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Effects of EDTA and DTPA on Lead and Zinc Accumulation of Ryegrass*

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ABSTRACT

The tailing ponds of lead-zinc mines are artificial environment pollution sources, and also important dangerous sources of heavy metal contamination in lead-zinc mining areas. To study the effects of Ethylene Diamine Tetracetic Acid (EDTA) and Diethylene Triamine Penlaacetic Acid (DTPA) on phytoremediation of lead-zinc mining area soil, two chelators (EDTA and DTPA) were used in enrichment plant ryegrass to improve the uptake of Pb and Zn from soil. The results showed that when the doses of 0, 0.5, 1 *and* 2 *mmol/kg EDTA and DTPA were used, the biomass of ryegrass (Lolium multiflorum Lam.) and its nutrient (N, P, K, Ca and Mg) content increased, whereas EDTA and DTPA with a dose of* 4 *mmol/kg decreased the biomass of ryegrass and its nutrient (N, P, K, Ca and Mg) content. EDTA and DTPA significantly enhanced the contents of Zn and Pb in ryegrass as compared with the control. As for Pb, the content of Pb in root and shoot reached a maximum of* 2730.54 *and* 2484.42 *mg/kg respectively when the dose of EDTA and DTPA was* 2 *mmol/kg. In the case of Zn, the content of Zn in root and shoot reached a maximum of* 2428.37 *and* 2010.43 *mg/kg respectively. The total Pb and Zn accumulations and translocation ratio in ryegrass had also been enhanced. The results indicated that EDTA and DTPA had great potential to be used for ryegrass to remedy Pb and Zn contamination soil of lead-zinc mining area, but should be used cautiously because of their environmental risks.*

Keywords: *EDTA, DTPA, Enrichment Plant, Ryegrass, Lead and Zinc*

1. Introduction

With the development of lead-zinc mineral resources, heavy metals were released and transferred into the ecological environment [1], which caused serious environmental problems. The contents of organic matter, nitrogen and phosphorus in the soil around the mine tailing area were reduced to levels that were 20% - 30% of those found in normal conditions [2,3]. As a result, it was urgent to apply ecological remediation methods in the lead-zinc mining area.

Recently, phytoremediation has appeared as an alternative reliable [4,5]. The use of hyperaccumulator species in continuous phytoextraction processes is limited by the low bioavailability of pollutants to the root uptake [6]. Some studies found that the application of chelating agents showed positive effects on increasing the solubility of heavy metals in soil, thereby enhancing phytoextraction [7-9], and enhancing the amount of bioavailable lead more than 100 times [10,11]. At present, most of the reported hyperaccumulators or accumulators have not had a very good effect on remedying heavy metals because of their small biomass and slow growth [12,13]. So, it could be a reliable practice to increase metal bioavailability, uptake and accumulation in the shoots of plants by applying chelator [9,14-16].

Annual ryegrass (*Lolium multiflorum* Lam.) is one kind of accumulator and graminaceous monocotyledon, and it has a strong regeneration ability and resistance to pests and diseases with its high biomass [17,18]. We aimed to access the influence of EDTA and DTPA on phytoremediation for contaminated soil of lead-zinc mining areas using ryegrass, providing a case of the use and promotion of chelator-induced phytoextraction in contaminated soil.

2. Materials and Methods

Annual ryegrass (*L. multiflorum* Lam.), ethylene diamine tetracetic acid (EDTA) and diethylene triamine penlaace-

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tic acid (DTPA) were selected as experimental plant and chelators. Soil was selected from the lead-zinc mining area of Tangjia Mountain which located in Hanyuan county, Sichuan province, China. The most relevant characteristics of the soil were (mean values) as follows, pH 6.83, organic matter 6.82 g/kg, total N 0.756 g/kg, total P 0.658 g/kg, total K 17.951 g/kg, available N 93.156 mg/kg, available P 3.426 mg/kg, available K 75.221 mg/kg CEC 5.368 cmol/kg, total Pb 2921.32 mg/kg, total Zn 1841.5 mg/kg, available Pb 378.79 mg/kg, available Zn 287.37 mg/kg.

The experiment was arranged in a randomized block design with two chelators of 5 levels and four replicates per treatment. Five concentrations of DTPA were 0 mmol/kg (A0), 0.5 mmol/kg (A1), 1 mmol/kg (A2), 2 mmol/kg (A3) and 4 mmol/kg (A4), and EDTA were 0 mmol/kg (B0), 0.5 mmol/kg (B1), 1 mmol/kg (B2), 2 mmol/kg (B3) and 4 mmol/kg (B4). After sieving (< 5 mm), 1.5 kilograms of dried soil were stored in plastic pots (15 cm×15 cm), and soil samples were mixed with fertilizers containing 100 mg/kg N in the form of urea, 80 mg/kg P as KH_2PO_4, and 100 mg/kg K as KCl. In all treatments, ryegrass was grown and placed in the artificial climate chamber with the environmental conditions, temperature 26˚C in day and 22˚C at night, humidity 75%, light intensity 20000 LX and illumination 12 hour per day. Each pot contained 3 seedlings. When the ryegrass had been growing for 2 months, two mixed chelator solutions were added to the soil. Plants were harvested after 14 days of adding chelator solutions and deactivated enzymes in 105˚C, and baked in the oven in 75˚C until constant weight.

Plant samples were nitrated by HCl—HNO_3—$HClO_4$, and the contents of Pb, Zn, P, K, Mg and Ca were determined by ICP—AES, and the content of N was determined by Kjeldahl nitrogen determination. The data was treated and analyzed by Microsoft Excel 2003 and SPSS 13.0.

3. Results and Analysis

3.1. Biomass and Root-shoot Ratio of Ryegrass

The results showed that, with a single chelator, the biomass of roots and shoots of ryegrass increased at first, and then decreased with the increasing concentrations of the chelator (**Table 1**), and the application of EDTA at the dose of 2 mmol/kg produced the maximum biomass of roots and shoots (1.49 and 1.66 g/plant respectively). When the dose of DTPA was 2 mmol/kg, the maximum biomass of root and shoot were 1.56 and 1.51 g/plant respectively. With the combined application of EDTA and DTPA, the trends in change of the biomass were similar to the application of a single chelator . For 2 mmol/kg dose of EDTA and DTPA, the maximum biomass of roots and shoots were 2.01 and 1.89 g/plant respectively. The root-shoot ratio of ryegrass showed the reverse law of the biomass. Whatever two chelators were used, they both showed significant effects ($P < 0.01$) on

Table 1. The effects of EDTA and DTPA on biomass and root-shoot ratio of ryegrass.

Treatments	Biomass		Root/Shoot
	Root (g/plant)	Shoot (g/plant)	
A0B0	1.10M	1.34P	0.82
A0B1	1.37I	1.55I	0.88
A0B2	1.21K	1.46K	0.83
A0B3	1.49G	1.66H	0.90
A0B4	1.10M	1.36N	0.81
A1B0	1.33I	1.43L	0.93
A1B1	1.62E	1.68G	0.96
A1B2	1.80C	1.81C	0.99
A1B3	1.71D	1.77E	0.97
A1B4	1.06N	1.35O	0.79
A2B0	1.40H	1.46K	0.96
A2B1	1.70D	1.71F	0.99
A2B2	1.81C	1.81C	1.00
A2B3	1.00O	1.28R	0.78
A2B4	1.90B	1.87B	1.02
A3B0	1.56F	1.51J	1.03
A3B1	1.99A	1.87B	1.06
A3B2	1.81C	1.78D	1.02
A3B3	2.01A	1.89A	1.06
A3B4	0.96P	1.26S	0.76
A4B0	1.38H	1.38M	1.00
A4B1	1.26J	1.31Q	0.96
A4B2	1.18L	1.26S	0.94
A4B3	1.07N	1.18T	0.91
A4B4	0.82Q	1.09U	0.75

Note: The data followed by uppercase letters indicate the difference at 1% level, the same as following tables.

the biomass of root and shoot of ryegrass.

3.2. Pb Content in Ryegrass Plant

With the increased EDTA dose, Pb content in the roots of ryegrass increased before a dose of 2 mmol/kg, and decreased when a dose of 4 mmol/kg was used (**Figure 1**). DTPA had approximately the same effects on Pb content in roots. When the dose of EDTA and DTPA was 2 mmol/kg, Pb content in roots reached the maximum of 2730.54 mg/kg, and it was 3.15 times higher than the control. The results also showed that EDTA increased Pb content more than DTPA did with the same dose. Both chelators showed significant effects ($P < 0.01$) on Pb content in roots.

EDTA and DTPA had approximately the same effect on the Pb content in shoots of ryegrass (**Figure 2**). Pb content in shoots reached the maximum of 2484.42 mg/kg, which was 2.15 times greater than the control, when the dose of EDTA and DTPA was 2 mmol/kg. EDTA enhanced Pb content in shoots more than DTPA did with the same dose. Both chelators showed significant effects ($P < 0.01$) on Pb content in shoots.

3.3. Zn Content in Ryegrass Plant

Zn content in the roots of ryegrass had the same variation with the doses of EDTA and DTPA increasing (**Figure 3**). When the dose of EDTA and DTPA was 4 mmol/kg, Zn content in roots reached the maximum of 2428.37 mg/kg, and it was 9.82 times greater than the control. EDTA enhanced Zn content in shoots more than DTPA at the same dose. Both chelators showed significant effects ($P < 0.01$) on Zn content in root.

EDTA and DTPA had approximately the same effect on the Zn content in shoots of ryegrass (**Figure 4**). When the dose of EDTA and DTPA was 4 mmol/kg, Zn content in shoots reached the maximum of 2010.43 mg/kg, and it was 5.14 times higher than the control. With the same dose, EDTA enhanced Zn content in shoots more than DTPA did. Both chelators showed significant effects ($P < 0.01$) on Zn content in shoot.

3.4. The Correlation between DTPA/EDTA and Pb and Zn Content in Ryegrass

The results of partial-correlation analysis showed that DTPA and EDTA didn't significantly correlate with Pb content in roots but did significantly correlate with Pb content in shoots (**Table 2**). The correlation between DTPA/EDTA and Zn content in roots and shoots were significant. The results indicated that two chelators had collaborative effects on Zn content in ryegrass plants.

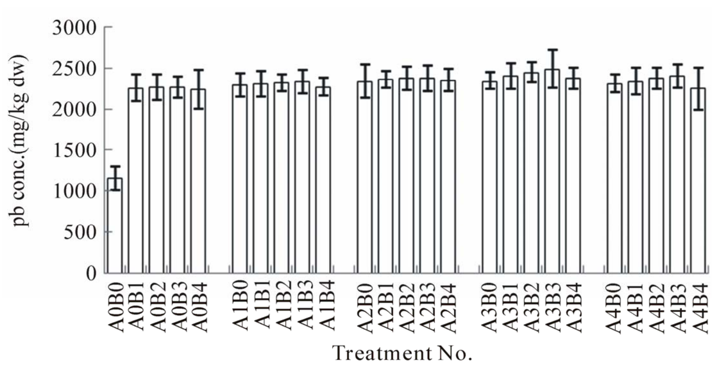

Figure 1. The effects of DTPA and EDTA on Pb content in the roots.

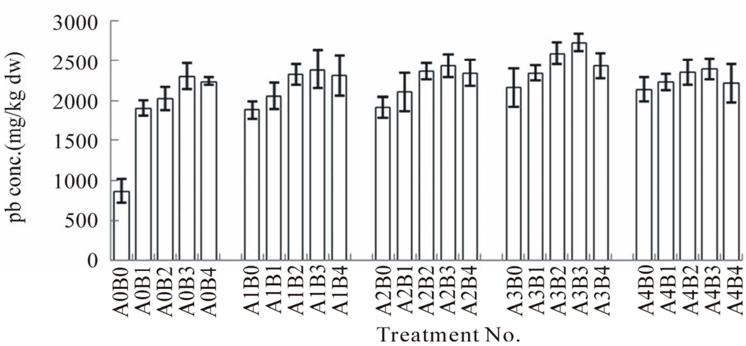

Figure 2. The effects of DTPA and EDTA on Pb content in the shoots.

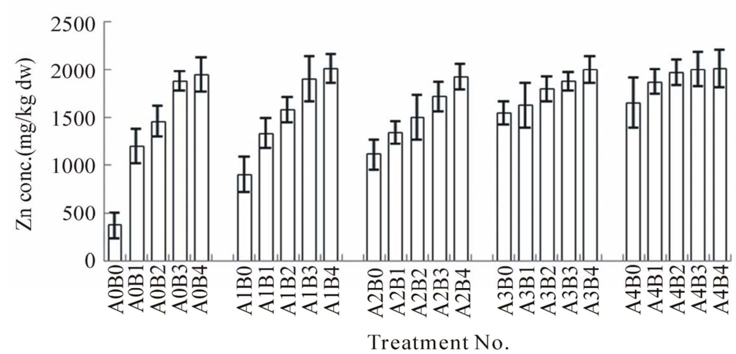

Figure 3. The effects of DTPA and EDTA on Zn content in the roots.

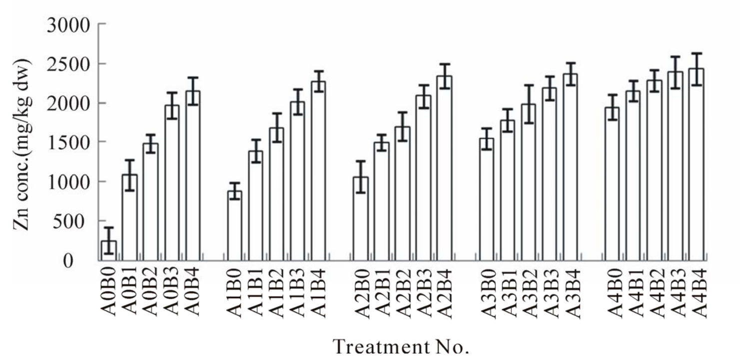

Figure 4. The effects of DTPA and EDTA on Zn content in the shoots.

Table 2. The partial-correlation coefficients of chelators and Pb/Zn content in ryegrass plant.

Chelators	Pb		Zn	
	Root	*Shoot*	*Root*	*Shoot*
EDTA	0.501	0.781**	0.648*	0.814**
DTPA	0.421	0.706*	0.801**	0.775**

Note: n = 9, r0.05 = 0.632, r0.01 = 0.765, * and ** showed the significant levels of 5% and 1%, respectively.

3.5. Total Pb and Zn Accumulation in Ryegrass Plants

DTPA and EDTA enhanced total Pb and Zn accumulation in roots and shoots compared with the control (**Table 3**). With the increase of one chelator dose, the total Pb and Zn accumulation in root and shoot increased at first and decreased at last. The maximum of total Pb and Zn accumulation in roots were 4.99 and 3.77 mg/plant respectively, and the maximum of Pb and Zn accumulation in shoots were 5.16 and 4.38 mg/plant respectively. The results showed that EDTA and DTPA had significant effects ($P < 0.01$) on the total Pb and Zn accumulation in ryegrass plants. Compared with the control, most of the Pb translocation ratios were enhanced, and all of the Zn translocation ratios were enhanced. This showed that DTPA and EDTA could promote transfer of Pb and Zn from roots to shoots.

3.6. Nutrients Contents in Ryegrass Plants

DTPA and EDTA enhanced the nutrient (N, P, K, Ca and Mg) contents in ryegrass plants compared with the control (**Table 4**). The nutrient contents increased at first and decreased at last when the dose of one chelator increased. The results showed that EDTA and DTPA had significant effects ($P < 0.01$) on the nutrient contents in ryegrass plants. This indicated that DTPA and EDTA could enhance the absorption of nutritional elements from soil to promote the growth of ryegrass plants.

4. Discussion

Previous studies found that in a certain range of concentrations, EDTA strongly inhibited plant growth [19]. In this paper, with increased EDTA and DTPA doses, the biomass in roots and shoots of ryegrass increased at first and decreased at last, and the nutrient (N, P, K, Ca and Mg) contents of ryegrass plants increased compared with the control, which could be due to the active effects of chelators on soil nutrients [20,21]. Meanwhile, EDTA may stimulate the heavy metal extraction from the soil [22], and reduced biological toxicityof heavy metals [23]. When the dose of EDTA and DTPA was 4 mmol/kg, the biomass in roots and shoots decreased. This could be due

Table 3. The effects of DTPA and EDTA on total Pb and Zn accumulation in ryegrass plant.

Treatments	Root		Shoot		Translocation ratio	
	Pb (mg/plant)	Zn (mg/plant)	Pb (mg/plant)	Zn (mg/plant)	Pb (%)	Zn (%)
A0B0	1.27O	0.41J	1.16K	0.33K	47.72	44.86
A0B1	3.09GHIJ	1.64GH	2.96GHI	1.68J	48.93	50.58
A0B2	2.74IJKLM	1.77GH	2.96GHI	2.16I	51.95	55.06
A0B3	3.38FGH	2.80C	3.83D	3.27BCDE	53.09	53.89
A0B4	2.46KLM	2.14EF	3.05GH	2.92DEFG	55.27	57.74
A1B0	3.05GHIJK	1.20I	2.69IJ	1.26J	46.89	51.18
A1B1	3.74DEF	2.16EF	3.46EF	2.33HI	48.05	51.83
A1B2	4.17BCDE	2.84C	4.20C	3.05CDEFG	50.19	51.76
A1B3	3.99CDE	3.25B	4.23C	3.56B	51.42	52.27
A1B4	2.40LMN	2.13EF	3.12GH	3.07CDEF	56.5	59.04
A2B0	3.27FGHI	1.55H	2.80HI	1.55J	46.15	49.99
A2B1	4.01CDE	2.28EF	3.60DE	2.56GHI	47.3	52.85
A2B2	4.29BCD	2.72CD	4.29C	3.08DEF	49.98	53.14
A2B3	2.37LMN	1.71GH	3.11GH	2.67FGH	56.74	60.91
A2B4	4.47ABC	3.66A	4.38BC	4.38A	49.5	54.49
A3B0	3.65EFG	2.40DE	3.27FG	2.33HI	47.24	49.21
A3B1	4.77AB	3.23B	4.38BC	3.33BCD	47.88	50.77
A3B2	4.42ABC	3.25FB	4.61B	3.53BC	51.05	52.06
A3B3	4.99 A	3.77A	5.16A	4.13A	50.82	52.32
A3B4	2.28MN	1.92FG	3.07GH	2.98DEFG	57.4	60.79
A4B0	3.19FGHI	2.27EF	2.95HI	2.68FGH	48.01	54.1
A4B1	2.95HIJKL	2.35E	2.92HI	2.81EFGH	49.73	54.43
A4B2	2.80HIJKLM	2.32E	2.97GHI	2.87DEFG	51.44	55.29
A4B3	2.57JKLM	2.14EF	2.83HI	2.82EFGH	52.43	56.8
A4B4	1.84N	1.65GH	2.42J	2.65FGH	56.77	61.62

Note: Translocation ratio (%) = Pb (Zn) accumulation in shoot / Pb (Zn) accumulation in root × 100%.

Table 4. The effects of EDTA and DTPA on nutrients contents of ryegrass plant.

Treatments	N (mg/g)	P (mg/g)	K (mg/g)	Ca (mg/g)	Mg (mg/g)
A0B0	13.2P	4.8N	20.9W	4.0M	1.0F
A0B1	19.8N	5.6K	28.7U	4.8J	1.3E
A0B2	24.7L	6.0I	36.1Q	5.0H	1.4D
A0B3	31.6ED	6.4F	41.4L	5.6D	1.5C
A0B4	32.7A	6.6D	45.7E	5.8B	1.7A
A1B0	17.4O	5.1M	26.6V	4.6L	1.3E
A1B1	21.6M	5.8J	30.8S	4.9I	1.4D
A1B2	26.5J	6.2H	38.7O	5.4E	1.5C
A1B3	32.0CD	6.5E	42.1K	5.7C	1.6B
A1B4	32.8A	6.8B	46.8B	5.9A	1.7A
A2B0	21.5M	5.4L	30.6T	4.7K	1.4D
A2B1	25.7K	6.0I	36.2P	5.3F	1.5C
A2B2	30.1G	6.4F	40.1M	5.6D	1.6B
A2B3	32.6AB	6.8B	44.7G	5.8B	1.7A
A2B4	32.8A	7.0A	47.0A	5.9A	1.7A
A3B0	27.4I	6.0I	34.2R	4.9I	1.5C
A3B1	29.1H	6.3G	38.7O	5.4E	1.6B
A3B2	31.1F	6.6D	42.6J	5.7C	1.7A
A3B3	32.8A	7.0A	46.4C	5.9A	1.7A
A3B4	32.7A	6.8B	46.0D	5.6D	1.6B
A4B0	31.2EF	6.2H	36.1Q	5.1G	1.6B
A4B1	31.8CD	6.4F	39.9N	5.6D	1.7A
A4B2	32.2BC	6.8B	43.2I	5.8B	1.6B
A4B3	32.6AB	6.7C	44.8F	5.6D	1.5C
A4B4	32.1C	6.5E	43.6H	5.4E	1.5C

to the biological toxicity of free chelators as well as the active effects of chelators on heavy metals [24,25]. Also, the trend of the root-shoot ratio indicated that the ecological adaptability of ryegrass plants had gradually increased.

Some scholars found that 60% of lead in soil was activated when 15 mmol/kg EDTA was added to acidic soil (pH 5.5). As EDTA had oxygen atoms with four electron pairs and nitrogen atoms with 2 electron pairs, it mainly existed in soil in the form of $H_2[EDTA]^{2-}$, and in this acidity range, heavy metal ions mainly existed in the form of bivalent as well. So, EDTA and heavy metal ions could form stable chelates [26,27]. DTPA and EDTA of organic acid ligands had a strong ability of chelation with the change of ligand and heavy metal elements, DTPA played a more stable effect on chelating with lead and zinc than that of EDTA [28]. Blaylock [29] found that DTPA and EDTA could enhance Pb and Cd accumulation in shoot of *Brassiajuncea* (1.6% and 1.0% respectively). Xu [30] showed that EDTA enhanced Zn accumulation in root and shoot of vetiver grass more than 7.3% and 37.4%. Combining with EDTA and DTPA, the shoot of *Thlaspi caerulescens* accumulated more Zn than the control [21]. Also, EDTA could improve the accumulation of Pb and Zn in *Chrysanthemum coronarium* and *Cirsium japonicum* [31,32], which had significant correlation with its concentrations [33]. Meanwhile, this study found that EDTA and DTPA enhanced the contents of Pb/Zn in root and shoot of ryegrass plant, which was consistent with the research of Wang [34], and increased total Pb/Zn accumulation and translocation ratio in ryegrass plant.

Bell [35] and Wenzel [36] reported that metal chelates could enter the root from the endodermis cleft and transfer into plants' stem and leaves. In the marking test of ^{14}C-EDTA-Pb, Blaylock [29] found that plants accumulated Pb more easily with chelators. Salt [6] found that chelation prevented precipitation and absorption of metals, thereby improving metals availability. But a proper concentration of EDTA which induced the accumulation of Pb was the key, and this may be due to the physiological mechanism of roots which controlled the transmembrane transportation. In this paper, with the same dose, EDTA enhanced total Pb and Zn accumulation in shoots more than DTPA, which could be due to its weak chelated ability and poor active ability.

5. Conclusions

EDTA and DTPA enhanced the biomass of ryegrass when the dose less than 4 mmol/kg, and enhanced Zn and Pb contents in roots and shoots of ryegrass. The total Pb and Zn accumulations, translocation ratio and nutrient (N, P, K, Ca and Mg) contents in ryegrass had also been enhanced. The partial correlation analysis showed that DTPA and EDTA didn't significantly correlate with Pb accumulation in roots and did significantly correlate with Pb accumulation in shoots of ryegrass, and the correlation between DTPA/EDTA and Zn accumulation in roots and shoots of ryegrass was significant. Therefore, EDTA and DTPA have great potential to be used for ryegrass to remedy Pb and Zn contaminated soil around lead-zinc mining areas, but should be used cautiously because of their environmental risks.

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