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Bioremediation of Cd-Contaminated Soil around Bauxite with Stimulants and Microorganisms

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Abstract: Heavy metal pollution in the soil around bauxite mines, especially cadmium pollution, is becoming more and more severe due to this mining becoming more frequent. Therefore, it is urgent to develop green and safe remediation technology. Biostimulants have been studied extensively, but their practical application is still challenging. In this study, the effects of humic acid (HA), glucose (GLU), and tetrasodium glutamate diacetate (GLDA), as well as their synergistic complex bacterial flora, on Cd-contaminated soil were analyzed. It has been shown that applying these three types of stimulants, individually or with complex bacterial flora, can enhance soil environment and quality. Nevertheless, the remediation efficacy of stimulants in combination with microbial communities surpasses that achieved through the use of stimulants alone. Among them, 1%GLU combined with complex bacterial flora had the best passivation effect on Cd, reducing the available Cd by 25%, followed by 0.5% GLU combined with complex bacterial flora and 0.5%HA combined with complex bacterial flora, which reduced the available Cd by 21.92% and 19.17%, respectively. The synergistic remediation method using stimulants and microorganisms can reduce the harm caused to the environment by conventional remediation methods and improve the effectiveness of soil remediation. It has broad application prospects in the field of bauxite-contaminated soil remediation.

Keywords: Cd-contaminated soil; stimulants; complex bacterial flora; bioremediation; soil enzyme activity

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1. Introduction

Bauxite mining and related industrial activities represent one of the important factors affecting the soil environment around mining areas. According to relevant data, approximately 1.5 t of bauxite slag is produced for every 1 t of alumina produced [1]. As of the end of 2022, China's cumulative inventory of bauxite slag had reached 1.6 billion tons [2]. The heavy metals in this slag will enter the surrounding soil of the mining area through natural diffusion, atmospheric sedimentation, rainwater erosion, and other channels, causing soil ecosystem imbalance [3,4]. Previous studies have shown that the soil around bauxite mines contains high concentrations of Cd, accompanied by metallic elements such as Cr and Mn [5,6]. Cd is highly toxic. Excessive Cd in soil can reduce soil quality and fertility, destroy soil microbial activity, affect crop growth and yield, and even threaten human life and health through the food chain [7,8].

At present, green and environmentally friendly biostimulant remediation technologies have been widely applied in Cd-contaminated sites [9]. Common stimulants include humic acid (HA), tetrasodium glutamate diacetate (GLDA), glucose (GLU), and so on. HA can improve the soil environment by chelating heavy metals and reducing their bioavailability

and migration in soil [10]. Gildas et al. found that humic acid can promote the transformation of Cd from an exchangeable state to a residual state [11]. GLDA can chelate with most metal ions, such as Pb, Cu, Zn, and Cd, to form water-soluble chelates, thereby activating heavy metals. Li et al. found through field experiments that GLDA can synergistically restore Cd-contaminated farmland with Alsophila spinulosa [12]. After GLU enters the soil as a stimulant, on the one hand, it can improve the soil respiration rate, increase the soil organic matter content [13], and increase the soil microbial count. On the other hand, it can promote the transformation of heavy metals from acid extractable forms to more stable forms, thereby achieving the remediation of heavy metal-contaminated soil by bauxite. Santini modified solid bauxite tailings with GLU and microorganisms, proving that using microorganisms to repair bauxite-contaminated sites is an effective remediation strategy [14]. Although these stimulants can promote the migration and transformation of heavy metals in contaminated sites to a certain extent, there are often challenges and controversies in their application due to the high cost and low treatment efficiency.

Therefore, to improve remediation efficiency, we treated Cd-contaminated soil with selected heavy metal-tolerant flora combined with stimulants. By adjusting the dosage of irritants, the remediation effect of combinations of different stimulants and complex bacterial flora on contaminated soil was analyzed. Moreover, the physicochemical properties and enzyme activity of soil were studied, and the remediation mechanism was discussed. The authors aim to provide a basis for practical applications for the remediation of contaminated soil around bauxite.

2. Materials and Methods

2.1. Source of Complex Bacterial Flora

The complex bacterial flora utilized in this study was obtained from a predominant microbial community previously selected by the research team from heavy metal-contaminated sites. (A soil suspension was prepared by mixing 10 g soil sample in 100 mL sterile water. Then, 2 mL of soil suspension was added to LB medium containing Cd. The culture was oscillated at 180 rpm at 30 °C. By gradually increasing the concentration of Cd^{2+} in the medium, the microflora was acclimated, and then the dominant microflora was obtained.) The bacterial flora was cultured in LB broth (BR, Shanghai Zhanyun Chemical Co., Ltd., Shanghai, China) containing 10 mg/L Cd. After DNA extraction, PCR amplification, fluorescence quantification, Illumina library construction, and Illumina sequencing (Biomarker Technologies, Qingdao, China), it was found that the top 5 bacterial strains at the genus level in this advantageous microbial community were *Bacillus*, *Paenibacillus*, *Delftia*, *Lysinibacillus*, and *Ligilactobacillus*, with a relative abundance of over 85%.

2.2. Preparation of Simulated Soil

A certain amount of $CdCl_3$ (AR, Shanghai Wokai Biotechnology Co., Ltd., Shanghai, China) heavy metal mother liquor was added into the tested soil and mixed well, so that the soil Cd content reached 10 mg/kg (the average concentration of Cd in the contaminated soil around bauxite). The simulated contaminated soil was aged at room temperature for 90 days. During this period, deionized water was added by weighing method every 3 days to keep the soil water content at about 60%. After natural air drying, the sample was ground through a 0.149 mm sieve and divided into several parts (each weighing 10.0 g) then set aside. The basic properties of the simulated soil are as follows (Table 1).

Table 1. The basic properties of the simulated soil.

Name	pH	Electrical Conductivity	Organic Matter	Cd
Simulated soil	6.53	1020 μs/cm	9.08 mg/kg	10.35 mg/kg

2.3. Remediation of Simulated Soil by a Single Stimulant

The simulated soil was added to 0.5% (*w*/*w*) HA (AR, Aladdin Biochemical Technology Co., Ltd., Shanghai, China), GLDA (AR, Shandong Yusuo Chemical Technology Co., Ltd., Linyi, China) and GLU (AR, Shanghai Zhanyun Chemical Co., Ltd., Shanghai, China), respectively, and the soil without stimulant was used as a blank control. Appropriate amount of sterile deionized water was added to the soil sample, stirred well, and incubated in the room for 49 days. Samples were taken every 7 days, and the samples were naturally air-dried and ground through a 0.149 mm sample screen. The pH value, electrical conductivity, organic matter content, metal occurrence form, microbial quantity and soil enzyme activity of each sample were determined. All samples were set with three sets of parallel samples.

2.4. Remediation of Simulated Soil by Combinations of Different Stimulants and Complex Bacterial Flora

This experiment consisted of 10 treatment groups (Table 2). In the distributed simulated soil, 3% of the complex bacterial flora was added; meanwhile, 0.1%, 0.5%, and 1% (*w*/*w*) of HA, GLDA, and GLU were added, respectively. The soil without complex bacterial flora and stimulant was used as blank control. Appropriate amount of sterile deionized water was added to the soil sample, stirred well, and incubated in the room for 49 days. The pH value, electrical conductivity, organic matter content, metal occurrence form, microorganism quantity, and soil enzyme activity of each sample were determined. All samples were set with three sets of parallel samples.

Table 2. Design of processing group.

Group	Processing Mode
CK	without complex bacterial flora and stimulants
T0	complex bacterial flora
T1	0.1% HA + complex bacterial flora
T2	0.5% HA + complex bacterial flora
T3	1% HA + complex bacterial flora
T4	0.1% GLU + complex bacterial flora
T5	0.5% GLU + complex bacterial flora
T6	1% GLU + complex bacterial flora
T7	0.1% GLDA + complex bacterial flora
T8	0.5% GLDA + complex bacterial flora
T9	1% GLDA + complex bacterial flora

2.5. Determination of Soil Properties

The soil moisture content, pH value, electrical conductivity, and organic matter were measured following the methods specified in the National Technical Regulations for Soil Analysis [15]. The number of soil microorganisms was determined by the plate-counting method (The number of colonies formed in each plate was recorded, and the total number of microbial colonies per gram of the original sample was calculated based on the dilution ratio). According to the method previously described by Han [16], the activities of sucrase, catalase, and urease were evaluated in this study.

Tessier continuous extraction method was used to determine the occurrence of metals in soil [17]. Different forms of Cd were extracted using different reagents and then determined by ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer, HKYT-799, Huake Yitong Analytical Instrument Co., Ltd., Beijing, China). For example, exchangeable Cd was extracted using $MgCl_2$, carbonate-bound Cd was extracted using NaAc, iron–manganese oxidation Cd was extracted using $NH_2OH{\bullet}HCl$, organic-bound Cd was extracted using HNO_3 and H_2O_2, and residual Cd was extracted using HNO_3, HCl, and $HClO_4$.

2.6. Statistical Analysis

Each set of experiments was carried out in triplicate. All the results are expressed by mean ± SD (standard deviation) values. All statistical analyses were performed by IBM SPSS Statistics 25.0 software.

3. Results and Discussion

3.1. Repairing Effect of a Single Stimulant on Soil

(1) Changes in physical and chemical properties of soil

The pH value of CK soil remained basically unchanged from day 0 to 49 (Figure 1a). The pH value of the soil in the GLDA treatment group showed a trend of first rising, then decreasing, and then rising again. This may be because GLDA is alkaline and will increase the pH value of the soil when added to the soil. At 7–28 days, GLDA stimulated soil microorganisms to produce organic acids, leading to a continuous decline in soil pH value [18]. At 28–49 days, GLDA in the soil undergoes a $-COO^- + H_2O = -COOH + OH^-$ reaction under microbial action, leading to an increase in soil pH. Since microorganisms easily use GLU for fermentation to produce acid and can be used as an electron donor to promote soil nitrification to produce H^+ [19], the soil pH value of the GLU treatment group slightly decreased from day 0 to 21. After that, the slow recovery was due to the consumption of H^+ in the mineralization process of GLU to produce carbon dioxide [20]. HA is weakly acidic as an organic acid, and functional groups such as -COOH can release H^+. Therefore, in the HA treatment group, the soil pH value slowly decreases with the increased time.

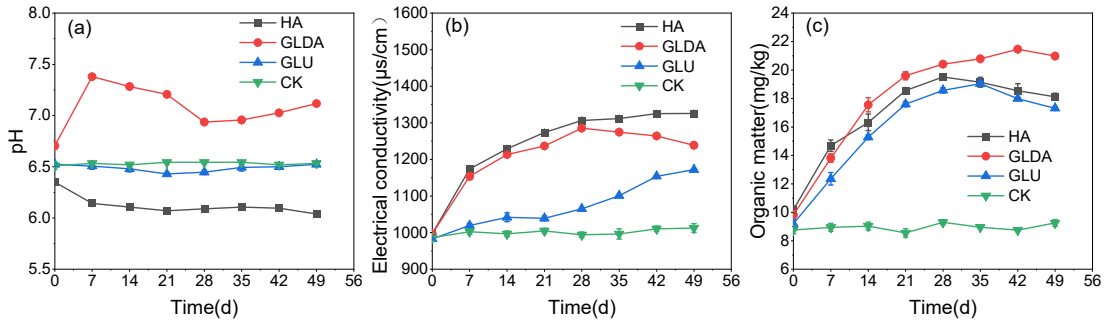

Figure 1. Changes in soil pH (**a**), electrical conductivity (**b**), and organic matter (**c**) with single stimulating agent treatment.

The soil electrical conductivity of all three treatment groups increased (Figure 1b). In the GLDA treatment group, soil electrical conductivity showed a trend of first increasing and then decreasing. After entering the soil, GLDA will analyze Cd on soil particles, activate Cd ions, and thereby increase soil electrical conductivity. Meanwhile, GLDA has biodegradability and gradually decomposes over time, resulting in a decrease in soil electrical conductivity at the later stages of the experiment [21]. In the HA treatment group, the soil electrical conductivity increased slowly and then tended to be stable. This phenomenon may be due to the deprotonation of functional groups such as the carboxyl and phenolic hydroxyl groups on humic acid, which increased the soil ion content and thus the soil electrical conductivity [6]. The soil electrical conductivity in the GLU treatment group increased slowly. This may be because GLU, as an electron donor, can promote electron transfer and improve soil electrical conductivity, but the specific reasons need further study.

The effects of three stimulators on the content of organic matter in Cd-contaminated soil are shown in Figure 1c. Compared with CK, the soil organic matter content in the treatment group was increased. At the beginning of the experiment, the organic matter content of the HA treatment group and GLDA treatment group increased; this may have been caused by HA and GLDA as organic matter. The content of organic matter in the GLU

treatment group increased, which may be due to the fact that glucose, as a single substance, is easily utilized by microorganisms, which increases the number of microorganisms, thus promoting an increase in organic matter content. In addition, humic acid is a porous substance, which helps to improve the soil aggregate structure, reduce soil organic carbon leaching, and increase the soil organic matter content [22]. When GLDA enters the soil, it may slow down the decomposition of organic matter in the soil and enable the accumulation of organic matter, which is similar to the research results of Li [18]. At the late stage of the experiment, the slight decrease in soil organic matter may be caused by the use of the stimulant by microorganisms.

(2) Transformation of soil Cd morphology

The effects of different stimulants on the chemical partitioning of Cd in soil are shown in Figure 2. From the graph, it can be seen that the chemical partitioning of Cd in CK shows little change with time. The decrease in the available state content and the increase in the stable state content indicate that the soil has a self-purification function, which can reduce the threat from Cd to the soil ecosystem, but its self-purification ability is limited. In the HA treatment group, the content of exchangeable Cd, carbonate-bound Cd, and iron–manganese oxidation Cd gradually decreased with the extension of time. In contrast, the content of other chemical partitioning of Cd gradually increased, indicating that HA can convert more active Cd into a stable state, thereby reducing the toxicity of Cd to microorganisms and the damage to the soil environment. In the GLU treatment group, the trend in the various chemical partitioning of Cd was similar to that in the HA treatment group. However, the passivation effect of HA is superior to GLU, because HA contains extremely rich functional groups, such as C=O, COOH-, or -OH, which provide a large number of binding sites for the fixation of Cd [23]. In the GLDA treatment group, the exchangeable Cd and carbonate-bound Cd content showed an increasing trend with time, while the residual Cd showed a decreasing trend. This suggests that GLDA can activate Cd and improve its bioavailability in soil. GLDA is a chelating agent with good solubility, which can react with Cd to resolve Cd from soil particles into a soil solution [24].

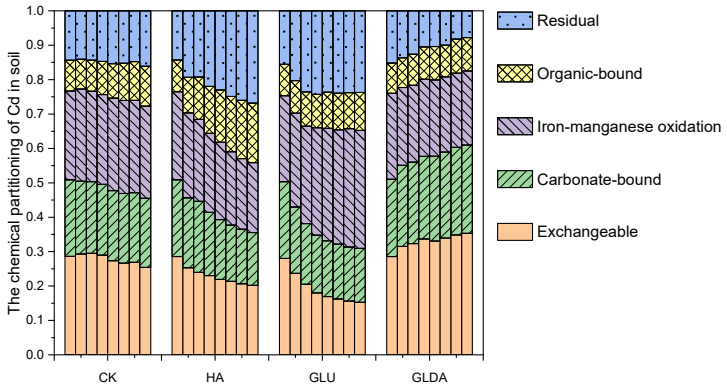

Figure 2. The chemical partitioning of Cd in soil with single stimulant treatment.

(3) Changes in soil enzyme activity

Soil enzymes are one of the components of soil and play an essential role in soil. Urease, sucrase, and catalase can catalyze the material cycle in soil, affecting soil structure and fertility. They represent one of the biological indicators of soil quality and a good indicator for evaluating heavy metal pollution. Therefore, the effects of HA, GLU, and GLDA on the activities of these three enzymes in contaminated soil were determined in this study. It can be seen from the analysis in Figure 3 that the activities of urease, sucrase, and catalase in CK were in a stable state. In the HA- and GLU-treated groups, the activity of these three enzymes increased with time. Many studies have found that soil enzyme activity is closely related to soil microorganisms [25]. The addition of stimulants improves the soil environment, promotes microbial respiration and nutrient cycling, and increases

the number of soil microorganisms. Therefore, urease, sucrase, and catalase activities were increased compared to the control group. In addition, this study found that the HA and GLU treatment groups had a better effect on improving soil enzyme activity than the GLDA treatment group. This is because the bioavailability of Cd in contaminated soil decreased after HA and GLU treatment, while GLDA treatment increased the bioavailability of Cd. The results show that Cd in soil can inhibit or inactivate enzyme activity in various ways, which can negatively affect the soil microflora. These methods often include poisoning microorganisms to reduce the generation of various enzymes, complexing with substrates, interacting with the protein active groups of enzymes, etc. [26].

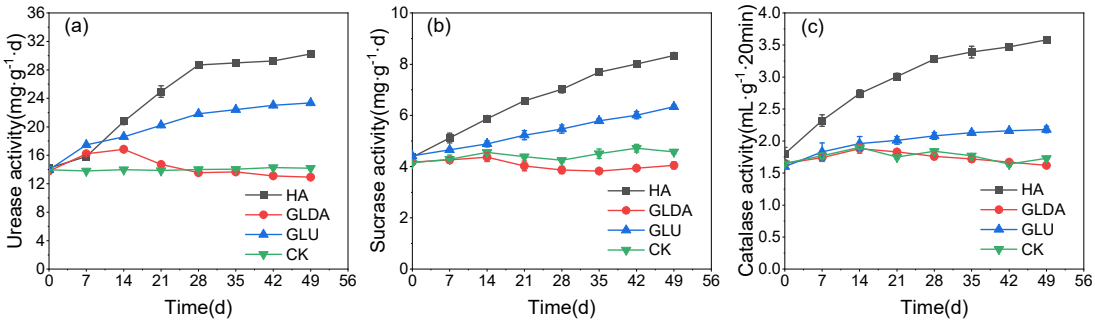

Figure 3. Changes in soil urease (**a**), sucrase (**b**), and catalase (**c**) activities with single stimulating agent treatment.

(4) Changes in soil microbial quantity

As shown in Figure 4, the number of soil microorganisms in CK remained basically unchanged. The number of soil bacteria and fungi increased in the HA and GLU treatment groups, indicating that adding stimulants can promote soil microorganisms' growth. The number of soil microorganisms in the HA treatment group was higher than in the other two treatment groups, indicating that HA had a better remediation effect on contaminated soil. HA has high surface activity and is easily adsorbed by cell membranes. When it obtains nutrient elements through complexation and chelation, these elements can be more easily absorbed and utilized by microorganisms during the adsorption process of HA [27]. Therefore, HA can promote the uptake of nutrients by microorganisms and provide more adequate nutrient support for the growth and reproduction of microorganisms. In addition, HA has the ability to optimize the soil microenvironment and create a suitable environment for the growth and reproduction of microorganisms by regulating the balance of gas phase and liquid phase substances in the soil, thus contributing to the reproduction and development of microorganisms [28]. The amount of soil bacteria and fungi in the GLDA treatment group was slightly lower than in the CK group. This may be because GLDA can activate Cd, and the increase in effective Cd content also enhances the toxic effect of Cd on microorganisms.

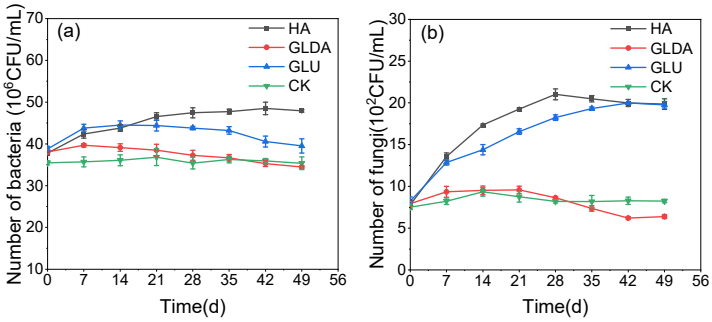

Figure 4. Changes in the number of bacteria (**a**) and fungi (**b**) in soil with single stimulator treatment.

3.2. The Effect of a Combination of Different Stimulants and Complex Bacterial Flora on Simulated Soil Remediation

(1) Changes in the physical and chemical properties of soil

Figure 5 shows the changes in pH value, electrical conductivity, and organic matter in contaminated soil with different treatments. As shown in Figure 5a, the decrease in soil pH in the T1, T2, and T3 treatment groups increased with the increase in HA addition, and there was a significant difference between T1 and the other two treatment groups ($p < 0.05$). The soil pH value was increased in the T4, T5, and T6 treatment groups, but the addition of GLU had little effect on the soil pH value, and there was no significant difference. The increase in soil pH in the T7, T8, and T9 treatment groups was increased with the increase in GLDA addition. There were significant differences between the T7 and T9 treatment groups ($p < 0.05$), while there were no significant differences between the T8 treatment group and other treatment groups. The electrical conductivity of soil can be improved by treatment with stimulant and combined bacteria (Figure 5b). In the T1, T2, and T3 treatment groups, the increase in soil electrical conductivity was 0.5% > 1% > 0.1% with different HA addition levels. In the T4, T5, and T6 treatment groups, the increase in soil electrical conductivity increased with the increase in GLU addition, and there were significant differences among different treatment levels ($p < 0.05$). In the T7, T8, and T9 treatment groups, different levels of GLDA addition resulted in an increase in soil electrical conductivity of 0.5% > 1% > 0.1%, and there was no significant difference between the T8 and T9 treatment groups. Treatment with stimulant combined with complex bacteria can also increase the content of soil organic matter (Figure 5c). In the T1, T2, and T3 treatment groups, the increase in soil organic matter increased with the increase in HA addition, and there was a significant difference between the T1 treatment group and the other two treatment groups ($p < 0.05$). In the T4, T5, and T6 treatment groups, the change of soil organic matter content was consistent with the change of electrical conductivity, and there was no significant difference between the different treatment levels. In the T7, T8, and T9 treatment groups, different levels of GLDA addition resulted in an increase in soil organic matter of 0.5% > 1% > 0.1%, and there was no significant difference between T9 and the other two treatment levels, but there was a significant difference between the T7 treatment group and the T8 treatment group ($p < 0.05$).

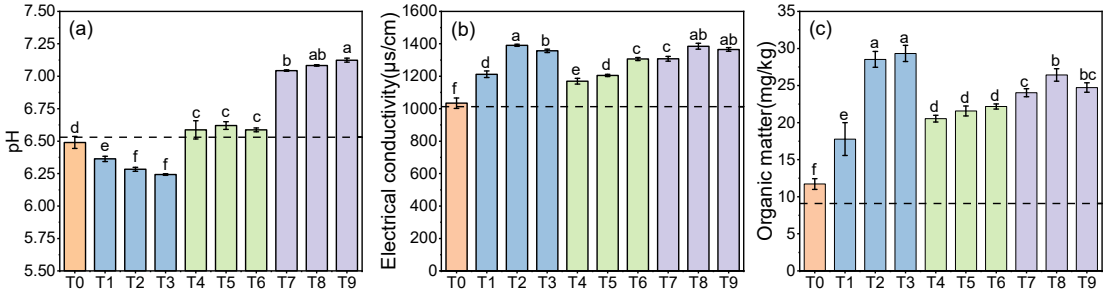

Figure 5. Changes in soil pH (**a**), electrical conductivity (**b**), and organic matter (**c**) after treatment with different stimulating agents and complex bacteria (the dashed line represents CK) (The difference is not significant if the same marked letter, and the difference is significant if the different marked letter).

In addition, compared with single-irritant treatment, the synergic combination of irritant and microbial communities could increase the soil pH, electrical conductivity, and organic matter content. This may be because the addition of stimulants can improve soil quality, provide a suitable living environment for its complex bacterial community, increase the growth level and activity of this complex bacterial community, and promote the restoration of this complex bacterial community in contaminated soil.

(2) Transformation of soil Cd morphology

The proportion of available (exchangeable and carbonate-bound) Cd in CK soil is as high as 45.52%, is easily absorbed by soil microorganisms, and leads to high risk (Figure 6). Compared with CK, the content of available Cd in soil decreased, the iron–manganese oxidation Cd changed little, and the organic-bound Cd and residual Cd increased after HA cooperative complex flora treatment. Among them, the content of available Cd in the T2 treatment group decreased by 19.17% compared with CK, and the residual Cd increased by 19%, indicating that the HA synergistic complex microbial community at this dose can reduce the bioavailability of Cd and transform soil Cd from effective to stable. The decrease in exchangeable Cd and carbonate-bound Cd in soil was greater with the addition of GLU after treatment with GLU. The content of iron–manganese oxidation Cd and residual Cd increased with the increase in GLU. In the T7, T8, and T9 treatment groups, the content of exchangeable Cd and carbonate-bound Cd in soil increased with the increase in GLDA, while the content of organic-bound Cd and residual Cd showed the opposite trend. In addition, among the treatment groups, the T6 treatment group had the best passivation effect on Cd, which could reduce the available Cd by 25%, followed by the T5 and T2 groups, which reduced the available Cd by 21.92% and 19.17%, respectively.

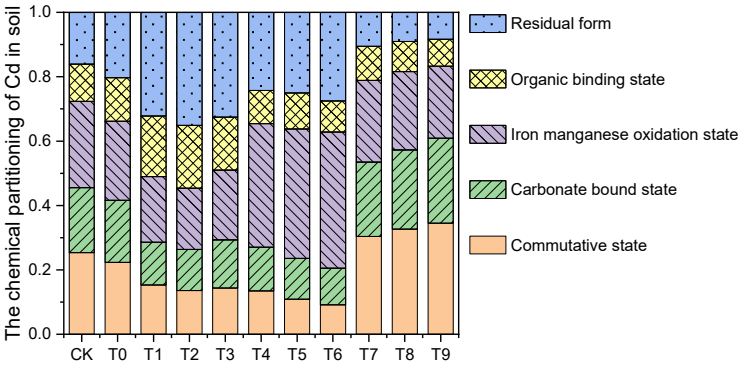

Figure 6. The chemical partitioning of Cd in soil after treatment with different stimulating agents and complex bacteria.

Both the HA synergistic complex microbial treatment group and the GLU synergistic complex microbial treatment group could passivate Cd, effectively reduce the content of available Cd, and increase the content of stable Cd in contaminated soil. In general, microorganisms in soil remove Cd from soil mainly through bioadsorption and bioaccumulation. Functional groups on the surface of bacterial cells (-COOH, -SO_3H, etc.) can fix Cd. In addition, Cd entered the bacterial cell through cation absorption mechanism, and the bacteria deposited Cd into the cell wall by modifying the cell wall–plasma membrane complex, thus achieving Cd enrichment [29]. HA and GLU, as organic compounds, increase the content of soil organic matter, promote the growth of microorganisms, make more microorganisms participate in the process of the passivation of heavy metals, and then reduce the content of exchangeable Cd in soil. Among all treatment groups, the T3 treatment group had the best passivation effect on Cd in polluted soil. This is because, compared with the GLU synergistic complex microbial treatment group, HA provides energy for soil microorganisms and utilizes its functional groups to undergo oxidation–reduction reactions with metal ions. By reducing metal ions, it becomes a stable insoluble complex, which is then fixed on the surface of irregular particles, thereby reducing the bioavailability of metal ions [30]. This is consistent with Liu's research [31]. GLDA combined with treatment with complex bacteria increased the content of available Cd in contaminated soil, indicating that GLDA has an activating effect on Cd and can convert stable Cd into an available state. Therefore, GLDA can be used as a biodegradable chelating agent for the phytoremediation of Cd-contaminated sites or as an eluent for the chemical leaching of contaminated sites.

In addition, with the same amount of stimulant, the treatment group with the addition of complex microbial communities showed a better Cd passivation effect. This is because the constructed complex flora itself has the ability to remove heavy metals. The addition of stimulants improves the flora's living environment, promotes complex flora growth, and allows more bacteria to participate in the process of passivating heavy metals in soil.

(3) Changes in soil enzyme activity

The urease activity in polluted soil was higher after HA cooperative complex bacterial flora treatment (Figure 7a). This is because HA contains N, and its addition can improve soil urease activity and promote the transformation and utilization of N by soil microorganisms. There were significant differences in soil urease activity among the T1, T2, and T3 treatment groups ($p < 0.05$). And the higher the concentration of HA, the higher the soil urease activity. In the T4, T5, and T6 treatment groups, soil urease activity increased with the increase in GLU concentration, which may be because GLU, as a carbon source, can be directly utilized by microorganisms. There was no significant difference between the T7, T8, and T9 treatment groups, indicating that the addition of GLDA and complex microbial communities had little effect on urease activity in contaminated soil.

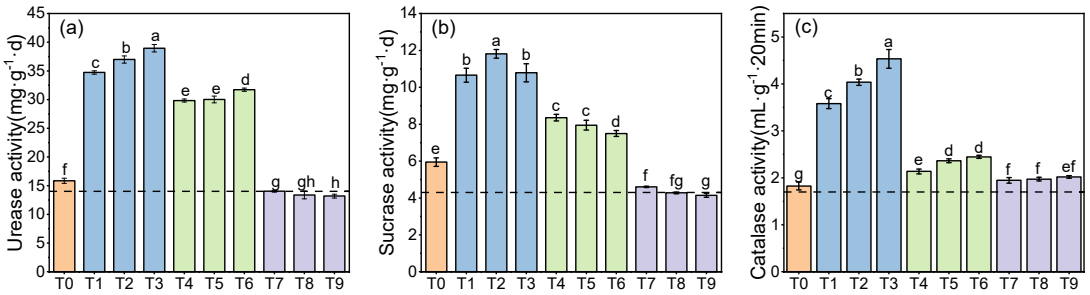

Figure 7. Changes in soil urease (**a**), sucrase (**b**), and catalase (**c**) activities after treatment with different stimulating agents and complex bacteria (the dashed line represents CK) (The difference is not significant if the same marked letter, and the difference is significant if the different marked letter).

Complex bacterial flora cooperating with three stimulants could enhance the activity of soil sucrase. However, both the HA synergistic complex microbial treatment group and the GLU synergistic complex microbial treatment group had a greater impact on the activity of soil sucrase (Figure 7b). In the T1, T2, and T3 treatment groups, the activity of soil sucrase first increased and then decreased with the increase in HA concentration. The sucrase activity in the T2 treatment group was the highest, and there was a significant difference compared with other treatment levels ($p < 0.05$). In the T4, T5, and T6 treatment groups, the soil sucrase activity decreased with the GLU concentration. There was little change in the soil sucrase activity in the T7, T8, and T9 treatment groups. In addition, compared with the single GLDA treatment, the soil sucrase activity increased after adding the complex flora, which may be attributed to the role of the complex flora.

The catalase activity in the polluted soil was significantly higher in the HA synergistic complex flora treatment group than in other treatment groups, and there was a significant difference ($p < 0.05$) (Figure 7c). One reason is that catalase is mainly derived from the secretions of soil microorganisms, and the number of soil microorganisms treated with the HA synergistic composite microbial community was higher than that in the other treatment groups. Another reason is that catalase activity is positively correlated with soil organic carbon, and the addition of HA increases the soil organic carbon content [32]. Compared with CK, the GLU synergistic microbial complex and GLDA synergistic microbial complex could enhance soil catalase activity, but the impact is relatively small.

(4) Changes in soil microbial quantity

Compared with CK, the number of bacteria, fungi, and actinomycetes in the different treatment groups all increased, and they increased with the increase in the stimulant

concentration (Table 3). This may be because the addition of irritants improved the soil environment polluted by heavy metals and promoted the growth of microorganisms. At the same time, the numbers of bacteria, fungi, and actinomycetes in all treatment groups were significantly different from those in the control group ($p < 0.05$), indicating that adding different irritants and complex bacteria to contaminated soil could increase the number of soil microorganisms and improve the quality of Cd-contaminated soil, to a certain extent. In addition, the number of bacteria, fungi, and actinomycetes in the HA cooperative complex flora treatment group was higher than in the other treatment groups, and there were significant differences ($p < 0.05$), indicating that HA cooperative complex flora treatment had a greater impact on the number of microorganisms in Cd-contaminated soil. Perhaps, this is because the addition of HA reduces the available Cd content in the soil, improves soil quality, and provides additional nutrients to microorganisms, promoting their growth and reproduction. After being treated with the GLDA synergistic compound microbial community, the number of microorganisms in the soil increased. This result indicates that the treatment provided a suitable environment for microbial growth, to a certain extent, but the amount of growth was small, which might be due to the toxic effect of Cd activated by GLDA on microorganisms.

Table 3. Changes in the number of soil microbes after treatment with different stimulating agents and complex bacteria. The difference is not significant if the same marked letter, and the difference is significant if the different marked letter.

Group	Number of Bacteria (10^6 CFU/mL)	Number of Fungi (10^2 CFU/mL)
CK	35.97 ± 1.43 e	8.85 ± 0.66 e
T0	37.01 ± 0.75 d	10.80 ± 0.53 d
T1	47.83 ± 1.29 a	19.69 ± 0.76 b
T2	48.26 ± 0.66 a	20.88 ± 0.46 ab
T3	48.71 ± 0.57 a	20.76 ± 0.83 ab
T4	42.56 ± 0.64 c	20.17 ± 0.45 ab
T5	42.92 ± 0.47 bc	20.49 ± 0.35 ab
T6	44.71 ± 0.80 b	21.21 ± 0.71 a
T7	38.00 ± 0.46 d	15.24 ± 0.29 c
T8	38.74 ± 0.39 d	15.32 ± 0.89 c
T9	39.02 ± 0.46 d	15.41 ± 0.82 c

4. Conclusions

Compared with the single-stimulant application, treatment with the stimulant combined with the bacterial community had a better effect on the remediation of contaminated soil. Following the synergistic formulation of microbial consortia with three stimulants, HA, GLU, and GLDA, soil properties were improved, and soil-related enzyme activity and microbial quantity were enhanced. The co-application of microbial consortia with either HA or GLU effectively mitigated Cd toxicity in soil, while the effect was reversed with GLDA co-application. Among them, the combination of 1%GLU and complex bacteria reduced the available cd by 25%, and the combination of 0.5%HA and complex strains reduced the available Cd by 19.17%. Additionally, combined with other indicators, in this study, the synergistic formulation of microbial consortia with HA exhibited the most effective remediation of Cd-contaminated soil, thereby facilitating the maintenance of ecological balance within soil ecosystems and having positive implications regarding the restoration of Cd-contaminated soils surrounding bauxite mines.

This study can provide a reference for the remediation of bauxite-Cd-contaminated soil, but only three irritants were studied for the remediation of contaminated soil via a synergistic combination of bacteria, and future research should focus on the remediation effects of combinations of irritants and synergistic combinations of bacteria in contaminated soil.

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