Effect of Different Washing Solutions on Soil Enzyme Activity and Microbial Community in Agricultural Soil Severely Contaminated With Cadmium

Yu Zhang

Nanjing University of Information Science and Technology

Chunfa Wu (✉ wchf1680@sina.com)

Nanjing University of Information Science and Technology <https://orcid.org/0000-0001-5058-7792>

Shaopo Deng

Nanjing Institute of Environmental Sciences

Jinlu Zhang

Nanjing University of Information Science and Technology

Jinyu Hou

Institute of Soil Science Chinese Academy of Sciences

Chong Wang

Nanjing University of Information Science and Technology

Zhaocong Fu

Nanjing University of Information Science and Technology

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Abstract

Soil enzyme activities and microbial communities have a good response to the remediation effect of heavy metal-contaminated soils. To evaluate the effect of three commonly used washing agents, ferric chloride (FC), ethylenediamine-tetra- methylenephosphonic acid (EDTMP) and ethylenediamine tetraacetic acid (EDTA) on soil enzyme activities and microbial community in cadmium (Cd)-contaminated agricultural soil collected around the mining area. The soil enzyme activities, microbial community, chemical forms of Cd and some physicochemical properties of the soil washed with different washing solutions were determined after two months incubation. The results showed that the three washing solutions had moderate removal efficiencies (22.83%-37.56%) for Cd in the tested soil and the breakdown product of EDTMP has a certain stabilizing effect on Cd. The geometric mean and the integrated total enzyme activity index showed that soil washing with FC and EDTA were more beneficial to the restoration of biochemical functions than that with EDTMP. The relative abundance of Gemmatimonadetes in the soil washed with EDTA was significantly higher than that with FC and EDTMP. The relative abundance of Firmicutes in the soil washed with EDTMP was significantly increased, and the relative abundance of Bacteroidetes in the soil washed with FC was significantly decreased. Pearson correlation analysis and redundancy analysis (RDA) indicated that the three washing solutions affected soil enzyme activities and microbial community by altering soil nutrient, total Cd concentration and Cd fractions in soils.

1 Introduction

It is well known that metal mining activity discharges large amounts of heavy metals (HMs) into the surrounding environment, including soils, and metal mining activity has become one of the major anthropogenic sources of HMs in soil. Due to the rapid development of metal mining and metal processing in recent decades, soil contaminated with HMs occurs widely in China (Luo et al. 2012, Mkhinini et al. 2020, Zhang et al. 2013a, Zhao et al. 2015). Cadmium (Cd) is a typical and common HM pollutant in agricultural soils surrounding mining areas (Du et al. 2020). Cd contamination in agricultural soils has become a serious challenge for food security in China due to the high mobility and bioavailability of Cd (Chen et al. 2018). Previous studies have shown that agricultural soils surrounding mining areas are always severely contaminated with Cd and other HMs (Helmfrid et al. 2015, Weerasundara et al. 2018), and it resulted in Cd concentrations in agricultural products generally exceeded the national standard (Du et al. 2020).

Chemical washing is one of the common remediation technologies that applied to agricultural soils contaminated with HMs including Cd (Wang et al. 2019a). It can permanently remove Cd from soils by the physical and chemical effects, such as desorption, chelation, dissolution, and fixation of the washing solutions (Rui et al. 2020). The common washing agents include inorganic washing agents, chelators, surfactants, etc. (Khalid et al. 2017, Wang &Chen 2019). Ferric chloride (FC) is a chloride solution, ethylenediamine tetraacetic acid (EDTA) is a chelating agent, and ethylenediamine-tetra-methylenephosphonic acid (EDTMP) is a photodegradable chelating agent. Previous studies showed that

the three washing agents have good Cd-extraction efficiency (Feng et al. 2020, Guo et al. 2016, Makino et al. 2016, Makino et al. 2008).

Soil enzyme activity is a potential indicator of soil health due to its high sensitivity to external interference (Tan et al. 2014). Sucrase, urease, acid phosphatase and catalase are common soil enzymes and their activities are closely related to soil carbon (C), nitrogen (N), phosphorus (P) cycling and soil redox capacity, respectively (Eivazi & Tabatabai 1977, Feyzi et al. 2020, Hu et al. 2014, Yang et al. 2016). The structure and diversity of soil microorganisms communities are also important biological indicators of soil health (Wang et al. 2019b). Previous studies have reported that soil Cd decreased the activities of soil enzymes and significantly affected the diversity of the microbial community (Wang et al. 2007, Xu et al. 2013). Therefore, soil enzyme activity and microbial community may have the potential for assessing the remediation effect of HMs contaminated soils.

Soil washing may not only remove some HMs pollutants in the soil but also lead to the loss of nutrients and major elements, the activation of soil HMs and the residue of washing agents, which may lead to significant changes in soil enzymes and microbial communities (Wei et al. 2020, Zhang et al. 2013b). Therefore, in the evaluation of remediation effect of HM-contaminated soil, we should not only consider the removal efficiency and the ecological risk of HMs, but also pay attention to the changes of soil enzyme activities and microbial community. Many studies on soil enzymes and microbial communities in soils contaminated with HMs have been conducted (Han et al. 2020a, Liu et al. 2020a, Njoku et al. 2020), however, to our knowledge, few studies have reported the effect of different washing solutions on enzyme activity and microbial community in agricultural soils contaminated with HMs. The objectives of this study were to evaluate the impact of the three washing agents (FC, EDTA and EDTMP) on the activities of the four soil enzymes (catalase, acid phosphatase, urease and sucrase) and microbial community and to study their potential influence factors.

2 Materials And Methods

2.1. Soil and washing agents

The test soils were collected from the topsoil (0–20 cm) of farmland near a non-ferrous metal smelter in Baiyin City, Gansu Province, China (104°14.622'E, 36°32.322'N). The soil samples were air-dried, ground and then sieved through a 2-mm sieve.

The concentration of Cd in the tested soil was as high as 40.82 mg kg⁻¹, far exceeds the risk intervention values (3.00 mg kg⁻¹) of the Soil Environmental Quality Risk Control Standard for Soil Contamination of Agricultural Land (GB 15618-2018), and the tested soil was severely contaminated by Cd.

FC and EDTA were purchased from Sinopharm Chemical Reagent Co., Ltd, and EDTMP was purchased from MERYER Co., Ltd. All the three agents were of analytical-reagent grade.

2.2 Soil washing and incubation procedures

1L Glass beakers containing 80 g of soil were prepared for batch washing experiments, followed by the addition of 10 mmol L⁻¹ EDTMP, FC, and EDTA solutions at a soil-solution ratio of 1:5 (w/v) that was selected based on the previous studies (Guo et al. 2018, Wei et al. 2020). Deionized water was added to the control treatment (CK). The beakers were shaken with an ultrasonic vibrating machine at 200 W for 45 min. After vibration, the supernatant was drained off. The washed soil was then rinsed with deionized water at a soil-solution ratio of 1:5 (w/v). Each experiment was replicated three times.

After washing, the soil was transferred to a 100 mL glass beaker and then incubated for two months according to previous studies (Rajaie et al. 2006, Wu et al. 2015a).

2.3 Soil analysis

After two months incubation, a proportion of soils was air-dried for soil analysis. Soil pH was determined with a pH meter (PHS-3C, INESA, China) at a soil-water ratio of 1:2.5. Soil conductivity (EC) was measured with a conductivity meter (DDS-307A, INESA, China) at a soil/water ratio of 1:5. Soil organic matter (SOM) was measured by the potassium dichromate oxidation-oil bath heating method (Nelson 1996). The cation exchange capacity (CEC) was analysed after soil extraction using ammonium acetate (Sumner & Miller 1996). The total nitrogen content (TN) was determined using the semi-micro Kjeldahl method (Bremner 1960). Soil total phosphorus content (TP) was measured using the Mo-Sb anti-spectrophotography method (Bao 2000). Soil available potassium (AK) was measured using the ammonium acetate extraction-flame photometric method (Bao 2000). The available P content (AP) was measured based on the method of Olsen et al. (Olsen 1954). The total Cd was measured using ICP-MS after digestion with HCl - HNO₃ - HClO₄.

2.4 Chemical forms of Cd

The chemical forms of Cd in the washed soil, including exchangeable (F1), bound to carbonates (F2), bound to Fe–Mn oxides (F3), bound to organic matter (F4) and residual (F5), were analysed using a modified Tessier's sequential extraction procedure (Zhu et al. 2018).

2.5 Soil enzymes

Soil catalase activity was measured by potassium permanganate titration, soil urease activity was measured by sodium phenol sodium hypochlorite colorimetry, soil sucrase activity was measured by the 3,5-dinitrosalicylic acid method, and soil acid phosphatase activity was analysed by sodium diphenyl phosphate colorimetry.

The effect of washing solutions on soil enzyme activities was evaluated using the geometric mean (GMean) index and the integrated total enzyme activity index (TEI) proposed by Tan et al. (Tan et al. 2014).

The GMean and the TEI were calculated using the following equations:

$$\text{GMean} = \sqrt[4]{\text{catalase} \times \text{acidphosphatase} \times \text{sucrase} \times \text{urease}} \quad (1)$$

$$TEI = \sum_{n=1}^i \frac{X_i}{\bar{X}_i} \quad (n=1,2,3,4) \quad (2)$$

where X_i is the activity of soil enzyme i and $\bar{X}_i$ is the mean activity of enzyme i in all samples.

2.6 Bacteria community

Microbial DNA was extracted from 0.5 g soil using a FastDNA spin kit, and the quality and concentration of extracted DNA were tested by a NanoDrop 1000 spectrophotometer (Thermo Fisher, Waltham, MA).

High-throughput sequencing was carried out by Shanghai Majorbio Bio-pharm Technology Co., Ltd. The primers 515F (5'-barcode-GTGCCAGCMGCCGCGG-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') were used to amplify the V4-V5 region of the bacterial 16S ribosomal RNA gene. According to the manufacturer's instructions, the amplified products were extracted from a 2% agarose gel and purified by an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.).

Sequences were clustered into operational taxonomic units (OTUs) of $\geq 97\%$ similarity using the Deblur denoising algorithm, which removes noise caused by sequencing errors (R. Cole's et al. 2009). The phylogenetic affiliation of each 16S rRNA gene sequence was analyzed by the RDP Classifier (<http://rdp.cme.msu.edu/>) at a confidence threshold of 70% (Caporaso et al. 2010).

2.7 Statistical Analyses

The Chao1 richness index and Shannon diversity index were evaluated using the phemap package (version 1.0.12) in R (version 1.30.1). Bioinformatic analysis was performed using the OmicStudio tools at <https://www.omicstudio.cn/tool>. One-way analysis of variance (ANOVA) and Tukey HSD were used to determine the statistical significance ($p < 0.05$) of differences between treatments. The correlation matrix of soil environmental factors, soil enzyme activities and bacterial diversity was drawn by Origin 2021.

3 Results

3.1 Soil properties

The physicochemical properties of the soils treated with different washing solutions are presented in Table 1. Compared with CK, the EC of soils washing with FC and EDTA significantly increased, however, the EC of soil washed with EDTMP significantly decrease. The concentrations of TN and TP in the soil washed with EDTMP were significantly higher than the other soils. However, compared with the CK, AK and AP were significantly decreased after washing with FC, EDTA and EDTMP. There was no significant difference in pH, CEC or SOM among all treatments.

Table 1 Selected physicochemical properties of the soil with different treatments

Data are presented as mean values $\pm$ SE ($n = 3$)

Soil properties	CK	FC	EDTA	EDTMP
Soil pH	7.44±0.07 ^a	7.29±0.13 ^a	7.41±0.07 ^a	7.25±0.13 ^a
EC, $\mu\text{S cm}^{-1}$	1287±0.94 ^c	1471±0.47 ^a	1307±0.94 ^b	1162±1.70 ^d
SOM, g kg^{-1}	15.1±1.64 ^a	14.14±0.85 ^a	14.37±0.52 ^a	16.27±0.90 ^a
CEC, cmol kg^{-1}	8.03±0.12 ^a	7.97±0.29 ^a	7.43±0.12 ^a	7.93±0.37 ^a
Total N, mg kg^{-1}	337±0.55 ^b	279±1.57 ^b	328±0.55 ^b	514±36.43 ^a
Total P, g kg^{-1}	0.82±0.01 ^b	0.87±0.04 ^b	0.85±0.03 ^b	2.80±0.28 ^a
Available K, mg kg^{-1}	194±1.63 ^a	178±1.70 ^b	177±0.82 ^b	168±1.63 ^c
Available P, mg kg^{-1}	60.68±4.10 ^a	42.14±2.79 ^b	46.93±2.68 ^b	37.45±2.07 ^b
Different letters indicate significant difference between treatments in the same soil at $P < 0.05$ following Tukey HSD test.				

3.2 Removal efficiencies and chemical forms of Cd

Table 2 Concentration of Cd in different chemical form in the soil with different treatments

Data are presented as mean values $\pm$ SE (n = 3)

	F1(mg kg^{-1})	F2(mg kg^{-1})	F3(mg kg^{-1})	F4(mg kg^{-1})	F5(mg kg^{-1})	Total Cd
CK	14.27±0.22 ^a	7.32±0.31 ^a	16.20±0.11 ^a	0.61±0.14 ^b	0.57±0.14 ^b	40.82±0.68 ^a
FC	7.00±0.23 ^c	6.12±0.09 ^b	13.83±0.28 ^b	0.61±0.08 ^b	0.55±0.06 ^b	31.50±0.59 ^b
EDTA	7.95±0.28 ^b	3.85±0.08 ^c	11.86±0.21 ^c	0.55±0.09 ^b	0.56±0.06 ^b	26.69±0.38 ^c
EDTMP	8.08±0.23 ^b	3.82±0.12 ^c	10.26±0.35 ^d	1.73±0.09 ^a	0.95±0.09 ^a	25.49±0.11 ^c

Different letters indicate significant difference between treatments in the same soil at $P < 0.05$ following Tukey HSD test.

In this study, three washing solutions and deionized water (as a control treatment) were used to simulate the washing of tested soil. The three washing solutions had significant removal effects on Cd after a short washing period. The removal efficiencies of EDTMP and EDTA were 37.56% and 36.04%, respectively, and were significantly higher than FC (22.83%) and CK (1.26%).

The chemical forms of Cd in the soils washed with different washing solutions are shown in Table 2. The speciation of Cd in the soils was mostly F3, F1 and F2, followed by F5, and F4. Compared to those in CK, the F1, F2 and F3 fractions of Cd in soil decreased after washing with the three washing solutions. However, after washing with EDTMP, the F4 and F5 fractions were significantly increased compared to CK.

3.3 Soil enzyme activities

The activities of enzymes in soils washed with different washing solutions are shown in Figure 1. Compared with CK, the activity of acid phosphatase in soil washed with FC was significantly increased, the activities of acid phosphatase and urease in soil washed with EDTA were significantly increased, and the activity of urease in soil washed with EDTMP was significantly increased but catalase decreased. The FC solution significantly increased the activity of soil acid phosphatase, the EDTA solution increased activities of acid phosphatase and urease, and the EDTMP solution increased the activity of sucrase activity but decreased catalase.

The mean GMean index values and the mean TEI values of all treatments were CK, 0.86 and 3.58; EDTMP, 0.87 and 3.81; EDTA, 1.03 and 4.26; and FC, 1.04 and 4.35, respectively.

3.4 Soil microbial community

Table 3 Alpha diversity indexes of bacterial communities

	CK	FC	EDTMP	EDTA
Chao1	11731	13497	13758	16568
Shannon	6.9	6.8	6.7	7.2

In this study, 120000 high-quality bacterial sequences were generated. The sequences were further clustered into 50430 OTUs with 97% similarity. The bacteria were from 41 phyla, 148 classes, 298 orders, 499 families and 864 genera. The coverage rate of bacteria was over 96%, which is suitable to represent the diversity of bacteria in all samples. As shown in Tab. 3, the FC, EDTA and EDTMP treatments increased the Chao1 index.

The differences in the relative abundance of microorganisms in the different treatments can be intuitively observed with a heat map (Fig. 3a). As shown in Fig. 3a, at the phylum level, the microbial community structure of the control and washed soil was mainly concentrated in Proteobacteria (26.9%-31.3%), Acidobacteria (12.8%- 16.8%), Gemmatimonadetes (8.1%-14.1%), Actinobacteria (9.0%-12.3%), Chloroflexi (11.7%-13.6%), Bacteroidetes (3.2%-5.9%), Planctomycetes (3.0%-4.5%), Firmicutes (0.8%-12.1%), and Nitrospirae (3.0%-4.5%). At the phylum level, the EDTA, FC and CK treatments had highly similar community composition, but the EDTMP treatment was different from them. The proportions of various bacterial phyla in each treatment were significantly different. Overall, the relative abundance of

Gemmatimonadetes in soil washed with EDTA was significantly higher than that with FC and EDTMP. The relative abundance of Firmicutes in soil washed with EDTMP significantly increased, and the relative abundance of Bacteroidetes in soil washed with FC significantly decreased (Fig. 3b).

LDA effect size (LEfSe) analysis examines the biomarkers for the soil bacterial communities (Fig. 4). The results indicated that 14 bacterial genera with an LDA level ≥ 4.0 differed. Firmicutes, Clostridia, Clostridiales, Symbiobacteriaceae and *Symbiobacterium* were biomarkers in the soil washed with EDTMP, Burkholderiales, Oxalobacteraceae and *Ramlibacter* were biomarkers in the soil washed with FC, and Gemmatimonadetes, MND1, Acidobacteria_6, Gemm_3 and iii1_15 were biomarkers in the soil washed with EDTA.

3.5 Relationship between environmental factors and microbial community

RDA results are used to show the relationship between environmental factors and soil microbial communities and the relationship between soil enzymes and the microbial community (Fig. 5). As shown in Fig. 5a, the first two axes of the RDA plot explained 25.52% and 18.86% of the variation in the soil microbial communities, respectively. TN and TP had a positive correlation with the EDTMP treatment and Firmicutes, Bacteroidetes and Verrucomicrobia. Soil pH had a positive correlation with the EDTA treatment, Gemmatimonadetes, Nitrospirae, Proteobacteria and Chlorobi. F2 and EC had a positive correlation with the FC treatment, Acidobacteria, Fibrobacteres, Chloroflexi and Actinobacteria. In addition, the correlations between Firmicutes and Bacteroidetes and the EDTMP treatment and between Gemmatimonadetes and the EDTA treatment were consistent with the LDA results (Fig. 4).

3.6 Relationship between activities of the soil enzyme and bacteria community

As shown in Fig. 5b, the first two axes explained 25.30% and 18.35% of the variation in the soil microbial communities, respectively. Urease and sucrase activities had a positive correlation with the EDTMP treatment, Firmicutes, Bacteroidetes and Verrucomicrobia. Acid phosphatase and catalase activities had a positive correlation with the FC treatment, Actinobacteria, Fibrobacteres, Acidobacteria, Proteobacteria, Chlorobi and Chloroflexi.

4. Discussion

4.1 Effect of washing solutions on Cd in soil

The concentrations of Cd in the soils washing with the FC, EDTA and EDTMP solutions were significantly lower than that in CK (Tab. 1), indicating that the three washing agents have certain removal effects on Cd. However, previous studies that showed EDTA could remove 93% of Cd at a soil-solution ratio of 1:5 for 2 h (Wang et al. 2018); FC could remove 62.9% of Cd at a soil-solution ratio of 1:2 for 1 h (Zhai et al. 2018), and EDTMP could remove 65.63% of Cd at a soil-solution ratio of 1:10 for 2 h (Feng et al. 2020).

The moderate removal rates of the three washing agents may be due to the high concentration of Cd in the soil and the relatively low concentration of washing agent in the washing solutions.

The Cd in the soils washed with the FC, EDTA, and EDTMP solutions as well as the CK was fractionated to further investigate how the chemical forms of Cd are affected by soil washing. F3, F4 and F5 generally exist in a stable state and have relatively small adverse effects on the environment; however, F1 and F2 have higher activity, which leads to greater environmental risk (Wu et al. 2015b, Yang et al. 2018, Zhang et al. 2017). The concentration of the active content of HMs in soil plays an important role in the ability of plants to absorb HMs. The higher the active content of HMs in soil, the easier it is for plants to absorb these HMs (Han et al. 2020a). Furthermore, a previous study has shown that the uptake of Cd is closely related to active fractions of Cd in soil (Li et al. 2017). In our study, all three washing solutions can significantly reduce the active components of Cd and reduce the environmental risk.

The EDTMP treatment significantly increased the F4 and F5 fractions, this can be attributed to the effect of the photodegradation of EDTMP. It has been proven that under experimental conditions, phosphonates are converted into orthophosphates, especially in the presence of iron and at acidic pH. EDTMP is photodegraded by UV into orthophosphates (Lesueur et al. 2005). Cd can precipitate with orthophosphates as $\text{Cd}_3(\text{PO}_4)_2$ in soils containing an excessive concentration of Cd (Mcgowen et al. 2001). In addition, EDTMP treatment significantly increased the activity of soil urease, which can catalyse the hydrolysis of urea to produce NH_4^+ and CO_3^{2-} and combine with Cd^{2+} to produce CdCO_3 , thereby reducing effective fractions of Cd in soil (Wang et al. 2020).

4.2 Effect of washing solutions on soil enzyme activities

Enzyme activity exhibits high sensitivity and rapid responses to soil quality and can be used as a performance index after soil washing (Beiyuan et al. 2018). Previous studies have confirmed that HMs can decrease the activities of soil sucrase, urease, phosphatase and catalase (Qin et al. 2020, Tang et al. 2020). Changes in soil physicochemical properties, such as SOM, nutrients, and pH, may be the main reasons for the changes in soil enzyme activities. Pearson correlation analysis showed that the activities of soil catalase and acid phosphatase were positively correlated with EC (Fig. 2), indicating that EC had a positive effect on enzyme activities which is consistent with the report by Tang et al. (Tang et al. 2020). The changes in the levels of soil nutrient elements such as N, P and K also significantly affected soil enzyme activities. For example, soil urease activity was positively correlated with the TN and TP ($P < 0.01$), which was consistent with previous studies (Im et al. 2015, Mkhinini et al. 2020, Qin et al. 2010). In addition, the chemical forms of Cd also strongly regulate soil enzyme activity. Soil catalase was negatively correlated with F4 ($P < 0.01$) and F5 ($P < 0.01$) and positively correlated with F3 ($P < 0.01$). Previous study demonstrated that moderate Cd stress can promote the activity of some soil enzymes and the promotion becomes inhibition when the concentration of Cd in soil is further increased (Wang et al. 2019a). In this study, the increase of some enzyme activities may be related to the concentration of Cd decreased to the level that promoted enzyme activity.

The geometric mean index can directly show the change of global enzyme activity (Xu et al. 2021), and it has been satisfactorily used to evaluate the quality of HMs-contaminated soils (Hinojosa et al. 2004, Lessard et al. 2014). TEI can be used to easily compare the combined enzyme activity and the quality of each soil sample (Tan et al. 2014). Compared with that in CK, the GMean index increased after washing with the three washing solutions. This indicated that chemical washing in our study might benefit the biochemical functions of agricultural soil contaminated with Cd. However, soil washing with EDTMP exhibited significantly decreased catalase activity, which led to a lower GMean index value than those of FC and EDTA. The result for the TEI index was the same as that for the GMean index, which indicated that these two indexes are reliable for the comprehensive evaluation of the global enzyme response after soil washing.

4.3 Effect of washing solutions on soil microbial community

After washing, the physiochemical properties of the soil changed, and the survival conditions for Cd-resistant dominant bacteria were disrupted, which made it difficult for some soil microorganisms to adapt to the new environment (Zhai et al. 2018). In this study, Proteobacteria were predominant in all treated soils, indicating that Proteobacteria were the most HMs-tolerant microorganisms in severely contaminated soils, which was consistent with a previous study (Eva-Maria et al. 2011). In addition, some researchers have suggested that Bacteroidetes may possess metal-resistant properties (Haller et al. 2011, Ni et al. 2016), Gemmatimonadetes have been identified to be associated with HM-contaminated soils (Ni et al. 2016), and Firmicutes also have a strong tolerance to extreme conditions (Hortal et al. 2013). RDA indicated that F2 had strong effects on the soil bacterial community. The bioavailable fraction, rather than the total content of HMs, is a major factor influencing bacterial community changes because bioavailable forms are easily used by surrounding microorganisms which was consistent with previous studies (Hou et al. 2017, Muhlbachova et al. 2015). Furthermore, the RDA results also indicated that TP and TN were two main factors affecting the microbial community. TN plays a key role in cell metabolic processes, such as energy metabolism, protein synthesis, and cell division (Liu et al. 2020b). RDA results showed that the relative abundance of Bacteroidetes was significantly positively correlated with TN, which was consistent with the previous study (Bian et al. 2018). Previous studies have also shown that changes in the P concentration in soil are the main factor leading to changes in microbial community composition (Wei et al. 2020). In addition, bacteria belonging to Proteobacteria and Firmicutes can use nutrient sources to increase soil quality (Han et al. 2020b). EDTA contains many sources of N and C, and EDTMP contains C, N and P, which may benefit HM remediation of agricultural soil.

The Chao1 index and Shannon index were used to evaluate the alpha diversity of the soil microorganism communities (Tab. 3). The Chao1 index is an indicator of species richness in ecology (Chao 1984), and a higher Chao1 indicates a larger number of species and a variety of species that exist in a sample. The Chao1 indexes of FC and EDTA were higher than that of EDTMP, indicating that the bacterial community richness under FC and EDTA was higher than that under EDTMP. The Shannon index is an abundance-based diversity index that is widely used in many disciplines (Chao et al. 2014), and a higher Shannon

index indicates higher community diversity within a sample. All the washed soil samples had a larger number of microbial taxa than the control sample. Nevertheless, the Shannon index was not significantly different between the washed and control soil samples, which was indicative of the stability of ecosystem productivity and the functions of the soil microbiome (Zheng et al. 2016).

Based on the above analysis, it is reasonable to conclude that the three washing solutions can significantly change the soil microbial community by changing the soil physicochemical properties and the concentration and fractionation of Cd.

4.4 The relationship between soil washing, soil enzyme and bacteria

In this study, soil washing may have affected enzyme activities in two ways. First, the washing agent may have had a direct impact on the activities of soil enzymes. Second, the washing agent may have caused changes in the soil bacterial community, resulting in changes in its secretions and metabolism and affecting its enzyme production behaviour. However, our results do not provide any information about the functions of these specific microorganisms. Therefore, it is necessary to use molecular biological methods to further analyse their function and to better understand the mechanism of the bacteria community regulating soil ecological functions after washing.

5 Conclusion

This work evaluated the impact of the three commonly used washing agents on soil enzyme activities and microbial community in agricultural soil severely contaminated with Cd. All three washing solutions had certain removal effects on Cd and their removal efficiency followed the order of EDTMP>EDTA>FC. All three washing solutions can significantly reduce F1, F2 and F3. In addition, the breakdown product of EDTMP had a certain stabilizing effect on soil Cd.

Soil enzyme activities had a significant change after soil washing. The activity of acid phosphatase in soil washed with FC was significantly increased, the activities of acid phosphatase and urease in soil washed with EDTA were significantly increased, and the activity of urease in soil washed with EDTMP was significantly increased but catalase decreased. The GMean index and the TEI showed that FC resulted in the best global enzyme response after soil washing. After soil washing, the Chao1 index of bacteria increased, and the microbial community structure changed. Proteobacteria, Firmicutes and Gemmatimonadetes can be used as markers for the soil microbial community response to soil washing, and TP, TN, EC and F2 were confirmed to be the four vital parameters that shaped the bacterial communities. These results could provide a new perspective on the assessment of the ecological effect of soil washing on agricultural soils severely contaminated with Cd.

Abbreviations

Cd, cadmium;

HMs, heavy metals;

EC, electrical conductivity;

SOM, soil organic matter;

CEC, cation exchange capacity;

FC, ferric chloride;

EDTA, ethylenediamine tetraacetic acid;

EDTMP, ethylenediamine-tetra-methylenephosphonic acid;

F1, exchangeable Cd;

F2, Cd bound to carbonates;

F3, Cd bound to iron and manganese oxides;

F4, Cd bound to organic matter;

F5, residual Cd.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Overarching research goals and aims were designed by Chunfa Wu. Material preparation, data collection and analysis were performed by Yu Zhang, Jinlu Zhang, Shaopo Deng, Jinyu Hou, Chong wang and Zhaocong Fu. The first draft of the manuscript was written by Yu Zhang and Chunfa Wu reviewed and commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Changes of soil enzymatic activities. (a)catalase, (b) acid phosphatase, (c) sucrase, (d) urease

Figure 2

Matrix of Pearson's rank correlation coefficients between soil environment factors and enzyme activities. Correlation coefficients (r values) are indicated by different colors and sizes of circle. Red represents positive correlation, blue represents negative correlation, and the larger the circle diameter is, the stronger the correlation is. * $p < 0.05$; ** $p < 0.01$

Figure 3

Heat map(a) and relative abundance of different bacteria(b) at phylum level. A rectangle represented a bacterium (at the phylum level). The redder the rectangle, the higher the relative abundance of bacteria. On the contrary, the bluer the rectangle, the lower the relative abundance

Figure 4

LEfSe and LDA analysis based on OTUs of four treatments. (a) Cladogram showed the biomarker microbes of the microbial lineages from domain to genus among four different treatments. Yellow dot represented the microbes with no statistical differences among four treatments; (b) LDA scores showing the significant bacterial difference between four treatments

Figure 5

Redundancy analysis (RDA) for the relationship between environmental factors and microbial community(a) and the relationship between activities of the soil enzyme and bacteria community(b). ● represents CK treatment; □ represents FC treatment; ⬠ represents EDTMP treatment and Δ represents EDTA treatment