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Effect of EDTA on phytoextraction of Pb by sunflower (*Helianthus Annuus* L.)

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Abstract
The study entitled, "Effect of EDTA on phyto extraction of Pb by sunflower (*Helianthus annuus* L.)" was undertaken by conducting a micro-plot experiment in the net house of Micronutrient Research Project (ICAR), Anand Agricultural University, Anand (Gujarat) during *kharif* season of the year 2015. The soil used for the experiment was alkaline in reaction with low in available P_20_5 and medium in K_20. The Pb status was 0.00025 mg kg^{-1} soil, which was spiked with 300 mg Pb kg^{-1} soil before 30 days of sowing for the purpose of artificial contamination for the study. Three levels of EDTA (3, 6 and 9 mmol kg^{-1} soil) and three periods of EDTA application (P_1: 6th, 7th and 8th weeks after sowing, P_2: 9th, 10th and 11th weeks after sowing and P_3: 12th, 13th and 14th weeks after sowing) and one absolute control were kept for the study. The lead content of seed, shoot and root was significantly more under E_9P_1, combination than the rest of the combination except E_6P_2 for seed and shoot and E_9P_2 for root. The uptake of Pb by shoot and total uptake were significantly more under E_9P_1 as compared to the rest except E_6P_2, while uptake by seed was maximum under E_6P_2. Remediation of Pb polluted soil is possible by using EDTA.

Keywords: lead, sunflower, root, shoot

Introduction
Soil contamination by heavy metals is a global environmental issue due to the rapid development of intensive agriculture and industry in many parts of the world. Elevated concentrations of heavy metal not only lead to reductions in the microbial activity and fertility of the soil, and in crop production (McGrath *et al*., 1995) [18], but also threaten human health through the food chain. The remediation of soil and water contaminated with heavy metals has become a challenging task facing regulators and scientific communities. Recently, phytoextraction, the use of plants to extract heavy metals from contaminated soils, has been receiving an increasing amount of attention. Lead can be accumulated in plant organs and agricultural products (Mahmoud and El-Beltagy, 1998) [17] and human body consequently enter human food chain (Wagner, 1993) [27]. As a result of consumption of food, lead accumulates in human body and it may cause renal failure, brain and liver damage and it can attack the nervous system and cause failing of sickness (Lucky and Kenugopal, 1997) [15]. Lead is one of the most difficult pollutants to control (Salt *et al*., 1998) [23]. Phytoextraction is a soil cleanup technology that uses the ability of metal accumulator plant to extract metals from contaminated soil with their roots and to concentrate these metals in above ground plant parts (DE Salt *et al.*, 1995; Chaney *et al.*, 1997; Lai *et. al.*, 2007 and Lai *et. al.*, 2008) [3, 12, 13, 22] and the metal-accumulating plant material can be safely harvested and removed from the site. Lead (Pb) is potential pollutant that readily accumulated in soil and sediments. Apart from natural weathering processes, lead contamination of the environment has resulted from industrial activities *viz.* mining and smelting processes, agricultural activities such as application of insecticide and municipal sewage and urban activities *viz.* use of lead in gasoline, paints and other materials (Sharma *et al.*, 2005) [24]. The Pb-contaminated soils are difficult to remediate with natural phytoextraction that utilizes hyper accumulators. In order to enhanced the availability of Pb in soil solution and its trance location from root to shoot, application of some chelating agents such as ethylene diamine tetraacetic acid (EDTA), diethylene trinitrilo pentaacetic acid (DTPA), nitrilotriacetic acid (NTA), ethylenediamine disuccinate (EDDA) have been proposed by various workers (Huang *et al.*, 1997; Meers *et al.*, 2005; Kumar *et al.*, 2011) [9, 19, 11]. EDTA is probably the most efficient chelate to increasing concentration of various metals especially lead in above ground plant tissues.

(Huang *et al.*, 1997; Vassil *et al.*, 1998; Meers *et al.*, 2005; Kumar *et al.*, 2011; Pal *et al.*, 2012) [9, 19, 11, 21]. In phytoextraction research, two main strategies can be identified. The first is the use of metal hyperaccumulating species (Baker *et al.*, 1994; Brown *et al*., 1994; Kumar *et al*., 1995) [11, 1, 2]. A second phytoextraction approach involves the use of high biomass producing species, such as sunflower (*Helianthus annuus*), and chemically enhancing their shoot levels to increase the removal efficiency. A number of soil amendments have been reported in literature which could render soil trace metals more phytoavailable, among which ethylene diamine tetraacetate (EDTA) has taken a predominant place (Cooper *et al*., 1999; Epstein *et al*., 1999; Shen *et al*., 2002) [4, 6, 25]. The plant species used for the enhanced phytoextraction experiment in this study is *Helianthus annuus*. In order to generate location specific information on "Effect of EDTA and on phytoextraction of Pb by Sunflower (*Helianthus annuus* L.)" the present study will be conducted during *kharif*, 2015 at Pot house of Micronutrient Research Project (ICAR), AAU, Anand.

Materials and Method

Rapid industrialization and urbanization have created enormous problems of environmental pollution due to disposal of large quantity of effluents. Reports indicate that the untreated and contaminated industrial effluents pollute the soils with heavy metals which need due to attention for remediation. Therefore, to meet the objectives as mentioned earlier, the present study was undertaken to study the effect of chelateor on phytoextraction for heavy metals uptake. The present investigation on "Effect of EDTA on phytoextraction of Pb by Sunflower (*Helianthus annuus*)" was undertaken by conducting a micro-plot study in 2015. Three levels of EDTA (3, 6 and 9 mmol kg^{-1} soil) and three periods of EDTA application (P_1: 6^{th}, 7^{th} and 8^{th} weeks after sowing, P_2: 9^{th}, 10^{th} and 11^{th} weeks after sowing and P_3: 12^{th}, 13^{th} and 14^{th} weeks after sowing) and one absolute control were kept for the study. After harvest of the crop, soil samples were collected and air dried in laboratory. The air dried soil samples were prepared by using wooden mortar and pestle and passed through 2 mm sieve. The soil samples were preserved in polythene bags for their chemical analysis later. The samples were washed with 0.2% detergent, 0.03 N HCl, single and double deionized water in a sequence and air dried. Then samples were dried in paper bags at 70° C till constant weight in a hot air oven and preserved for further analysis. The dried plant samples were cut and ground in a stainless steel blade mixer and were preserved in polythene bags for further analysis. The concentration of micronutrient and heavy metal in plant were expressed in terms of mg kg^{-1}. Nutrient uptake was calculated by using yield and nutrient content data. The following formula was used to compute the nutrient uptake.

$$\text{Nutrient uptake for Micronutrient (g plot}^{-1}) = \frac{\text{Nutrient content (mg kg}^{-1}) \times \text{Yield (g plot}^{-1})}{1000}$$

Result and Discussion

Pb content in plant and its uptake

The perusal of data given in Table 4.3 reveal that the Pb content and in plant was significantly influenced by both levels of EDTA and period of its application, even comparison of the rest of the treatments with control showed significant variations in Pb content in different parts of the plant, which is graphically presented in Fig.4.3 and Fig.4.4. Among the different levels of EDTA, E_9 achieved significantly the highest Pb content in seed (150.85 mg kg^{-1}) and root (539.70 mg kg^{-1}), whereas in shoot Pb content was noted significantly higher under EDTA level E_6 (626.82 mg kg^{-1}) than E_3, but it was at par with E_9 (605.32 mg kg^{-1}).

The effect of different periods of application of EDTA was found significant for Pb content in seed, shoot and root. Among the different periods, P_2 recorded significantly the highest Pb content in seed (175.69 mg kg^{-1}) and root (479.36 mg kg^{-1}). The Pb content in shoot was found significantly higher under period P_1 (667.38 mg kg^{-1}) than P_3 (241.60 mg kg^{-1}), but it was on par with P_2 (646.26 mg kg^{-1}). Comparison of control with rest of the treatments also showed significant differences. The significantly higher Pb content was observed with the rest of the treatments than control.

The interaction effect of E×P was significant for Pb content in seed, shoot and root (Table 4.3a, 4.3b and 4.3c). The combination of E×P indicated that the combination of E_9P_1 recorded significantly higher values for Pb content in seed (205.26 mg kg^{-1}), shoot (964.53 mg kg^{-1}) and root (619.77 mg kg^{-1}), which was at par with Pb content in seed (192.52 mg kg^{-1}) and shoot (956.41 mg kg^{-1}) under combination of E_6P_2 and Pb content of root (598.87 mg kg^{-1}) under combination of E_9P_2.

Pb uptake

The perusal of data given in Table 4.3 reveal that the Pb uptake by seed, shoot, root and total uptake was significantly influenced by both levels of EDTA and periods of its application, even comparison of rest of the treatments with control showed significant variations in Pb uptake by various parts of the plant.

Among the different levels of the EDTA, E_6 noted significantly higher Pb uptake in seed (21.97 mg $plot^{-1}$) than E_3 (14.54 mg $plot^{-1}$), but it was at par with E_9 (21.74 mg $plot^{-1}$). The Pb uptake by shoot was significantly more under E_9 (298.20 mg $plot^{-1}$) than E_3 (148.56 mg $plot^{-1}$), but it was on par with E_6 (277.32 mg $plot^{-1}$). Among the different levels of the EDTA, E_9 achieved significantly the highest Pb uptake by root (16.15 mg $plot^{-1}$) and total uptake (336.09 mg $plot^{-1}$). The application periods of EDTA indicated that P_2 reported significantly the highest Pb uptake by seed (27.82 mg $plot^{-1}$), shoot (316.69 mg $plot^{-1}$), root (14.99 mg $plot^{-1}$) and total uptake (359.50 mg $plot^{-1}$).

The comparison of control with rest of the treatments showed higher Pb uptake by seed, shoot and root as well as total uptake as compared to control.

The interaction effect of E×P was significant for Pb uptake by seed, shoot and total uptake by sunflower plant (Table 4.3d, 4.3e and 4.f). The E_6P_2 combination noted significantly higher Pb uptake by seed (30.44 mg $plot^{-1}$) than the rest of the combinations, but it was on par with E_9P_2 (29.21 mg $plot^{-1}$) and E_9P_1 (27.24 mg $plot^{-1}$). The Pb uptake by shoot (455.91 mg $plot^{-1}$) was significantly more than rest of the treatment combinations except E_6P_2 (440.27 mg $plot^{-1}$) combination. The total uptake of Pb also followed the similar trend, wherein E_9P_1 (499.17 mg $plot^{-1}$) registered significantly higher Pb uptake than rest of the combinations barring E_6P_2 (440.27 mg $plot^{-1}$) combination.

The Pb content in different plant parts was increased with the increased levels of EDTA. The rise in Pb content in seed and root was 79.58 per cent and 118.91 per cent, respectively under E_9 over E_3. In case of shoot it was 94.00 per cent under E_6 over E_3. The Pb uptake was also increased as the levels of EDTA increased. The rise in the Pb content and uptake in

plant parts due to effect of EDTA on Pb, which increased the bioavailability of Pb in soil and thus lead accumulated in the plant. This results are supported by Omar *et al.* (2015) [20], Hovseyan and Greipsson (2007) and Liphadzi and Khirkham (2009) [14] in sunflower, Kumar *et al.* (2011) [10] and Pal *et al.* (2012) [21] in mustard, Hovsepyan and Greipsoon (2007) Ghasemi-Fasaei (2012) [7] and Ebrahimi (2013) [5] in maize.

The influence of periods of EDTA application was significant for Pb content and uptake by different plant parts of sunflower. The data indicated that maximum content and uptake of Pb by all the plant components were under P_2 (application of EDTA at 9, 10 and 11 weeks after sowing) period of application. The phytoextraction of lead was improved under mid period of application because application of EDTA in early growth period of plant reduced plant growth and dry weight of plant because of Pb toxicity. The results reveled that application of EDTA at P_2 (9^{th}, 10^{th} and 11^{th} week after sowing) is the right period for bioaccumulation of lead and its removal by plant. The similar finding was also noted by Sinegani and Khalilikhah (2008) in sunflower, who also reported that the application of EDTA before seeding was inferior to its application after sowing. Between the periods (10 and 30 days after sowing), application at 30^{th} day was better in extraction of Pb than 10 days after sowing.

The increase in Pb content and uptake in plant in rest of the treatments over control was due to the application of EDTA. The EDTA increased the Pb concentration in soil solution which was absorbed by the plant root and translocated in shoot. Similar results were also reported by Madrid and Khirkham (2002) [16].

Table 4.3: Effect of EDTA levels and its application periods on Pb content and uptake in plant

Treatments	Pb content (mg kg^{-1})			Pb uptake (mg plot^{-1})			
	Seed	Shoot	Root	Seed	Shoot	Root	Total
EDTA Levels (mmol kg^{-1} soil)							
E_3	84.00	323.10	246.61	14.54	148.56	9.25	172.35
E_6	138.58	626.82	414.46	21.97	277.32	13.12	312.41
E_9	150.85	605.32	539.70	21.74	298.20	16.15	336.09
S. Em. ±	2.88	11.10	11.86	0.79	7.76	0.65	7.38
C.D. at 5%	8.49	32.74	34.99	2.32	22.89	1.91	21.76
Application periods							
P_1	145.60	667.38	395.76	20.81	290.04	10.88	321.73
P_2	175.69	646.26	479.36	27.82	316.69	14.99	359.50
P_3	52.14	241.60	325.65	9.63	117.34	12.66	139.63
S. Em. ±	2.88	11.10	11.862	0.79	7.76	0.65	7.38
C.D. at 5%	8.49	32.74	34.99	2.32	22.89	1.91	21.76
Control Vs. Rest							
Control	10.71	172.51	127.19	2.13	109.12	7.23	118.47
Rest Treatment	124.48	518.41	400.25	19.42	241.36	12.84	273.62
S. Em. ±	3.70	14.26	15.24	1.01	9.97	0.83	9.48
C.D. at 5%	10.91	42.07	44.97	2.981	29.42	2.45	27.96
Interaction E x P	Sig.	Sig.	Sig.	Sig.	Sig.	NS	Sig.
C.V. (%)	7.6	6.9	9.5	13.3	10.2	15.8	8.6

Table 4.3a: Interaction effect of EDTA levels and its application periods on Pb content (mg kg^{-1}) in seed

EDTA levels / Application Periods	E_3	E_6	E_9
P_1	63.02	168.52	205.26
P_2	148.30	192.52	186.24
P_3	40.67	54.70	61.06
S. Em. ±	4.99		
C.D. at 5%	14.71		

Table 4.3b: Interaction effect of EDTA levels and its application periods on Pb content (mg kg^{-1}) in shoot

EDTA levels \ Application Periods	E_3	E_6	E_9
P_1	348.08	689.51	964.53
P_2	455.79	956.41	526.58
P_3	165.44	234.52	324.85
S. Em. ±	14.26		
C.D. at 5%	42.07		

Table 4.3c: Interaction effect of EDTA levels and its application periods on Pb content (mg kg^{-1}) in root

EDTA levels \ Application Periods	E_3	E_6	E_9
P_1	204.74	362.77	619.77
P_2	263.65	575.55	598.87
P_3	271.44	305.05	400.46
S. Em. ±	11.86		
C.D. at 5%	34.99		

Table 4.3d: Interaction effect of EDTA levels and its application periods on Pb uptake (mg plot^{-1}) in seed

EDTA levels \ Application Periods	E_3	E_6	E_9
P_1	11.41	23.78	27.24
P_2	23.81	30.44	29.21
P_3	8.40	11.70	8.79
S. Em. ±	1.36		
C.D. at 5%	4.02		

Table 4.3e: Interaction effect of EDTA levels and its application periods on Pb uptake (mg plot^{-1}) in shoot

EDTA levels \ Application Periods	E_3	E_6	E_9
P_1	161.81	252.41	455.91
P_2	208.09	440.27	301.72
P_3	75.77	139.28	136.96
S. Em. ±	13.44		
C.D. at 5%	39.65		

Table 4.3f: Interaction effect of EDTA levels and its application periods on Total Pb uptake (mg plot^{-1}) in plant

EDTA levels \ Application Periods	E_3	E_6	E_9
P_1	180.53	285.48	499.17
P_2	242.28	486.95	349.27
P_3	94.24	164.82	159.83
S. Em. ±	12.77		
C.D. at 5%	37.69		

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