Effects of \( \text{Cr}^{3+} \) stress on chromium chemical speciation distribution and bacterial community structure in the *Coix lacryma-jobi* L. constructed wetlands

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**ABSTRACT**

In this study, micro *Coix lacryma-jobi* L. vertical flow constructed wetlands with 0, 10 and 30 mg/L \( \text{Cr}^{3+} \) (in the form of \( K_2\text{Cr}_2\text{O}_7 \)) in the water sources were constructed to study the purification efficiency of chromium-contaminated wastewater, bacterial community diversity, chromium chemical speciation distribution and other indicators. The results showed that the constructed wetlands had high purification efficiency for chromium-contaminated wastewater. The removal rate of \( \text{Cr}^{3+} \) reached 99.61% and 95.45% in 10 and 30 mg/L \( \text{Cr}^{3+} \) treatment. The bacterial community index was unaffected by \( \text{Cr}^{3+} \) treatment; however, the bacterial richness index declined dramatically as \( \text{Cr}^{3+} \) treatment concentration increased. The bacterial community structure was influenced by the substrates pH, moisture content, organic matter and total chromium content. The abundance of *Burkholderiaceae (uncultured)* and *Saccharimonadales (uncultured)* were positively correlated with non-residual chromium content. The results contributed to understand the effect of chromium on bacterial community structure by considering the chromium chemical speciation.

**1 Introduction**

Chromium (Cr) is considered as a major environmental pollutant that is mostly found in the form of trivalent chromium \( [\text{Cr}^{3+}] \) and hexavalent chromium \( [\text{Cr}^{6+}] \) in the environment [1]. \( \text{Cr}^{6+} \) is mostly found in soil and water as \( \text{CrO}_4^{2-} \) or \( \text{Cr}_2\text{O}_7^{2-} \). \( \text{Cr}^{6+} \) is more harmful to organisms than \( \text{Cr}^{3+} \) because of its high mobility, toxicity and bioavailability [2]. The widespread use of Cr and its derivatives in numerous industrial processes, such as leather tanning, metallurgy and electroplating, is the main source of chromium contamination [3–7]. Wastewater from chromium-involved industries typically contains high Cr concentration, and the majority of these wastewaters are discharged directly into the environment without treatment, resulting in major environmental issues such as groundwater contamination, water eutrophication and soil health deterioration [8–10]. As a result, treating Cr-contaminated wastewater effectively is a concern for human health and worldwide environmental security.

The use of physicochemical technologies to remove Cr from wastewater (e.g. adsorption, membrane filtration and ion exchange) has several drawbacks, including high cost, high generation of toxic chemical sludge and susceptibility to secondary contamination [11], constructed wetland treatment technology, on the other hand, has the advantages of high efficiency, cheap cost and environmental friendliness, and can efficiently remove Cr from wastewater, attracting a lot of interest from researchers [12–14]. Plant uptake, microbial activity and substrate adsorption are all part of the Cr removal mechanism in a constructed wetland, which is a complicated physical, chemical and biological process.

During the treatment of Cr-contaminated wastewater in constructed wetlands, plants can grow in substrates with high Cr content and absorb Cr from them, accumulating Cr in the roots and above ground [15,16]. Alelu found that P. purpureum constructed wetlands could remove 99.38% of Cr from tannery wastewater, and the roots of the plants could accumulate large amounts of Cr up to 193.75 ± 36.09 mg/kg, 12.25 ± 1.77 and 20.00 ± 1.87 mg/kg for stems and leaves, respectively [17]. However, when compared to plant uptake, it has been discovered that the substrates adsorbed more Cr. For example, Sinha discovered that in *Tradescantia pallida* constructed wetlands, \( \text{Cr}^{6+} \) absorption by the substrates was the predominant channel for \( \text{Cr}^{6+} \) elimination, with 67–69% of total Cr absorbed by the substrates [18]. In constructed wetlands, the contribution of plants to Cr removal is less important than the substrates, and Cr adsorption by the substrates is an important method for the treatment of Cr-contaminated wastewater. The migration behavior of heavy metals in the environment is intimately related to their chemical morphological
transformation. According to the five-step extraction method of Tessier [19], heavy metals in soil can be classified into five forms: exchangeable (F1), carbonate (F2), Fe-Mn bound (F3), organic (F4) and residual (F5) forms. Among them, the exchangeable (F1) and carbonate (F2) are easily mobile and absorbed by microorganisms in the environment, while the Fe-Mn bound (F3) and organic (F4) can be converted from inactive to active forms and directly or indirectly bioavailable when soil physicochemical properties are changed [Huang et al., [6]]. Furthermore, the residual form is immobile in the environment and is permanently entrenched in the soil [20]. The chemical speciation transformation of heavy metals in the soil is influenced by a variety of factors, including the total content of heavy metals, soil physicochemical properties and microbial activity [21,22]. The bioavailability of heavy metals varies depending on their chemical forms. Therefore, studying the content of different forms of heavy metals will help to gain insight into heavy metal effects on soil microbial community.

Microorganisms are particularly sensitive to environmental changes and are used as sensitive indicators of environmental stress (D. Huang et al., 2017). On the one hand, heavy metal pollution impacted the metabolic and ecological functions of microorganisms, as well as altered the variety and structure of soil microbial community [23,24]. Sun found that Cr\(^{6+}\) stress suppressed most metabolic pathways and functional genes in microbial communities [25]. Deng discovered that heavy metal pollution reduced the abundance of bacteria and fungi in agricultural soil and affected community structure [26]. On the other hand, previous studies have shown that microbial community were intimately related to the chemical speciation of heavy metals in soil [27]. Somenahally discovered that As speciation transformations in As-contaminated rice fields were associated with microbial activity [28]. Hao found that changes in the bacterial community of Cd-contaminated soils were correlated with changes in Cd-speciation [29]. Most of the existing studies have focused on the direct correlation between total Cr content and microbial community in soil. Cr is the major heavy metal related to the regulation of the bacterial community. Unlike other heavy metals, Cr produces different effects in discrepant chemical forms. However, there are few studies on the correlation between the Cr chemical speciation and soil microbial community. Studying the relationship between the Cr chemical speciation and microbial community will help us understand how to reduce the biological availability and toxicity of chromium.

In this study, the micro-vertical flow constructed wetlands were built for the treatment of simulated Cr-contaminated wastewater containing different Cr\(^{6+}\) concentrations to investigate (1) the characteristics of substrates microbial community in constructed wetlands under different concentrations of Cr\(^{6+}\) stress and (2) the transformation of different Cr chemical speciation and their relationship with substrates microbial community to provide the theoretical basis for the treatment of Cr-contaminated wastewater and the bioremediation of Cr\(^{6+}\) pollution.

2 Material and methods

2.1 Experimental design

The study was carried out in 2020 at the College of Agriculture, Guangxi University, Nanning, Guangxi (southern China), which is located at N22°50′28.41″ N108°17′9.00″ E. The area has a humid subtropical monsoon climate with an average annual temperature of 22.0°C, and an average annual rainfall of 1300–2000 mm. In this paper, Coix lacryma-jobi L. is a wild wet species in Guangxi that is uncontaminated by heavy metals and tolerant of humidity and drought. Referring to the method of Li [30] to construct the micro-vertical flow constructed wetland system: a plastic bucket with a top diameter of 71.0 cm, a bottom diameter of 45.0 cm, and a height of 61.0 cm was filled with pebbles (2–5 cm in diameter) and fine sand (0–0.6 mm in size) about 10 cm high and 40 cm thick from the bottom to the top, for a total height of 50 cm.

2.2 Pot experiment

Six uniformly grown Coix lacryma-jobi L. seedlings were planted in each bucket with the uniform interval of single plants, 1/2 Hoagland nutrient solution was used as constructed wetlands intake water. When the Coix lacryma-jobi L. grew to 20 cm, 0, 10 and 30 mg/L of Cr\(^{6+}\) (in the form of K\(_2\)Cr\(_2\)O\(_7\)) were added to different water sources respectively. According to Li [30], the intermittent water intake method was adopted, and the water stayed for 3 days, and then dried for 4 days, in a 7-day cycle. The treatment lasted for 12 weeks.

2.2 Sample collection

At 84 days after Cr\(^{6+}\) treatment, plant samples were obtained. The water in the constructed wetlands was dried before the samples were taken, the plants were carefully hauled out, and the soil on the roots was gently brushed off before being returned to the laboratory. The plant samples were separated into roots, stems and leaves, cleaned with ultrapure water and swabbed dry, and the roots were soaked twice (15 min/time) in 20 mmol-L\(^{-1}\) Na\(_2\)EDTA to remove Cr adsorbed on the root surface. The cleaned plants samples were killed at 105°C for 30 minutes and then baked at 80°C until a constant weight was achieved. The dried samples were then weighed and crushed.
2.4.1 Total Cr extraction and determination

2.4.1.1 Cr content determination in plants roots, stems and leaves

According to the method of Wang [31], 0.3000 g of dried plant samples were accurately weighed and soaked overnight in nitric acid and perchloric acid (V/V = 4:1). The volume was fixed to 50 mL with 0.2% dilute nitric acid after digestion was done. A 0.45 μm water filtration membrane was used for filtering. Inductively coupled plasma emission spectrometry (ICP-5000, Beijing, China) was used to assess total Cr content, with a detection limit of 0.001–0.008 mg/L.

2.4.2 Substrates Cr contents determination

According to the method of Xiao [32] with improved procedure, 0.3000 g of matrix sample with 100 mesh sieve was weighed into a 50 mL Teflon boiling tube, 2 mL of HCl was added to soak for about 1 h, and then 3 mL of HNO₃ and HF were added separately, boiled at 130°C in a graphite boiling furnace for 3 h. After cooling down, 1 mL of HClO₄ was added and the mixture was continued to boil for 1 h. The temperature was then reduced to 120°C. Then, the lid was opened and the acid was drained out until all that is left is a drop of colorless sticky solid at the bottom of the tube; if it is yellow, distilled water was added and the acid drainage was kept. After cooling, it was adjusted to 50 mL by adding distilled water and was filtered by passing through 0.45 μm water filtration membrane. The content of chromium was determined by inductively coupled plasma atomic emission spectrometer (ICP-5000, Beijing, China).

2.5 Determination of chemical speciation of chromium in the substrates

According to the method of Tessier [19], five chemical forms were determined including exchangeable (F1), carbonate (F2), Fe-Mn bound (F3), organic (F4) and residual (F5) forms. Details of the extraction process can be found in the references [19]. The extracts obtained from each step were used for the determination of Cr content by inductively coupled plasma emission spectrometer (ICP-5000, Beijing, China).

2.6 Determination of Cr⁶⁺ content in wetlands effluent

The effluent Cr⁶⁺ content was measured by the diphenylcarbazide method [34].

2.7 Extraction of substrates DNA and analysis of microbial community structure

Substrate samples of DNA were extracted using a DNA isolation kit (FOREGENE, Chengdu, China) according to the manufacturer’s instructions. Nanodrop 2000 (Nanodrop Technologies, USA) was used to determine the concentration and absorbance of extracted DNA, and qualified and pollution-free DNA was used for subsequent steps [35].

PCR primers 338 F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806 R (5′-GGACTACHVGGGTWTCTAACT-3′) were selected to target the V3+ V4 region of the 16S rRNA gene of bacteria for microbial community analysis. The mixed DNA samples were sent to BMK Biotechnology Technology Co., Ltd. (Beijing, China) for small fragment library construction and paired-end sequencing using Illumina HiSeq 2500 (2 × 250 paired-end) sequencing system to determine the changes in bacterial communities in the substrates.

2.8 Statistical analysis

The experimental data were presented as mean ± SD. Origin 2021 and Microsoft Excel were used to analyze
and plot the data. Multiple comparisons of significant differences (P < 0.05) were performed using Duncan’s test, and calculations and statistical analyses were performed using SPSS 22.0 software.

3 Results

3.1 Cr accumulation in Coix lacryma-jobi L. and Cr distribution in different parts of the plants

The Cr contents of different parts (roots, stems and leaves) of Coix lacryma-jobi L. in constructed wetlands are shown in Table 2. Coix lacryma-jobi L. accumulated 280.94 ± 13.88, 67.05 ± 0.66 and 78.85 ± 5.88 mg/kg of Cr in roots, stems and leaves, respectively, after 30 mg/L Cr⁶⁺ treatment. Under 10 mg/L Cr⁶⁺ treatment, the Cr content of roots, stems and leaves were 91.82 ± 3.00, 3.97 ± 0.10 and 18.68 ± 0.45 mg/kg, respectively. For roots, stems and leaves, the differences between 30 mg/L Cr⁶⁺ treatment and 10 mg/L Cr⁶⁺ treatment were significant (P < 0.05). The Cr accumulation in Coix lacryma-jobi L. increased significantly with the increase of Cr⁶⁺ concentration.

The distribution of chromium in different parts of the plants is shown in Figure 1. The distribution of chromium in roots, stems and leaves under 10 mg/L Cr⁶⁺ treatment was 80.20%, 3.47% and 16.32%, respectively. The Cr accumulation rate in roots of 10 mg/L Cr⁶⁺ treatment reached 80.20%, which was much higher than that of 30 mg/L Cr⁶⁺ treatment (65.80%) and 0 mg/L Cr⁶⁺ treatment (52.44%). The Cr accumulation in roots was much greater than that in stems and leaves, indicating that roots were the main part of Cr accumulation.

As shown in Table 3, the Cr absorbed by plants was 32.65 ± 0.51 mg/kg and the Cr adsorbed by substrates was 90.77 ± 6.19 mg/kg under 10 mg/L Cr⁶⁺ treatment, whereas the Cr absorbed by plants and absorbed by substrates was 84.07 ± 3.31 mg/kg and 237.17 ± 21.15 mg/kg under 30 mg/L Cr⁶⁺ treatment, respectively. Under 10 and 30 mg/L Cr⁶⁺ treatments, the percentage of Cr absorbed by plants was 27.21% and 25.21%, respectively, of the total Cr input into the constructed wetland, whereas the Cr adsorbed by the substrates was 70.06% and 65.88%, respectively. The results indicated that the amount of Cr absorbed by plants under 10 and 30 mg/L Cr⁶⁺ treatment was substantially less than that adsorbed by the substrates.

3.2 Bacterial community diversity and richness under Cr⁶⁺ stress

To investigate the effect of Cr⁶⁺ stress on bacterial community diversity and abundance, high-throughput sequencing was performed for each treatment, generating a total of 46639–51466 sequences and obtaining 37433 high-quality sequences. As shown in Figure 2, the slope of the dilution curve of each sample was close to saturation, indicating that the sequencing depth of the samples basically met the requirements and was sufficient to reflect the vast majority of microbial species in the samples. Venn diagrams of OTU are shown in Figure 3, with a total of 2629 OTUs for each treatment, 1625, 1327 and 1180 OTUs for the 0, 10 and 30 mg/L Cr⁶⁺ treatments, respectively, and 848, 327 and 345 OTUs unique to each treatment, with a common detection of 394 OTUs.

In this study, the ACE, Chao1, Shannon and Simpson indices were used to assess bacterial diversity and richness. Table 4 shows that as the concentration of Cr⁶⁺ treatment increased, the bacterial richness index decreased, with the highest bacterial richness under the 0 mg/L Cr⁶⁺ treatment, followed by 10 mg/L treatment, and the lowest under 30 mg/L treatment. The 0 mg/L Cr⁶⁺ treatment had a greater diversity index than the other two treatments, but the difference was not significant. All treatments had a coverage index of
or above, reflecting that the sequencing results represent the real situation of microorganisms in the samples.

There were 28 phyla, 73 orders, 198 families, 198 genera in all bacterial communities. As shown in Figure 4, the dominant phyla were **Proteobacteria** (34.06%–45.31%), **Chloroflexi** (7.05%–9.82%), **Bacteroidetes** (7.67%–10.42%), **Cyanobacteria** (3.65%–16.89%) and **Actinobacteria** (3.76%–6.12%).

At the phylum level, the bacterial composition was basically similar across **Cr**<sup>6+</sup> treatments, among which the abundance of **Chloroflexi**, **Bacteroidota** and **Actinobacteria** increased with the increase of **Cr**<sup>6+</sup> treatment concentration. **Cyanobacteria** abundance was the highest under 10 mg/L **Cr**<sup>6+</sup> treatment, which was significantly higher than that under 0 and 30 mg/L **Cr**<sup>6+</sup> treatment.

**Figure 5** shows the hierarchical clustered heatmap of bacterial communities at the genus level for different **Cr**<sup>6+</sup> treatments. **Comamonadaceae (unclassified)** (4.34%–10.16%), **Sphingomonas** (2.44%–4.76%) and **Cyanobacteriales (unclassified)** (1.79%–6.08%) dominated in abundance.

At the genus level, **Comamonadaceae (unclassified)** was the most abundant genus among all treatments, with the highest abundance at 30 mg/L **Cr**<sup>6+</sup> treatment, followed by 0 mg/L, and the lowest abundance at 10 mg/L. At 10 mg/L **Cr**<sup>6+</sup> treatment, the abundance of **Sphingomonas** and **Cyanobacteriales (unclassified)** was highest, followed by 30 mg/L and 0 mg/L.

### 3.3 Physicochemical characteristics of substrates and their correlation with bacterial community structure

The physicochemical characteristics of the substrates are shown in **Table 5**. This study found that with the increase of **Cr**<sup>6+</sup> stress, the total chromium content of 0, 10 and 30 mg/L **Cr**<sup>6+</sup> treatment decreased significantly, while the substrates pH, moisture content (MC) and organic matter (OM) increased significantly (P < 0.05).
RDA analysis was carried out with the top 10 flora and environmental factors, as shown in Figure 6. The first and second axes, which might better reflect the major environmental factors impacting bacterial community, could explain 62.45% and 36.35% of the differences in bacterial community, respectively. MC had a greater effect on bacterial community structure, followed by total Cr content and OM, and substrates pH had the least effect. MC, OM and pH values were positively correlated with bacterial community, while total Cr content was negatively correlated with bacterial community.

3.4 Total Cr content and Cr chemical forms in the substrates

A five-step sequential extraction method was performed to study the fractional distribution of metals in substrates. As shown in Figure 7, the residual form (F5) dominated in all treatments, accounting for 81.69%, 77.04% and 76.12% of the total Cr content in the 0, 10 and 30 mg/L Cr$^{6+}$ treatments, respectively, and 18.31%, 22.96% and 23.88% in the non-residual forms. The Fe-Mn bound (F3) accounted for 11.13%, 15.71% and 16.70% of the total content, respectively. Under 0, 10 and 30 mg/L Cr$^{6+}$ treatments, the organic (F4) accounted for 6.31%, 3.66% and 2.78%, respectively, whereas the percentages in the exchangeable (F1) and carbonate (F2) are very low, 0.74%, 2.71% and 3.28% in the exchangeable (F1), and 0.13%, 0.88% and 1.12% in the carbonate (F2), respectively.

3.5 Relationship between Cr chemical forms in the substrates and bacterial community structure

The correlation between the genus-level bacteria in the top 50 abundance and the content of five Cr chemical forms (F1, F2, F3, F4 and F5) is shown in Figure 8. These genus-level bacteria can be divided into two groups.

In the first groups, the abundance of Rhodobacteraceae (uncultured), Geobacter, Bryobacter and Ellin6067 was positively correlated with F4 and F5 contents and negatively correlated with F1, F2 and F3 contents. Furthermore, the abundance of Anaeromyxobacter, Haliangium, Anaerolineae (uncultured) and MND1 was positively correlated with F4 content. The abundance of Blastocatellaceae (uncultured) and
Rhodocyclaceae (uncultured) was negatively correlated with F1 content.

In the second groups, Azoarcus, Nocardioides, Acidimicrobiia (uncultured), Piscinibacter, Ideonella, Inhella, Chloroflexaceae (uncultured) and env. OPS_17 (uncultured) abundance were positively correlated with F1, F2 and F3 contents and negatively correlated with F4 and F5 contents. In addition, the abundance of Burkholderiaceae (uncultured) and Saccharimonadales (uncultured) was positively correlated with F1 content, and the abundance of Chloronema was negatively correlated with F4 content. The abundance of Blastocatellaceae (uncultured) and Rhodocyclaceae (uncultured) in the first group was negatively correlated with the non-residue form content; while the abundance of Burkholderiaceae (uncultured) and Saccharimonadales (uncultured) in the second group was positively correlated with the non-residue form content.

Figure 5. Hierarchical clustered heatmap showing the relative abundance of each taxonomic genus in substrates. Changes in bacterial community compositions were depicted by the color intensity ranged from −2 to 2 (blue to red).

Table 5. The substrate physicochemical properties.

| Cr⁶⁺ treatment (mg/L) | pH      | MC (%) | OM (g/kg) | Total Cr (mg/kg) |
|----------------------|---------|--------|-----------|-----------------|
| 0                    | 6.56 ± 0.06 a | 11.03 ± 0.14 a | 8.08 ± 0.13 a | 20.17 ± 0.57 c  |
| 10                   | 6.42 ± 0.03 b | 10.16 ± 0.04 b | 6.97 ± 0.18 b | 108.07 ± 6.19 b |
| 30                   | 6.30 ± 0.06 c | 9.84 ± 0.03 c | 5.32 ± 0.31 c | 275.17 ± 21.15 a |

Different letters in the same column indicate that the differences between substrates samples are significant at p < 0.05.
Figure 6. RDA analysis of bacterial community in different substrates. Group a, b and c represents 0, 10 and 30 mg/L Cr⁶⁺ treatment, respectively.

Figure 7. The distribution of total chromium content and its occurrence state of substrates. Exchangeable chromium content (F1), carbonate bound chromium content (F2), iron manganese oxide bound chromium content (F3), organic bound chromium content (F4) and residual chromium content (F5). The data are expressed as the mean ± SD of three replications. The different letters on the bar chart represent the difference between treatments based on the Dunnett test at the 5% level (P = 0.05).
3.6 Purification effect of the *Coix lacryma-jobi* L. constructed wetlands on Cr-contaminated wastewater purification

Figure 9 shows the effluent Cr\(^{6+}\) removal rate during the operation of the constructed wetlands. At influent Cr\(^{6+}\) concentration of 10 mg/L, the Cr\(^{6+}\) removal rate of the constructed wetlands was 93.79%–99.61%, while at 30 mg/L, the removal rate was 88.18%–95.45%. The Cr\(^{6+}\) removal rate under 10 and 30 mg/L Cr\(^{6+}\) treatment decreased with the extension of Cr\(^{6+}\) treatment time.

4 Discussion

On the one hand, wetland plants can provide energy and carbon sources for organisms through root secretions, and on the other hand, they can also provide the necessary nutrients as well as a large attachment surface for microorganism growth and a sorption surface for pollutants. Previous studies have shown that heavy metals absorbed by plants are mainly concentrated in the roots when constructed wetlands are used to treat heavy metals polluted wastewater. It is consistent with the past research results, the Cr content accumulated by the roots of *Coix lacryma-jobi* L. was significantly greater than that of the stems and leaves in this study, and its accumulation accounted for 65.8%–80.2% of the total plant accumulation, indicating that the roots of *Coix lacryma-jobi* L. were the primary site of chromium accumulation [36,37]. Previous studies had shown that *Coix lacryma-jobi* L. is an ideal wetland plant for the purification of Cr-contaminated...
wastewater and has a high Cr tolerance, which is supported by the findings of this study [30]. In addition, this study also discovered that the substrates adsorbed more Cr than plants uptake and that the substrates were the most important abiotic factor impacting the effectiveness of constructed wetlands in treating Cr-contaminated wastewater, similar to previous studies [38]. This could be because Cr is less mobile in most circumstances, is easily absorbed by the substrates, and the amount of Cr that plants can absorb is restricted.

Soil physicochemical properties, such as pH and organic matter, are closely related to the Cr chemical speciation distribution and the Cr availability in the soil [39–41]. Soil pH affects Cr chemical speciation distribution in the soil, which has an impact on the Cr availability [22]. Soil organic matter was important for metal transport, transformation, etc., and its ability to change the Cr chemical speciation distribution, hence affecting the Cr availability [42,43]. The effect of moisture content on the Cr chemical speciation is mainly reflected in the solubility of the exchangeable and carbonate forms, which can be dissolved in water or soil solutions and directly or indirectly absorbed and utilized by organisms, so the moisture content also affects the Cr availability. In this study, the lower the substrates pH, water content and organic matter content in each treatment, the higher the total Cr content and nonresidual Cr content, and the greater the biological Cr availability, similar to previous studies [44]. It may be because Cr-contaminated wastewater has a harder time binding to the substrates, whereas Cr is more bioavailable, toxic and easily absorbed and used by organisms. The substrates provide a site for microbial attachment and activity, and the Cr availability in the substrates can also influence microbial growth [45].

Soil microbial community is extremely sensitive to changes in the external environment, and changes in soil physicochemical properties’ qualities can also affect microbial diversity and abundance [46,47]. Cr-tolerant bacteria can only grow in a pH range of 5.5–8.5 and cannot grow beyond that [48,49]. Microorganisms can get energy and nutrients from soil organic materials, which helps them develop [50]. Microorganisms cannot grow without water, and moisture content directly affects the growth and metabolism of microorganisms. High Cr content in soil can inhibit microbial activity and alter the microbial community structure [51]. Similar to the above studies, MC, OM and substrates pH all exhibited positive correlations with the bacterial community in this study, but total Cr content indicated a negative correlation with the bacterial community.

Environmental factors are also important factors that influence microbial community structure [52]. Several studies have found that soil pH, MC, OM and total Cr content all influence bacterial community structure, with total Cr content had the greatest effect on the change of bacterial community [53,54]. Similar to the above studies, total Cr content, substrates pH, MC and OM were the main factors affecting the bacterial community structure in this study. However, the toxicity of Cr to microorganisms depends largely on the chemical speciation in which it is present, and the total Cr content does not correctly reflect the toxicity of Cr to microorganisms [55]. Most previous studies have focused on the relationship between total Cr
content and bacterial community, with little attention has been paid to the impact of Cr chemical speciation on bacterial community structure. Microorganisms can drive Cr\textsuperscript{6+} reduction, affect Cr chemical speciation conversion, reduce the non-residual Cr content and increase the precipitation of Cr in the residual form as a way to reduce Cr toxicity, but the total Cr content remains constant during this process \cite{56}. Therefore, it is necessary to investigate the relationship between microorganisms and Cr chemical speciation distribution. In this study, more correlations were found between non-residual Cr content and genus-level bacterial abundance than residual Cr content, suggesting that non-residual Cr content is more important than residual Cr content in elucidating the interaction between the bacterial community and Cr chemical speciation. Among the top 50 genus-level bacteria in terms of abundance, the abundance of Burkholderiaceae (uncultured) and Saccharimonadales (uncultured) was positively correlated with non-residual Cr content; the abundance of Blastocatellaceae (uncultured) and Rhodocyclaceae (uncultured) was negatively correlated with non-residual Cr content. There was no significant difference in the bacterial diversity index and significant difference in the richness index among the treatments, indicating that the bacterial richness varied widely between the treatments, with the highest bacterial abundance found in the 0 mg/L treatment, followed by the 10 mg/L treatment and the lowest in the 30 mg/L treatment. The 30 mg/L Cr\textsuperscript{6+} treatment had the lowest bacterial richness and the highest non-residual Cr content, which could be due to the weaker Cr bioimmobilization by bacteria, with less Cr\textsuperscript{6+} flowing in the substrates being converted to residual Cr and more non-residual Cr, which is more toxic to bacteria. In addition, this study also found that the residual Cr content was the highest in each treatment, indicating that the majority of the Cr\textsuperscript{6+} entering the wetlands was adsorbed by the substrates and transformed to residual Cr precipitated in the substrates due to the microorganism induction. This study identified several bacterial populations associated with non-residual Cr content, which could be used as a reference for future studies on microbial remediation of Cr-contaminated wastewater.

The main purpose of constructing the Coix lacryma-jobi L. constructed wetlands is to treat Cr-contaminated wastewater, and the effectiveness of wastewater treatment is closely related to plant uptake, substrate adsorption and microbial activity \cite{57}. In this study, it was found that the Cr\textsuperscript{6+} removal rate of 10 mg/L Cr\textsuperscript{6+} treatment was higher than that of 30 mg/L Cr\textsuperscript{6+} treatment during wetland operation, most likely because the 10 mg/L Cr\textsuperscript{6+} treatment was less toxic to organisms and more suited for plant growth and microbial activity. In addition, the Cr\textsuperscript{6+} removal rate of 10 and 30 mg/L Cr\textsuperscript{6+} treatment gradually decreased with increasing treatment time, which might be due to poor Cr\textsuperscript{6+} treatment effect as plants roots limited their growth and development after accumulating large amounts of Cr. When the hexavalent Cr concentration in the influent water is between 10 and 30 mg/L, this constructed wetland has a good effect on Cr removal from wastewater. This constructed wetland can be used as a secondary treatment for the high-concentration Cr-contaminated wastewater, to meet the wastewater discharge criteria.

5 Conclusion
In this study, a significant increase in Cr content in Coix lacryma-jobi L. plants was found under 30 mg/L Cr\textsuperscript{6+} stress. The precipitation and adsorption of chromium by constructed wetland substrates were more important than plant uptake in terms of the purification effect on Cr-contaminated wastewater. Based on 16S rRNA high throughput sequencing, this study found that bacterial community displayed higher richness in 0 and 10 mg/L Cr\textsuperscript{6+} treatment than those in 30 mg/L Cr\textsuperscript{6+} treatment, while bacterial diversity was not significantly different among all treatments. Substrates pH, MC, OM and total Cr content were the key factors that had the great impact on bacterial community. More correlations between non-residual Cr and genus-level bacteria were observed. Among genus-level bacteria, the abundances of Burkholderiaceae (uncultured) and Saccharimonadales (uncultured) were positively correlated with the content of non-residual Cr; the abundances of Blastocatellaceae (uncultured) and Rhodocyclaceae (uncultured) were negatively correlated with the content of non-residual Cr. Compared with total Cr content, non-residual Cr content could impact a more important role in understanding bacterial community interaction with environments.

Disclosure statement
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