Effects of Application of NTA and EDTA on Accumulation of Soil Heavy Metals in Chrysanthemum

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Abstract. In order to find out the effect of non-bio chelating agent EDTA and bio-chelating agent NTA on soil heavy metal pollution, the effects of different ratio of chelating agent NTA and EDTA on soil heavy metals (Pb, Cu and Cd), the effects of chelating on content of chlorophyll and vitamin C and the degree of soil nutrient loss were evaluated. The results showed: that the contents of Pb and Cd were the highest in the roots of Chrysanthemum in the treatment (EDTA / NTA = 2: 1). The treatment (EDTA / NTA = 1: 1) was the best chelating agent ratio for the synergistic effect, which can significantly promoted the Chrysanthemum on heavy metal Cu uptake and transport to aboveground. Chrysanthemum were inhibited by all chelating agents treatments, while the content of chlorophyll and vitamin C of the physiological indexes were reduced. In the treatment (EDTA = 1), chlorophyll SPAD, vitamin C content reached a minimum of 36 and 38mg · 100g⁻¹, respectively. The nutrient element TN in the leachate were gradually decreased with the time, and the tenth day was significantly lower than the leaching rate of the first day (p <0.05) in the treatments (NTA = 1, EDTA / NTA = 1: 2). The nutrient element TN was decreased most, reaching 51.6%, and the activation effect was decreased significantly in the fifteenth day in treatment (NTA = 1), Treatment(EDTA / NTA = 1: 1)> treatment (EDTA = 1) >treatment (EDTA / NTA = 2: 1) >treatment (EDTA / NTA = 1: 2)> treatment (NTA = 1). Treatment (EDTA / NTA = 2: 1) was recommended for the chelating agent ratio with the better physiological parameters, the more heavy metal extraction and the less nitrogen and phosphorus loss.

1. Introduction
In 2014, the Ministry of Environmental Protection released the "Bulletin of the State of Soil Pollution Survey" showing that the situation of soil heavy metal pollution in China is grim [1].

However, the availability and mobility of heavy metals in soils were low and difficult to absorb by plants, so it limits the extraction efficiency of plants [2]. Therefore, a series of artificial and natural chelating agents have been developed to increase soil heavy metal activity. For example, chelator EDTA (ethylenediaminetetraacetic acid) can significantly increase the concentration of water-soluble Cd and Pb in soil, and promote the absorption of Pb, Zn and Cd in rape, corn and wheat. However, artificial chelating agents were often difficult to biodegradable, and have a high activation of heavy metals in the soil, staying in the soil for long periods of time, increasing the risk of soil elemental infiltration [4]. In contrast, the natural chelator NTA (diethyltriacetic acid) degradation time was short and high degree of environmental friendliness. NTA biodegrades into CO₂ and NH₃ two weeks later. In addition, from the price point of view, the price of about 200 yuan per kilogram of NTA compared to the price of thousands of yuan per 100 milliliters of the natural chelating agent EDDS (ethylenediamine disuccinic acid), it was easier to promote the practical application.
Chrysanthemum as a kind of vegetables with short growth period and large planting area, although the absorption of heavy metals did not reach the requirements of hyperaccumulator, it grew fast, high enrichment of heavy metal[3]. Therefore, Chrysanthemum coronarium L was selected as the main research object in this paper. The content of Chlorophyll and vitamin C of Chrysanthemum were analyzed after different concentrations of EDTA and NTA were applied to polluted soil. Find out the effects of different ratios of EDTA and NTA on the extraction of heavy metals in soils and potential environmental risks. While ensuring the improvement of the extraction efficiency of chelating agents, reduced the utilization of chelating agents and the loss of nutrients, and the theory is provided for chelating agents to promote phytoremediation of heavy metal contaminated soils Basis and practice reference.

2. Materials and methods

2.1 Experimental Materials
The test soil was selected from farmland soil polluted by vehicle exhaust on both sides of the national road Hemuwu Road (106 ° 25'52 "E, 30 ° 9'27" N) in Chongqing. Twenty-three soil samples were collected, and the five-point mixed sampling method was used to collect the soil samples with 0-20 cm surface layer. Cool and ventilated place after natural dry, over 4mm sieve spare. 2 kg soil samples were weighed in each pot, and fast-acting urea (80 g) and potassium dihydrogen phosphate (60 g) were added to provide plants with sufficient nutrients. The soil was mixed repeatedly and loaded into basins. Sowing balance after 15 days. Soil physical and chemical properties in Table 1.

| Physical and Chemical Properties | Content | Physical and Chemical Properties | Content |
|----------------------------------|---------|----------------------------------|---------|
| pH                               | 6.87±0.17 | CEC (cmol/kg)                   | 35.7±2.3 |
| Organic matter (g/kg)            | 14.5±2.67 | Cd (mg/kg)                      | 17.9±3.1 |
| Total nitrogen content(g/kg)     | 1.86±0.01 | Cu (mg/kg)                      | 89±35   |
| Total phosphorus content(g/kg)   | 1.42±0.8  | Pb (mg/kg)                      | 613±18  |

Note: The above data sa mean ± standard deviation

Chrysanthemum coronarium L with fast growth (harvesting period of 36-50 days), heat-resistant and cold-tolerant, high biomass and high disease resistance and heavy metal tolerance were selected.

2.2 Experimental methods
2kg soil put in every pot, mixed soil before sowing and timely spraying water. Potted plants assigned to the growth of 10 consistent basil seedlings. Weigh the next day of growth, added evaporation of water 100 ~ 150mL, to ensure the growth of plants. Soil moisture remained at 20%. Make sure the experiment room temperature at 18 ~ 25 ℃, humidity control at 60% to 80%. The different ratio EDTA and NTA treatments, as shown below, with each being repeated three times.

a. Control group (CK): add deionized water
b. Treatment of EDTA (EDTA = 1): Administration of 1.6 mmol / kg (EDTA)
c. Treatment (EDTA / NTA = 1: 1): 0.4 mmol / kg (EDTA) + 0.4 mmol / kg (NTA)
d. Treatment (EDTA / NTA = 2: 1): 0.8 mmol / kg (EDTA) + 0.4 mmol / kg
e. Treatment (EDTA / NTA = 1: 2): 0.4 mmol / kg (EDTA) + 0.8 mmol / kg (NTA)
f. Treatment NTA (NTA = 1): 1.6 mmol / kg (NTA)

After 38 days of planting (10 days before harvest), biomass of Chrysanthemum was accumulated and was added EDTA and NTA at the same time. Chrysanthemum continue to grow 10 days. Then, the biomass of aboveground and root were harvested. Fresh samples were cleaned by tap water and ultrapure water, 80 °C oven dried, crushing over 100 mesh sieve.

Nutrient loss experiment
Artificial pure water was used to simulate rainwater leaching on the first day, the seventh day, the tenth day and the fifteenth day after adding chelating agent in pot experiment. Simulated the average annual rainfall of Chongqing water poured 300 mL, evenly added into the basin within 1 hour. Due to the short duration of the experimental simulation, the vegetation
cut-off and rain-period evaporation are ignored. The leachate was collected in 500 mL glass bottles and the filter paper was filtered to determine the content of nutrients TN and TP.

2.3 Determination method
Chlorophyll of Chrysanthemum: Hand-held chlorophyll meter (TYS-A, China) was used during the day.

Vitamin C of leaves: Weigh 3g of fresh leaves, the use of 2,6-dichlorophenol indophenol colorimetric method, the upper xylene extract was measured absorbance.

Content of Pb, Cu, Cd of Chrysanthemum: H₂SO₄-H₂O₂ digestion, atomic absorption method (Shimadzu-AA6800) determination.

Total nitrogen (TN) of leachate: semi-trace Kjeldahl method; total phosphorus (TP) was determined by sulfuric acid-perchloric acid digestion and molybdenum-antimony colorimetric assay.

In the experiment, plant-like shrubs and leaves GBW07602 as quality control reference material.

3. Results and Discussion

3.1 Effects of Chelating agent on the growth of Chrysanthemum
Before the application of chelating agent, Chrysanthemum sowing, budding, seedlings grow normally. The application of chelating agents with different ratio all affected the biomass of Chrysanthemum, as shown in Table 2.

Chelator application time was very important. High concentrations of chelating agents were added all at once before harvesting, since the plants have accumulated a relatively large amount of biomass and can extract more metals after a short treatment time. In this experiment, after adding chelating agents with different concentrations at a time, the edge of leaves of Chrysanthemum began to witness wilting, necrosis and black spots on the leaves to some extent. The accumulation of biomass in roots were decreased (p <0.05).

Table.2 The Chrysanthemum biomass and reduction after the application of chelating agent

| Treatments | Leaf Bioaccumulation/g | Bioreduction/ % | Root Bioaccumulation/g | Bioreduction/ % | Root/Leaf |
|------------|------------------------|-----------------|------------------------|-----------------|------------|
| CK         | 0.3746                 | 0               | 0.0718                 | 0               | 0.19       |
| E=1        | 0.2103                 | 46.72           | 0.0355                 | 51.6            | 0.17       |
| E/N=1:1    | 0.286                  | 36.51           | 0.0518                 | 30.1            | 0.18       |
| E/N=1:2    | 0.1975                 | 37.65           | 0.0324                 | 29.86           | 0.16       |
| E/N=2:1    | 0.1825                 | 33.25           | 0.0419                 | 33.42           | 0.23       |
| N=1        | 0.204                  | 29.74           | 0.0428                 | 35.62           | 0.21       |

3.2 Effects of Chelating agent on the chlorophyll and vitamin C
As can be seen from Fig 1, the Vc content of Chrysanthemum CK group without applying chelating agent was 62 mg · 100 g⁻¹. With the application of chelating agent, the physiological index of Chrysanthemum showed an overall downward trend. The content of vitamin C in Chrysanthemum reached 50mg · 100g⁻¹, which was the highest among all chelating agents added, which was 20.0% less than CK. Treatment (E=1), the content of vitamin C in Chrysanthemum reached the lowest 38mg · 100g⁻¹, 38.7% lower than the blank group.

3.3 Effects of Chelating agent on the accumulation of heavy metal in Chrysanthemum

The effects of chelating agent EDTA and NTA on soil heavy metal extraction from Chrysanthemum were shown in Fig. 2. The results showed that the contents of Pb and Pb in roots and roots were significantly changed (P <0.05) with the application of EDTA and NTA (P <0.05). When the chelating agent was not applied, the content of Pb in root of Chrysanthemum was 1151.85mg / kg. When the treatment was E/N = 2:1, the content of heavy metal Pb in root of Chrysanthemum was the highest, reaching 4421.55mg / kg, which was 3.8 times that of the control group. The highest concentration of 843.7mg / kg was 3.8 times that of the control group. The root absorption of heavy metal Pb from roots of Chrysanthemum was significantly higher than that of shoots.

Figure 2 Pb 、Cd and Cu concen of Chrysanthemum in different chelating agent ratio
Compared with no added chelating agent, the content of heavy metal Cd in root and shoot of Chrysanthemum increased with different levels of chelator. Under the treatment of chelating agent (E/N=1:1, N=1), the increase of Cd content in the plants was less than that in the treatments (E/N = 1:2) and (E/N =2:1) Cd content showed a more substantial increase. The content of Cu in Chrysanthemum increased significantly with the application of chelator, and the contents of shoots and roots changed significantly. The total Cu content of chrysanthemum with no chelating agent was only 1351.98 mg / kg, and the Cu content of the plant reached 8388.45 mg / kg at the time of E/N = 1:1, which was 6.2 times of that of the control group (E / N = 1:1), the content of Cu in shoots and roots was close, which indicated that the chelating agent with the lowest dosage and the synergistic effect was the best, which significantly promoted the absorption of heavy metal Cu and the shoot transport, Thus reducing the toxicity of heavy metal ions to plant roots and increasing the tolerance to heavy metals.

It can be seen from the above that the synergistic application of EDTA and NTA helps the plants to absorb heavy metals and transport them to the aboveground parts. On the one hand chelators activate metal ions in the soil to enhance their bioavailability; on the other hand, chelation and complexation of metal ions contribute to the formation of Pb, Cu and Cd chelates in the plant from the roots. The use of chelating agent with different proportions of treatment, the accumulation characteristics of heavy metals in Chrysanthemum Table 3 shows. After applying chelating agents with different proportions, the accumulations of aerial parts of Chrysanthemum were quite different. Among them, the accumulative rate of Pb was the highest (24.89%) on the ground (E/N = 1:2) had the highest accumulation rate of aboveground parts, accounting for 50.45% and 58.62% respectively.

Table 3 Enrichment characteristics of Heavy metal in Chrysanthemum

| Treatments | Pb (%) | Cd (%) | Cu (%) | Bioaccumulation factor (BCF) | Pb | Cd | Cu | Transfer coefficient (S/R) |
|------------|--------|--------|--------|----------------------------|----|----|----|---------------------------|
| CK         | 12.93  | 23.98  | 8.59   | 0.06 | 0.52 | 0.01 | 0.10 | 0.75 | 0.09                     |
| E=1        | 13.16  | 40.75  | 17.94  | 0.15 | 1.24 | 0.02 | 0.15 | 0.69 | 0.22                     |
| E/N=1:1   | 19.19  | 50.45  | 58.62  | 0.19 | 1.69 | 0.05 | 0.15 | 1.02 | 0.35                     |
| E/N=1:2   | 24.89  | 42.71  | 21.80  | 0.20 | 2.67 | 0.04 | 0.12 | 0.32 | 0.28                     |
| E/N=2:1   | 16.03  | 34.21  | 19.82  | 0.14 | 1.46 | 0.04 | 0.19 | 0.52 | 0.10                     |
| N=1       | 14.49  | 31.47  | 28.18  | 0.13 | 1.32 | 0.02 | 0.17 | 0.46 | 0.39                     |

Accumulation rate of heavy metals in shoots (%) = accumulation of heavy metals in shoots / accumulated weight of plants in heavy metals * 100%

Bioaccumulation Coefficient Factors (BCF) = heavy metal content above ground / heavy metal content in soil

Transport coefficient (S / R) = The content of heavy metal aboveground / The content of heavy metal in root

As can be seen from Table 3, the BCFs of Pb, Cd and Cu of Chrysanthemum significantly changed when different proportions of EDTA and NTA were applied. Bioconcentration factor (BCF) was the ratio of heavy metal concentration in plants to the concentration of heavy metals in the soil environment. It represents the accumulation trend of heavy metals in plants. The bioconcentration factors of Pb and Cd were the highest under the treatment (E/N = 1:2), which were 3.3 times and 5.1 times that of the control without adding chelating agent respectively. The bioaccumulation coefficient of Cu with no chelating agent was 0.01, and the highest bioaccumulation coefficient (E/N = 1:1) was 5.0 times of that without chelating agent.
The effects of different ratio of EDTA/NTA application on the loss of total nitrogen and total phosphorus in soil

The TP loss of the treatment (N=1, E/N = 1: 2, E/N = 2:1) decreased with the nutrient element TP in the leachate. The treatment of TP (E=1, E/N=1) continued to increase with the time. The maximum loss occurred on the seventh day and maintained at a relatively high level of TP loss. The amount of TP loss. Chelating agent EDTA was a strong ligand, in addition to activating heavy metals, while N, P and other nutrients loss occurred.

4. Conclusion
1. Adding chelating agents all inhibited the growth of Artemisia selenges, and decreased the contents of chlorophyll and vitamin C in Chrysanthemum. In the treatment (E = 1), chlorophyll SPAD, vitamin C content reached the lowest 36 and 38mg : 100g⁻¹.  
2. The chelating agent can improve the contents of Pb, Cu and Cd in roots and shoots of Chrysanthemum to different degrees. Among them, the content of Pb and Cd in the roots of Chrysanthemum was the highest when treated with E/N = 2:1. Treatment (E/N = 1:1) significantly promoted the absorption of Cu and the translocation of shoots to the plants of Chrysanthemum.  
3. Treatment (N=1, E/N = 1:2) with the passage of time, the amount of TN in the leachate decreased gradually and was significantly lower on the tenth day than that on the first day (p <0.05) On the 15th day, the TN loss decreased most (51.6%) in the treatment (N = 1), and the chelator activation was significantly reduced.

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