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The Effect of Microbial Compound Fertilizer on the Heavy Metal Binding Forms and Enzyme Activity in Soil

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Abstract: Nowadays, heavy metal pollution in soil caused by human production activities is increasingly serious. The heavy metal ions in soil inhibit plant growth and endanger human health as they can disrupt the physicochemical properties of soil. However, the elimination of heavy metals in soil is so difficult that more and more researchers are studying effective soil conditioners. The negatively charged groups in microbial communities can bind with heavy metal ions in the soil to remove them. In this paper, Cr- and Cd-polluted soils were used to simulate heavy-metal-polluted soil, and microbial compound fertilizer (MOF) was used as a soil conditioner for removing Cr and Cd in soil. The effects of different additive amounts of MOF on the physicochemical properties, the concentration of metal binding forms in soil and the enzyme activity of soil were investigated. The results showed that when the addition amount of fertilizer was 10%, the improvement effect on Cr- and Cd-polluted soils was the best. Compared with polluted soils without MOF addition, the physicochemical properties of MOF-treated polluted soils improved significantly, the concentration of effective forms of heavy metals decreased significantly, and the concentration of organic and residual forms as well as soil enzyme activity increased significantly. This indicates that the addition of MOF can increase the activity of soil microbial communities and soil fertility, and has the ability to remediate heavy-metal-polluted soil. MOF is expected to become an efficient soil conditioner for heavy metals.

Keywords: microbial compound fertilizer; heavy-metal-polluted soil; physicochemical properties; heavy metal binding forms; soil enzyme activity

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1. Introduction

Since the 20th century, human industrial activities such as metallurgy and chemical production, automobile exhaust emissions, soil dust, and fuel combustion [1,2] have led to heavy metals being deposited into the atmosphere [3], the water sources through irrigation [4], solid waste [5], agricultural products [6], and entering the soil. These heavy metal ions, through the natural energy cycle of substances, are absorbed by plants and then by the human body, posing a serious threat to human health. For example, Cr easily penetrates cell membranes, triggering the accumulation of reactive oxygen species within cells, which can alter cell structures and lead to diseases such as lung cancer, birth defects, and decreased reproductive capabilities [7]. Cd inhibits the normal metabolism of key enzymes in the body and causes DNA damage, potentially inducing mutations and carcinogenesis [8]. The heavy metals in soil exist in various forms with different toxicity, and their long-term accumulation damages the physicochemical properties of the soil, resulting in it being difficult to eliminate them [9]. It is reported that Cr- and Cd-polluted soils occupy a significant proportion of the total polluted soil. Cr-polluted soil accounts for more than 11% in the United States, 14% in Japan, and over 5% in China [10,11]. Among all heavy metal-polluted soils, Cd has the highest over-standard rate, which accounts for 7% in China [12]. Therefore, the improvement of heavy-metal-polluted soil has attracted widespread attention in society.

Microbial organic fertilizer contains a high amount of plant active substances and enzymes, and it is rich in organic matter and various microelements. Microbial organic fertilizer has the function of increasing soil nutrients, improving soil physical and chemical properties, enhancing crop yield and quality, and neutralizing or decomposing toxic substances [13–16]. The microorganisms can remove heavy metal in soil through interactions such as chelation, complex formation, covalent adsorption and ion exchange [17–20]. This is because the negatively charged groups in microbial communities can bind with the heavy metal ions in the surface layer of the soil [21]. There are five forms of heavy metal binding in the soil: exchangeable state, carbonate state and Fe-Mn oxide state, organic matter binding state, and residual state. The stability of the first three forms is poor, while the last two forms are very stable [22]. The damage caused by heavy metal pollution mainly comes from the unstable forms of heavy metals. Stable microorganisms can transform heavy metal ions into the latter two forms or accumulate them in their bodies, thereby reducing the concentration or toxicity of heavy metals in the soil [23]. The studies conducted by Wang et al. [24] have demonstrated that the microbial community comprising *Rhodococcus qingshengii*, which metabolizes abscisic acid, significantly contributes to the remediation of heavy-metal-polluted soil, facilitating the restoration of soil to a normal condition and markedly enhancing plant yields. Victor et al. [25] found that a composite bacteria mixture formed by mixing the *Achromobacter denitrificans*, *Klebsiella oxytoca*, and *Pseudomonas fluorescens* in a ratio of 1:1:2 can reduce the Pb content by 32%, the As content by 15%, the Hg content by 13%, the Ni content by 31%, and the Cd content by 25% in the soil. Bybin et al. [26] investigated the impact of heavy metals, including As, Pb and Cd, on the survival and biofilm formation of *Bacillus megaterium*. They discovered that these three heavy metals form strong compounds with the biofilm matrix substances and the high-molecular-weight organic compounds formed during the sporulation process of *Bacillus radicosus*. Thus, it can immobilize heavy metals, thereby increasing the resistance of bacteria to high concentrations of heavy metals, such as As, Pb, and Cd. In order to remediate Cr- and Cd-polluted soil, we take the model of Cr- and Cd-polluted soil as the research object, use microbial composite fertilizer (MOF) containing *Bacillus subtilis*, *Bacillus licheniformis*, *Streptomyces* enzyme and *Aspergillus* enzyme as a soil conditioner, and investigate the effects of different additive amounts of MOF on the physicochemical properties of soil, the concentration of heavy metal binding forms in soil, and soil enzyme activity.

2. Materials and Methods

2.1. Materials

The test soil was sourced from Caofeidian District, Tangshan City, Hebei Province, on 3 March 2023. It was an unfertilized soil with a relatively dry and loose texture. After removing stones and weeds, the test soil was air-dried in the laboratory, and the particles finer than 100 mesh were collected, mixed thoroughly, and stored in sealed containers. The urease and sucrose activity of the test soil were 0.2117 mg/g and 14.9118 mg/g, respectively. The basic physicochemical properties of test soil are shown in Table 1.

Table 1. The basic physicochemical properties of test soil.

Element	Ammonium Nitrogen (ppm)	Rapidly Available Phosphorus (ppm)	Available Potassium (ppm)	Organic Matter (g/kg)	pH	Cr (ppm)	Cd (ppm)
Content	4.71 ± 0.09 ^{f}	43.34 ± 1.11 ^{b}	17.30 ± 0.56 ^{d}	31.38 ± 1.48 ^{c}	7.72 ± 0.03 ^{e}	60.19 ± 2.18 ^{a}	0.22 ± 0.01 ^{g}

Note: Lowercase letters indicate significant differences in the physicochemical properties of soils ($p < 0.5$).

The test microbial fertilizer used MOF, which appeared as large-grained clustered black block. The urease and sucrose activity of MOF were 8.9805 mg/g and 56.7826 mg/g, respectively. The basic physicochemical properties of test soil are shown in Table 2.

Table 2. The basic physicochemical properties of microbial composite fertilizer (MOF).

Element	Ammonium Nitrogen (ppm)	Rapidly Available Phosphorus (ppm)	Available Potassium (ppm)	Organic Matter (g/kg)	pH	Cr (ppm)	Cd (ppm)
Content	98.73 ± 4.41 ^{c}	363.26 ± 8.98 ^{b}	1482 ± 86.53 ^{a}	372.07 ± 18.62 ^{b}	7.52 ± 0.03 ^{d}	79.35 ± 5.00 ^{c}	0.34 ± 0.01 ^{d}

Note: Lowercase letters indicate significant differences in the physical and chemical properties of soils ($p < 0.5$).

2.2. Instruments

A pH meter, model PHSJ-3F (INESA Scientific Instrument Co., Ltd., Shanghai, China), was used to measure the pH value of soil samples. A soil nutrient tester, model TPY-6ZD (Zhejiang Topu Yunnong Technology Co., Ltd., Hangzhou, China), was used to analyze the contents of ammonium nitrogen, rapidly available phosphorus and available potassium in soil samples. A flame atomic absorption spectrophotometer, model TAS-990 (Beijing General Instrument Co., Ltd., Beijing, China), was used to measure the content of Cr and Cd in Cr- and Cd-polluted soil samples.

2.3. Preparation of Heavy-Metal-Polluted (Cr and Cd) Soil Model

The nitric acid chromium solution and nitric acid cadmium solution were, respectively, added to 150 g of test soil at concentrations of 360 mg Cr/150 g soil and 54 mg Cd/150 g soil, which exceeded by two times the national standard value of heavy metals [27]. The soils containing Cr and Cd were covered with plastic films for 20 days to form a Cr- and Cd-polluted soil model. Starting from the 8th day, the polluted soil samples were collected every 5 days by a five-point sampling method, then air-dried, ground, and sifted to collect soil particles finer than 100 mesh for determining the concentration of five heavy metal binding forms in the Cr- and Cd-polluted soil.

2.4. Soil Amendment Experiment

Briefly, 0 g, 1.5 g, 4.5 g, 7.5 g, and 15 g of MOF were, respectively, added into five portions of 150 g heavy-metal-polluted soil. Deionized water was used to maintain the soil moisture in the containers at 50% of saturation, and the containers were sealed with plastic film and left for 20 days. Subsequently, the basic physicochemical properties, concentration of heavy metal binding forms, and soil enzyme activity of the amended soil were determined under different concentrations of MOF.

2.5. Determination of Basic Physicochemical Properties of Soil and MOF

The basic physicochemical properties of soil and MOF include the pH value and the content of organic matter, ammonium nitrogen, available phosphorus, available potassium and heavy metal (Cr and Cd). The soil pH value was determined by using the potentiometric method [28], the content of organic matter was determined by using the ignition method [29], and the content of ammonium nitrogen, available phosphorus, available potassium was determined by using a soil nutrient analyzer.

2.6. Determination of the Contents of Heavy Metal Binding Forms in Soil

According to the Tessier five-step extraction method [30,31], the content of heavy metal binding forms in the soil, including exchangeable state, carbonate bound state, Fe-Mn oxide state, organic matter binding state and residual state, was sequentially and chemically extracted and determined by using flame atomic absorption spectrophotometry [32]. The total Cr or Cd content in polluted soil samples is the sum of the five individual fractions' contents.

2.6.1. Exchangeable State

In addition, 1 g of soil sample was added to a centrifuge tube, and 8 mL of $MgCl_2$ $6H_2O$ (1 mol/L) was added into the tube. The mixture was oscillated for 1 h at 25 ± 2 °C (200 r/min), then centrifuged for 10 min (4000 r/min). The supernatant was filtered through a 0.22 μm microfiltration membrane and then diluted to 50 mL, which was filtered again. The filtrate was used to measure the content of exchangeable state by using flame atomic absorption spectrophotometry.

2.6.2. Carbonate-Bound State

At 25 ± 2 °C, 8 mL of sodium acetate (1 mol/L) was used to extract the carbonate bound state from the residue obtained from the previous step. The pH value of solution was adjusted to 5.0 with acetic acid, which was then oscillated for 8 h (200 r/min), and centrifuged for 10 min (4000 r/min). The supernatant was filtered through a 0.22 μm microfiltration membrane and diluted to 50 mL. Then, the diluent was used to measure the content of carbonate-bound state by using flame atomic absorption spectrophotometry.

2.6.3. Fe-Mn Oxide State

In order to extract the Fe-Mn oxide, 20 mL of acetic acid solution containing 0.04 mol/L hydroxylamine hydrochloride was added into the residue obtained from the previous step. The extraction step was performed at 96 ± 3 °C for 4 h. Then, the extractive was centrifugated for 10 min (4000 r/min). The supernatant was filtered through a 0.22 μm microfiltration membrane and diluted to 50 mL. Then, the diluent was used to measure the content of Fe-Mn oxide state by using flame atomic absorption spectrophotometry.

2.6.4. Organic Matter Binding State

Furthermore, 3 mL of HNO_3 (0.02 mol/L) and 5 mL of H_2O_2 (30%) were added into the residue obtained from the previous step sequentially. The pH value of the mixture solution was adjusted to 2 by using HNO_3. Then, it was heated at 85 ± 2 °C for 2 h and oscillated once every 30 min during the period. After adjusting the pH value of the mixture to 2 again with 5 mL of H_2O_2, the mixture was heated at 85 ± 2 °C for 3 h and oscillated once every 30 min during the period again. After cooling to room temperature, 5 mL of NH_4Ac (3.2 mol/L) and 20 mL nitric acid solution (20%, v/v) were added into the mixture to 20 mL. The diluent was oscillated for 30 min (210 r/min), and centrifuged for 10 min (4000 r/min). The supernatant was filtered through a 0.22 μm microfiltration membrane and diluted with deionized water to 50 mL. Then, the diluent was used to measure the content of organic matter binding state by using flame atomic absorption spectrophotometry.

2.6.5. Residual State

Firstly, the total content of Cr or Cd in a 1 g soil sample was measured, and then the contents of the four forms mentioned above were successively subtracted from the total content of Cr and Cd to obtain the residual state content of Cr or Cd.

2.7. Determination of Urease Activity of Soil

Soil samples were collected using a five-point sampling method from Cr- and Cd-polluted soil, which were then thoroughly mixed. The urease activities of soil and MOF were determined by using the phenol-sodium hypochlorite colorimetric method [33].

2.8. Determination of Sucrase Activity of Soil

Soil samples were collected using a five-point sampling method from Cr- and Cd-polluted soil, which were then thoroughly mixed. The sucrase activity of soil and MOF was determined by using the 3,5-dinitrosalicylic acid colorimetric method [34].

2.9. Statistical Analysis

All data represent the mean values of three parallel experiments and are expressed as mean ± standard deviation to indicate normal distribution. The SPSS 26.0 statistical package (IBM Corp., Armonk, NY, USA) was utilized to conduct one-way analysis of variance with Duncan's multiple range test ($p < 0.05$) on these data.

3. Results and Analysis

3.1. Effects of MOF on the Physicochemical Properties of Cr- and Cd-Polluted Soil

3.1.1. pH Value of Soil

The pH value of soil affects the bio-availability, the migration of heavy metals in soil–plant systems, chemical reactions in soil and the remediation effects of polluted soil. Therefore, the pH value of soil is crucial for the growth and development of plants. We investigated the effect of the additive amount of MOF on the pH values of soils polluted by Cr and Cd. The results are shown in Table 3. From Table 3, it can be observed that adding MOF can significantly reduce the pH value of Cr- and Cd-polluted soil ($p < 0.05$), compared with not adding MOF. As the additive amount of MOF gradually increased from 0 to 10%, the pH values of both Cr- and Cd-polluted soils gradually decreased, approaching 7. The reduction rate of pH values in the Cr-polluted soil increased from 1.47% to 3.73%, while in Cd-polluted soils, it increased from 1.17% to 5.34%. This indicates that MOF has the ability to adjust the pH value of soil towards neutrality (pH = 7). When the additive amount of MOF reached 10%, the pH values of both Cr- and Cd-polluted soils decreased the most, by 3.73% and 5.34%, respectively. Therefore, the optimal additive amount of MOF for improving the pH value of soil is 10%.

Table 3. Influence of additive amount of microbial composite fertilizer (MOF) on the physicochemical properties of Cr- and Cd-polluted soil.

Heavy-Metal-Polluted Soil	Amount of MOF (%)	pH	Organic Matter (g/kg)	Ammonium Nitrogen (ppm)	Rapidly Available Phosphorus (ppm)	Available Potassium (ppm)
Cr	0	7.50 ± 0.12 ^{a}	26.56 ± 1.16 ^{e}	23.55 ± 0.82 ^{e}	18.21 ± 0.87 ^{d}	123.2 ± 6.09 ^{d}
	1	7.39 ± 0.05 ^{a,b}	32.66 ± 1.81 ^{d}	28.56 ± 1.17 ^{d}	22.53 ± 0.44 ^{c}	135.66 ± 6.26 ^{c,d}
	3	7.43 ± 0.12 ^{a,b}	51.49 ± 2.59 ^{c}	32.22 ± 1.21 ^{c}	22.86 ± 0.61 ^{c}	143.77 ± 7.35 ^{b,c}
	5	7.28 ± 0.03 ^{b,c}	66.33 ± 2.95 ^{b}	39.97 ± 1.53 ^{b}	26.42 ± 1.04 ^{b}	152.68 ± 7.49 ^{b}
	10	7.22 ± 0.04 ^{c}	85.63 ± 3.75 ^{a}	48.73 ± 2.14 ^{a}	31.13 ± 1.25 ^{a}	169.52 ± 10.15 ^{a}
Cd	0	7.68 ± 0.14 ^{a}	27.14 ± 1.34 ^{e}	14.19 ± 0.57 ^{e}	11.55 ± 0.37 ^{e}	82.32 ± 3.70 ^{d}
	1	7.59 ± 0.10 ^{a,b}	35.16 ± 1.97 ^{d}	19.89 ± 1.03 ^{d}	14.56 ± 0.55 ^{d}	98.97 ± 4.88 ^{c}
	3	7.47 ± 0.11 ^{b,c}	55.62 ± 2.97 ^{c}	27.26 ± 1.33 ^{c}	17.82 ± 0.83 ^{c}	107.32 ± 4.96 ^{b,c}
	5	7.33 ± 0.05 ^{c,d}	72.33 ± 3.10 ^{b}	33.18 ± 1.88 ^{b}	21.66 ± 1.06 ^{b}	111.43 ± 6.02 ^{a,b}
	10	7.27 ± 0.02 ^{d}	92.33 ± 4.20 ^{a}	40.88 ± 2.05 ^{a}	25.52 ± 1.23 ^{a}	119.97 ± 5.33 ^{a}

Note: Lowercase letters indicate significant differences in the physical and chemical properties of soils ($p < 0.5$).

3.1.2. Organic Matter Content

The influence of varying MOF additive amounts on the organic matter content of the two types of polluted soils was investigated. The results are listed in Table 3. As detailed in Table 3, the organic matter contents of MOF-treated Cr- and Cd-polluted soils were significantly higher than that of the untreated soils ($p < 0.05$). It is evident that the organic matter content in both soil types increased progressively and significantly with an increasing MOF additive amount. Specifically, for Cr-polluted soil, the growth rates of organic matter content increased from 22.97% to 222.40%, whereas for Cd-polluted soil, it rose from 29.55% to 240.20%. This suggests that MOF is capable of enhancing soil fertility in both soil types, with a more profound effect observed in Cd-polluted soil. When the additive amount of MOF reached 10%, the organic matter content in the Cr- and Cd-polluted soils reached its peak, increasing by 222.40% and 240.20%, respectively. Therefore, it is concluded that the optimal MOF additive amount for maximizing soil organic matter content is 10%.

3.1.3. Ammonium Nitrogen Content

The impact of the additive amount of MOF on the ammonium nitrogen content of the two types of polluted soils was investigated. The results are presented in Table 3. As shown in Table 3, adding MOF can significantly increase the ammonium nitrogen content of Cr- and Cd-polluted soils ($p < 0.05$) compared with not adding MOF. With the increase in the additive amount of MOF, the ammonium nitrogen content in Cr- and Cd-polluted soils gradually increased. The growth rates of ammonium nitrogen content in Cr- and Cd-polluted soils increased from 21.27% to 106.92% and from 40.17% to 188.09%, respectively. It is evident that the growth rate of ammonium nitrogen content in Cd-polluted soil was significantly higher than that in Cr-polluted soil. This indicates that the ammonium nitrogen content of polluted soils significantly increases with the increase in additive amount of MOF, especially in Cd-polluted soil. It can be concluded that MOF can enhance soil fertility, with a stronger effect observed in Cd-polluted soil. When the additive amount of MOF reached 10%, the ammonium nitrogen content in Cr- and Cd-polluted soils increased the most, by 106.92% and 188.09%, respectively. Therefore, the optimal additive amount of MOF for improving the ammonium nitrogen content soil is 10%.

3.1.4. Rapidly Available Phosphorus Content

The effect of the additive amount of MOF on the rapidly available phosphorus content of the two types of polluted soils was investigated, and the results are shown in Table 3. As depicted in Table 3, the rapidly available phosphorus content of MOF-treated Cr- and Cd-polluted soils was significantly higher than that of the untreated soils ($p < 0.05$). The rapidly available phosphorus content in Cr- and Cd-polluted soils increased with the increase in the additive amount of MOF. The growth rates of rapidly available phosphorus content in Cr- and Cd-polluted soils ranged from 23.72% to 70.95% and from 26.06% to 120.95%, respectively. It can be observed that the rapidly available phosphorus content in both types of Cr- and Cd-polluted soils increased with the increase in the additive amount of MOF. This indicates that MOF has the ability to increase the fertility of both types of polluted soils. Additionally, the growth rate of available phosphorus content in Cd-polluted soil was significantly higher than that in Cr-polluted soil. It is suggested that MOF has a stronger effect in increasing the fertility of Cd-polluted soil compared to that of Cr-polluted soil. When the additive amount of MOF reached 10%, the rapidly available phosphorus content of Cr- and Cd-polluted soils increased the most, by 70.95% and 120.95%, respectively. Therefore, the optimal additive amount of MOF for improving the rapidly available phosphorus content of soil is 10%.

3.1.5. Available Potassium Content

The impact of additive amount of MOF on the available potassium content of the two types of polluted soils was investigated. The results are shown in Table 3. The growth rates of available potassium content in Cr- and Cd-polluted soils increased from 10.11% to 37.60% and 20.23% to 45.74%, respectively. From Table 3, it can be observed that adding MOF can significantly increase the available potassium content of Cr- and Cd-polluted soils ($p < 0.05$), compared with not adding MOF. With the increase in MOF concentration, the available potassium content in both types of polluted soils gradually increased with the increase in additive amount of MOF. Additionally, the available potassium content of Cd-polluted soil was significantly higher than that in Cr-polluted soil. This indicates that MOF has the ability to increase soil fertility, and its effect is stronger on Cd-polluted soil than Cr-polluted soil. When the MOF concentration reached 10%, the available potassium content in Cr- and Cd-polluted soils increased the most, by 37.60% and 45.74%, respectively. Therefore, the optimal additive amount of MOF for improving the available potassium content of soil is 10%.

3.2. Effect of MOF on the Heavy Metal Binding Forms of Cr- and Cd-Polluted Soils

Samples were taken from the Cr- and Cd-polluted soils after adding MOF on the 5th, 10th, and 15th days. These samples were used to measure the concentrations of five heavy metal binding forms such as exchangeable state, carbonate-bound state, Fe-Mn oxide state, organic matter binding state, and residual state.

3.2.1. Exchangeable State

The changes in the concentrations of exchangeable states in Cr- and Cd-polluted soils after adding MOF for the 5th, 10th, 15th day with different concentrations of MOF are shown in Table 4. From Table 4, it can be seen that compared with the treatment without MOF, adding MOF can significantly reduce the exchangeable content of heavy metals in Cr- and Cd-polluted soil, with Cd showing a more significant reduction ($p < 0.05$). With increasing time after adding MOF and increasing the additive amount of MOF, the concentrations of exchangeable state in Cr- and Cd-polluted soils gradually decrease, increasing the stability of Cr- and Cd-polluted soils. This indicates that MOF can reduce the concentration of exchangeable state in Cr- and Cd-polluted soils. When the MOF concentration is 10%, the reduction in exchangeable state content is the highest in Cr- and Cd-polluted soil, with reductions of 2.54% and 29.53%, respectively. Therefore, the optimal concentration of MOF for reducing the exchangeable state content in Cr- and Cd-polluted soil is 10%.

Table 4. Effect of additive amount of microbial composite fertilizer (MOF) on the concentration of five heavy metal binding forms in Cr- and Cd-polluted soils for the 5th, 10th and 15th day.

Heavy-Metal-Polluted Soil	**Time (Day)**	**Amount of MOF (%)**	**Concentration (mg/kg)**				
			Exchangeable State	**Carbonate-Bound State**	**Fe-Mn Oxide State**	**Organic Matter Binding State**	**Residual State**
Cr	0	0	9.84 ± 0.40^{a}	13.62 ± 0.48^{a}	53.44 ± 1.77^{a}	6.52 ± 0.02^{a}	8.62 ± 0.77^{a}
	5	0	9.89 ± 0.37^{a}	13.70 ± 0.51^{a}	53.74 ± 1.81^{a}	6.55 ± 0.04^{e}	8.50 ± 0.74^{d}
		1	9.92 ± 0.43^{a}	12.52 ± 0.46^{b}	$52.91 \pm 1.60^{a,b}$	8.75 ± 0.06^{d}	$9.26 \pm 0.05^{c,d}$
		3	9.84 ± 0.42^{a}	10.62 ± 0.10^{c}	$50.81 \pm 1.22^{b,c}$	9.12 ± 0.07^{c}	10.09 ± 0.09^{c}
		5	9.79 ± 0.33^{a}	9.57 ± 0.09^{d}	48.97 ± 1.09^{c}	9.34 ± 0.08^{b}	12.79 ± 0.49^{b}
		10	9.77 ± 0.29^{a}	8.45 ± 0.06^{e}	45.29 ± 0.91^{d}	9.53 ± 0.10^{a}	13.98 ± 0.53^{a}
	10	0	9.79 ± 0.28^{a}	13.92 ± 0.54^{a}	55.94 ± 1.82^{a}	6.18 ± 0.03^{d}	8.47 ± 0.06^{e}
		1	9.67 ± 0.19^{a}	12.11 ± 0.39^{b}	51.25 ± 1.53^{b}	9.97 ± 0.47^{c}	9.38 ± 0.08^{d}
		3	9.69 ± 0.16^{a}	10.11 ± 0.15^{c}	$49.06 \pm 1.32^{b,c}$	11.30 ± 0.17^{b}	11.25 ± 0.20^{c}
		5	9.69 ± 0.19^{a}	9.08 ± 0.12^{d}	48.43 ± 1.11^{c}	11.26 ± 0.23^{b}	14.00 ± 0.63^{b}
		10	9.61 ± 0.10^{a}	8.03 ± 0.06^{e}	43.08 ± 0.88^{d}	12.86 ± 0.42^{a}	15.97 ± 0.88^{a}
	15	0	9.97 ± 0.45^{a}	14.26 ± 0.61^{a}	57.29 ± 1.91^{a}	6.04 ± 0.04^{e}	8.33 ± 0.04^{e}
		1	9.88 ± 0.44^{a}	12.18 ± 0.42^{b}	48.00 ± 1.03^{b}	9.67 ± 0.20^{d}	9.49 ± 0.14^{d}
		3	9.75 ± 0.24^{a}	10.16 ± 0.16^{c}	45.04 ± 0.88^{c}	11.27 ± 0.28^{c}	11.90 ± 0.26^{c}
		5	9.66 ± 0.21^{a}	9.23 ± 0.12^{d}	40.77 ± 0.74^{d}	12.37 ± 0.45^{b}	14.75 ± 0.48^{b}
		10	9.59 ± 0.16^{a}	7.18 ± 0.03^{e}	33.04 ± 0.61^{e}	14.25 ± 0.52^{a}	17.89 ± 0.60^{a}
Cd	0	0	12.26 ± 0.35^{a}	2.65 ± 0.08^{a}	2.07 ± 0.03^{a}	0.11 ± 0.03^{a}	2.53 ± 0.08^{a}
	5	0	12.71 ± 0.39^{a}	2.05 ± 0.03^{a}	2.14 ± 0.05^{b}	0.06 ± 0.01^{d}	2.52 ± 0.08^{d}
		1	11.74 ± 0.35^{b}	2.05 ± 0.02^{a}	2.26 ± 0.06^{b}	0.09 ± 0.01^{c}	$2.58 \pm 0.10^{c,d}$
		3	11.37 ± 0.31^{b}	1.53 ± 0.01^{b}	2.81 ± 0.09^{a}	0.14 ± 0.02^{b}	$2.73 \pm 0.11^{b,c}$
		5	10.03 ± 0.24^{c}	1.29 ± 0.02^{c}	2.84 ± 0.08^{a}	0.25 ± 0.02^{a}	2.84 ± 0.10^{b}
		10	10.25 ± 0.29^{c}	1.11 ± 0.02^{d}	2.87 ± 0.11^{a}	0.27 ± 0.01^{a}	3.22 ± 0.11^{a}
	10	0	12.90 ± 0.42^{a}	1.47 ± 0.04^{a}	1.98 ± 0.06^{a}	0.06 ± 0.00^{a}	2.50 ± 0.11^{a}
		1	10.93 ± 0.26^{b}	1.38 ± 0.06^{b}	2.18 ± 0.09^{b}	0.09 ± 0.03^{a}	2.65 ± 0.10^{b}
		3	$10.47 \pm 0.20^{b,c}$	1.27 ± 0.02^{c}	2.24 ± 0.07^{c}	0.11 ± 0.01^{b}	2.64 ± 0.08^{c}
		5	10.31 ± 0.19^{c}	0.71 ± 0.01^{d}	2.59 ± 0.06^{c}	$0.26 \pm 0.03^{b,c}$	2.98 ± 0.12^{c}
		10	8.52 ± 0.12^{d}	0.64 ± 0.04^{e}	2.98 ± 0.10^{d}	0.27 ± 0.02^{c}	3.56 ± 0.14^{c}
	15	0	13.42 ± 0.49^{a}	1.04 ± 0.03^{a}	1.74 ± 0.03^{c}	0.06 ± 0.01^{c}	2.47 ± 0.09^{e}
		1	10.11 ± 0.15^{b}	$1.00 \pm 0.01^{a,b}$	2.92 ± 0.08^{b}	0.09 ± 0.01^{c}	2.77 ± 0.12^{d}
		3	9.54 ± 0.13^{c}	0.98 ± 0.03^{b}	3.04 ± 0.09^{b}	0.16 ± 0.03^{b}	3.02 ± 0.13^{c}
		5	9.36 ± 0.08^{c}	0.85 ± 0.04^{c}	3.04 ± 0.10^{b}	0.28 ± 0.03^{a}	3.35 ± 0.13^{b}
		10	8.64 ± 0.11^{d}	0.81 ± 0.01^{c}	3.25 ± 0.10^{a}	0.33 ± 0.05^{a}	3.82 ± 0.16^{a}

Note: Lowercase letters indicate significant differences in the concentrations of five heavy metal binding forms in soils ($p < 0.5$).

3.2.2. Carbonate-Bound State

When soil samples are dissolved in acetic acid, the most sensitive to pH is the carbonate-bound state. MOF can adjust the pH value of Cr- and Cd-polluted soils, which were originally alkaline, gradually tending towards neutral, as mentioned above. The neutral soil environment can inhibit the carbonate-bound state in Cr- and Cd-polluted soil.

The changes in the concentrations of carbonate-bound states in Cr- and Cd-polluted soils after adding MOF on the 5th, 10th, 15th days with different concentrations of MOF are shown in Table 4. From Table 4, it can be observed that compared with the treatment without MOF, adding MOF can significantly reduce the carbonate-bound state content of heavy metals in Cr- and Cd-polluted soil ($p < 0.05$). The overall trend of concentrations of the carbonate-bound states gradually decreases with the extension of addition time of MOF and the increase in additive amount of MOF. This indicates that MOF can reduce the concentrations of the carbonate-bound states of Cr- and Cd-polluted soils. When the additive amount of MOF is 10%, the reduction in the concentrations of carbonate-bound states is the highest in Cr- and Cd-polluted soils, reaching 47.28% and 69.43%, respectively. Therefore, the optimal concentration of MOF for reducing the concentrations of carbonate-bound states in the soil is 10%.

3.2.3. Fe-Mn Oxide State

The changes in the concentrations of Fe-Mn oxide states in Cr- and Cd-polluted soils after adding MOF on the 5th, 10th, 15th days with different concentrations of MOF soils can be observed in Table 4. From Table 4, it can be seen that compared with the treatment without MOF, adding MOF can significantly reduce the Fe-Mn oxide state content of Cr in Cr-polluted soil ($p < 0.05$). With the extension of the addition time of MOF and the increase in additive amount of MOF, the overall trend of concentration of Fe-Mn oxide state gradually decreases in Cr-polluted soil and slightly increases in Cd-polluted soil. Therefore, it can be concluded that MOF can reduce the concentration of Fe-Mn oxide state in Cr-polluted soil and increase the concentration of Fe-Mn oxide state in Cd-polluted soil. When the concentration of MOF is 10%, the reduction in the concentration of Fe-Mn oxide state in Cr-polluted soil and the increase in the concentration of Fe-Mn oxide state in Cd-polluted soil are the greatest, with changes of -38.17% and 57.00%, respectively, compared with test soil. Thus, the optimal additive amount of MOF for improving the concentration of Fe-Mn oxide state in Cr- and Cd-polluted soils is 10%.

3.2.4. Organic Matter Binding State

Heavy metals can enter into or bind to organic matter particles in different forms, and can complexate with organic matter or form sulfides, thereby forming relatively stable organic sulfides and sulfide-bound states that are less readily absorbed and utilized by organisms.

The changes in the concentrations of organic matter binding state in Cr- and Cd-polluted soils with different additive amount of MOF on the 5th, 10th, 15th days are shown in Table 4. From Table 4, it can be observed that compared with the treatment without MOF, adding MOF can significantly increase the organic matter binding state content of heavy metals in Cr- and Cd-polluted soils ($p < 0.05$). With the extension of the addition time of MOF and the increase in the additive amount of MOF, the overall trends of concentrations of organic matter binding state in Cr- and Cd-polluted soils significantly increase, thereby enhancing the stability of the two heavy metals in the soil. It can be concluded that MOF can increase the concentrations of organic matter binding state in Cr- and Cd-polluted soils. When the additive amount of MOF is 10%, the increase in the concentrations of organic matter binding state in Cr- and Cd-polluted soils is the highest, achieving 118.56% and 200%, respectively. Therefore, the optimal additive amount of MOF for increasing the concentration of organic matter in the soil is 10%.

3.2.5. Residual State

The residual state of heavy metal mainly comes from natural minerals, which are biologically ineffective and stably exist in crystalline mineral lattice such as quartz and clay minerals. The changes in the concentrations of residual state in Cr- and Cd-polluted soils with different additive amounts of MOF on the 5th, 10th, 15th days are shown in Table 4. From Table 4, it can be seen that compared with the treatment without MOF, adding MOF can significantly increase the residual state content of heavy metals in Cr- and Cd-polluted soils ($p < 0.05$). The overall trends of the concentrations of residual state in Cr- and Cd-polluted soils significantly increase with the extension of addition time of MOF and the increase in additive amount of MOF, thereby enhancing the stability of the two heavy metals in the soil. It can be concluded that MOF can increase the concentration of residual state in Cr- and Cd-polluted soils. When the additive amount of MOF is 10%, the concentrations of residual state in the Cr- and Cd-polluted soils are the highest, with increments of 107.54% and 50.99%, respectively. Therefore, the optimal concentration for increasing the concentration of residual state is 10%.

3.3. Impact of MOF on the Enzyme Activity of Cr- and Cd-Polluted Soils

3.3.1. Urease Activity

The effects of additive amount of MOF on the urease activity of Cr- and Cd-polluted soils are shown in Table 5. From Table 5, it can be seen that compared with the treatment without MOF, adding MOF can significantly increase the urease activity in Cr- and Cd-polluted soils ($p < 0.05$). As the additive amount of MOF increased, the urease activity of Cr- and Cd-polluted soils increased from 84.21% to 563.16% and from 90.48% to 595.24%, respectively. This indicates that the urease activity of Cr- and Cd-polluted soils increases with the increase in the additive amount of MOF. When the MOF concentration is 10%, the greatest increase in urease activity is observed, achieving 563.16% and 595.24% for Cr and Cd, respectively. Therefore, the optimal additive amount of MOF for enhancing the urease activity of Cr- and Cd-polluted soils is 10%. It can be concluded that the application of MOF can restore the urease activity of Cr- and Cd-polluted soils.

Table 5. Effect of additive amount of microbial composite fertilizer (MOF) on the urease and sucrase activity of Cr- and Cd-polluted soils.

Soil Sample	Amount of MOF (%)	Urease Activity (mg/g)	Sucrase Activity (mg/g)
Cr-polluted soil	0	0.19 ± 0.01^{e}	13.44 ± 0.52^{d}
	1	0.35 ± 0.01^{d}	16.38 ± 0.61^{c}
	3	0.49 ± 0.02^{c}	17.85 ± 0.71^{b}
	5	0.68 ± 0.03^{b}	19.10 ± 0.86^{b}
	10	1.26 ± 0.05^{a}	23.51 ± 1.07^{a}
Cd-polluted soil	0	0.21 ± 0.02^{e}	14.91 ± 0.70^{d}
	1	0.40 ± 0.03^{d}	16.59 ± 0.99^{c}
	3	0.58 ± 0.03^{c}	18.27 ± 1.02^{c}
	5	0.77 ± 0.04^{b}	22.04 ± 1.00^{b}
	10	1.46 ± 0.07^{a}	26.65 ± 1.17^{a}

Note: Lowercase letters indicate significant differences in the urease and sucrase activity of Cr- and Cd-polluted soil ($p < 0.5$).

3.3.2. Sucrase Activity

The effects of additive amount of MOF on the sucrase activity of Cr- and Cd-polluted soils are shown in Table 5. From Table 5, it can be observed that compared with the treatment without MOF, adding MOF can significantly increase the sucrase activity in Cr- and Cd-polluted soils ($p < 0.05$). As the additive amount of MOF increased, the sucrase activity of Cr- and Cd-polluted soils, respectively, increased from 21.88% to 74.93% and from 11.27% to 78.74%. When the additive amount of MOF is 10%, the greatest increase in sucrase activity is observed, achieving 74.93% and 78.74% for Cr and Cd, respectively. Therefore, the optimal additive amount of MOF for enhancing the sucrase activity of soil is 10%. It can be concluded that the application of MOF can restore the sucrase activity of Cr- and Cd-polluted soils.

4. Conclusions

This study simulated Cr- and Cd-polluted soils and investigated the effects of additive amounts of MOF on the physicochemical properties, the concentration of heavy metal binding forms and the enzyme activity of the soils. The results are summarized as follows:

MOF can improve the physicochemical properties of soil and enhance the soil's fertility. The improvement effect on Cr- and Cd-polluted soils becomes more significant with increasing the additive amounts of MOF. The optimal improvement effect is observed when the additive amount of MOF is 10%. Compared to Cr- and Cd-polluted soils without MOF addition, the pH value of the improved soil decreased by 3.73% and 5.34%, respectively. The organic matter content increased by 222.40% and 240.20%, respectively. The ammonium nitrogen content increased by 106.92% and 188.09%, respectively. The rapidly available phosphorus content increased by 70.95% and 120.95%, respectively. The available potassium content increased by 37.60% and 45.74%, respectively.

MOF can reduce the concentration of heavy metal binding forms (exchangeable state, carbonate-bound state and Fe-Mn oxide state) in soil and increase the organic matter binding state and residual state in soil. The improvement effect on Cr- and Cd-polluted soils becomes more significant with increasing the additive amount of MOF. The optimal improvement effect is observed when the additive amount of MOF is 10%. Compared to the Cr- and Cd-polluted soils without MOF addition, the concentration of exchangeable state decreased by 2.54% and 29.53%, respectively. The concentration of carbonate-bound state decreased by 47.28% and 69.43%, respectively. The concentration of Fe-Mn oxide state increased by -38.17% and 57.00%, respectively. The concentration of organic matter binding state increased by 118.56% and 200%, respectively. The concentration of residual state increased by 107.54% and 50.99%, respectively.

MOF can enhance the activity of native microbial communities and improve the soil's fertility. MOF significantly enhances the enzyme activity of the soil. The enzyme activity of Cr- and Cd-polluted soils gradually increases with increasing the additive amount of MOF. The optimal improvement effect is observed when the MOF concentration is 10%, the results of which are that urease activity increased by 563.16% and 595.24%, respectively, while sucrase activity increased by 74.93% and 78.74%, respectively.

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