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Phytoremediation of Pb polluted soils through *Chrysanthemum indicum* L. with the help of EDTA and Vermicompost

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ABSTRACT
The pot experiment was conducted to grow Chrysanthemum indicum L. to investigate the phytoremediation potential of Pb contaminated soils with the help of EDTA and Vermicompost. The result show the combined application of EDTA (5 mmol kg^{-1}) and vermicompost (10 g kg^{-1}) enhanced the accumulation of Pb in root and shoot up to 42.21, 20.67 mg kg^{-1} dry weight of plant. However, the accumulation of Pb increased by increasing the concentration Pb [applied in the form of [$Pb(NO_3)_2$] in soil from 0 to 40 mg kg^{-1} up to 0.68 to 42.21 and 0.33 to 20.67 mg kg^{-1} in root and shoot respectively. Maximum root and shoot growth observed in T_6 treatment (10 mg kg^{-1} Pb, 5 mmol kg^{-1} EDTA + 10 g kg^{-1} vermicompost) 16.34, 42.82 cm and minimum in T_{10} treatment (only 40 mg kg^{-1} Pb) 9.10, 29.42 cm respectively. Root and shoot biomass were found maximum in T_6 treatment applied 10 mg kg^{-1} Pb, EDTA (5 mmol kg^{-1}) and vermicompost 10 g kg^{-1} up to 1.28, 4.27and minimum in T_{10} (only 40 mg kg^{-1} Pb) applied treatment 0.71, 2.25 g pot^{-1} . The concentration of Pb in root was found higher than shoot. The combined application of EDTA (5 mmol kg^{-1}) and vermicompost (10 g kg^{-1}) in treatment T_3 show the maximum translocation factor 0.63 and bioaccumulation factor in T_9 (20 mg kg^{-1} Pb, 5 mmol EDTA and 10 g vermicompost) 0.46 followed by T_{12}, T_{11} and T_{10} treatments.The result of present study reveals that the Chrysanthemum indicum had phytoremediation potential of Pb polluted soils and EDTA and vermicompost are the enhancers of phytoremediation.
Keywords: *Bioaccumulation, Lead, Phytoremediation, Pollution, Translocation, Vermicompost*

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INTRODUCTION

To feed the rapidly growing population, agriculture is being expanded on more and more land and large industries are being setup to fulfill the requirements of mass population and developments of big cities for secure residence for all. It has been causing many environmental problems, which is the biggest challenge for modern civilization. Among the various environmental problems the soil pollution is a serious problem which is created by unscientific farming, excessive use of chemicals and chemical fertilizers, large quantities of waste material generated in urban areas dumped in cultivated land without proper treatment, nuclear power plants and military activities. When these activities run continuously long time the amount of pollutants increased beyond the threshold level in soil and huge amount of pollutants make undesirable changes in physical, chemical and biological properties of soil resulting decreases soil fertility. When soil fertility decreases ultimately crop production are reduced then create the shortage of food. The availability of food is reduce due to which starvation spreads and when there is starvation, it does not come alone, it brings many problems, so it is very important that the problem of soil pollution is eliminated.

Soil pollutants can be divided into two main groups: inorganic and organic. The main components of inorganic pollutants are heavy metals, such as lead, arsenic, cadmium, copper, zinc, nickel, and mercury, which are continuously added into environments via disposal of urban sewage sludge and industrial wastes in agricultural soils and via agrochemical usage [16]. These pollutants, together with radionuclide are released through various human activities (mining and milling of nuclear fuel, testing of nuclear weapons, occasional nuclear disasters), accumulate in the soils, and can be readily passed in to human beings through the food chain due to soil-to-plant transfer of metals and radionuclide's [16, 26]. In addition, vast areas of soils in the world are polluted with organic pollutants, mainly pesticides, petroleum hydrocarbons, polycyclic aromatic hydrocarbons, and explosives.

Lead is the important heavy metals among the different heavy metals that easily accumulate in soils. The potential sources of lead contamination in environments are natural processes, industrial activities, mining, agricultural activities and urban areas related activities. Lead have important place in changed human life. Contamination of soils with lead increased day to day and no any chances of reduction of lead contamination in soil in future without proper management of soil and wastes lead. The concentration of lead in cultivated areas has been increase near urban areas. The increased lead concentration enters in human and animal food chain through consumption of that plants which can grown in lead contaminated soils. Conventional techniques used for management of contaminated soils, namely chemical, physical, and microbiological methods are costly to install and operate. A sustainable economically, environmental friendly, socially acceptable technology has been developed which called phytoremediation means use of green plants for cleanup of soil, water and environment. Phytoremediation has six types of remediation techniques but only two phytostabilization and phytoextraction are used for in-situ phytoremediation of heavy metals polluted soils [30]. Chelating agents and genetic engineering improve the phytoextration potential of green plants by modification in plants characteristics and physiology. The total concentration of lead in soil is high but availability of lead for accumulation in plant is very low due to strong binding of lead with organic matter and oxides of Fe and Mn. The various synthesized chelating agents likes EDTA, HEDTA and NTA were used to heavy metals polluted soil to enhanced the mobility and bioavailability of heavy metals in soils [12] and also to improve the amount of heavy metals accumulated in plants [6]. However, at high concentration of chelating agents in soil harmful to the plants [8].

The application of compost and vermicompost in lead contaminated soil is reported to improve the fertility status and physico-chemical properties of soils and facilitate the phytoremediation of heavy metals polluted soil. The vermicompost improve the growth habit and biomass production capacity of plant by which more accumulation of metals from soil and develop resistance against metal toxicity [31]. The available waste material used for preparing vermicompost and these vermicompost used as phytoremediation enhancer.

The application of phytoremediation techniques for heavy metal pollution control, however, has several constraints that require further research on plant and site-specific soil conditions. Phytoremediation is dominantly confined to the area covered by the root systems of growing plants, and as the consequence, plant have low root system do not efficiently phytoremediation of contaminated soils. In addition, non-perennial plants, particularly those with slow growth and low biomass production require a long time for remediation. Environmental conditions also determine the efficiency of phytoremediation as the survival and growth of plants are adversely affected by extreme environmental conditions, toxicity, and the general conditions of soil in contaminated lands [24]. Finally, various types of soil and water pollutants require various types of plants for phytoremediation and additional techniques that enhance the phytoremediation potential of plants.

Chrysanthemum indicum L. is an ornamental plant commonly called Indian chrysanthemum (Guldaudi) the family of Asteraceae. The Chrysanthemum plant is grown as ornamental plant in Indian continent and other parts of the globe. These plants have fast growing habit, attractive flower and short life. C. indicum plant is most suitable to grown in heavy metal contaminated soils near urban areas. Chrysanthemum plant is a fertilizer responsive plant which requires large amount of macro and micro nutrients and these nutrients applied through different sources likes chemical fertilizer, organic manures. Organic manures play key role in nutrient availability, improve physiochemical properties of soil and enhancer of phytoremediation. Vermicompost have higher amount of various organic acids which facilitate the chemical and biological activities in soil.

With this perspective regarding problems associated with lead contamination in soil and its possible solution through phytoremediation. The main objectives of present study are to investigate the phytoremediation potential of *Chrysanthemum indicum* plant with application of EDTA and Vermicompost.

MATERIAL AND METHODS

Experimental layout

The pot experiment was conducted at Sheila Dhar institute of soil science, Prayagraj (UP). The Prayagraj is located in junction of Ganga and Yamuna rivers and having alluvial soil deposited by these rivers. The physicochemical properties of initial soil and vermicompost present in table-01. Healthy Chrysanthemum plants were planted in pots having 5 kg capacity. In pot experiment used Pb, EDTA and Vermicompost in different doses through twelve treatments and each treatment replicated thrice in completely randomized block design. The treatment details have been present in table-02. Lead was applied in the form of lead nitrate [Pb $(NO3)_2$] solution in soil at the rate of 0, 10, 20 and 40 mg kg^{-1} of soil. EDTA was applied in to the soil in the form of Na_2-EDTA at the rate of 0 and 5mmol kg^{-1}. Well prepared Vermicompost applied in pot soils before planting of *Chrysanthemum indicum* at the rate of 10 g kg^{-1}. Soil moisture maintains by frequent irrigation. *Chrysanthemum indicum* plant harvested after 65 days of planting.

Collection of Soil samples

To take an inclusive soil sample, the field is divided in uniform plots keeping in view the geographical situation of the field. The soil sample were collected carefully from each uniform plots 2 m apart from field margin. The soil texture is determined by pipette method [7].

Analysis of lead in soil

The determination of total Pb in soil, by take one gram of soil sample was mixed with 5 ml of HNO_3 (16 M, 71 %) and 5 ml of $HClO_4$ (11 M, 71 %) then soil mixture was heated until dry of sample then added hot distilled water. The dry sample filtrate by filter paper and final sample volume maintain 50 ml the filtrate sample was used to analysis of Pb by Atomic Absorption Spectrophotometer (AAS). For the determination of available Pb from soil, take 5 gram soil was mixed with 20 ml DTPA solution {Di-ethyl-tri-amine-penta acetic acid (DTPA) solution [1.97 g (0.05 M) DTPA powder, 13.3 ml (0.1 M) Tri-ethanol amine and 1.47 g (0.01 M) $CaCl_2$ were dissolved in distilled water [20] and maintain 1 liter sample after adjusting the pH 7.3] was added and the contents were shaken for 2 h and then filtered through Whatman filter paper No. 42. The clean filtrate was used for the estimation of Pb by the spectrophotometer.

Determination of Soil pH

Soil pH was determined with 1:2.5 soil–water ratios using digital pH meter at the Laboratory of Sheila Dhar Institute of Soil Science, University of Allahabad, Prayagraj, Uttar Pradesh, India. Double distilled water was used for the preparation of all solutions.

Determination of Organic carbon

For the determination of organic carbon take fine grind one gram soil sample and digested with 10 ml of 1 N potassium dichromate ($K_2Cr_2O_7$) solution and 20 ml of concentrated sulphuric acid (18 M, 96 %). The soil solution was shaken for 2 minutes and kept for half an hour and then diluted with 200 ml of distilled water. Then addition of 10 ml of Ortho-phosphoric acid (15 M, 85 %) and 1 ml of diphenylamine indicator were in solution. The solution became deep violet in colour and further it was titrated against N/2 ferrous ammonium sulphate solution, till the violet colour changed to purple and finally to green [7].

Determination of Cation exchange capacity (CEC)

The CEC of soil was determined by using neutral 1 N ammonium acetate solution. A known weight of soil (5 g) was shaken with 25 ml of the acetate solution for 5 min and filtered through Whatman filter paper No. 42 [7].

Determination of Total nitrogen

The total nitrogen in soil was determined by take 1 g fine soil sample and digested with 10 ml of digestion mixture containing sulphuric acid and selenium dioxide. Salicylic acid was also added in solution to include the nitrates and nitrites. The digestion carried out till the soil colour changed to white. The N in the digest was estimated by using micro-Kjeldahl method, Glass Agencies, Ambala, India [7].

Determination of Total phosphorus

For the determination of total phosphorus , 10 g fine grind soil sample was taken with 4 ml $HClO_4$ (11 M, 71 %) in a 50-ml beaker covered with watch glass and put on a hot plate and digestion was carried out till the soil colour changes to white. 10 ml HNO_3 (16 M, 71 %) was added to the filtrate solution. Ammonia was added to saturate the solution. Then 30 ml standard ammonium molybdate solution was added in the solution to extract the total phosphorus content from soil [18].

Plant sampling and analysis

Plants were harvested after 65 days of transplanting. Plant samples were firstly washed by tap water than 0.2% detergent solution, 0.1 N HCL, de-ionized water and double distilled water. Plant samples then soaked with tissue paper, air dried for 2-3 days in clean environment, placed in clean paper envelopes, dried in hot air oven at a temperature of 45°C and grind in to a fine powder. Plant dry biomass weight was recorded. 1g of ground plant material was digested with15 ml of tri-acid mixture containing concentrated HNO_3 (16 M, 71 %), H_2SO_4 (18 M, 96 %) and $HClO_4$ (11 M, 71 %) in (5:1:2). The composite was heated on hot plate at low heat (60°C) for 30 min, and the volume was reduced to about 5 ml until a transparent solution was obtained. After Cooling, 20 ml distilled water was added and the content was filtered through Whatman filter paper No. 42 [18]. The Pb was determined by Atomic Absorption Spectroscopy (GBC Avan AAS. Australia).

Table 01: Physico-chemical properties of initial soil

Parameters	Soil	Vermicompost
Sand (%)	55.54±0.14	
Silt (%)	20.32±0.11	
Clay (%)	24.25±0.02	
PH	7.7±0.17	6.70±0.13
EC (dsm^{-1}) at 25°C	0.29±0.02	1.08±0.01
Organic carbon (%)	0.58±0.03	13.45±0.04
CEC [C mol (p^+)/kg]	20.6±0.02	87.00±0.03
Total N (%)	0.08±0.01	1.23±0.02
Total P (%)	0.039±0.01	0.48±0.01
Total Pb (mg/kg)	9.68±0.08	0.15±0.05
DTPA-extractable Pb (mg/kg)	1.57±0.02	0.06±0.04

± Values indicate standard deviation having three replications, EC electrical conductivity, CEC cation exchange capacity, DTPA diethyl tri-amine penta-acetic acid

Table 02: Treatment combinations used in pot experiment at Sheila Dhar Institute of soil science, Prayagraj

Symbols	Treatment combination
T_1	Pb (0 mg kg^{-1})+0 mmol kg^{-1} EDTA
T_2	Pb (0 mg kg^{-1}) + 5 mmol kg^{-1} EDTA
T_3	Pb (0 mg kg^{-1})+5 mmol kg^{-1} EDTA+ 10 g kg^{-1} vermicompost
T_4	Pb (10 mg kg^{-1})+0 mmol kg^{-1} EDTA
T_5	Pb (10 mg kg^{-1})+5 mmol kg^{-1}EDTA
T_6	Pb (10 mg kg^{-1})+ 5 mmol kg^{-1} EDTA+10 g kg^{-1} vermicompost
T_7	Pb (20 mg kg^{-1})+0 mmol kg^{-1} EDTA
T_8	Pb (20 mg kg^{-1})+5mmol kg^{-1} EDTA
T_9	Pb (20 mg kg^{-1})+5 mmol kg^{-1} EDTA+10 g kg^{-1} vermicompost
T_{10}	Pb (40 mg kg^{-1})+0 mmol kg^{-1} EDTA
T_{11}	Pb (40 mg kg^{-1})+5 mmol kg^{-1} EDTA
T_{12}	Pb (40 mg kg^{-1})+5 mmol kg^{-1} EDTA+10 g kg^{-1} vermicompost

EDTA=Ethylene diamine tetra acedic acid

Translocation factor

Translocation factor (TF) was use to determine the efficiency of phytoremediation. TF is an indication of the capacity of particular plant to movement of meals from root to areal portion of plant [23]. It is represented by the formula:

$$\text{Traslocation Factor} = \frac{\text{Metal concentration in shoot}}{\text{Metal concentration in root}}$$

Bioaccumulation factor

Bioaccumulation factor (BAF) can be used to evaluate the heavy metals accumulation efficiency in plants by comprising the concentration in plant and soil [2, 21].

$$\text{Bioaccumulation Factor} = \frac{\text{Metal concentration in shoot}}{\text{Metal concentration in soil}}$$

Statistical analysis

The pot experiment was conducted as a factorial randomizes block design with each treatment replicated thrice. Statistical analysis of data was done following analysis of variance (ANOVA), when the ANOVA was significant that mean were separated using least significance difference (LSD), at $P \leq 0.05$ level of significance.

RESULT AND DISCUSSION

Root and shoot growth affected by Pb, EDTA and Vermicompost

The result present in Fig.-01 indicate that the combined application of 10 mg kg^{-1} Pb with 5 mmol kg^{-1} EDTA and Vermicompost (10 g kg^{-1}) in treatment (T_6) enhanced root and shoot length up to 16.34,42.82 cm respectively which was observed higher over the all treatments followed by T_9 (15.53, 41.62), and T_{12} (15.10, 41.00) treatments. Application of Pb at 10 mg kg^{-1} along with EDTA or Vermocompost or both caused root and shoot elongation from 11.10,35.61 to 16.34, 42.82 cm respectively. However combined application of EDTA (5 mmol kg^{-1}) and Vermicompost (10 g kg^{-1}) enhanced the root and shoot growth in all Pb (10, 20, 40 mg kg^{-1}) concentration. The application of 40 mg kg^{-1} Pb without EDTA and Vermicompost in treatment (T_{10}) was reduced the root and shoot growth which was observed minimum 9.10, 29.42 cm. the results clearly indicates that lead has inhibitory effect on root and shoot growth when the concentration of Pb more than (20 mg kg^{-1}) Reduction in root growth is possible due to restriction in cell division and cell elongation [22]. The symptoms of Pb toxicity in root of plants including reduced the number of root hairs and suppress the root growth in comparison to plant grown in uncontaminated soils. The present study indicate that application of Pb up to 20 mg kg^{-1}did not affect the root and shoot growth of plant but increases the concentration of Pb up to 40 mg kg^{-1} or more without addition of EDTA and Vermicompost reduced the root and shoot growth. It may be due to increases in antioxidant activities of plants at lower level of lead (17, 14). The present study indicates that the ameliorative effect of EDTA and Vermicompost was observed higher in moderate to higher Pb contaminated pots.

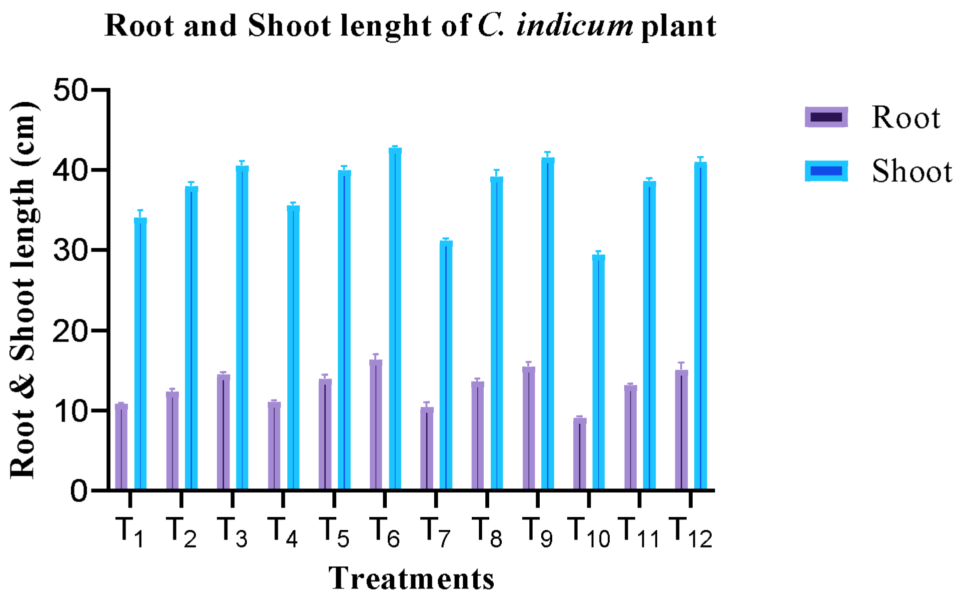

Fig.o1: Effect of Pb, EDTA and Vermicompost on growth of *Chrysanthemum indicum* plant

Effect of Lead, EDTA and Vermicompost on root and shoot biomass

The result present in Fig.02 indicated that the combined application of 10 mg kg^{-1} Pb with EDTA (5 mmol kg^{-1}) and Vermicompost (10 g kg^{-1}) in (T_6) produced maximum root and shoot dry biomass up to extent of 1.28, 4.27 g pot^{-1} respectively. Application of EDTA and Vermicompost at different level of Pb 10, 20, 40 mg kg^{-1} treated pots produced root and shoot dry mass of 1.28, 1.20, 0.97 and 4.27, 4.20, 3.80 in comparison to non amended treatments (T_4, T_7, T_{10}) 0.74, 0.71, 0.70 and 2.30, 2.25, 2.23 g pot^{-1} respectively. The increases the concentration of Pb in soil above the (20 mg kg^{-1}) decreased the root and shoot biomass of plant. The present study clearly indicated ameliorative effect of vermicompost on biomass production (5, 25). The application of only Pb at 40 mg kg^{-1} in (T_{10}) reported the minimum dry biomass of root and shoot up to 0.70 and 2.23 g pot^{-1} showing maximum reduction in root and shoot biomass in comparison to 10 mg kg^{-1} Pb in (T_4) and 20 mg kg^{-1} Pb in (T_7) treatments 0.74, 0.71 and 2.30, 2.25 g pot^{-1} respectively because the concentration Pb in root environment reach the toxic level.

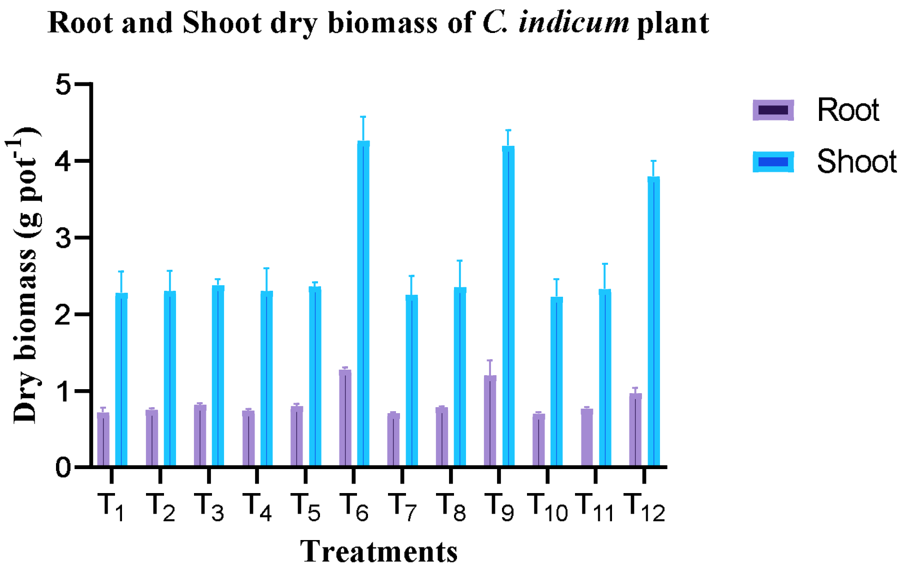

Fig.02: Effect of Pb, EDTA and Vermicompost on dry biomass of *Chrysanthemum indicum* plant

Effect of EDTA and Vermicompost on accumulation of Pb by *Chrysanthemum indicum* plant

The data present in Fig. 03 indicated that addition of lead up to 0-40 mg kg^{-1} significantly improve the accumulation of Pb in root and shoot of *C. indicum* L. plant which were observed 0.68-32.46 and 0.33-17.87 mg kg^{-1} respectively. Roots were observed high accumulation of Pb as compared to shoots. The concentration of Pb in plant is proportionate to the concentration of Pb in soil. The plant roots accumulate higher amount of metals and less amount transport to the shoot. Similar pattern of metal accumulation has been reported to accumulate rapidly in the roots and small amounts translocated to the other parts of plant [4]. The combined application of 40 mg kg^{-1} Pb along with EDTA (5 mmol kg^{-1}) and Vermicompost (10 g kg^{-1}) in (T_{12}) observed maximum concentration of Pb 42.21 and 20.67 mg kg^{-1} in root and shoot respectively. [13] has previously show the lead phytoextration can be enhanced with addition of chelating agents to the soil and that of several chelating agents tested, EDTA was shown to be most effective Pb^{+2} chelator. [33] also determine the EDTA was superior to other chelating agents for increasing the mobility of lead in highly weathered soils. Higher accumulation of Pb in which treatments the combined application of Pb, EDTA and Vermicompost might to be ascribed due to lowering of soil pH as a result of production of various organic acids. Lowering of soil pH promoted the mobility of Pb in the investigated soil [1].Application of vermocompost in soil to mobilize the Pb in soil by producing various organic acids having lower molecular weight, responsible for formation of organic complexes. Vermicompost enhanced phytoextraction of Pb as a high source of organic matter through production of phytochelatin. The application of vermicompost in soil enhanced the mobility of lead through the formation of soluble metal organic complexes [37]

Chelating of heavy metal ions has been identified as an important factor in metal accumulation by plants. Results from this study show that EDTA can promote Pb accumulation in *C. indicum* plant. Most of the increased lead accumulation after the chelate treatments could be explained as an effect of enhancing Pb solubility [19].The application of EDTA chelating agent mobilized large quantities of lead in soil which then diffuses down its concentration gradient into plant root and can be taken up by mass flow mechanism. Recent studies have shown that lead accumulation in plant parts is correlated with formation of Pb-EDTA complex and this complex is major form of lead absorbed and translocated by plant [10, 3]. The present study indicated enhanced Pb accumulation in root and shoot of plants when lead was applied in combination with EDTA and Vermicompost. Thus, phytoaccumulation potential of *C. indicum* L.was proved and its potential was improved by the application EDTA and Vermicompost.

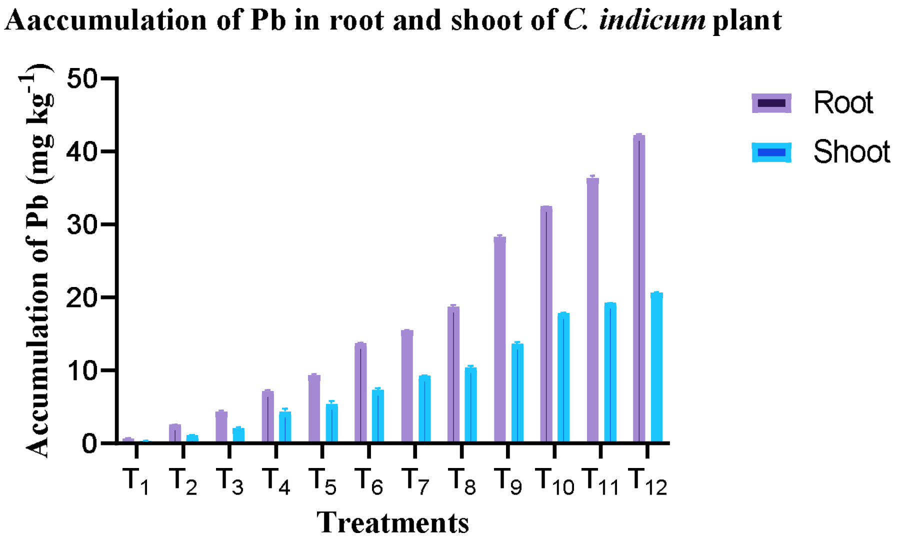

Fig.03: Effect of EDTA and Vermicompost on accumulation of Pb in root and shoot of *Chrysanthemum indicum* plant

Translocation Factor (TF) and Bioaccumulation Factor (BAF)

The translocation and bioaccumulation factors has been graphically present in the Fig.04 indicate that higher TF has been found in T_3 (0.63) followed by T_4 (0.61), T_7 (0.59) and T_5 (0.58) treatments and minimum TF has been found in T_2 (0.42) followed by T_1, T_9, and T_{12} (0.48) treatments. The maximum BAF has been found in T_9 (0.46) followed by T_{12} (0.41), T_{11} (0.38) and T_6 (0.37) treatments and minimum BAF was found in T_1 (0.03) followed by T_2 (0.11), T_3 (0.21) and T_4 (0.22) treatments. The EDTA (5 mmol kg^{-1}) and Vermicompost (10 g kg^{-1}) applied in soil enhance the solubility of metals resulting uptake of metals in plant increases so increase the bioaccumulation factors. The translocation of heavy metals in plants from root to shoot is enhanced by EDTA and Vermicompost. Bio-concentration factors (BCF) have been widely used to estimate the potential of plants for phytoremediation purpose [35, 38]. Lead translocation predominantly into the root apoplast and thereby in a radial manner across the cortex and accumulates near the endodermis. The endodermis acts as a partial barrier to the movement of lead from root to shoot. This may be reported that the higher lead accumulation in roots as compared to shoots [34]. It has been suggested that metal chelate complex may enter the root at breaks in the root endodermis and casparian strip and be rapidly transported to shoots [27].

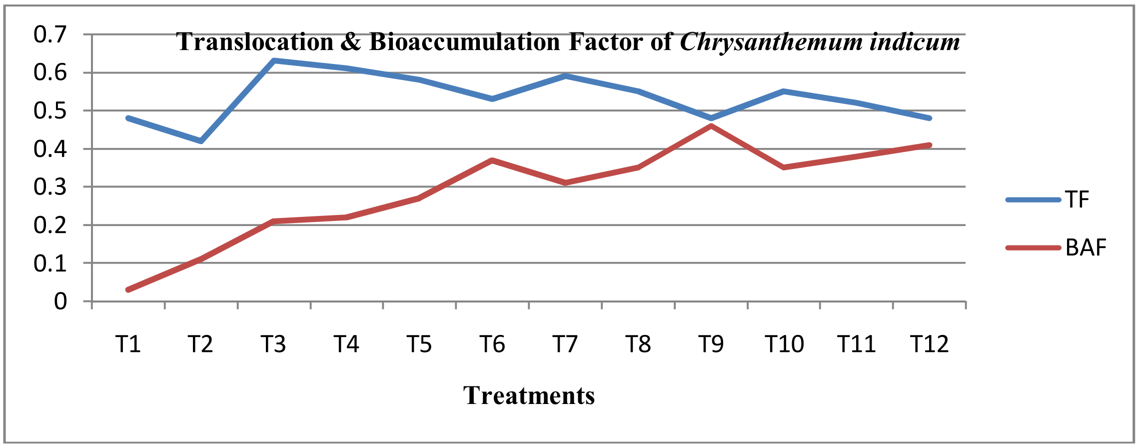

Fig.04: Translocation and Bioaccumulation factor of *Chrysanthemum indicum*

CONCLUSION

Concentration of Pb less than 20 mg kg^{-1} promotes the growth of *C. indicum* L. but the concentration of Pb above the 20 mg kg^{-1} retarded the plant growth. The concentration of Pb in root was found higher than shoot. The application of EDTA and Vermicompost enhanced the remediation efficiency in Pb contaminated soils. Phytoremediation of heavy metals contaminated soils through ornamental plant

(*Chrysanthemun indicum*) reduce the heavy metals toxicity in soil and risk to the human and animal health because ornamental plant do not consume by human and animal. *Chrysanthemum indicum* also has good potential for the accumulation of heavy metals in its roots and shoots; particularly it is a hyper accumulator of Pb. Therefore, it can be used for phytoremediation of heavy metals from contaminated soils. Increases the concentration of Pb in soil also increases the accumulation of Pb but does not increase the proportion in which the concentration of Pb in soils increased. Using EDTA and Vermicompost together enhance the phytoremediation potential and also increase the bioaccumulation and translocation factor. The results clearly indicate that the *C. indicum* is a suitable ornamental plant for phytoremediation of Pb contaminated soil. The biggest advantage of phytoremediation is its low cost. A better understanding of the biochemical process involve in plant heavy metal uptake, transport, accumulation and resistance will help in systematic improvements in phytoremediation using molecular genetics approaches. Another approach for improving the high potential of phytoremediation in to introduce genes responsible for accumulation and resistance from wild show growing plants to fast high growing high biomass plant species.

CONFLICT OF INTEREST

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

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