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## Effect of chelating agent and chemical fertilizer on uptake of zinc by sunflower

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### Abstract

Pot experiment was conducted in greenhouse to investigate the effect of chelating agent and chemical fertilizer on zinc uptake by Sunflower. Pots were treated with different EDTA (0, 5, 7 and 9 mM/kg) and NH<sub>4</sub>Cl (0, 100, 200 and 300 mg/kg) concentrations three to four weeks after sowing. The results showed that chelating agent and ammonium chloride alone and their interaction had significant effect on zinc uptake. The data revealed that maximum zinc uptake was observed by high concentration of chelating agent and ammonium chloride alone with respect to control. The best treatment for maximum uptake of zinc was noted in pots applied with combination of 9 mM EDTA/kg and 300 mg/kg NH<sub>4</sub>Cl with respect to EDTA and NH<sub>4</sub>Cl alone and control. The results indicated that the study crop can tolerate heavy metal concentration and chelating agent and ammonium chloride fertilizer had potential to promote the uptake of zinc.

**Keywords:** Zinc, chelating agent (EDTA), chemical fertilizer (NH<sub>4</sub>Cl), sunflower

### Introduction

High biomass producing plant species, such as *Helianthus annuus*, have potential for removing large amounts of trace metals by harvesting the aboveground biomass if sufficient metal concentrations in their biomass can be achieved. However, the low bioavailability of heavy metals in soils and the limited translocation of heavy metals to the shoots by most high biomass producing plant species limit the efficiency of the phytoextraction process. Large biomass production and accelerate metal uptake rate and translocation into aerial parts are critical factors to achieve a viable metal phytoextraction. The success of phytoremediation depends upon the ability of a plant to uptake and translocate heavy metals, which is a function of the specific phenotype and genotype (Chen *et al.* 2003)<sup>[1]</sup>. Several studies have documented that chelating agents such as ethylenediamine tetra-acetic acid (EDTA), *N*-(2-hydroxyethyl)-ethylenediamine tetra-acetic acid (HEDTA), and citric acid (CA) can be used to increase metal mobility, thereby enhancing phytoextraction (Huang *et al.* 1997, Elless and Blaylock 2000, Turgut *et al.* 2004, Liphadzi and Kirkham 2006)<sup>[5, 4, 9, 8]</sup>. In view of phytoremediation, the best effect would provide soil amendments that are able to facilitate plant biomass, yield and its metal uptake. In this regard, nitrogen compounds frequently used for agriculture purpose may be a promising way of increasing hyperaccumulator capability to remove metals from polluted soil. Chelating agents have been shown to desorb heavy metals from the soil matrix into the soil solution, and to facilitate metal translocation from roots to shoots. The effect may vary from plant and metal species. Chelating agents are frequently used to increase the bioavailability of heavy metals, thus enhancing their uptake by plants, though this may also decrease their biomass because of the toxicity. Chelating agents assisted phytoremediation has been demonstrated as a viable treatment for the remediation of heavy metal contaminated soil and sediment. The chelant is mainly to improve the solubilization of target metals from the soil, and render the availability of plant uptake and translocation (Yeh and Pan, 2012)<sup>[10]</sup>. The objectives of this study aimed to investigate the effect of chelating agent and chemical fertilizer to determine the extent of sunflower to uptake Zn from soil.

### Materials and methods

#### Pot-culture experiment

Five kg soil was transferred to plastic pots (cm height and cm diameter). The soil was artificially contaminated with zinc at the rate of 200 mg / kg in the form of ZnSO<sub>4</sub>.7H<sub>2</sub>O

before sowing. After 5 days of heavy metal application, the seeds of sunflower were sown in each pot. After growing for 3-4 weeks, a dose of 0, 5, 7 and 9 mM / kg of chelating agent (EDTA) and 0, 100, 200 and 300 mg / kg of chemical fertilizer (NH<sub>4</sub>Cl) respectively were applied to soil. Data on heavy metal concentration was collected after harvesting of crop. The pots were watered to reach 75% of the field water holding capacity and maintained this humidity by daily watering throughout the cultivation.

**Treatment details**

The experiment was conducted in pots using factorial design (4×4) with two factors of four levels and three replications.

**Table 1:** Treatment combination for pot experiment

Treatment combination	NH <sub>4</sub> Cl(0 mg / kg)	NH <sub>4</sub> Cl(100 mg / kg)	NH <sub>4</sub> Cl(200 mg / kg)	NH <sub>4</sub> Cl(300 mg / kg)
EDTA (0 mM / kg)	C <sub>0</sub> F <sub>0</sub> (T <sub>0</sub> )	C <sub>0</sub> F <sub>1</sub> (T <sub>1</sub> )	C <sub>0</sub> F <sub>2</sub> (T <sub>2</sub> )	C <sub>0</sub> F <sub>3</sub> (T <sub>3</sub> )
EDTA (5 mM / kg)	C <sub>1</sub> F <sub>0</sub> (T <sub>4</sub> )	C <sub>1</sub> F <sub>1</sub> (T <sub>5</sub> )	C <sub>1</sub> F <sub>2</sub> (T <sub>6</sub> )	C <sub>1</sub> F <sub>3</sub> (T <sub>7</sub> )
EDTA (7 mM / kg)	C <sub>2</sub> F <sub>0</sub> (T <sub>8</sub> )	C <sub>2</sub> F <sub>1</sub> (T <sub>9</sub> )	C <sub>2</sub> F <sub>2</sub> (T <sub>10</sub> )	C <sub>2</sub> F <sub>3</sub> (T <sub>11</sub> )
EDTA (9 mM / kg)	C <sub>3</sub> F <sub>0</sub> (T <sub>12</sub> )	C <sub>3</sub> F <sub>1</sub> (T <sub>13</sub> )	C <sub>3</sub> F <sub>2</sub> (T <sub>14</sub> )	C <sub>3</sub> F <sub>3</sub> (T <sub>15</sub> )

**Plant heavy metal analysis**

The plants after harvesting were first washed with tap water, distilled water and finally by double distilled water to remove attached soil, separated into roots, stem and leaves, shredded and dried. The vegetation was dried first at room air temperature and then in an oven at 65°C. Dried plant parts were then crushed and powdered separately. Powdered plant samples were then put separately in well washed dried and suitably labeled flasks. A di-acid mixture comprised of concentrated HNO<sub>3</sub> and HClO<sub>4</sub> was used. To one gram of plant material, 5 ml of concentrated HNO<sub>3</sub> was added and kept overnight. Then next day, 12 ml of di-acid mixture (conc. HNO<sub>3</sub> + HClO<sub>4</sub> in the ratio of 3:1) was added and digested on hot plate till white reddish brown fumes of perchloric acid comes out. Plant samples slowly begin to dissolve and digest in di-acid mixture. After few hours, plant samples dissolved completely in the digestion mixture and the solution was then evaporated until only about 2 ml was left in the flask. After cooling double distilled water was added and the samples were filtered through Whatman No. 42 filter paper. Digested samples were then transferred for heavy metal analysis using Atomic Absorption spectrophotometer (Jackson, 1973)<sup>[6]</sup>.

**Results and discussion**

The results of the experiment presented in Table 2 showed that the effect of chelating agent and chemical fertilizer and their interaction significantly affect the zinc uptake by different parts of sunflower. Zinc uptake was found maximum in roots followed by stem and leaves. The data revealed that in case of chelating agent alone, the maximum uptake 4.91, 4.21 and 2.40 ppm was recorded in C<sub>3</sub> (9 mM EDTA/kg) and minimum 1.60, 1.26 and 0.67 ppm was recorded in C<sub>0</sub> (0 mM EDTA/kg). The data also suggested that maximum zinc uptake 3.66, 3.05 and 1.75 ppm in case of chemical fertilizer alone was found in roots, stem and leaves respectively in F<sub>3</sub> (300 mg / kg NH<sub>4</sub>Cl) and minimum 2.87, 2.38 and 1.33 ppm respectively were recorded in F<sub>0</sub> (0 mg / kg NH<sub>4</sub>Cl), but in case of interaction effect of both EDTA and NH<sub>4</sub>Cl; it was observed that the maximum uptake of zinc 5.21, 4.46 and 2.62 ppm was found in roots, stem and leaves respectively in T<sub>15</sub> (9 mM EDTA/ kg + 300 mg NH<sub>4</sub>Cl / kg) and minimum 0.72, 0.58 and 0.26 ppm respectively were recorded in T<sub>0</sub> (no

**A. Chelating agent levels Symbol**

- 1. EDTA (0 mM / kg) C<sub>0</sub>
- 2. EDTA (5 mM / kg) C<sub>1</sub>
- 3. EDTA (7 mM / kg) C<sub>2</sub>
- 4. EDTA (9 mM / kg) C<sub>3</sub>

**B. Chemical fertilizer levels Symbol**

- 1. NH<sub>4</sub>Cl (0 mg / kg) F<sub>0</sub>
- 2. NH<sub>4</sub>Cl (100 mg / kg) F<sub>1</sub>
- 3. NH<sub>4</sub>Cl (200 mg / kg) F<sub>2</sub>
- 4. NH<sub>4</sub>Cl (300 mg / kg) F<sub>3</sub>

chelant and chemical fertilizer). The data clearly revealed that both increasing concentration of chelating agent and ammonium chloride increased zinc uptake. From the study it was clear that the chelating agent in combination with ammonium chloride boosted the zinc uptake more than the chelating agent and ammonium chloride alone. The results indicated that the sunflower can tolerate heavy metal concentration and EDTA and NH<sub>4</sub>Cl had potential to promote the uptake of zinc. Combined effect of 9 mM EDTA and 300 mg / kg NH<sub>4</sub>Cl was found best for maximum uptake of zinc. The results were also in accordance with (Lesage *et al.*, 2005)<sup>[7]</sup> who found that heavy metal concentrations in harvested shoot of *Helianthus annuus* increased with EDTA concentration. Similar results were also shown by (Ebrahimi *et al.*, 2015)<sup>[3]</sup> who stated that increased dose of EDTA increased the Zn content in the roots and in the shoots of plant. (Chen *et al.*, 2017)<sup>[2]</sup> also found that the combinations of ammonium fertilizers and chelating agent treatments demonstrated heavy metal uptake capacity greater than the control.

**Table 2:** Effect of chelating agent and chemical fertilizer on uptake of zinc by root, stem and leaves of Sunflower.

Treatment combination	Root	Stem	Leaves	
T <sub>0</sub>	0.72	0.58	0.26	
T <sub>1</sub>	1.65	1.34	0.63	
T <sub>2</sub>	1.89	1.46	0.83	
T <sub>3</sub>	2.14	1.64	0.94	
T <sub>4</sub>	2.58	2.01	1.20	
T <sub>5</sub>	2.83	2.21	1.37	
T <sub>6</sub>	2.98	2.39	1.41	
T <sub>7</sub>	3.15	2.54	1.48	
T <sub>8</sub>	3.56	2.97	1.65	
T <sub>9</sub>	3.77	3.21	1.71	
T <sub>10</sub>	3.93	3.35	1.84	
T <sub>11</sub>	4.15	3.54	1.96	
T <sub>12</sub>	4.62	3.97	2.20	
T <sub>13</sub>	4.76	4.10	2.32	
T <sub>14</sub>	5.04	4.32	2.46	
T <sub>15</sub>	5.21	4.46	2.62	
CD (P=0.05)	Due to C	0.027	0.020	0.022
	Due to F	0.027	0.020	0.022
	Due to I (C*F)	0.053	0.041	0.043

## Conclusion

The most important advantage of using *Helianthus annuus* in a phytoextraction practice may be its easy cultivation and large biomass. EDTA alone was better than NH<sub>4</sub>Cl in enhancing zinc and stimulates the transport efficiency of zinc from roots to the shoot tissues. The combinations of chelating agent and ammonium fertilizer treatments demonstrated that zinc uptake capacity was greater than the treatments alone and the control. From the study it was clear that chemical fertilizer should be used with chelating agent in order to have a good biomass of plant and maximum removal of heavy metal. In this study, sunflower was the best plant species to carry out phytoextraction of zinc due to its hyperaccumulator characteristics.

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