproducibility

The between run precision of the method was determined from replicate analyses of samples in a similar way as was done for repeatability. Three batches of sample preparation and analysis were carried within three days. Finally, the RSD of the results was calculated [39].

3.3.7 Date analysis

Results were analyzed using Microsoft Excel 2007. A one tailed significance test was carried out to compare result in respective treatment at 95% confidence level. This confidence level was also used in confidence interval of our result. Results of this study were stated in mg/kg of plant dry wt. The concentrations of Pb and Cr in ppm from the

calibration curve were converted to mg/kg of dry weight of sample by the expression shown below [38].

$$\text{Concentration (mg/kg)} = \frac{\text{Concentration (mg/L)} \times \text{Extract volume (L)}}{\text{Sample weight (kg)}}$$

The translocation factors (TF) of the metals in all the vegetable were determined by the ratio of metal concentration in plant shoot to metal concentration in root [11].

4. RESULTS AND DISCUSSION

4.1 Instrument operation parameters

Parameters used for the determination of Pb and Cr using FAAS were optimized separately. The optimized parameters which were used for the determination of the two metals are summarized in table 1 below.

Table.1 FAAS operation parameters

Parameters	Optimized values for Pb and Cr determination	
	Pb	Cr
Wave length (nm)	283.3	357.6
Slit width (nm)	1.2	0.2
Burner height (mm)	6.0	8.0
Current (mA)	4.0	5.0

Fuel flow (L/h)	65	90
Gas/Oxi	0.138	0.213

4.2 Physicochemical properties of soil samples

The two soil samples were analyzed for their Cr and Pb contents and other parameters (pH, organic matter, cation exchange, electrical conductivity and texture) prior to green house experiment**.** The results obtained are given in table 2 below.

Table.2 Physicochemical properties of the soil samples

Properties	Sample	
	Soil-1 (S_1)	Soil-2 (S_2)
Pb concentration	34.58 ± 1.49	41.78 ± 2.13
Cr concentration	9.89 ± 0.79	11.17 ± 0.97
pH	6.32 ± 0.14	5.48 ± 0.12
(OM)-%(w/w)	4.24 ± 0.19	7.78 ± 0.21
(CEC)-meq/100g soil	27.90 ± 0.14	29.30 ± 0.42
EC(µs/m)	81.4 ± 1.33	136.3 ± 1.86
Texture	CL	CL

Note: CL=clay loam

The soil samples were found to be different in all the parameters determined except texture. S_2 was more acidic and contains more organic matter and exchangeable cations than S_1. Both soil samples had the same texture (clay loam). S_2 had insignificant variation in Pb and Cr content than S_1.

4.3 Method validity test

Method validity tests were carried out for the determination of Pb and Cr in soil and plant samples. The parameters that have been tested and the results obtained are summarized in table 3. As shown the table, the percent recoveries for both Cr and Pb were between 96 and 105%, well within the acceptable 60 - 115 % range [40], the repeatabilities less than 5 % and the reproducibilities below 10% [39].

Table.3 method validity test parameters

Parameters	Soil sample		Plant sample	
	Pb	Cr	Pb	Cr
MDL (mg/kg)	0.49	0.041	0.981	0.161
Recovery (%)	101.31	96.37	99.70	104.08
Repeatability (RSD)	2.48	4.13	2.43	3.85
Reproducibility (RSD)	5.67	8.27	4.21	7.45

Generally the results obtained for data quality test have revealed that the analytical method utilized in this study provides statistically acceptable accurate and precise data.

4.4 Plant available Pb and Cr in soil

Results of the plant available concentrations of Pb and Cr in the soil samples of the various treatments are given in table 4. As shown in the table, the plant available Pb in PES_1 is significantly ($p < 0.05$) greater than in PS_1.

Table.4 Plant available Pb and Cr

Soil samples		Metal concentration (mg/kg)	
		Pb	Cr
S_1	CS_1	8.21 ± 0.79	2.40 ± 0.31
	PS_1	22.34 ± 0.92	53.68±1.02
	PES_1	39.87 ± 1.19	59.73 ± 1.08
S_2	CS_2	9.73 ± 0.89	3.94 ± 0.80
	PS_2	128.37 ± 2.39	43.57 ± 0.91
	PES_2	134.69 ± 2.41	45.12± 0.99

There was a significant difference ($p < 0.05$) between the available Pb and Cr concentrations of PS_1 and PES_1. However, this is not true in S_2. Plant available Cr is found to be greater than Pb in S_1 and Pb is found to be more available in S_2 than in S_1. Availability of Cr was observed to increase in PS_1 when compared with CS_1.

4.5 Plant sample analysis

4.5.1 Plant dry matter

Large enough biomass production is one indicator of the resistively of plants to a given level of toxicity relative to non toxic level [11]. Therefore, the dry weights of the roots and shoots of the plant samples were determined after oven-drying. The root and shoot parts of the plant samples were washed with tap water and rinsed with distilled water after collection from the green house. The dry weight of each sample was then determined after oven drying at 70 ^{0}C for 48 hr. The results obtained are given in table 5.

Table.5 Dry matter contents of the root and shoot parts of the plant samples (g/pot)

Soil samples		Plant sample								
		LC	LP	LPE	KC	KP	KPE	SC C	SC P	SC PE
S_1	Shoot	6.13	4.11	4.45	6.20	5.85	5.03	8.91	8.14	9.95
	Root	0.52	0.30	0.28	1.62	1.25	0.99	1.70	1.37	1.66
S_2	Shoot	3.53	3.30	3.84	5.48	5.42	4.80	8.17	8.11	9.11
	Root	0.15	0.20	0.15	0.99	0.95	0.82	1.23	1.30	1.37

Note; **:** LC, KC and SC C = lettuce, kale and Swiss chard grown in the controls respectively. LP, KP and SC P = lettuce, kale and Swiss chard grown in the polluted soils respectively. LPE, KPE and SC PE = lettuce, kale and Swiss chard grown in the polluted and spinach extract treated soils.

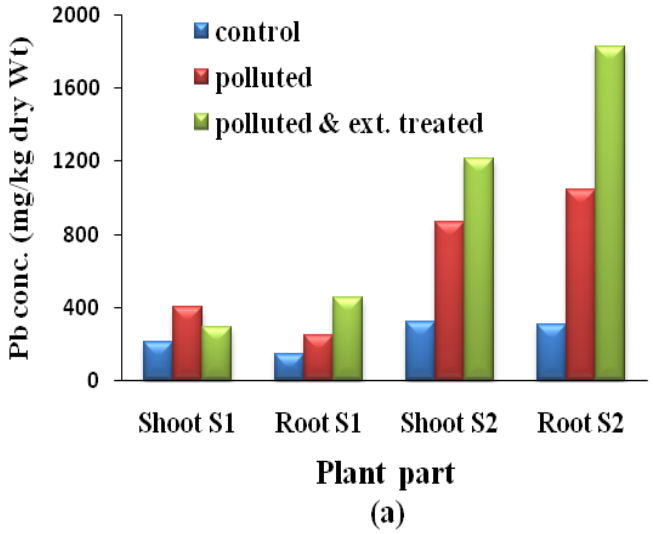
As shown in table 5, variation in dry matter content of a given plant sample was observed between treatments, between plant varieties and soil type within a given treatment. The decrease in the biomasses of the plants grown in the polluted soils of both S_1 and S_2 relative to the controls could be due to the toxicity of the metals. Zia-Ur-Rehman et al. found that *Albizia lebbeck* shoot and root growth was reduced by an increase in Pb dose. This was suggested to be due to accumulation of lead in soil which physically blocks water uptake from root to shoot and is related with the rate of photosynthesis [41]. The variation in the dry matter content between different plant species within a given treatment could be due to natural difference between the plant species. The shoot and root dry matter contents of all the plants grown in S_2 were smaller than their respective masses in S_1. As has been observed in the physico-chemical properties of the soils, S_2 is more acidic than S_1. It is known that, as soil acidity increases the solubility of ions, such as Al^{3+}, adsorbed on soil surfaces increases. Availability of such ions to plants could be toxic and may reduce plant growth [42]. This could be the reason for the decrease in the dry matter contents of the plants in S_2 relative to that of S_1.

Growth enhancement was observed on lettuce and Swiss chard when spinach extract was added to the polluted soils. Similar result has been reported by Prijambada and Proklamasiningsih in which dry matter of Soybean (*Glycine max)* was increased by the addition of malic acid and lactic acid in to the soil to reduce toxicity of Al^{3+} which is the main factor limiting plant growth in soil. As a result plants were able to grow without any toxicity symptom [43]. In the present study, the increase in biomass production could attribute to a decrease in the Al^{3+} toxicity due to organic acids that could exist in the extract.

4.5.2 Lead concentration in plant tissues

Lettuce:

The concentration of Pb in the roots and shoots of lettuce grown in the various treatments is given in fig 4. As shown in the figure, the concentration of Pb in the roots and shoots of lettuce grown in the controls is less than in that of lettuce grown in PSs and PESs. Similar results have been reported by Panich-pat and Srinives. E [22] and Reda et al. [44] in a green house experiment on Pb and other metals accumulation of rice and other plants from soils containing moderate to high amounts of heavy metals. The reason attributed for the higher accumulation of Pb and other metals in the shoots and roots of the plants grown in the PSs was an increase in the plant available fractions of the metals in the soil around the root zone. Our results of the plant available fractions of Pb in the various treatments are in agreement with this idea. The PESs were found to have the highest plant available fraction of Pb followed by the PSs soil and the CSs has the least content. Lettuce was able to accumulate a maximum of about 450 mg/kg in its root in S_1 with spinach extract amendment. Although spinach extract amendment has improved root accumulation of lead the total amount of Pb determined didn't show significant enhancement.



(a)

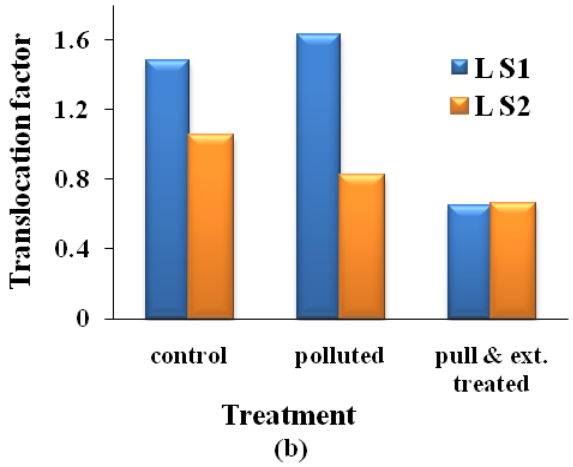


Fig. 4 (a) Pb accumulation in lettuce and (b) Translocation factor

Note: Shoot S_1 and Shoot S_2 = Shoot parts of lettuce grown in S_1 and S_2 respectively. Root S_1 and Root S_2 = Root parts of lettuce grown in S_1 and S_2 respectively. LS_1= lettuce grown in S_1, LS_2 = lettuce grown in S_2

The trend in Pb accumulation in the shoots of lettuce grown in the various treatments of S_1 was found to be different from that of S_2. The order of Pb level in the shoots of lettuce was PS_1 (404.8 mg/kg) > PES_1 (294.56 mg/kg) > CS_1 (209.52 mg/kg). The decrease in shoot Pb accumulation in PES_1 might not due to less availability of Pb. Because Mahmood has reported that LMWOAs have particular importance in metal mobilization and uptake in soil at high pH. This is due to the increase in metal-organic complex stability [7]. But it could have attributed to the lower transportation of Pb complex formed by xylem to the shoot than uncomplexed Pb. As it has been reported by Robinson et al., Chelate amendment could result in metal mobility in soil. But solubilization doesn't necessarily lead to bioaccumulation. It may cause decrease in accumulation provided that the metal complex formed due to the chelate amendment is unable to pass though root membrane of the plant and less transportation by xylem in a given soil condition [45]. In our study, significant enhancement of plant available Pb was obtained (table. 4) in S_1 by the addition of spinach extract. Shoot accumulation observed in S_2 amended with extract could result from the lower probability of metal complex formation at lower pH there by decreases the chance to leaching due to lower pH.

Kale:

Results of the Pb analysis in the shoot and root parts of the kale plant in the various treatments are given in fig.5. As shown in the figure, kale grown at CS_1 and PS_1 was found to accumulate more Pb in its root than shoot. This can be observed from the TF value less than 1 in fig.5 (b). However, shoot accumulation by kale grown in PES_1 was found to be enhanced significantly ($p<0.05$). This was positively related to the increase in available Pb PES1. The more available Pb in S_2 obtained was positively related to the uptake of kale. Higher root Pb accumulation than shoot was observed by Orhue and Inneh on *Celosia argentea*. They have concluded that this condition could be resulted from ability to restrict heavy metal to root as a mechanism to block the translocation to shoot as a result of excluder plant characteristics [46]. The enhancement of Pb accumulation in shoot of kale in our result is in agreement to the results reported by Mahmood. It has been suggested that this enhancement could be due to complexing ability of LMWOAs there by enhance the translocation to the shoot [7]. However, shoot Pb accumulation of kale grown in PES_2 was not enhanced. The enhancement of Pb shoot accumulation in S_1 which is slightly acidic could be resulted from the increase in the metal-organic complex stability at the pH value which is close to neutral. On the other hand, the inhibition of Pb accumulation in shoot of kale grown in PES_2 could be the instability of the complex formed with the organic acids in the spinach extract used for amendment at a relatively lower pH than S_1 [7]

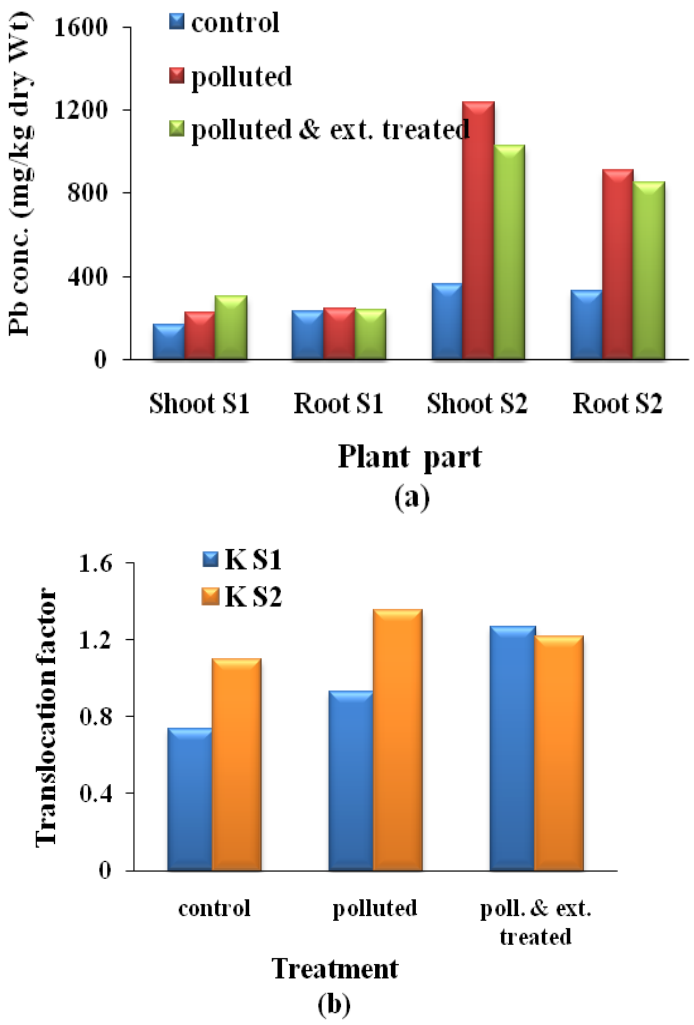


Fig. 5 (a) Pb accumulation in kale and (b) Translocation factor

Note: Shoot S_1 and Shoot S_2 = Shoot parts of kale grown in S_1 and S_2 respectively. Root S_1 and Root S_2 = Root parts of kale grown in S_1 and S_2 respectively. KS_1= kale grown in S_1, KS_2 = kale grown in S_2

The decrease in shoot and root accumulation of Kale grown in PES_2 could have resulted from inability of the metal mobilized in the given soil to pass through the root membrane and lower translocation. Robinson et al. have reported that Chelate amendment could result in metal mobility in soil. Ni solubilization in soil was enhanced by chelating agents. But the uptake Ni by *Berkheya coddii* plant has been decreased. This implies solubilization doesn't necessarily lead to bioaccumulation. It may cause decrease in plant uptake provided that

the metal complex formed due to the chelate amendment is unable to pass though root membrane of the plant and less transportation by xylem in a given soil condition [45]. TF of kale grown in PES_1 was found to be enhanced (> 1).

Swiss chard:

Results of the Pb analysis in the shoot and root parts of Swiss chard are given in fig. 6. As shown in the figure, Swiss chard grown in S_1 was able to accumulate more Pb in its shoot than in its root in all treatments. This shows Pb is easily translocated from root to shoot in Swiss chard. An enhancement of shoot accumulation was observed in Swiss chard grown in PES_1 as compared to that of the Swiss chard grown in the PS_1 (313.28 mg/kg and 283.04 mg/kg respectively). The increase in shoot accumulation by Swiss chard in PES_1 could have resulted from the enhanced complex formation at higher pH as it is explained in case of kale. A similar enhancement effect of organic acid (citric acid) on Mo phytoextraction in higher soil pH has been reported by Rodriguez. Accordingly the enhancement of shoot accumulation was attributed to the water soluble complexes and acidification the soil that lead to metal mobilization [47].

The TF of Swiss chard grown in the PS_1 and PS_2 were observed to decrease relative to that grown in the controls. With the addition of spinach extract however, TF increased significantly in both soils ($p < 0.05$). The significant increase in TF in both soils might be due to the increase in metal mobility in soil and root to shoot metal transport enhancement by LMWOAs [7, 47]. The minimum TF in both PSs could have resulted from higher root accumulation rendering toxicity of metal. In the study of U, Mo and As mobilization

described above, the accumulation of the metal by shoot was higher in less polluted soil. The main reason suggested for small accumulation in polluted soil was the toxicity effects of multiple metal contaminants on plant metabolism and function [47].

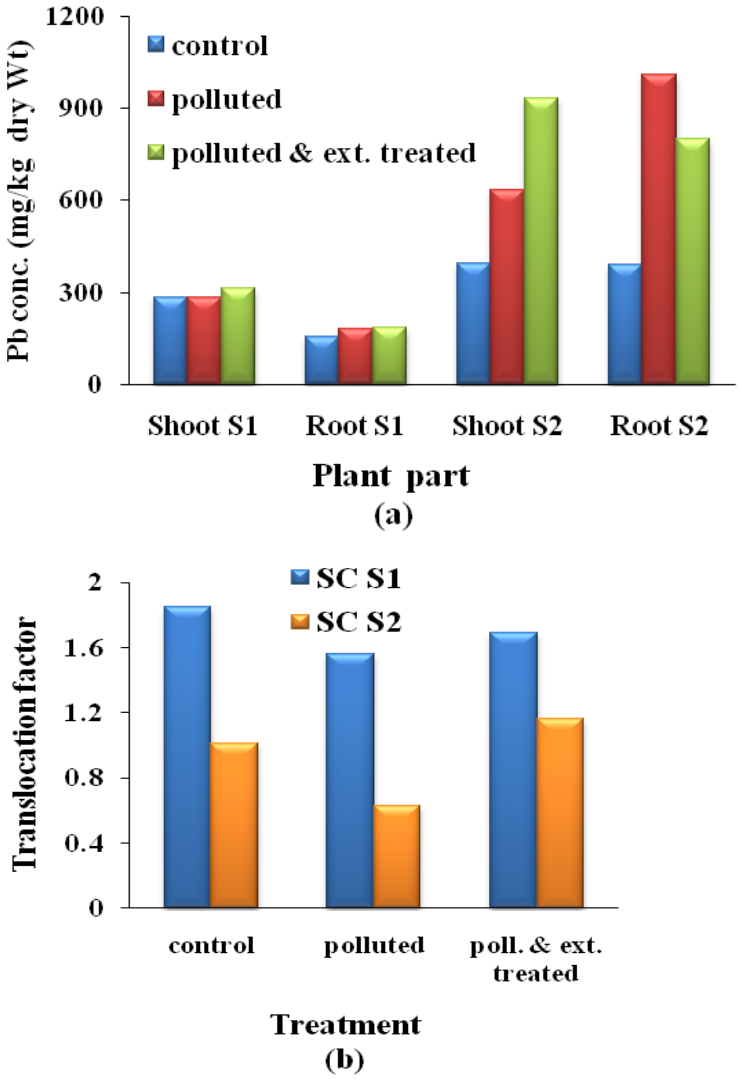


Fig. 6 (a) Pb accumulation in Swiss chard and (b) Translocation factor

Note: Shoot S_1 and Shoot S_2 = Shoot parts of Swiss chard grown in S_1 and S_2 respectively. Root S_1 and Root S_2 = Root parts of Swiss chard grown in S_1 and S_2 respectively. SC S_1= Swiss chard grown in S_1, SC S_2 = Swiss chard grown in S_2

The accumulation of Pb in Swiss chard grown in S_2 was higher than in S_1 in all treatments. Many adsorption sites are pH dependent according to Chao and Sanolone. They describe that, at more acidic condition, metal solubility is enhanced due to decrease the negative

sites for cation adsorption on clay surface, organic matter, Iron and Manganese oxides in soil [48]. In our study, the acidic nature of S_2 (pH = 5.48) could attribute to the higher accumulation of Pb by increasing the competing ions in the soil than in S_1. Organic matter contains Water soluble and particulate portions. The water soluble part facilitates metal mobility by adsorbing itself on clay surface [49]. The higher OM content in S_2 that may contain water soluble portion could lead to the high accumulation than in S_1. Shoot Pb accumulation was significantly enhanced ($p < 0.05$) in Swiss chard grown in the PES_2. The TF of Swiss chard grown in PS_2 was smaller due to high root accumulation. Increase in TF value in extract treated soil of both samples might have resulted from the complexation of the metal with the content of the spinach extract added [7].

Shoot accumulations of Pb in the vegetables investigated were compared within treatment of each S_1 and S_2 as shown in fig.7. Swiss chard was able to accumulate more Pb than Kale and lettuce at CS_1. Lettuce shoot accumulation was larger than kale and Swiss chard grown in PS_1. The relative shoot accumulation of Pb in PES_1 by the three plants indicated significant enhancement in kale. Swiss chard was also found to accumulate similar amount of Pb in CS_1 and PS_1. All of the vegetable grown PES_1 were able to accumulate similar amount of Pb in their shoot.

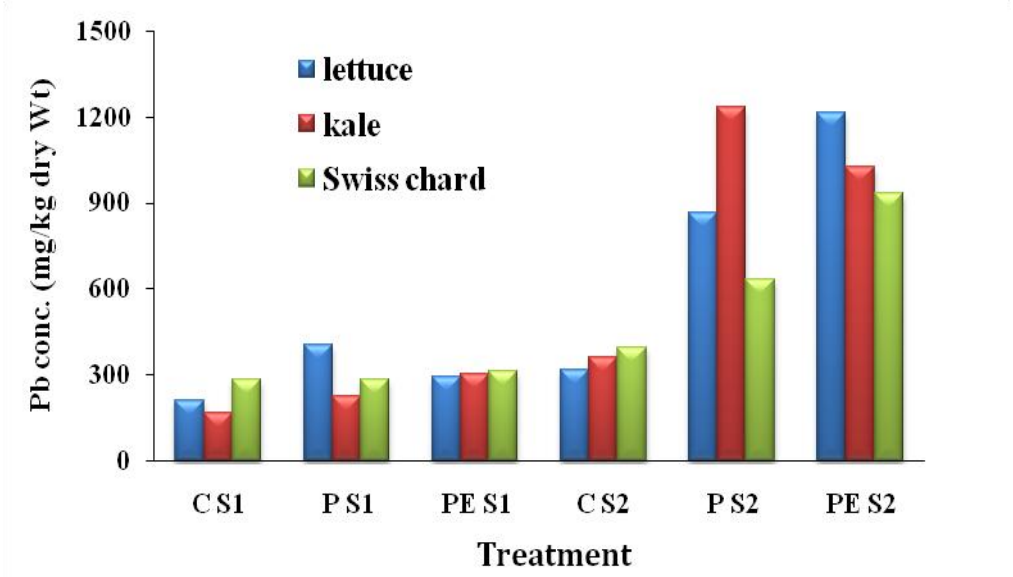


Fig. 7 Pb accumulation in shoots of lettuce, kale and Swiss chard grown in S_1 and S_2

Plant responses to metals and accumulation can differ strongly as complex interactions exist, particularly in the rhizosphere, between soil components and metals and genetic back ground of the plants [50]. In the present study, the maximum accumulation of Pb in Swiss chard shoot at CS_1 could be due to the hairy and long root system which results uptake of the metal at less toxic level in a depth of soil solution and translocate to shoot. A study conducted on effect of root in metal uptake by Ogbonna.P and Ukiwe, showed that, the higher range of Fe and Ni accumulation in leaves of woody plants was significantly greater than Iron concentration obtained in leafy vegetables which could probably the rooting ability of woody plants that enhanced uptake of metals leached into lower layers of the soil [51]. Higher Pb shoot accumulation by lettuce than kale and Swiss chard in PS_1 could have resulted from the toxicity effect of metals dissolved in soil solution to the long rooted plants (kale and Swiss chard) than short rooted (lettuce) [18].

The amount of Pb accumulated in shoots of the plants in the various treatments of S_2 showed a different trend than observed in S_1. In the control, Swiss chard was able to accumulate more Pb in its shoot followed by kale and lettuce. The higher Pb accumulation in Swiss chard in CS_2 confirms the ability of long rooted plants to accumulate more than short rooted (Green house Experiment observation) provided that it tolerates the given concentration [51]. In PS_2, kale was found to accumulate the highest amount of Pb in its shoot and Swiss chard the least. Unlike in control, the smaller value of Swiss chard Pb accumulation in shoot grown in PS_2 could be attributed to the multi metal toxicity effect of added Cr and Pb solution than lettuce and kale [47]. In the present study, plant available Pb in PS_1 was 2.7 times greater than in CS_1. But in PS_2, it was 13.2 times greater than that of CS_2. PS_2 and PES_2 had similar concentration of available Pb. This was in a similar trend to the shoot accumulation (fig.7). Moreno-Jimenez et al reported that, phytoavailability of metals was better predicted from the available fraction than the total metal concentration [52].

In PES_2, Pb accumulation in shoot was better in lettuce followed by kale and Swiss chard. Shoot accumulation of Pb enhancement was found to be significant ($p< 0.05$) in S_2 by lettuce and Swiss chard. A decrease in shoot accumulation of Pb by kale was observed in PES_2. But kale was able to concentrate more Pb in its shoot in PS_2. The variation in Pb accumulation between the two treatments for kale relative to the other two plants could be attributed to its genetic variation, interaction of rhizosphere with the metal and metal with the plant. Similar result has been reported by Dumat et al. on three cultivars of *Pelargonium*. Accordingly, the maximum Pb accumulator plant was not the same in two soils [50]. This shows the difference in plant response to a give soil condition.

In acidic soil, metal solubility can be enhanced by a decrease in the negative sites for cation adsorption on clay surface, organic matter, Iron oxide and manganese oxides in soil [48]. In our study, the lower pH of S_2 could enhance Pb shoot accumulation in all the plants compared to S_1. Organic matter contains Water soluble and particulate portions in which the water soluble part facilitates metal mobility [49]. The lower concentration of Pb in all plants in control soil in this study could be attributed to lower concentration of Pb in the soil solution [44]. Generally all plant species were able to accumulate Pb differently in both soil Samples. Plants grown in S_1 which is less acidic accumulate Pb in their shoot less than those grown in S_2 in all corresponding treatments.

The calculated Pb translocation factors of lettuce, kale and Swiss chard in the various treatments of S_1 and S_2 are given in fig.8. Swiss chard was able to translocate more Pb followed by lettuce CS_1 and PES_1. Higher translocation of Pb in lettuce and Swiss chard relative to other leafy vegetables was reported by other researchers [4]. Ogbonna.P and Ukiwe reported that, the higher accumulation of Fe and Ni in leafs of woody plant could result from the rooting ability of the plant to the depth [51].

In PS_1, translocation of Pb by lettuce and Swiss chard was larger than that of kale. In case of S_2 however, translocation of Pb in kale was significantly greater than that of Swiss chard and lettuce. Such variations of plant responses to metal accumulation could result from

genetic variation which determine the uptake capacity, transporter selectivity etc in the given soil condition and the interaction of the metal with soil components [50].

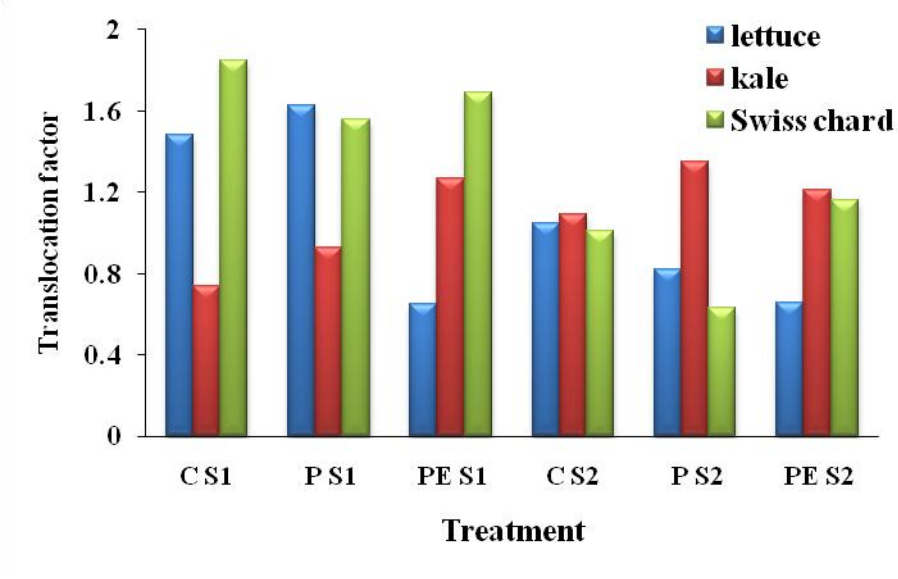


Fig. 8 Pb translocation factors of the three vegetables grown in the various treatments of S_1 and S_2

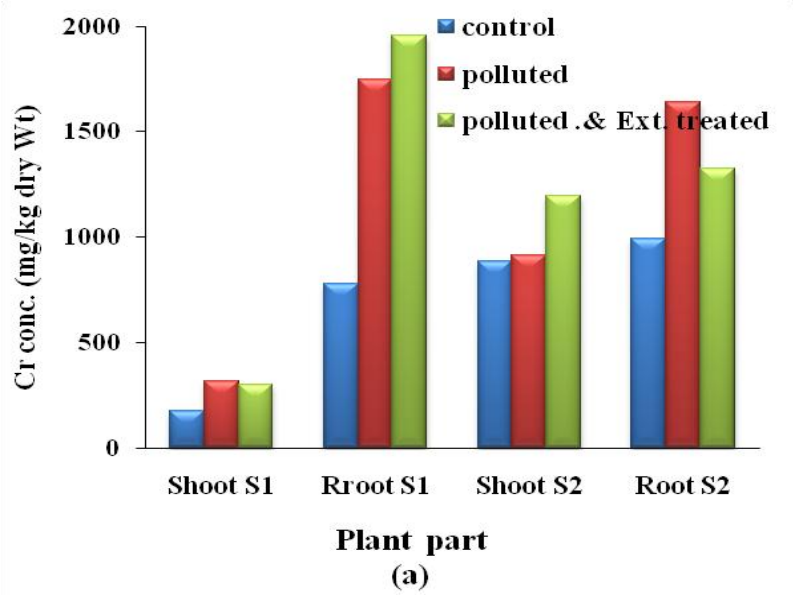
In PS_2, the translocation of Pb was greater in kale than lettuce and Swiss chard. Swiss chard and kale had higher TF values in PES_2 relative to lettuce. The variation in TF between the plant species could be attributed to the difference in permissivity of plant roots to the dissolved metal and uptake capacity [45]. Relative to S_1 which is less acidic, TF value of Pb in S_2 was not enhanced by the extract except in Swiss chard.

The decrease in TF of Pb in kale grown in PES_2 could have resulted from inability of the metal-organic complex that might be formed by the addition of the spinach extract to pass through the root membrane. This was confirmed by the result reported by Robinson et al. which shows a decrease in Ni accumulation in *Berkheya coddii* plant despite the Chelate amendment resulted in metal mobility in soil [45].

4.5.3 Chromium concentrations in plant tissues

Lettuce:

Chromium concentrations in the root and shoot parts of lettuce grown in both S_1 and S_2 are summarized in fig.9 below. As it is shown in the figure, the concentrations of Cr accumulated in shoot of lettuce grown in S_1 were less than that of root for each corresponding treatment. The maximum accumulation of Cr in root than in shoot in plants has been supported by Haki- Hsun and co-workers [53]. This fact is attributed to the fact that translocation of high Cr to the shoots of most plants may cause death. To reduce the toxicity effect of Cr, plants accumulate in their root and hinder translocation. This could be a natural response of plant to toxicity. Similar suggestions were give by Arun [54, 55]. The addition of spinach extract results to an increase in root accumulation in S_1, and this leads to a decrease in TF value. This result coincides with the finding reported by Haki-Hsun, the addition of LMWOAs like oxalic acid enhances root uptake [53]. The result obtained on available Cr in S_1 which indicates a significant increase by the addition of spinach extract was positively correlated to the enhanced root accumulation of all the vegetables under investigation.



(a)

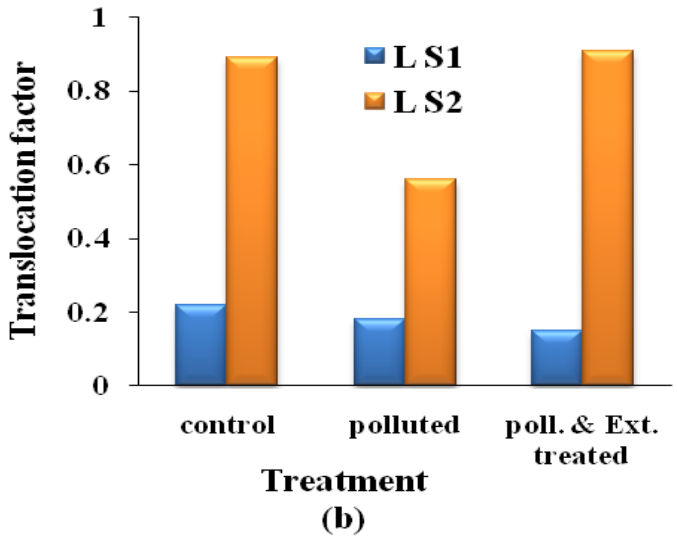


Fig. 9 (a) Cr accumulation in lettuce and (b) Translocation factor

Note: Shoot S_1 and Shoot S_2 = Shoot parts of lettuce grown in S_1 and S2 respectively. Root S_1 and Root S_2 = Root parts of lettuce grown in S_1 and S_2 respectively. LS_1= lettuce grown in S_1, LS_2 = lettuce grown in S_2

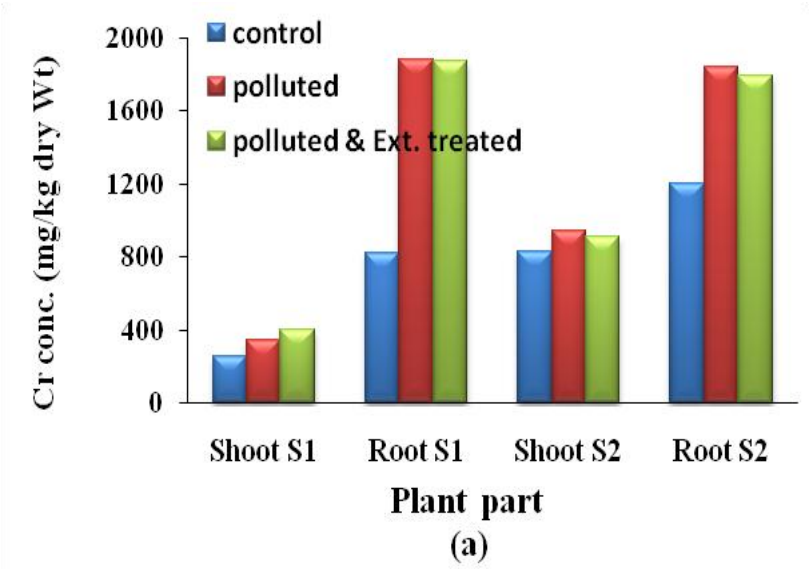
The accumulation of Cr in root of lettuce in S_2 was greater than in shoot. However, shoot Cr gets more than 1000 mg/kg when it was amended with spinach extract. The effect of soil pH has been reported by Chao and Sanolone that the adsorption of hexavalent Cr on soil surface is limited to the positive sites of the surface, the number of which decreases with an increase in soil pH. This adsorption takes place due to its anionic nature [48]. In our result smaller root accumulation in lettuce grown in PS_2 and PES_2 relative to S_1 could attribute to the adsorption of Cr^{6+} added in to the soil in the form of dichromate on the positive sites of soil surface. The higher Cr root accumulation in CS_2 compared to CS_1 could have resulted from the lower toxicity of the back ground Cr concentration thereby fast uptake by root.

The higher shoot and root Cr accumulation in polluted soil in both soils relative to control could have resulted from an increase in the amount of dissolved Cr in the soil due to the externally applied chromium in to the soil. Reda and co-workers have found that metal root and shoot accumulation by *Zea mays, Sorghum bicolor, Helianthus annuus, Conyza*

*discoridies and Cynodon d*actylon grown in soils containing moderate to high amounts of heavy metals is high. The reason for this fact was suggested to be the increase in the plant available metal concentration around the root zone which has been confirmed in separate experiment [44]. Similarly a research carried out by Saggoo and Arneet, on garden soil treated with different level of Cr solution showed a clear trend of increase in the amount of chromium accumulation in plants with increase in the quantity of Cr added to soil [26].

Kale:

Results of Cr analyzed in the shoot and root parts of kale grown in both soil samples are shown in fig.10. From the figure, we can see that root Cr accumulation in Kale grown in S_1 was greater than in the shoot. The higher root Cr accumulation of kale than shoot could be attributed to the immobilization of Cr on root to protect itself from toxicity effect as it has been explained in case of lettuce [53, 54]. In addition to this, Arun and co-workers reported that the limited Cr translocation to shoot could be due to the lack of any specific mechanism in the plant for Cr transport from root to shoot due to its toxicity to plant growth [55].



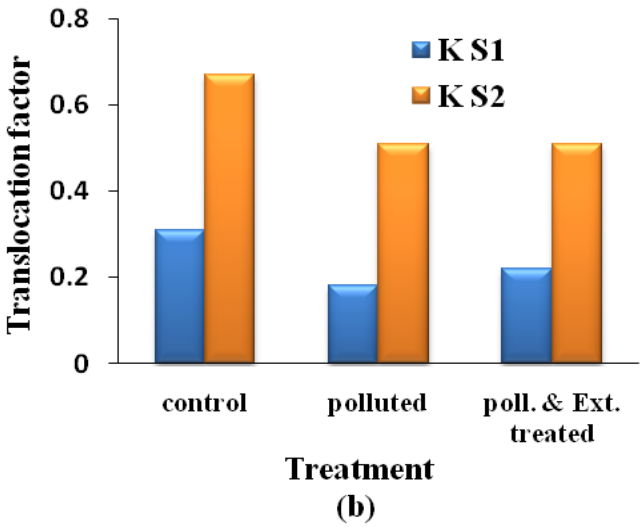


Fig. 10 (a) Cr accumulation in kale and (b) Translocation factor

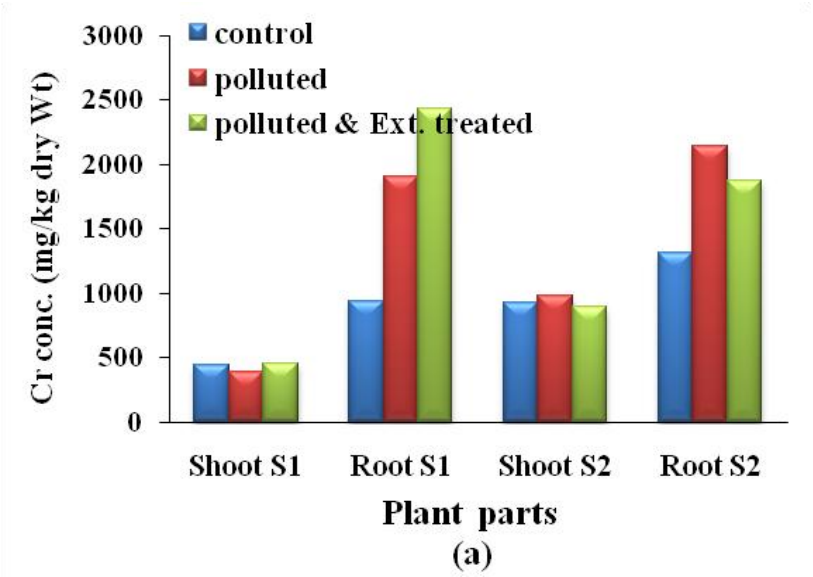
Note: Shoot S_1 and Shoot S_2 = Shoot parts of kale grown in S_1 and S2 respectively. Root S_1 and Root S_2 = Root parts of kale grown in S_1 and S_2 respectively. KS1= kale grown in S_1, KS2 = kale grown in S_2

The addition of spinach extract enhances shoot accumulation but not significantly. The lower pH of S_2 enhances Cr adsorption on positive exchange sites of the soil surface. This could reduce toxicity effect of Cr to plant by decreasing the available Cr concentration. This exhibits translocation of Cr which is restricted by the plant to survive on higher level of Cr [53, 54, 55].

The inhibition of Cr uptake and translocation to shoot of kale in PES_2 could have attributed to the enhanced reduction of Cr (VI) to Cr (III) by LMWOAs present in the spinach extract used for amendment. It is reported by Naidu and co-workers that, in soils high in organic matter, Cr (VI) reduction is rapid regardless of the soil pH. Soluble LMWOAs have shown to be effective reductants of Cr (VI). In addition to reducing Cr (VI) to Cr (III) which is less mobile, many LMWOAs form Cr-organic complexes which can eventually complex with manganese oxides. This renders Cr immobile and less accumulation [15, 56].

Swiss chard:

Results of Cr analysis in the roots and shoots of Swiss chard grown in the various treatments of S_1 and S_2 are shown in fig.11. In these results we can see that, Cr accumulation in the shoots of Swiss chard shows a similar trend with that of the other plants. The root of Swiss chard was able to accumulate more Cr than that of its shoot in both S_1 and S_2. The higher Cr accumulation in roots of Swiss chard could be attributed to the lack of root to shoot Cr transport mechanism in plants as it has been explained by Arun et al. [55]. Similarly, it was explained that Cr translocation to shoot was limited and it immobilized in root vacuoles. This is the mechanism plant use to survive in toxic level of Cr. This immobilization protects the plat from toxicity effect of the metal [53, 54]. In both soil Cr accumulation increment was observed with pollution in Swiss chard similar to lettuce and kale.



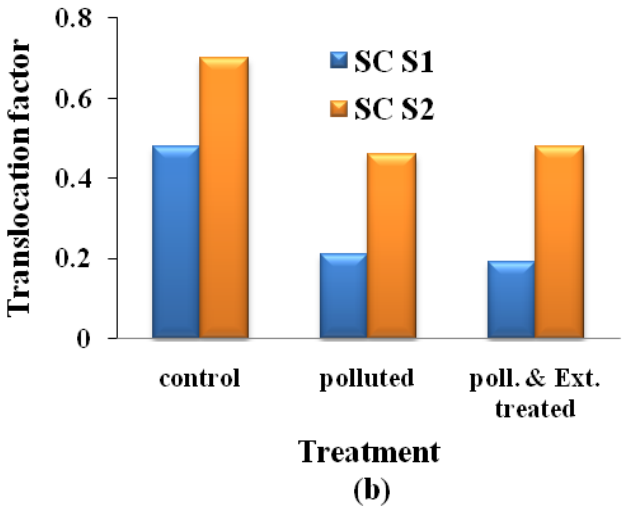


Fig. 11 (a) Cr accumulation in Swiss chard and (b) Translocation factor

Note: Shoot S_1 and Shoot S_2 = Shoot parts of Swiss chard grown in S_1 and S_2 respectively. Root S_1 and Root S_2 = Root parts of Swiss chard grown in S_1 and S_2 respectively. SC S_1= Swiss chard grown in S_1, SC S_2 = Swiss chard grown in S_2

Swiss chard in CS_1 has been found to accumulate more Cr in its shoot than the one grown in PS_1. The smaller Cr accumulation in shoot at PS could be due to toxicity effect of multi metal contamination on plant metabolism [53]. Cr accumulation in shoot and root of Swiss chard grown in S_2 was relatively greater than that of in S_1 except in the presence of spinach extract. In case of S_2, the higher Cr adsorption on positive sites due to low pH decreases the toxicity effect of Cr; there by possible translocation. This leads to the higher shoot accumulation [48].

Chromium concentrations in both shoot and root of Swiss chard were found to decrease in the presence of extract in S_2. The inhibition of Cr accumulation in Swiss chard tissues in S_2 could be due to the reduction of Cr (VI) to Cr (III) at higher OM [56]. The reduction in Cr accumulation in shoot of Swiss chard in PES_2 might be due to Cr-organic complexes formation with LMWOAs which can eventually complexes with manganese oxides as indicated by Naidu and co-workers [56]. The inability of Cr that could be complexed with

organic acids in PES_2 to pass through root membrane could affect metal uptake. The result that has been reported by Robinson and co-workers on Ni solubilization by chelating agent uptake by Berkheya coddii plant confirms that solubilization may not necessarily lead to bioaccumulation as it has been explained in case of Pb [45].

The concentrations of Cr in the shoots of lettuce, kale and Swiss chard in all treatment of both S_1 and S_2 are summarized in fig.12 below. Lettuce grown in S_1 was able to accumulate Cr in its shoot less than kale and Swiss chard in all treatments, whereas Swiss chard accumulates more than kale and lettuce. It was tried to explain the effect of rooting system on metal uptake in case of Pb above. Accordingly, plants with longer roots were able to uptake higher Fe and Ni than that of short root [51]. Our result in almost all of the treatments confirms this finding except in PES_2. This exceptional trend could be resulted from the toxicity effect of the complexed Cr to the plant with long rooting system.

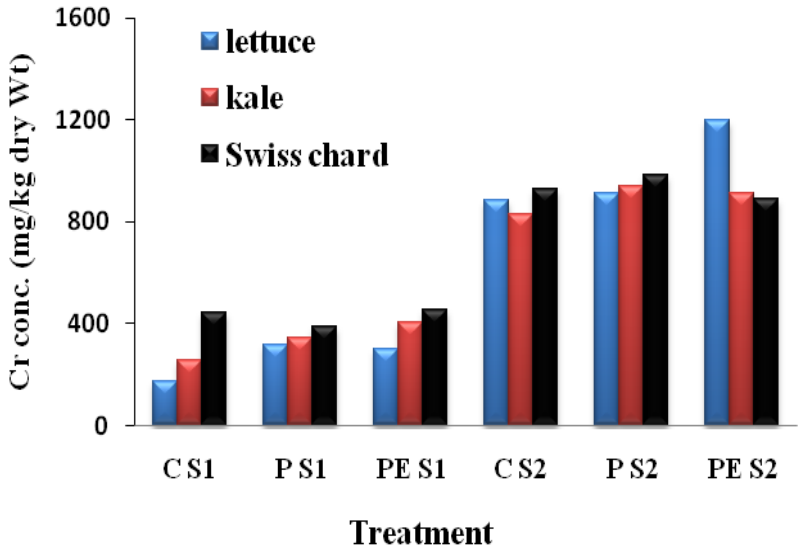


Fig. 12 Cr accumulation in the shoots of lettuce, kale and Swiss chard grown in S_1 and S_2

The accumulation of Cr in shoot of the three vegetable species was comparatively higher in S_2 than S_1. The less available Cr in S_2 (table.4) could be due to the reduction of Cr (VI) to less mobile Cr (III) in soil with high OM [56]. This reduces the toxicity stress and enhances translocation. Except lettuce grown in PES_2, all accumulations were bellow 1000 mg/kg. A

significant shoot accumulation enhancement was observed in lettuce with addition of extract. But in $PS_{2,}$ there was no significant variation in shoot accumulation between the vegetables. The enhancement in shoot accumulation could be due to the detoxification of Cr by the added extract though complexation and an increase in translocation [7, 55]. Plant responses to metals and accumulation can differ strongly as complex interactions exist, particularly in the rhizosphere, between soil components and metals and genetic back ground of the plants [50]. In our study these complex interactions and genetic variation of the vegetables investigated were shown in their metal accumulations and translocations in different soils and at the same soil in different treatments.

Chromium TF of the three vegetables investigated were compared in each treatment of both soil samples. As it is shown below in fig.13, TF of all plants in all treatment and both soil samples were less than 1. Relatively, TF of the three plants was greater in CS_1 than PS_1 and PES_1. This could be due to restricted upward transport of Cr in these plants body at higher concentration [17, 47]. At low Cr soil concentrations, Cr has high transfer mobility from roots to shoots, and when roots take up additional Cr from soils, transfer efficiency from roots to shoots declines. This may be a survival mechanism for the plant in environments with high Cr levels [53]. Lack of Cr transport mechanism from root to shoot of the vegetables to prevent toxicity could be the reason for the observed low translocation [55]. The addition of spinach extract was not able to enhance translocation significantly.

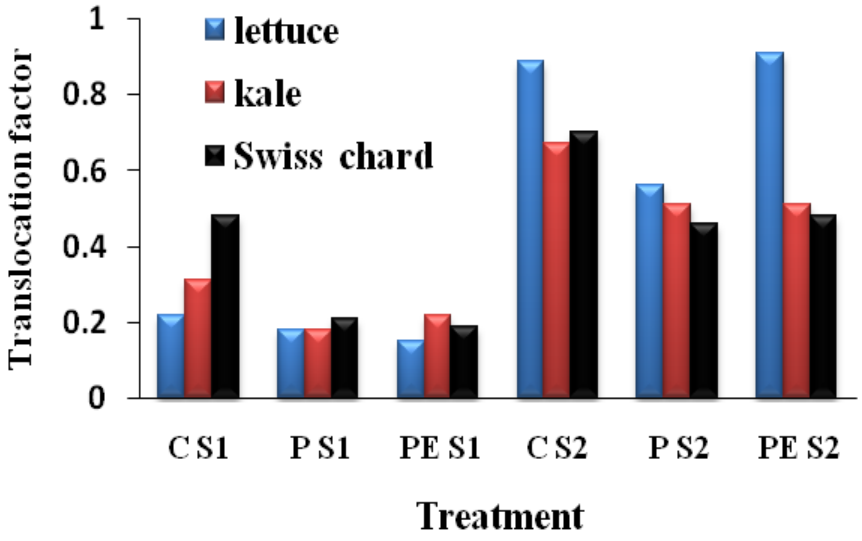


Fig.13 Cr translocation factors of the three vegetables grown in the various treatments of S_1 and S_2

The vegetables grown in S_2 had TF greater than those grown in S_1 in all respective treatments. The TF of lettuce in CS_2 and in PES_2 were significantly ($P < 0.05$) greater than those of kale and Swiss chard. The decrease in TF with increase metal concentration in soil could be by the same reason occurred in S_1 except lettuce. Unlike the other two plants, the higher TF in PES_2 could have resulted from the lower toxicity effect of Cr on the short rooting system of the plant. In each treatment of S_2, a decrease in TF indicates the increase in toxicity effect on the longer rooted plant thereby inhibits Cr translocation in to shoot.

The translocation factors of the three plants for Pb and Cr in all the treatments of both soil samples are summarized in fig. 14 blow. Except in lettuce grown in PES_2, in which the TF for Cr is greater than that of Pb, TF values of Pb in all the three plants grown in all the treatments of S_1 and S_2, were greater than those of Cr.

Pb TF of lettuce and Swiss chard were greater in S_1 than S_2 except in PES. Lettuce grown in PES had higher Cr TF in S_2 than Pb in S_1 and any other treatment.

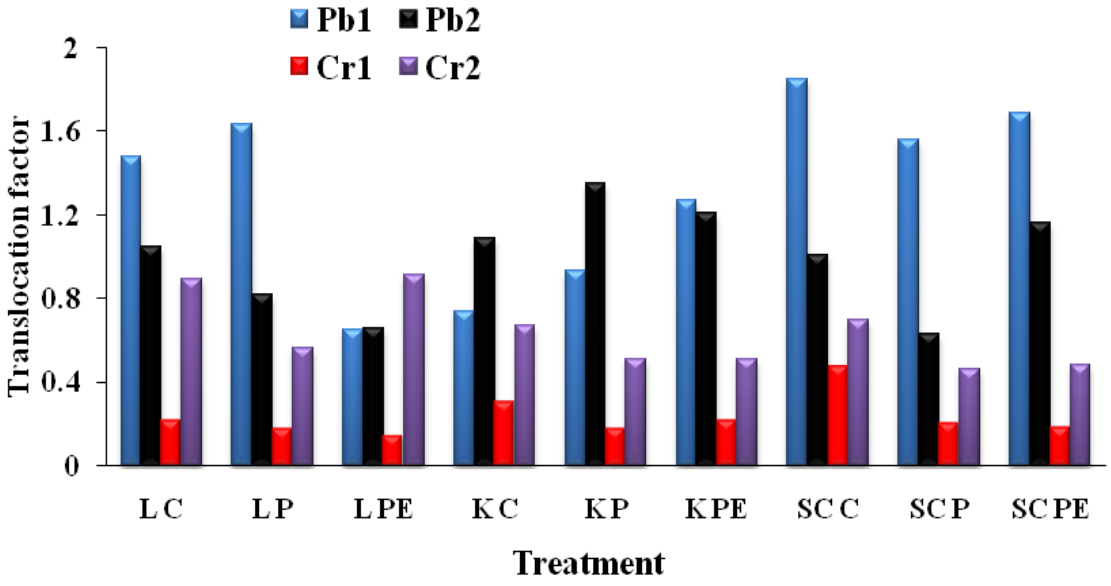


Fig. 14 Translocation factors of lettuce, kale and Swiss chard for both Pb and Cr in all the treatments.

Note: LC, KC and SC C = lettuce, kale and Swiss chard grown in the controls respectively. LP, KP and SC P = lettuce, kale and Swiss chard grown in the polluted soils respectively. LPE, KPE and SC PE = lettuce, kale and Swiss chard grown in the polluted and spinach extract treated soils.

Unlike in the case of Pb, the TF values of all the three plants for Cr in S_2 were significantly ($P < 0.05$) greater than those of corresponding treatments in S_1. The adsorption of Cr (VI) on positive sites of soil surface and Iron oxide in acidic S_2 and rapid reduction of Cr (VI) to Cr (III) which is less toxic in S_2 with high OM reduces the toxicity stress of Cr on the plants [56]. The reduced stress of Cr in S_2 could enhance the translocation of Cr from root to shoot than in S_1. This leads to the higher TF of Cr in S_2 than in S_1. TF values of Pb in S_1 (less acidic) were relatively higher than in S_2 (moderately acidic) except in kale. TF calculated for Pb was not as much soil type dependent. Such variations of plant responses to metal accumulation and translocation could result from genetic variation which determine the uptake capacity, transporter selectivity etc in the given soil condition and the interaction of the metal with soil components [50].

5. CONCLUSION

It has been indicated that plant species which are suitable for the phytoextraction of Pb and Cr are those which accumulate more than 1000 mg kg^{-1} in their shoot with a TF value greater than 1. In our study, the amount of lead accumulated by each of the plants grown in S_1 has been found to be less than this value. Moreover, the total amount lead accumulated by each plant in a given treatment in S_2 has been found to be greater than that of lead accumulated by the same plant in its respective treatment in S_1. The use of spinach extract amendment has been found to enhance shoot accumulation of lead in lettuce and Swiss chard in S_2 significantly ($P < 0.05$). However, the accumulation of lead in kale

including its TF has been found to decrease with spinach extract amendment than when not amended.

Therefore, taking into account the amount of lead accumulated by the plants and their translocation factors, lettuce, with more than 1000 mg kg^{-1} Pb accumulated in soils having similar physico-chemical condition as S_2 when treated with spinach extract and a TF value of greater than one in polluted soil having similar physico-chemical condition as S_1 without spinach extract is a good phytoextractant for Pb. Kale has also been found to be a good phytoextractant for Pb under soil conditions similar to S_2 either with spinach extract amendment or without amendment. The amount of Pb accumulated in shoot of kale and its TF value however, is better without spinach amendment. Swiss chard, although the total amount of Pb it has accumulated is lower than kale and lettuce, it has also been found to translocate more Pb PES_2. However, it's TF in PS_2 (0.63) is much less than in that of PES_2 (1.16). This fact also shows that, Swiss chard could serve as a good phytoextractant in S_2 type soils with spinach extract amendment. Kale in soils having similar physico-chemical condition as S_1 with spinach extract, S_2 with and without spinach extract amendment could be used as phytoextractant of Pb due to its higher TF.

In both soil types, all the plants were found to uptake a relatively high amount of chromium although slightly higher values were recorded in S_2 for all treatments. A large portion of the metal was not translocated to the shoot of the plant in both soil types. The TF values of the plants for Cr in all the treatments have shown an increase in S_2 relative to their TF values in respective treatments in S_1. Swiss chard has shown a 1.5 to 2.5 times increase, kale 2 to 3 times increase and lettuce 3 to 6 times increase. Although the TF value of lettuce for Cr in S_2 is slightly less than 1, it has been found to be a good phytoextractant for Cr than the other two plants in S_2 type soils with spinach amendment.

6. RECOMMENDATIONS

Plants investigated (lettuce, kale and Swiss chard) were found to have different accumulation and translocation capacity towards Pb and Cr in a given soil. The following issues require further investigation for the practical application of the plant as phytoextractant.

Spinach extract amendment of the soils was found to enhance Pb accumulation and translocation more than Cr by the plants investigated. However, the level of optimum

quantitative amendment for a better accumulation and translocation requires further investigation.

The extent of accumulation and translocation of the plants may vary with a difference in the level of pollution. Therefore, the phytoextraction capacities of the plants need to be investigated by varying the level of pollution of the soils.

The time interval by which these plants can accumulate and translocate more metal has to be studied by varying the harvesting time of the plants

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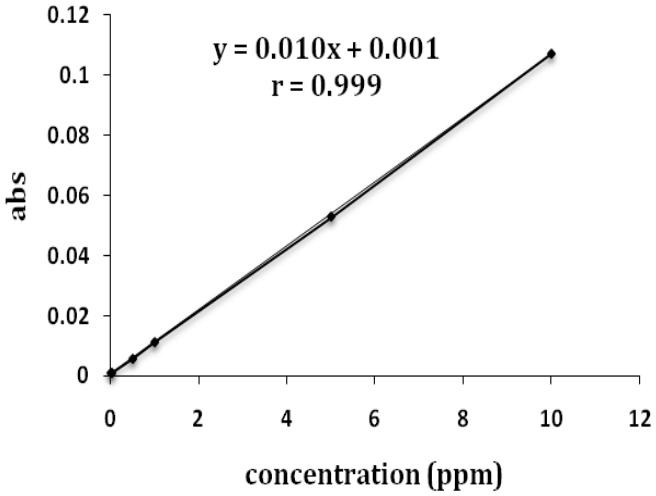
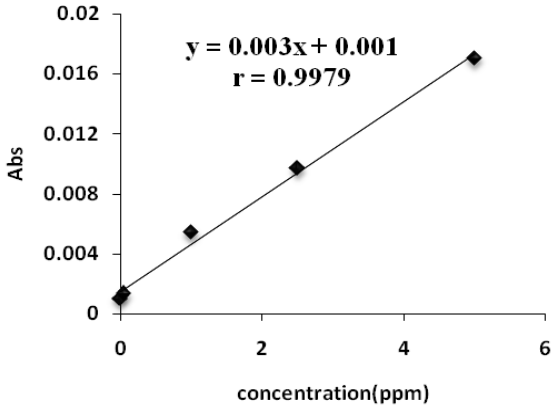
Appendix – A Pb and Cr Calibration carves



(a) Calibration carve of lead



(b) Calibration curve of Chromium

Appendix-B Lead and chromium concentration in vegetables

Lead concentration of plant tissue grown in S_1 and TF value

Plant	Treatment	plant part		Translocation factor (TF)
		Shoot	Root	
Lettuce	Control	209.52±4.61	141.40±6.73	1.48
	Polluted	404.80±3.90	248.40±4.77	1.63
	Pol.Ex	294.56±4.12	451.20±1.17	0.65
Kale	Control	169.66±5.45	229.78±3.08	0.74
	Polluted	228.26±4.82	245.62±3.57	0.93
	Pol.Ex	304.28±4.88	239.20±3.17	1.27
Swiss-chard	Control	284.80±4.95	153.80±6.09	1.85
	Polluted	283.04±5.07	182.00±6.14	1.56
	Pol.Ex	313.28±3.46	184.92±6.37	1.69

Lead concentration of plant tissue grown in S_2 and TF value

Plant	Treatment	Plant part		Translocation factor (TF)
		Shoot	Root	
Lettuce	Control	318.36±3.55	303.52±4.39	1.05
	Polluted	865.60±6.21	1044.40±6.97	0.82
	Pol.Ex	1213.20±8.06	1826.40±9.23	0.66
Kale	Control	362.92±4.36	330.20±4.67	1.09
	Polluted	1235.20±8.65	909.80±6.58	1.35
	Pol.Ex	1027.80±7.39	851.00±6.39	1.21
Swiss-chard	Control	394.16±4.70	391.84±4.98	1.01
	Polluted	635.20±5.31	1010.80±7.12	0.63
	Pol.Ex	933.60±6.79	801.60±6.43	1.16

Cr concentration of plant tissue grown in S_1 and TF value

Plant	Treatment	plant parts		Translocation factor (TF)
		Shoot	Root	
Lettuce	Control	174.80±6.22	781.60±1.35	0.22
	Polluted	317.20±5.04	1744.67±6.65	0.18
	Pol.Ex	299.73±5.31	1955.33±7.58	0.15
Kale	Control	257.33±5.57	819.33±2.21	0.31
	Polluted	344.40±4.98	1883.33±6.98	0.18
	Pol.Ex	404.40±4.54	1871.33±6.87	0.22
Swiss-Chard	Control	446.00±4.39	938.00±2.79	0.48
	Polluted	390.13±5.01	1900.67±7.19	0.21
	Pol.Ex	454.27±4.27	2439.33±8.75	0.19

Cr concentration of plant tissue grown in S_2 and TF value

Plant	treatment	plant parts		Translocation factor (TF)
		Shoot	Root	
Lettuce	Control	885.20±3.18	994.80±3.93	0.89
	Polluted	914.00±2.51	1640.67±6.17	0.56
	Pol.Ex	1196.67±4.26	1323.33±5.18	0.91
Kale	Control	828.00±2.34	1206.00±4.39	0.67
	Polluted	940.13±2.81	1842.00±7.07	0.51
	Pol.Ex	912.27±2.69	1792.67±6.96	0.51
Swiss-chard	Control	926.13±2.67	1314.00±5.10	0.70
	Polluted	981.73±3.84	2142.00±7.94	0.46
	Pol.Ex	889.47±2.53	1871.33±6.87	0.48