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Induced accumulation of lead and cadmium in sunflower (*Helianthus annus.L)* under heavy metals contaminated coastal saline soil

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ABSTRACT

To evaluate the ability of sunflower (Helianthus annus.L) for accumulating lead (Pb) and cadmium (Cd) from an industrially polluted coastal saline sandy loam soil using various organic and inorganic soil amendments along with recommended dose of NPK fertilizers an experiment was conducted at pot culture yard, Department of Soil Science and Agricultural Chemistry,Faculty of Agriculture, Annamalai University. From the experimental results, it was observed that sunflower exhibited significant differences with respect to accumulation of Pb and Cd against amendments tested. Higher shoot concentration of Pb and Cd (0.72, 0.57; 0.94, 0.97 mg kg^{-1}) and the root concentration (1.78, 2.32; 0.35, 0.32 mg kg^{-1}) were recorded in the treatment RDF + EDTA at 45 DAS and at harvest stages, respectively. Remediation efficiency of sunflower increased by application of RDF + EDTA through enhanced solubility of Pb and Cd in soil (2.97, 2.86 and 0.97,1.05 mg kg^{-1}) and thus increased Pb and Cd accumulation in root and shoot of sunflower both at 45 DAS and at harvest. Whereas, the application of RDF+ FYM or press mud reduced the extractability of Pb and Cd in soil and thus restricted the accumulation of Pb and Cd in sunflower. The results indicate that RDF + EDTA enhanced the accumulation of Pb and Cd by sunflower.

Key words*:* Cadmium, EDTA, FYM, lead, Phytoaccumulation, remediation efficiency

INTRODUCTION

Contamination of soil and water by toxic heavy metals (HMs) have become a serious environmental problem leading to a reduction in plant growth and development and risk to human health via the food chain (Rizwan *et al.*,2016; Shaheen and Rinklebe, 2015). It has been widely reported that HMs are toxic to plants and reduce plant growth, biomass, grain yield, and crop quality. Excessive heavy metal concentrations in plants decrease seed germination, photosynthesis and mineral nutrient uptake. Heavy metals cause oxidative stress in plants via the production of reactive oxygen species (ROS) (Jabeen *et al.*2016) Phytomanagement is the use of vegetation possibly in combination with other technologies such as phytoremediation, phytomining and biofortification for mitigating any environmental risk associated with contaminated soils in a cost-effective way. Phytomanagement of heavy metal-contaminated soils is an environmentally friendly and cost-effective technique. Among the current developed methods for phytoremediation, phytoextraction is a technique in which different plants (Hyper accumulators) remove toxic metals from soil, which subsequently accumulate in different parts of the plant. The success of phytoextration depends upon the presence of plant species that can tolerate the accumulation of HMs without significantly affecting plant growth and productivity. Polluting heavy metals are emitted from various sources as solid, liquid and as fine particles directly into atmosphere and ultimately deposited on the surface of land and water bodies. The dangers associated with heavy metals and toxic ions present in untreated effluents, sewage sludge and sewer water, causing phytotoxicity to crops and food chain contamination have become a matter of great social and scientific concern.. Thus, the issues of contamination of agricultural soils and chemical residues in agricultural produce have become increasingly important in recent years due to increased public awareness and concern for food and land quality.

Here cadmium (Cd) and lead (Pb) gained more attention because of their wide spread and high toxicity to plant functions and lead inhibits metabolic process such as nitrogen assimilation, photosynthesis, respiration, water uptake and transcription, respectively. Cadmium (Cd), recognized as one of the most hazardous elements, is not essential for plant growth. The main source of Cd contamination is industries involved in protective plating on steel and

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production of various alloys, pigments, stabilizers for plastic, Ni-Cd dry batteries and other miscellaneous items including photovoltaic cells and control rods for nuclear reactors. The other sources of Cd contamination come from mining, ore dressing and smelting of cadmium sulphide ore. Like Cd, excessive build up of Pb due to mining, smelting, manure, sewage sludge and vehicle exhausts. During the past decades, the annual widespread release of heavy metals reached 22,000 t (metric ton) for Cd and 7, 38,000 t for Pb (Singh and Tewari, 2003).

Sunflower (Helianthus annuus L.) has been considered a suitable candidate for bioaccumulation and phytoremediation of HMs. In addition, the sunflower has a greater potential for HMs uptake and tolerance than other crops such as maize, ricinus, alfalfa, and mustard have (Niu,2007).Roots are the main pathway through which HMs gain access to epical parts. HMs uptake by sunflowers varies with soil properties and its cultivars. HMs concentration in soil solution is the most important factor responsible for HM uptake by plants (Kolbas *et al.*, 2014). A number of studies have shown that metal uptake and translocation increase in sunflowers with increasing metal concentration in the growth medium (Chaves *et al.*, 2011). After uptake by plants, HMs reach the xylem and then translocate to the shoots. Cadmium (Cd) uptake and rate of Cd loading in the xylem sap increase with increasing Cd^{2+} concentration in the soil solution. The translocation factor, the ratio of the metal concentration in the shoots to that in the roots, which indicates the phytoextraction potential of these sunflower species (Adesodun *et al.*,2010) and sunflower is a promising phytoaccumulator to remove heavy metals from contaminated soils. In this study we aimed at studying the role of sunflower plants in phytoremediating soils contaminated with the most common and toxic heavy metals *viz.*, lead (Pb) and cadmium (Cd).

MATERIALS AND METHODS

The pot culture experiment was carried out using sunflower as a test crop in sandy loam soil at pot culture yard in the Department of Soil Science and Agricultural Chemistry, ***t***Annamalai University, Annamalainagar during July – October, 2017.Contaminated *Typic udipsamments* containing 9.60 mg kg^{-1} and 76.10 mg kg^{-1} of total cadmium and lead concentration and DTPA extractable concentration of cadmium was 0.75 mg kg^{-1} and the lead was 1.56 mg kg^{-1} presented respectively. The experimental soil was sandy loam nature with the pH, EC,CEC and organic carbon (7.81, 0.72 d Sm^{-1}, 13.52 c mol (P^{+}) kg $^{-1}$ and 0.41 g kg^{-1}) respectively. The available NPK status of soil were low, medium and low (178.2 kg ha^{-1}, 11.3kg ha^{-1} and 146.8 kg ha^{-1}) respectively. The experiment was laid out in completely randomised block design, which comprised of eight soil amendments as treatments with recommended dose of N, P, K (60: 90: 60 kg ha^{-1}) with three replications. The experimental soil was filled with cement pot of 30 cm height according to the experimental design. The contaminated soil was amended with (**T_2** - RDF + Farm yard manure, **T_3** - RDF + Pressmud, **T_4 -** RDF **+**EDTA, **T_5 -** RDF + Lime, **T_6 -** RDF + gypsum, **T_7 -** RDF + Potassium Humate, **T_8 -** RDF + Zeolite, **T_1 -** Control (100% RDF)) different amendments (organic and inorganic) selected for the study were applied as per the treatment schedule. Sunflower seeds (Sunbred hybrid) were sown and one healthy seedling was maintained up to 95 days. Experimental pots were maintaining at 60 % of water holding capacity of the soil. The soil and plant samples at 45^{th} day and at harvest were taken and processed for analysis. Soil pH was measured conductivity was measured in the same suspension using a conductivity meter and cation exchange capacity by neutral normal ammonium acetate method (Jackson,1973). Organic carbon content was determined by modified Chromic acid wet digestion titration method. The available nitrogen was determined by alkaline permanganate method Available phosphorus (using 0.5 M $NaHCO_3$ of pH 8.5) was quantified by the spectrophotometer method (Olsen *et al.*,1954). Available potassium (using neutral normal ammonium acetate extract) was determined by Flame photometer.

Analysis of heavy metals in soil and plant: Soil and plant samples (1 g) were digested after adding tri-acid mixture (HNO_3, H_2 SO_4, and $HClO_4$ in 5:1:1 ratio). Concentrations of heavy metals in the filtrate of digested soil and plant samples were estimated by using Inductively Coupled Plasma optical Emission Spectrometer (Model-ICP-OES-5110, Agilent).

To evaluate the ability of metal phytoextraction / phytoaccumulation by sunflower, the following parameters were considered: a) plant biomass (dry weight of roots and shoots), b) metal concentration in plant tissues (roots and shoots), c) translocation factors (TFs) calculated as the metal in shoots to the metal in roots ratio suggested by Zhixin *et al.*(2009) and d) bioconcentration factors (BCFs and ER) of shoots and roots calculated as the ratio between metal concentration in plant tissues (shoots or roots, respectively) and total metal initial soil concentration suggested by Elouear *et al.*(2016). The data of heavy metal concentrations and soil properties of different amendments were subjected to one way analysis of variance (ANOVA). All the statistical tests were performed using SPSS software.

RESULTS AND DISCUSSION

The possibility of using crops as phytoremediants depends on the accumulation and distribution of metals among their morphological organs. Plants characterized by high biomass production and intensive heavy metals accumulation in shoots and roots can be used as phytoremediants. The availability of heavy metals in the soil depends on chemical speciation of the metal, soil pH and other soil chemical and physical properties (Smith, 2009).

Physico-chemical properties of the soil

Phsico-chemical properties of metal alleviated soil are presented in Table 1.The higher pH, EC and CEC, SOC values were observed in RDF + lime (T_5) and RDF+FYM (T_2) amended pots, respectively. This might be attributed to the effect of lime on heavy metal contaminated soils duly increased soil pH which depends on pH of liming material and Ca^+ concentration in soil solution (Rehman *et al.*,2015) and also addition of lime have positive momentum of increasing soil pH and EC. Whereas the higher values of CEC and SOC were attributed duly by the addition of FYM which increased adsorption sites by increasing negative charges on the soil surface and reducing competing metal cations (Alamgir *et al.*, 2011). Further, addition of soil organic carbon might be due to accumulation plant residual lignin in FYM directly flows to the structural and metabolic soil carbon pool and increased biologically active soil organic carbon such as microbial biomass carbon and mineralizable carbon (Chang *et al.*,2007)). Lower values of pH, EC were observed in RDF + EDTA (T_4) amended pots at both stages. This could be ascribed due to the EDTA's chelating effect (induces variable charge) on growth medium and thus reduces both pH and EC. While, the lower values of CEC and SOC were recorded by RDF +lime amended pots. The reduction in CEC and SOC content in lime applied treatments might be caused by lime effect on reducing pH, EC and increasing soluble salts contents. Ever since, both EDTA and lime application did not influence this pool. Similar results were reported that manure was the most effective in increasing POM-C when compared with urea or without urea or other soil amendments (Mando *et al.*, 2005). The N fertilization through organic manures stimulates microbial activity and enhances C turnover, which explains the negative values of C stabilization in the fertilizer N applied plots which was hinder by the RDF+ lime applied treatment.

Table 1: Effect of organic and inorganic amendments on pH, EC (dS m^{-1}), CEC (cmol (p^+) kg^{-1}) and SOC in heavy metal alleviated soil

Treatments /Parameters	45 DAS				Harvest			
	pH	EC	CEC	SOC	pH	EC	CEC	SOC
T_1- Control (100% RDF)	7.79	0.64	13.93	0.48	7.70	0.60	14.46	0.57
T_2- RDF + Farm yard manure	7.60	0.58	15.53	0.65	7.53	0.51	16.13	0.73
T_3- RDF + Pressmud	7.80	0.72	15.15	0.57	7.78	0.65	15.78	0.68
T_4- RDF +EDTA	7.25	0.35	13.37	0.40	7.13	0.27	14.02	0.49
T_5- RDF + Lime	8.37	0.95	8.24	0.08	8.35	0.87	7.75	0.08
T_6- RDF + Gypsum	7.61	0.50	12.66	0.24	7.51	0.41	13.32	0.37
T_7- RDF + Potassium humate	7.75	0.80	11.41	0.29	7.71	0.76	11.98	0.29
T_8- RDF + Zeolite	8.33	0.85	9.40	0.10	8.30	0.80	8.25	0.10
SEd±	0.29	0.02	0.38	0.01	0.32	0.03	0.38	0.01
CD (P=0.05)	0.62	0.06	0.80	0.03	0.68	0.05	0.81	0.03

Heavy metal concentrations in the plants

Lead and cadmium concentrations in sunflower plant in metal alleviated soil are presented in Table 2. Among the various organic and inorganic soil amendments tried, the maximum lead (Pb) and cadmium (Cd) concentration in shoot and root at 45 DAS and at harvest were recorded in RDF+ EDTA (T_4) amended pots of 0.72, 1.78; 0.81, 2.32 mg kg^{-1} and 0.94, 0.35; 0.97, 0.32 mg kg^{-1}). This was significantly followed in following order of RDF +K-humate > RDF + zeolite > RDF +lime > RDF + gypsum > RDF alone (control) > RDF + press mud = RDF +FYM amended treatments. The maximum values Pb and Cd in shoot and root of sunflower accumulated by EDTA amended one was due to that factors which causing accumulation of lead and cadmium were not only related with soil (PH, EC, CEC, SOC and soil type) but also plants (rhizospheric processes, plant variety or cultivar/hybrid and its uptake mechanisms) and amendments effect on metal adsorption/desorption, precipitation and or solidification processes (Shittu *et al.*, 2015). Where in this treatment, the chief mechanism of accumulating Pb and Cd were identified as chelating effect of EDTA by forming of stable complexes. Further, increased absorption of Pb and Cd from soil into the roots of sunflower and then to shoots attributed by chelating effects of EDTA along the chelating agents produced by the sunflower plant roots. Results showed that this treatment had relatively low effect on Pb concentration in shoot organs of sunflower. It may be concluded that EDTA can solubilise soil Pb and Pb-EDTA can be easily absorbed by roots of plant. Suthar *et al.* (2013) reported that addition of chelating agent and the consequent formation of element–chelate complexs prevent precipitation and sorption of the elements in the soil. Whereas the lower values of Pb and Cd (0.09, 0.11; 0.03, 0.6 mg kg^{-1} and 0.19, 0.08; 0.15, 0.05 mg kg^{-1}) were observed in the treatment received RDF + FYM (T_4). This may be attributed due to increased sorption sites by FYM through addition of organic carbon, enhancing the CEC and humic substances in the soil (Singh and Agrawal, 2013) thus HMs may get adsorbed, precipitated or more options of forming complex with Pb and Cd which results in higher immobilization of lead and cadmium.

Table 2: Effect of organic and inorganic amendments on phytoaccumulation of lead (Pb) and cadmium (Cd) (mg kg^{-1}) by sunflower

Treatments /Parameters	45 DAS				Harvest			
	Shoot		Root		Shoot		Root	
	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd
T_1-Control (100% RDF)	0.25	0.39	0.73	0.13	0.27	0.42	0.76	0.10
T_2-RDF + Farm yard manure	0.09	0.19	0.11	0.08	0.03	0.15	0.06	0.05
T_3- RDF + Pressmud	0.13	0.25	0.25	0.09	0.07	0.21	0.10	0.06
T_4- RDF +EDTA	0.72	0.94	1.78	0.35	0.81	0.97	2.32	0.32
T_5-RDF + Lime	0.51	0.65	1.21	0.21	0.57	0.69	1.63	0.19
T_6-RDF + Gypsum	0.37	0.52	1.01	0.17	0.48	0.56	1.28	0.14
T_7-RDF + Potassium humate	0.63	0.80	1.52	0.31	0.70	0.84	2.01	0.27
T_8-RDF + Zeolite	0.55	0.67	1.32	0.27	0.61	0.71	1.72	0.23
SEd±	0.03	0.05	0.09	0.01	0.04	0.04	0.11	0.01
CD (P=0.05)	0.07	0.10	0.19	0.04	0.09	0.10	0.24	0.03

Soil DTPA extractable heavy metal concentration (Pb and Cd)

Among the treatments tried, higher values of DTPA extractable Pb and Cd (3.98 and 1.19; 3.85 and 1.31 mg kg^{-1}) were observed in RDF +EDTA (T_4) amended pots. This might be attributed due to enhanced Pb-EDTA and Cd-EDTA complex formation that brought the metals from sorbed sites to the soil solution and increases the bioavailability of Pb , Cd and other heavy metals by forming water –soluble- chelate complexes and addition of chelating agent and consequent formation of element–chelate complexs prevent precipitation and sorption of the elements in the soil (Suthar *et al.*,2013). Whilst, the lower values of DTPA extractable Pb and Cd (0.75 and 0.57; 0.62 and 0.51 mg kg^{-1}) were observed in the treatment amended with RDF+FYM (T_2) at 45 DAS and at harvest, even though it registered low pH values compared to all other treatments except RDF+EDTA applied one (Table 3).This might be attributed due to increased sorption sites by FYM through addition of organic carbon, enhancing the CEC and humic substances in the soil. By which HMs may get adsorbed, precipitated or more options of forming complex with Pb and Cd in sandy loam soil texture having pH < 8.5.The low CEC of the soil attests to its low metal immobilization which may result in increased elemental concentration in soil solution (Lucchini *et al.*, 2014).Higher values of CEC and SOC were observed in FYM amended treatment throughout the crop growth, which results in lower DTPA extractable lead (Pb) and cadmium (Cd).

Table 3: Element concentrations of lead (Pb) and cadmium (Cd) (mg kg^{-1}) in heavy metal alleviated soil

Treatments /Parameters	45 DAS		Harvest	
	DTPA		DTPA	
	Pb	Cd	Pb	Cd
T_1-Control (100% RDF)	1.47	0.80	1.35	0.77
T_2-RDF + Farm yard manure	0.75	0.57	0.74	0.57
T_3- RDF + Pressmud	0.87	0.61	0.62	0.51
T_4- RDF +EDTA	3.98	1.19	3.85	1.31
T_5-RDF + Lime	2.97	0.97	2.86	1.05
T_6-RDF + Gypsum	2.29	0.91	2.14	0.99
T_7-RDF + Potassium humate	3.57	1.12	3.45	1.23
T_8-RDF + Zeolite	3.12	1.04	3.01	1.15
SEd±	0.08	0.02	0.07	0.03
CD (P=0.05)	0.17	0.06	0.16	0.06

Remediation efficiency of sunflower

The rate of metal uptake is greatly influenced by plant species, the transfer factor of the metals by each plant species are desirable for classification of the plants phytoaccumulation, phytostabilization and phytoextraction potentials (Ayari *et al.*, 2010). In the present study, phytoaccumulation efficiency of sunflower was investigated using organic and inorganic soil amendments along with NPK fertilizers and found bioconcentration factor (BCF),transfer factor(TF) and remediation efficiency (ER) from the soil and plant heavy metal analytical values (Table 4). BCF values with respect to Pb and Cd are < 1 indicating that the concentration of the elements does not exceed its content in the soil at both stages. The BCF values of Cd were higher than that of Pb in the trial at both stages of crop. This might be due to plants do not take up large quantities of soil lead (Pb) (Raymond *et al.*,2011) and its low mobility compared to cadmium which is highly mobile in both soil and plant (Radulescu *et al.*,2013). Further, considerable differences between the BCF values obtained for the organic and inorganic amendments treated in the contaminated soil, which elucidated that compared to organic amendments in accumulating heavy metals by sunflower, the inorganic amendments found to be superior. According to the BCF values obtained, the experimental materials used could be arranged in the following order for Pb and Cd are $T_4 > T_7 > T_8 > T_5 > T_6 > T_1 > T_3 > T_2$ both at 45 DAS and at harvest, respectively. Based on the BCF values, application of EDTA could be a better option for enhancing the bioconcentration of Pb and Cd compared to other amendments tried with sunflower. The values of TF showed the amount of movements of metals absorbed from root system to above ground part of the plant it involves photochemistry in metal transport and storage. TF values vary from one plant species to another and from one heavy metal to another (Obasi *et al.*, 2013).

In general TF, does not exhibit the same pattern of BCF. Metal translocation indicates the ability of amendments to affect metal transfer from roots to shoots. If the TF values are more than 1 suggesting that heavy metals are readily transported from the root to shoot (Elouear *et al.*, 2016). The results of present study showed that application of RDF +FYM and or RDF + press mud led to an increased transfer factor for Pb, however their TF values were less than 1, which indicates an enhanced ability of plants to accumulate heavy metals in the roots and not to the aerials of sunflower compared to other amendments.

Table 4: Effect of organic and inorganic amendments on Bio Concentration Factor (BCF), Transfer Factor (TF) and Remediation Efficiency (ER) of sunflower

Treatments /Parameters	45 DAS		Harvest		45 DAS		Harvest		45 DAS		Harvest	
	BCF				TF				ER			
	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd
T_1-Control (100% RDF)	0.014	0.394	0.015	0.394	0.34	3.00	0.36	4.20	0.11	1.62	0.39	5.67
T_2-RDF + Farm yard manure	0.003	0.205	0.001	0.152	0.82	2.38	0.50	3.00	0.06	0.66	0.05	1.45
T_3- RDF + Pressmud	0.006	0.258	0.002	0.205	0.52	2.78	0.70	3.50	0.08	0.80	0.12	1.93
T_4- RDF +EDTA	0.036	0.977	0.045	0.977	0.40	2.69	0.35	3.03	0.68	11.06	2.48	34.74
T_5-RDF + Lime	0.025	0.652	0.032	0.667	0.42	3.10	0.35	3.63	0.11	1.41	0.67	7.80
T_6-RDF + Gypsum	0.020	0.523	0.026	0.530	0.37	3.06	0.38	4.00	0.22	3.35	0.96	11.03
T_7-RDF + Potassium humate	0.031	0.841	0.039	0.841	0.41	2.58	0.35	3.11	0.27	3.96	1.27	16.46
T_8-RDF + Zeolite	0.027	0.712	0.034	0.712	0.42	2.48	0.35	3.09	0.31	4.11	1.14	14.0

Whilst, the TF values for Cd showed a different trend such as the application RDF+ lime, RDF+ gypsum and RDF alone applied treatments showed higher TF values for Cd. TF values of Cd are three folds of Pb. This might be due to higher mobility of Cd in plants (roots to shoots) than Pb, which mostly retained in roots itself influenced by these amendments. These results are in conformity with those Smical *et al.*, 2008 who reported that metals distribution in plant seems to be controlled by some mechanism and this suggests the existence of some boundaries and/or change in the metal chemical state.

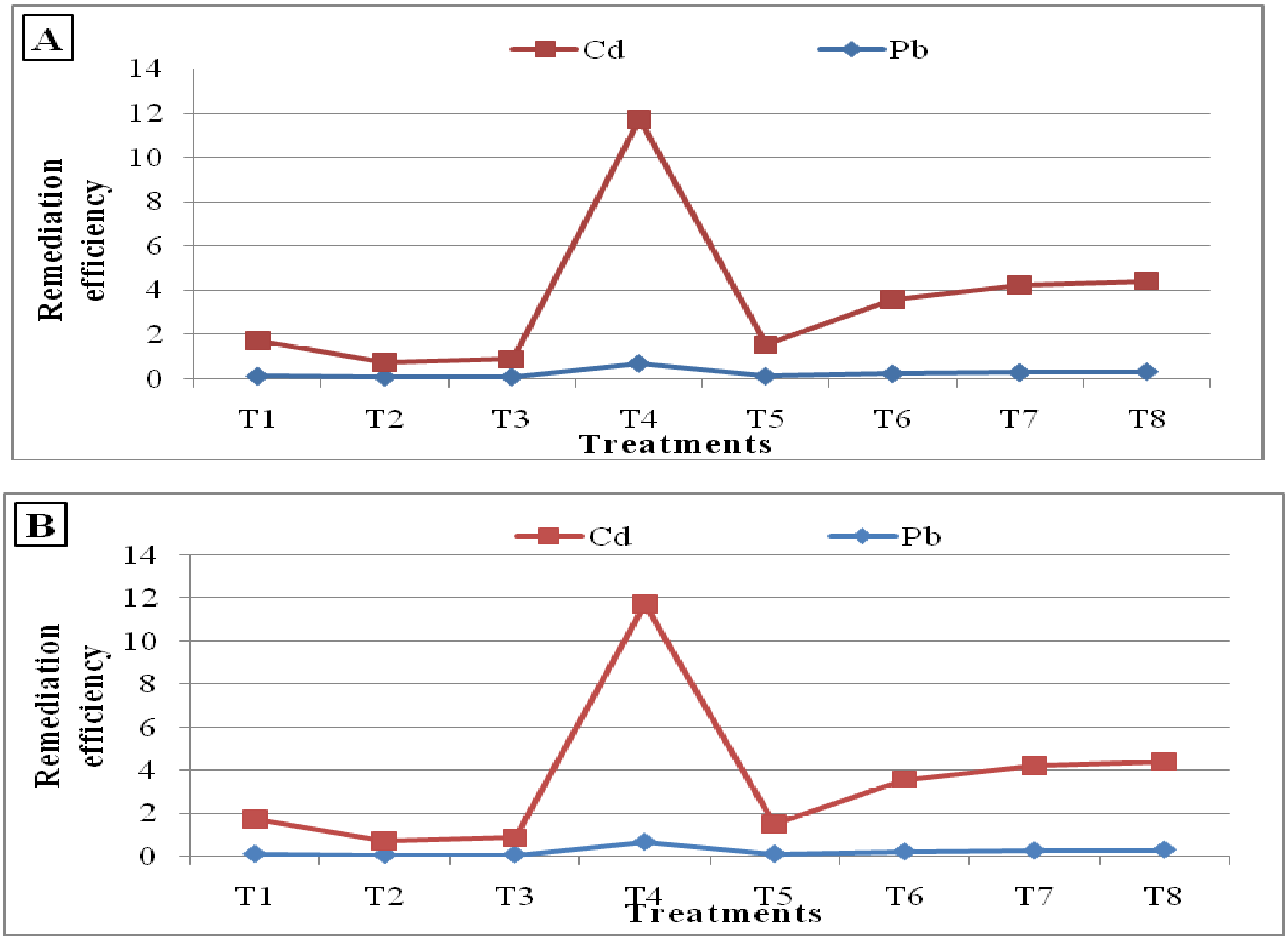

Fig.1. Remediation efficiency sunflower for Pb and Cd by (A) 45 DAS (B) Harvest

Remediation efficiency (ER) can be describe amount of heavy metal uptake (Concentration in aerials multiplied with its weight) by plants from the soil (concentration in soil multiplied with soil weight).From the ER values it can be decide that the accumulating factor or efficiency of plant species. Higher the ER values higher the remediation efficiency (Elouear *et al.*, 2016). Present investigation results showed that application of RDF + EDTA (T_4) highest ER values of 0.68, 2.48 and 11.06, 34.74, whereas the lowest ER values (0.06, 0.05 and 0.66, 1.45) were observed in RDF+ FYM (T_2) amended treatments for Pb and Cd at 45 DAS and at harvest, respectively (Fig.1).

Results from this study proved that the addition of soil organic amendments, such as FYM, press mud reduced the phytoaccumulation of metals, inorganic amendments addition such as lime, gypsum, zeolite optimally increased the phytoaccumulation and application of chelates EDTA and K-humate along with recommended dose of NPK fertilizers are drastically increased the phytoaccumulation of metal contaminated soil. It can be concluded that the use of amendments and chelates enhanced accumulation of heavy metals in contaminated soils by the increase of pH and organic matter content and enhanced biochemical properties.

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