Fraction Transformation of Cr in *Leersia hexandra* Swartz Constructed Wetland

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Abstract. In this paper, the fraction distribution of Cr contaminated wastewater were treated by *Leersia hexandra* Swartz constructed wetlands. The results showed that with the Cr-contaminated wastewater enter the wetland, the wastewaters transformed to different forms respectively, and residuals fraction mainly were existed in the matrix and in plant bodies. The residuals present in all zones turned up at first and then decreased; the reducible fraction showed a gradual decreased in the wetland; and the weak acid extractable did not change much. The fraction of Cr in *L. hexandra* roots increased first and then decreased in different zone, of which the most obvious changes were in weak acid extractable fraction. The fraction of weak acid extractable and residue in the stems of *L. hexandra* increased first and then decreased in different zone, while the ethanol fraction decreased gradually. Residue fraction and ethanol fraction in different zone gradually decreased in the *L. hexandra* leaves, while the weak acid extractable showed the first increase and then decrease.

1. Introduction

After heavy metals got into the soil through a series of physical and chemical reactions, the formation of different chemical forms, and showed different activities. The biological toxicity of heavy metals was not only related to their total amount, but also to a greater extent determined by their morphological distribution. Different forms of heavy metal had different environmental effects, which directly affected the toxicity and migration of heavy metals and the circulation in nature. The form and transformation of heavy metals in soils was of great significance for studying the environmental effects of heavy metals and the remediation of soils contaminated by heavy metals[1-2].

Hexavalent chromium [Cr (VI)] was considered a hazardous water pollutant. Thus, the removal of Cr (VI) was necessary for the protection of the environment [3]. The two common oxidation fractions of Cr were present in the environment; i.e. Cr (III) and Cr (VI).Cr (III) was considered to be a trace element essential for the proper functioning of living organisms. It was reported to be responsible for the control of glucose and lipid metabolism in mammals [4-5]. Cr (III) was difficult to dissolve in water and considered stable in natural environment. Cr (IV) was highly water soluble and known to be highly toxic including carcinogenicity [6].

Leersia hexandra Swartz (*L. hexandra*) was grass, which grown in temperate regions, propagates very rapidly with high growth density and biomass per unit area. The plant was also known to be capable of accumulating a variety of heavy metals[7].Constructed wetlands (CWs) were engineered

biogeochemical systems that mimic the natural wetland in remediation process, where a complex system of plants, microorganisms, and soil substrate act together to treat contaminated water. Constructed wetlands had the advantages of high efficiency, low investment, low running cost, was widely used in the treatment life, a variety of types such as industrial wastewater, was developing rapidly in recent years, ecological wastewater treatment technology [8].

2. Materials and Methods

2.1. The Experimental Materials

2.1.1. Plant. The *L. hexandra* used in this study were obtained from the paddy field of Yanshan Campus of Guilin University of Technology, Guilin, China.

2.1.2. Experimental Constructed Wetland. A constructed wetland with the dimensions of 1.3 m (L) ×0.7 m (W) ×0.5 m (H) was used in the study.
The length of the wetland inflow area was 0.1 m, the length of the plant area is 0.4 m, each for the following three compartments (plant areas). Each compartment was filled with gravels in 0.1 m thickness on the bottom, then topped with 0.3 m Thick layer of red soil. Cut view of the constructed wetland was depicted in Figure 1.
Gravels, which were obtained from a construction team in Guangxi, have the particle size of 30-60 mm, and the red soil, which was collected from the campus of Guilin University of Technology, has particle size of 0.9 - 3.9 mm.
Overall layout of the CW is shown in Figure 1. As shown in Figure 1, the constructed wetlands consists of water inlet zone, three plant areas number 1, 2 and 3 and an outlet; Zone 1 was the wetland primary stage, zone 2 was the wetland middle stage and zone 3 was the wetland last stage. What flow in the CW was shown by arrow. A, B, C and D in the figure were the sampling ports for water samples designated as No.1 effluent, No. 2 effluent, No.3 effluent and the final effluent, respectively. The influent to the CW was an artificial Cr-polluted wastewater with Cr (VI) concentration of 20 mg/L.$K_2Cr_2O_7$, was used in preparation of the artificial wastewater.

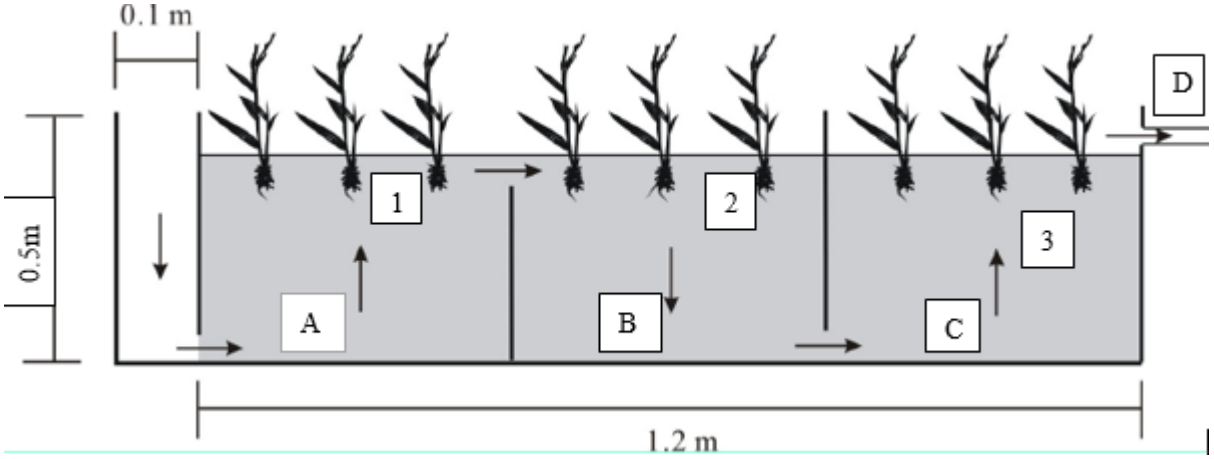

Figure 1. Schematic of wetland setup used in this study. The arrow represents direction of water flow.

2.2. Method

2.2.1. Collection and treatment of plant samples. After 180 days of normal operation of the constructed wetland, the *L. hexandra* were harvested, and the roots, stems and leaves of the plants were collected. The collected plants were repeatedly rinsed with tap water, and washed three times with ultrapure water, then sonicted for 15 min in an ultrasonic cleaner, finally washed three times with deionized water in order to remove trace amount of metal attached on the plant bodies. The washed

plant samples were divided into three parts: root, stem and leaf. Each part was deactived at 105 °C for 30 minutes and then dried to constant weight at 70 °C.

2.2.2 Collection and treatment of soil sample

Soil samples were collected in the CW for 180 days, and air-dried in a cool place for more than fifteen days until constant weight, and sieved by 100 mesh sieves.

2.3. Analysis of Cr in the Wetland Matrix

Cr extraction of matrix was constructed by the sequential BCR extraction method, and the acid extractable chromium content was determined in each sample as follows [2].

(1) 1.00 g of prepared soil sample in a cetrifuge tube (100 mL) was added with 40 mL of 0.11 M CH_3COOH, then the tubers were shaken by a reciprocal shaker at 250 rpm and at 25 °C for 16 h. The centrifuge tubes were centrifuged at 4,000 rpm for 15 min, and each supernatant was transferred to a 50 mL volumetric flack. Ultrapure water was added to bring the volume to 50.0 mL. The concentration of Cr in the diluted sample was determined by atomic absorption spectrophotometer. 20 mL of distilled water was added to the residual soil sample, shaken for 15 minutes, and centrifuged at 4,000 rpm for 15 minutes. Then, water phase was discarded,and the remaining solid sample was kept for the next analysis.

(2) Determination of chromium content in reducible (oxidation bound) state. The remaining soil samples from above procedure (1) were added with 40 mL mixture of 0.5 M hydroxylamine hydrochloride and 0.05 M HNO_3, and shaken on a reciprocal shaker at 250 rpm and at 25 °C for 16 h, and then centrifuged at 4,000 rpm for 15 min. Finally, the supernatant in the centrifuge tube was transferred to a 50 mL volumetric flask and diluted with ultrapure water to 50.0 mL. Cr concentration was determined by atomic absorption spectrometry. Residual solid sample was kept for the next analysis.

(3) Determination of chromium content in oxidation (organic combination) state. 10 mL of H_2O_2 was added to the remaining soil sample, pH was adjsuted to 2 ~ 3 with HNO_3, then the tube was left at room temperature for 1 h (with occasional stirring by a glass rod), then kept on 85 °C water bath for 1 h with a cap. Heating continued without cap until the volume was reduced to no more than 3 mL. then 10 mL of H2O2 was added to the tube for additional heating on the water bath for 1 h with cap, then without cap until the volume was reduced to 1 mL. After cooling, the mixture was added with 1.0 M ammonium acetate solution (pH = 2.0) to 50mL, shaken continuously for 16 h, and finally centrifuged to obtain binding form Cr test solution.

(4) Determination of residual state content. Used triacid nitrification to analysis and test the total amount for the after determination solution, Subtract respectively the acid extractable content, oxidation state content and organic binding state content is the residual content.

2.4. Analysis of Cr in the Wetland Plants

The chemical forms of Cr in the *L. hexandra* sample specimens grown in the constructed wetland were studied by two-step continuous extraction method.

(1) Ethanol extractable Cr fraction. 1.0 g dry plant sample was put into 100 mL centrifuge tube and added with 10 mL 80% ethanol. The prepared tube were shanken continuously for 16 h at 25 °C and at 160 rpm, then centrifuged at 4,000 rpm for 10 min. The supernatant was transferred to a 150 mL Erlenmeyer flask, and then remaining residue was added with 10 mL of ethanol extractant, shanken for 2h under the same conditions as above, then centrifuged for 10 min to obtain supernatant which was added to the Erlenmeyer flask. This procedure was repated twice or three times and all the supernatants obtained were transferred to the 150 mL Erlenmeyer flask , and heated and concentrated on a hot plate at 140 °C for 2 h. A small funnel on the Erlenmeyer flask was used for a reflux effects; 2.0 mL of concentrated HNo3 was added during the concentration. When the solution was concentrated to near dryness, the Erlenmeyer flask was removed from the hot plate and cooled to room temperature, then added with deionized water to a 25.0 ml and transferred to a cuvette for analysis.

(2) Hydrochloric acid extractable Cr fraction. The residue from procedure (1) was added with 10.0 mL of 0.6 M HCL extractant followed by the same extraction procedure as described in (1).

(3) Residue state Cr fraction. The residue of procedure (2) was digested in HNO3-H2O2 system.

The contents of heavy metals in each prepared sample were determined by ICP and atomic fluorescence spectrometry.

3. Results and Discussion

3.1. Cr matrix form Distribution in Constructed Wetlands

The distribution of Cr in various forms in the CW was shown in Figure 2. With the operation of the CW, chromium-containing wastewater entered into the wetland through the gravel area in Zone 1and flows through the CW subject to a series of functions of the wetland matrix, plants and microorganisms (Figure 1). As a result of these functions, a part of chromium in the wastewater was removed by precipitation, adsorption and absorption.

As can be seen in figure 2, the fraction of residual Cr in the CW was the highest among the four fractions investigated, which implies that the aqueous chromium in the wastewater entering the CW could be adsorbed, complexed and/or transformed into the forms other than aqueous by the mineral particles in the soil. The acid extractable fraction, reducible fraction and oxidizable fraction were all less than 20% or less of the fraction. As shown in the Figure 2, the reducible form of chromium in Zone 2 had increased. Other forms of Cr in Zones 1, 2 and 3 had basically decreased as the wastewater flowed throught the CW. The reduction of the hydrochloric acid extractable fraction of Cr is considered to be related to the change between oxidizable and reducible and environment. With the operation of chromium-bearing heavy metal wastewater in CWs, the heavy metal-containing wastewater entering the CWs changed in the matrix of constructed wetland. Except for the reducible fraction of chromium heavy metal of zone 2 had an increase than zone 1, other forms of Cr content of heavy metals were Zone 1> Zone 2> Zone 3. The reduction of the fraction of heavy metal chromium-weak acid extractable bound fraction were related to the changes of oxidizable and reducible morphological contents and the environment.

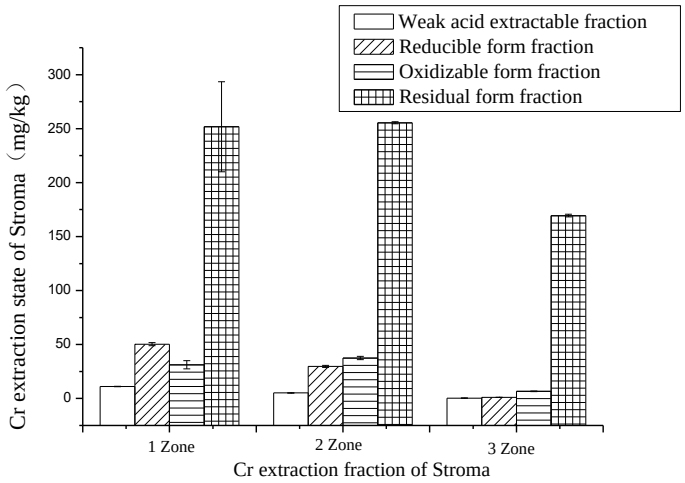

Figure 2. Distribution of Cr in the Constructed Wetland Material

3.2. The Distribution of Cr in Constructed Wetlands Plants

The distribution of Cr with in the roots of the plant grown in the CWs is shown in Figure 3 a. In the wetland, the distribution of Cr in the roots of *L. hexandra* was in the order of weak acid extractable fraction > residual fraction> ethanol fraction. Fractions of Cr in the roots ethanol, hydrochloric acid extractable, and residual forms in the CW plants increased from Zones 1 to 2 and then decreased from Zones 2 to 3, especially, changes in ethanol fractions are obvious(Figure 1 a).

The distribution of Cr in the stems of *L. hexandra* grown in the CW observed is shown in Figure 3 b. It can be seen from the figure, fraction of Cr in the stems of *L. hexandra* were the highest in Zone 2 in the three forms studied. It was also observed that Cr fraction in the stems were in the order of residual > ethanol extractable > hydrochloric acid extractable fractions in each zone. In all the forms of Cr

studied, the fraction of Cr in the stems of *L. hexandra* was highest in the residual form in Zone 2 (Figure 3 b). In the CW study with the influent of chromium contained wastewater, the Cr distribution data that Zone 2 had the higher Cr fractions than Zone 1 and 3 in the sequence. The content of residual and hydrochloric acid extractable fractions increased from Zone 1 to 2,and the decreased from Zone 2 to 3 like in Cr in the stems. While the ethanol extractable fractions decreased from Zone 1, 2 and 3 in this sequence.

The distribution of Cr in the leaves of *L. hexandra* is shown in Figure 3 c. From the figure, it can be seen that residual Cr in the leaves of *L. hexandra* grown in a zone of CW was more than that hydrochloric acid extractable and ethanol extractable fractions in the same zone. The residual fraction was the highest in Zone 1 and decreased as the wastewater flowed in Zone 2 and Zone 3. Residual form was found present in Zone 1 accounting for 88.14% of the total amount of residual form of Cr in the leaves. The residual fraction of Cr in the leaves decreased from in Zone 1 to in the Zone 2 and Zone 3. The content of total Cr form to district 57.80%, hydrochloric acid extractable form increased in Zone 2 compared with that in Zone 1indicating Cr absorbed by *L. hexandra* leaves in Zone 2 was transformed-into hydrochloric acid extractable state, while ethanol extractable content and residual form of Cr in Zone 1 decreased in Zones 2 and 3. Only hydrochloric acid extractable fraction increased in Zone 2 from Zone 1, but decreased in Zone 3.

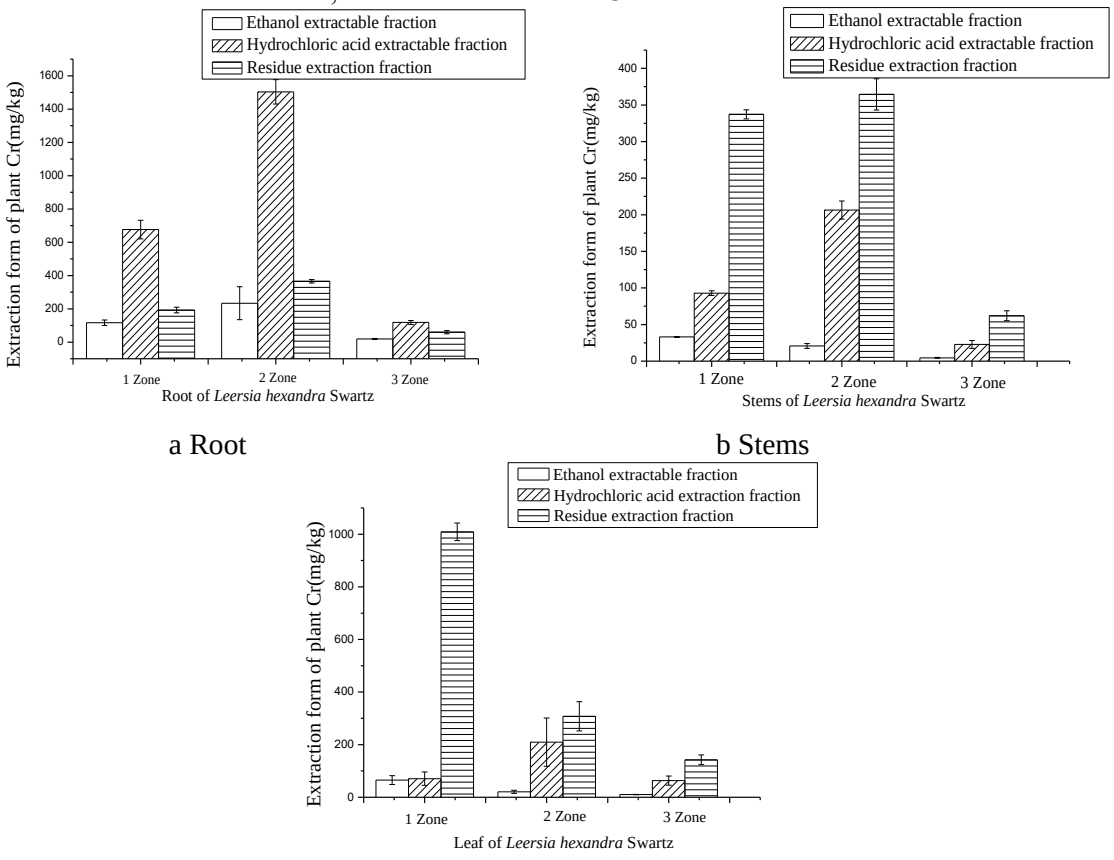

Figure 3. Distribution of Cr in the *L. hexandra* grown in the CW

It was observed in the study that Cr in the CW was mainly in residual form, followed by the reducible and oxidizable forms. These results are in agreement with the data reported by Zhang Xinyan[9] on Cr behaviors in CW. Cr contaminated wastewater into the soil, Cr reducible content with the wetland operation, its concentration decreased. The research by You Shaohong[10] showed that in the CW to maintain the stability of the system had played an important role. At the same time, it was also observed that pH, organic matter and microbial population in the CW system all affect on the accumulation of Cr in the CW matrix. It has been investigated and reported by Zhang[9] that addition

of humic acid into the CW matrix enhances significantly transfer of exchangeable and carbonate forms to organic fraction.

4. Conclusion

In the construction wetland, the residual form of Cr mainly in the CW matrix, while the presence weak acid extractable forms of Cr changes little in the CW. In the parts of *L. hexandra* grown in the CW over the period of 180 days, the hydrochloric acid extractable form of Cr in the roots is in the hightest amount, while Cr in the residual state is mainly in the stems and leaves. It is concluded that *L. hexandra* in the CW can increase Cr accumulation in the matrix and plant resulting in an enhanced removal of aqueous Cr in the influent.

5. References

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