Effect of Edta on the Growth and Cadmium Accumulation of Orychophragmus Violaceus

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Abstract: In this paper, a pot experiment was conducted to study the effect of EDTA on the growth and cadmium accumulation of Orychophragmus violaceus. The results showed that the plant height, the dry weight of the overground part, the chlorophyll content, the root activity and the malondialdehyde content of Orychophragmus violaceus of the experimental group were significantly different from those of the control group. When the concentration was 3.0 mmol · kg-1EDTA, the cadmium content in the overground part of Orychophragmus violaceus was the highest. The average value was 96.21 μg · g-1, which was 396.62% higher than that of the control group. It had a significant effect on the growth of Orychophragmus violaceus, and it could improve the accumulation of cadmium in the overground part with the best effect.

1. Introduction

With the rapid development of China's economy, soil pollution caused by cadmium, mercury, arsenic, copper and other heavy metals is particularly serious; among them, cadmium has the characteristics of stability, accumulation and difficult to remove [1-2].

EDTA (Ethylene Diamine Tetraacetic Acid) has a high chelating efficiency for various metal ions in soil. Because EDTA is stable in the environment, it becomes a commonly used chelating agent for heavy metals in soil [3-4]. EDTA can significantly activate zinc, copper, cadmium and lead in soil, thus increasing the absorption and enrichment of these metals by plant roots [5]. At present, EDTA has been applied to strengthen the induced remediation effect of ryegrass, oil seed rapes and other crops grown on lead and chromium contaminated soil [6-9], since it can effectively improve the accumulation of heavy metal ions in crops.

February Orchid (*Orychophragmus violaceus*) is a kind of annual or biennial herbaceous plant, which is native to China. They are widely cultivated in the north and south of China as a kind of ornamental flower; wild February orchids can also be found. In previous studies, *Orychophragmus violaceus* revealed its characteristics as a super cadmium accumulating plant, and it has good ability to remove cadmium from soil with the aid of chelating agent. The urban soil is generally land use for greening. As an ornamental plant, *Orychophragmus violaceus* can not only beautify the environment, but also remove the cadmium in the shallow layer of urban soil. Meanwhile, it does not enter the food chain. This experiment can provide a method for the application of EDTA in other ornamental plants which can repair heavy metal contaminated soil.

2. Research Materials and Methods

2.1 Plant Materials and Test Soil

In the experiment, the *Orychophragmus violaceus* plants are annual. They were purchased from the seedlings wholesale in Ganzhou, Jiangxi Province; the sandy loam used was purchased from Chunnian Horticulture Company in Meishan, Sichuan Province. The basic physical and chemical properties of the tested soil are as follows. The pH value was 6.6; organic matter was 1.2%; total nitrogen was 1 g · kg^{-1}; available phosphorus was 9 g · kg^{-1}; available potassium was 95 g · kg^{-1}.

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The soil contained trace of cadmium.

2.2 Test Method

2.2.1 Test Design

Detail information is shown in Table 1. No reagent was added to CK0 soil. Add 50 mg · kg^{-1} $CdCl_2 \cdot 2.5H_2O$ to CK1 and group A soil, mix them well and let them stand for one month, and add EDTA compound stress to group A soil at the same time. No cadmium was added to group B soil; only EDTA was added. A. The concentration gradient of EDTA in group A and B was 1.5, 3.0, 4.5 and 6.0 mmol · kg^{-1}. Three flowerpots were treated each time; there were 2.5 kg of soil per flowerpot. Five plants of *Orychophragmus violaceus* were planted in each pot. The plant height, dry weight of the overground part, chlorophyll, root activity and malondialdehyde index were measured every 10 days, and cadmium accumulation in the overground part of *Orychophragmus violaceus* in group A was measured after 40 days.

Table 1 Different Treatment Methods for Orychophragmus Violaceus Growth Conditions.

Treatment	Processing method
CK0	reagent blank
CK1	50 mg · kg^{-1} $CdCl_2 \cdot 2.5H_2O$
A1	50 mg . kg^{-1} CdCl2 · 2.5H2O + 1.5 mmol · kg^{-1} EDTA
A2	50 mg · kg^{-1} CdCl2 · 2.5H2O + 3.0 mmol · kg^{-1} EDTA
A3	50 mg · kg^{-1} CdCl2 · 2.5H2O + 4.5 mmol · kg^{-1} EDTA
A4	50 mg · kg^{-1} CdCl2 · 2.5H2O + 6.0 mmol · kg^{-1} EDTA
B1	1.5 mmol · kg^{-1} EDTA
B2	3.0 mmol · kg^{-1} EDTA
B3	4.5 mmol · kg^{-1} EDTA
B4	6.0 mmol · kg^{-1} EDTA

2.2.2 Index Determination Method

The plant height is the distance from the base of plant to the top of main stem, namely the growing point of the main stem. The data is measured with tapeline and recorded every 10 days. The dry weight of the above ground part was measured with an electronic balance after drying. After the above ground parts were washed with distilled water, they were killed out at 105 degrees for 20 minutes, then dried in the oven at 70 degrees to constant weight. Then the mass was measured. Chlorophyll was determined by spectrophotometry [10]; root activity was measured by TTC colorimetry [10]; malondialdehyde was measured by spectrophotometry [10].

The accumulation of cadmium in the overground part was determined by inductively coupled plasma mass spectrometry [11]. The overground part and the underground part of the *Orychophragmus violaceus* plant were separated; the overground part was washed with distilled water, then it was killed out at 105 degrees for 20 minutes, and then dried to constant weight in the oven at 70 degrees. The researchers then ground the dried plant samples into powder after weighing, and then send them to Chengdu Huayang Bona Biotechnology Co., Ltd. for measurement after sifting through mesh.

2.3 Data Statistics and Analysis

WPS Excel and DPS 7.05 were used for data processing and analysis.

3. Results and Analysis

3.1 Height of Orychophragmus Violaceus

It can be seen from table 2 that the average plant height of *Orychophragmus violaceus* in CK0 group is significantly lower than that of other treatments, and there are significant differences between each treatment and the control group ($p < 0.05$). The height of *Orychophragmus violaceus* in group B increased gradually with the increase of EDTA concentration. In group A the plant height increased first and then decreased, but the value was still higher than that of CK1. On the

15th, 30th, 45th and 60th day, the plant height of group B3 was the highest; the figures were 14.1, 30.2, 34.4 and 36.3cm respectively. Compared with the figures of 11.3, 25.1, 28.8 and 30.4cm of CK0, they were significantly higher by 24%, 20%, 19% and 19% respectively. With the increase of treatment time, the height of orchid also increased.

Table 2 Height of Orychophragmus Violaceus under Different Treatments.

Treatment	*Orychophragmus violaceus* height/cm			
	10 d	20 d	30 d	40 d
CK0	11.3 ± 0.6d	25.1 ± 1.1d	28.8 ± 0.5e	30.4 ± 0.7d
CK1	13.2 ± 0.4abc	29.5 ± 0.8ab	32.7 ± 0.4bc	33.6 ± 0.6bc
A1	12.1 ± 0.5cd	27.2 ± 0.6c	30.6 ± 0.7d	32.2 ± 0.6bc
A2	13.3 ± 0.3ab	28.7 ± 0.5ab	31.7 ± 0.6cd	33.4 ± 0.7bc
A3	13.5 ± 0.6ab	29.7 ± 0.7ab	32.4 ± 0.8bc	34.2 ± 0.5ab
A4	12.2 ± 0.4cd	28.5 ± 0.8bc	32.1 ± 1.1bc	34.5 ± 0.6ab
B1	12.6 ± 1.1bc	28.3 ± 0.5ab	31.8 ± 0.7cd	33.4 ± 0.3bc
B2	12.6 ± 0.5bc	29.4 ± 1.0ab	32.6 ± 0.8bc	34.3 ± 0.7ab
B3	14.1 ± 0.4a	30.2 ± 0.6a	34.4 ± 0.6a	36.3 ± 0.4a
B4	13.7 ± 0.4a	29.5 ± 0.8ab	33.3 ± 0.7ab	35.2 ± 0.8a

Note: different small letters in the same column indicate significant difference ($p < 0.05$). Different capital letters showed very significant difference ($p < 0.01$), the same as following tables.

3.2 Dry Weight of Above-Ground Parts of Orychophragmus Violaceus

It can be seen from table 3 that the average overground dry weight of *Orychophragmus violaceus* in CK0 group was significantly lower than that in other treatments, and there was significant difference between each treatment and the control group ($p < 0.05$). In group B, the dry weight of overground part increased gradually with the increase of EDTA concentration, while in group A, the dry weight increased first and then decreased, but it was still higher than the control group. At the 10th, 20th, 30th and 40th day, the dry weight of *Orychophragmus violaceus* in B3 group was the heaviest; the figures were 456.64, 624.34, 689.39 and 715.82 mg respectively, which increased 89%, 39%, 32% and 31% compared with the figures of 241.55, 446.79, 521.82 and 545.19 mg of CK0. It can be seen that EDTA had significant effect on the dry weight of the overground part of *Orychophragmus violaceus*. With the increase of treatment time, the dry weight of the overground part of *Orychophragmus violaceus* increased.

Table 3 Dry Weight Of Above-Ground Parts of Orychophragmus Violaceus under Different Treatments.

Treatment	*Orychophragmus violaceus* dry weight/mg			
	10 d	20 d	30 d	40 d
CK0	241.55 ± 7.51f	446.79 ± 10.27g	521.82 ± 8.59f	545.19 ± 11.21f
CK1	263.34 ± 7.70e	458.37 ± 10.78f	535.31 ± 10.03ef	561.53 ± 9.46e
A1	298.33 ± 8.57d	501.82 ± 11.19d	574.25 ± 7.12d	600.75 ± 11.76cd
A2	337.56 ± 8.37c	540.82 ± 9.22c	619.04 ± 10.94b	642.72 ± 10.03b
A3	285.82 ± 10.64d	521.29 ± 13.19cd	592.89 ± 9.94c	618.14 ± 7.18c
A4	256.39 ± 6.63ef	473.44 ± 8.17e	548.18 ± 11.27e	575.54 ± 9.21d
B1	335.51 ± 12.26c	542.74 ± 11.54c	621.66 ± 8.69b	645.47 ± 8.18b
B2	410.32 ± 8.07b	603.11 ± 9.56b	684.27 ± 7.54a	710.45 ± 10.53a
B3	456.64 ± 7.03a	624.34 ± 10.51a	689.39 ± 7.70a	715.82 ± 5.65a
B4	400.27 ± 10.32b	596.24 ± 15.61b	683.07 ± 9.41a	706.99 ± 13.88a

3.3 Chlorophyll of Orychophragmus Violaceus

It can be seen from table 4 that the content of chlorophyll a in B3 group was the highest at 10th and 40th days; the figures were 2.40 and 1.19 mg · cm^2 respectively, which were significantly different from 2.16 and 0.89 mg · cm^2 of CK0 (p <0.05) with an increase of 11% and 33% respectively. At the 20th day, the highest content of chlorophyll a was 1.54 mg · cm^2 in group A1, which was significantly different from CK0 (p <0.05), and was 19% higher than that of CK0 (1.29 mg · cm^2). At the 30th day, the highest content of chlorophyll a was 1.35 mg · cm^2 in CK1 group, which was significantly different from CK0 (p <0.05), and 25% higher than the 1.08 mg · cm^2 of CK0 group. On the 10th, 20th and 40th days, the content of chlorophyll b was highest in B3 group; the figures were 1.27, 0.56 and 0.37 mg · cm^2 respectively, and significantly different from CK0 (p <0.05). They were 12%, 36% and 94% higher than CK0's 1.13, 0.41 and 0.19 mg · cm^2 respectively. At the 30th day, the content of chlorophyll b in group A1 was 0.46 mg · cm^2, which was significantly higher than that in group CK0 (p<0.05), and 31% higher than that of group CK0 (0.35 mg · cm^2). On the 10th, 20th, 30th and 40th days, the total amount of chlorophyll in A1 group was the highest, which was 4.71, 2.57, 2.24 and 2.07 mg · cm^2 respectively. The figures were significantly different from those of CK0 (p <0.05), and were 8%, 17%, 7% and 28% higher than those of CK0 (4.36, 2.19, 2.08 and 1.61 mg · cm^2). In general, with the increase of EDTA concentration, the chlorophyll content of *Orychophragmus violaceus* increased gradually; with the increase of treatment time, the chlorophyll content of each treatment decreased gradually.

Table 4 -1 Chlorophyll a Content of Orychophragmus Violaceus in Different Treatments

Treatment	Chlorophyll a content of *Orychophragmus violaceus*/mg · cm^2			
	10 d	20 d	30d	40 d
CK0	2.16 ± 0.13b	1.29 ± 0.04bc	1.08 ± 0.05c	0.89 ± 0.11b
CK1	2.23 ± 0.13ab	1.49 ± 0.05ab	1.35 ± 0.03a	1.15 ± 0.07a
A1	2.42 ± 0.09a	1.54 ± 0.06a	1.34 ± 0.06a	1.19 ± 0.06a
A2	2.31 ± 0.11ab	1.35 ± 0.05b	1.16 ± 0.05b	1.07 ± 0.05ab
A3	2.24 ± 0.07ab	1.34 ± 0.11b	1.17 ± 0.03b	1.03 ± 0.08ab
A4	2.20 ± 0.08ab	1.31 ± 0.03bc	1.08 ± 0.11bc	0.99 ± 0.06b
B1	2.34 ± 0.05ab	1.37 ± 0.03b	1.11 ± 0.07bc	1.03 ± 0.12ab
B2	2.29 ± 0.06ab	1.31 ± 0.04b	1.20 ± 0.04ab	1.07 ± 0.07ab
B3	2.40 ± 0.09a	1.47 ± 0.09ab	1.26 ± 0.10ab	1.19 ± 0.04a
B4	2.29 ± 0.04ab	1.35 ± 0.06b	1.15 ± 0.06b	1.10 ± 0.04ab

Table 4 -2 Chlorophyll B Content of Orychophragmus Violaceus in Different Treatments

Treatment	Chlorophyll b content of *Orychophragmus violaceus*/mg · cm^2			
	10 d	20 d	30 d	40 d
CK0	1.13 ± 0.12b	0.41 ± 0.04b	0.35 ± 0.04b	0.19 ± 0.06b
CK1	1.28 ± 0.04a	0.51 ± 0.05ab	0.43 ± 0.05ab	0.36 ± 0.06a
A1	1.30 ± 0.06a	0.50 ± 0.07ab	0.46 ± 0.06a	0.40 ± 0.04a
A2	1.21 ± 0.04ab	0.57 ± 0.09a	0.41 ± 0.07ab	0.27 ± 0.05ab
A3	1.24 ± 0.07ab	0.48 ± 0.07ab	0.38 ± 0.05b	0.29 ± 0.04ab
A4	1.13 ± 0.09b	0.45 ± 0.06b	0.37 ± 0.07b	0.23 ± 0.03b
B1	1.28 ± 0.07a	0.45 ± 0.10ab	0.40 ± 0.06b	0.30 ± 0.04ab
B2	1.23 ± 0.11ab	0.50 ± 0.09ab	0.37 ± 0.09ab	0.29 ± 0.05ab
B3	1.27 ± 0.06a	0.56 ± 0.07a	0.43 ± 0.03ab	0.37 ± 0.06a
B4	1.24 ± 0.09ab	0.48 ± 0.07ab	0.38 ± 0.06b	0.27 ± 0.07ab

Table 4 -3 Total Chlorophyll Content of Orychophragmus Violaceus under Different Treatments

Treatment	Total chlorophyll content of *Orychophragmus violaceus*/mg · cm^2			
	10 d	20 d	30 d	40 d
CK0	4.36 ± 0.04c	2.19 ± 0.07c	2.08 ± 0.03bc	1.61 ± 0.05c
CK1	4.68 ± 0.05ab	2.51 ± 0.04a	2.20 ± 0.07a	2.05 ± 0.10a
A1	4.71 ± 0.06a	2.57 ± 0.05a	2.24 ± 0.10a	2.07 ± 0.04a
A2	4.65 ± 0.03ab	2.34 ± 0.06b	2.14 ± 0.09b	1.88 ± 0.08ab
A3	4.52 ± 0.06bc	2.31 ± 0.05b	2.10 ± 0.06bc	1.83 ± 0.12ab
A4	4.55 ± 0.03bc	2.23 ± 0.04bc	2.11 ± 0.04b	1.64 ± 0.08c
B1	4.59 ± 0.07b	2.33 ± 0.04b	2.15 ± 0.10ab	1.87 ± 0.05ab
B2	4.61 ± 0.04b	2.30 ± 0.09b	2.16 ± 0.12ab	1.81 ± 0.07ab
B3	4.69 ± 0.09ab	2.46 ± 0.05ab	2.24 ± 0.05ab	2.07 ± 0.08a
B4	4.57 ± 0.05bc	2.31 ± 0.06b	2.16 ± 0.08b	1.79 ± 0.04bc

3.4 Root Activity of Orychophragmus Violaceus

It can be seen from table 5 that with the increase of EDTA concentration, the root activity of

Orychophragmus violaceus gradually decreased. At the 10th, 20th, 30th, and 40th days, the root activity of CK 0 was the highest, which was 4.933, 3.698, 3.449 and 2.513 mg TPF · g^{-1}FW · h^{-1} respectively. The root activity was the least in group A4. The difference between A4 group and CK0 group was significant ($p < 0.05$), which was 4.530, 3.114, 2.983 and 1.968 mg TPF · g^{-1}FW · h^{-1} and was 8%, 15%, 13% and 21% lower than that of the CK0 group. With the increase of treatment time, the root vigor of *Orychophragmus violaceus* decreased gradually.

Table 5 Root Activity Of Orychophragmus Violaceus in Different Treatments.

Treatment	Root activity/mg TPF · g-1FW · h-1			
	10 d	20 d	30 d	40 d
CK0	4.933 ± 0.148a	3.698 ± 0.167ab	3.449 ± 0.172a	2.513 ± 0.793a
CK1	4.861 ± 0.194ab	3.811 ± 0.137a	3.358 ± 0.157a	2.457 ± 0.928a
A1	4.749 ± 0.183bc	3.536 ± 0.110bc	3.079 ± 0.128bc	2.041 ± 0.108bc
A2	4.730 ± 0.121bc	3.441 ± 0.131c	3.011 ± 0.206cd	1.997 ± 0.124bc
A3	4.661 ± 0.253cd	3.348 ± 0.188cd	2.869 ± 0.118d	2.047 ± 0.109bc
A4	4.530 ± 0.153d	3.114 ± 0.195d	2.983 ± 0.140cd	1.968 ± 0.116c
B1	4.625 ± 0.182cd	3.587 ± 0.160bc	3.364 ± 0.176a	2.148 ± 0.103bc
B2	4.743 ± 0.175bc	3.251 ± 0.118d	3.126 ± 0.235bc	2.237 ± 0.099bc
B3	4.853 ± 0.157ab	3.623 ± 0.176b	3.258 ± 0.196ab	2.191 ± 0.155b
B4	4.750 ± 0.106bc	3.066 ± 0.125f	3.006 ± 0.133cd	2.028 ± 0.181bc

3.5 Malondialdehyde Content of Orychophragmus Violaceus

It can be seen from table 6 that the content of malondialdehyde increased gradually with the increase of EDTA concentration. On the 10th, 20th, 30th and 40th days, the contents of MDA were the highest in group A4 , and the difference with the control group was significant ($p < 0.05$). The figures were 0.0151, 0.0241, 0.0245 and 0.0229 μmol · g^{-1}FW respectively. Compared with the figures of 0.0121, 0.0147, 0.0184 and 0.0205 μmol · g^{-1}FW of the CK0 group, the malondialdehyde content increased by 24%, 63%, 33% and 12% respectively. Compared with the figures of 0.0127, 0.020 5, 0.0192 and 0.0201 μmol · g^{-1}FW of the CK1, the figures increased by 18%, 17%, 27% and 13% respectively. With the increase of treatment time, the content of malondialdehyde in *Orychophragmus violaceus* increased gradually.

Table 6 Malondialdehyde Content Of Orychophragmus Violaceus in Different Treatments.

Treatment	Malondialdehyde content/μmol · g-1FW			
	10 d	20 d	30d	40 d
CK0	0.012 1 ± 0.004 6d	0.014 7 ± 0.003 8d	0.018 4 ± 0.001 8c	0.020 5 ± 0.001 6d
CK1	0.012 7 ± 0.004 1c	0.020 5 ± 0.001 2c	0.019 2 ± 0.002 3c	
A1	0.014 3 ± 0.006 0b	0.021 2 ± 0.002 1bc	0.022 6 ± 0.001 9b	0.022 7 ± 0.001 5c
A2	0.013 8 ± 0.003 7b	0.023 2 ± 0.002 5ab	0.023 5 ± 0.003 7ab	0.023 1 ± 0.002 4bc
A3	0.015 4 ± 0.004 6a	0.022 6 ± 0.001 7a	0.022 8 ± 0.002 9b	0.023 9 ± 0.003 1abc
A4	0.015 1 ± 0.002 9a	0.024 1 ± 0.002 6a	0.024 5 ± 0.004 1a	0.022 9 ± 0.004 4a
B1	0.012 8 ± 0.003 6c	0.022 9 ± 0.003 1abc	0.023 3 ± 0.002 5ab	0.023 0 ± 0.001 9bc
B2	0.013 0 ± 0.002 1c	0.021 7 ± 0.001 7abc	0.0237 ± 0.002 1b	0.023 2 ± 0.003 5bc
B3	0.014 0 ± 0.003 5b	0.023 0 ± 0.003 1a	0.023 5 ± 0.001 8ab	0.024 2 ± 0.002 2ab
B4	0.014 7 ± 0.004 6b	0.023 5 ± 0.001 5a	0.024 1 ± 0.002 4ab	0.024 5 ± 0.001 8ab

3.6 Cadmium Accumulation in Aerial Parts of Orychophragmus Violaceus

It can be seen from table 7 that with the increase of EDTA concentration, the cadmium accumulation of *Orychophragmus violaceus* increased first and then decreased, and the difference between treatments was very significant ($p < 0.01$). Compared with CK1, the Cadmium accumulation in groups A1, A3 and A4 increased by 161.86%, 347.38% and 327.61% respectively. In treatment A2, namely adding EDTA with the concentration of 3.0 mmol · kg-1, the effect of Cadmium accumulation was the best. The average value of Cadmium accumulation in the above ground part was 96.21 μg · g, which was 396.62% higher than that of CK1 of 19.37 μg · g-1.

Table 7 Accumulation of Cadmium in Aerial Parts of Orychophragmus Violaceus.

Treatment	Measurements/μg · g-1
CK0	2.05 ± 0.05F
CK1	19.37 ± 0.19E
A1	50.73 ± 0.30D
A2	96.21 ± 1.301A
A3	86.67 ± 0.99B
A4	82.84 ± 1.23C

4. Conclusion

The application of EDTA in soil can effectively extract heavy metal ions, increase the absorption efficiency of plant roots for chelate formed by EDTA and heavy metal ions, thus greatly accelerating the transfer of heavy metal ions from roots to the above ground parts of the plant, and increase the accumulation of heavy metal ions in plants [11-12]. The results are consistent with those of Song Wang, Jianv Liu and other scholars [13]. EDTA application can significantly enhance ($p < 0.05$) the accumulation of cadmium in the overground part of *Orychophragmus violaceus;* the difference is that the accumulation of cadmium in the overground part of *Orychophragmus violaceus* first increases and then decreases in the test data. It is speculated that it is a protective mechanism of *Orychophragmus violaceus*; it decreases when the concentration of activated heavy metal ions in the soil increases. The root transfers heavy metal ions to the upper ground part, in order to reduce the toxicity of heavy metal ions to the upper ground part [14]. The effects of EDTA on plant heights, dry weights of the above ground parts, chlorophyll contents, root activity and malondialdehyde contents were significant ($p < 0.05$). Moderate cadmium stress, EDTA treatment or the combination of the two can improve the biomass of *Orychophragmus violaceus; Orychophragmus violaceus* can reduce the concentration of cadmium ion, EDTA or their chelates in vivo by increasing its biomass, which is consistent with the experimental results of Jingwen Wang and other scholars [15] who apply EDTA to Houttuynia cordata to treat polluted soil. The results showed that *Orychophragmus violaceus* can remedy moderate cadmium contaminated land with the help of EDTA. It is suitable for soil remediation in urban green land; moderate cadmium stress and EDTA treatment have a relatively positive impact on the growth of *Orychophragmus violaceus.*

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