Effect of multiple washing operations on removal of heavy metals from an alkaline farmland soil and the strategy for agricultural reuse

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Abstract

Little study carried out the soil washing experiments using pot experiments to simulate *in-situ* soil washing operation, particularly for the alkaline soil. This study explored the effect of multiple washing operation using pot experiments on the removal efficiencies of heavy metals from the alkaline farmland soil and the reuse strategy of the washed soil for safe agricultural production. The results showed that the removal efficiencies of Cd, Pb, Cu and Zn after the seven times washing with the mixed chelator (EDTA, GLDA and citric acid) were 41.1%, 47.1%, 14.7% and 26.5%, respectively, which were close to those from the results of EDTA treatment. For the alkaline soil studied, the 2nd washing with the mixed chelator most effectively removed heavy metals owing to the activation of heavy metals after the first washing operation. The mixed chelator more effectively increased the proportion of stable speciation of heavy metals and maintained soil nutrients such as nitrogen content in soil than EDTA did, indicating the little disturbance of alkaline soil quality after being washed by the mixed chelator. After application of amendment into the washed soil, there was no visible difference in biomass weight of crops from the soil washed with different agents, indicating that the inhibitory effect of both washing agents on the plant growth was effectively alleviated. The Cd and Pb in *Z. mays* were below the threshold of Hygienical Standard for Feeds of China (GB 13078 – 2017) (1 and 30 mg·kg⁻¹). Moreover, after three times of cropping, the concentrations of available heavy metals in the soil washed with the mixed chelator were lower than those in the washed soil with EDTA, indicating the value and potential of agricultural reuse of alkaline farmland soil being washed with the mixed chelator here.

1 Introduction

The national survey of soil contamination in China reported that about 16.1% of the sampling sites was over the standard and one-sixth of the farmland was contaminated with heavy metals (Chen et al. 2014). Heavy metals were the primary pollutants to affect the soil environmental quality in China, particularly the farmland soil (State of China's Ecological Environment, 2021). Numerous studies have found that the potential risk of heavy metals in farmland soil is related to the soil properties, especially soil pH (Zeng et al., 2011; Orr et al., 2020; Mikel et al., 2022). When the levels of heavy metals in alkaline and acidic soils are comparable, the potential risk of heavy metals in the alkaline soil was lower than that in the acidic soil (Tang et al., 2015). Most researches on the remediation of heavy metals-contaminated soils focused on the acid soil and neutral soil (Ma et al., 2020; Wu et al., 2020; Jia et al., 2021). In fact, the alkaline soils that contaminated by toxic heavy metals should not be ignored because the high levels of heavy metals in the alkaline soils also have the negative impact on the growth and productivity of crops and health risk through food chain (Gluhar et al., 2019b; Wang et al., 2020b). Therefore, the remediation of alkaline soil contaminated with heavy metals also needs to be paid enough attention to ensure the enough and safe food production.

Various remediation strategies have been developed according to the immobilization mechanism (i.e., organic amendment, inorganic amendments) or removal mechanism (i.e., adsorption, complexation, ion exchange, chemical precipitation, oxidation-reduction) (Nejad et al. 2017; Beiyuan et al. 2018). Among the remediation measures, soil washing is one of the most efficiently chemical treatment techniques for removal of heavy metals from soil (Yoo et al. 2018). The chelating agents, especially Ethylenediaminetetraacetic acid (EDTA), had a high activity and removal of multiple heavy metals from soils with various pH values (Udovic et al., 2012; Manouchehri et al., 2006). Nevertheless, the removal of heavy metals from the alkaline soils by chelating agents was more difficult than acid soil (Alberto et al. 2015; Sun et al., 2020). For example, the removal percentage of Pb in the alkaline soil (828 mg·kg⁻¹ of Pb) by EDTA was 67%, which was evidently lower than the removal percentage of Pb (80%) in the acidic soils (673 mg·kg⁻¹ of Pb) by EDTA (Gluhar et al. 2019a). Therefore, it is necessary to take measures to improve the removal efficiencies of heavy metals from alkaline soils by chelating agents.

It has been reported that various factors such as washing times, flow rate and composition of chelating agent were important for increasing removal efficiency of heavy metals from soils (Guo et al. 2018a; Liu et al. 2019; Yan et al. 2021). Our previous study found that multiple washing processes could promote the removal efficiencies of heavy metals from an alkaline-contaminated soil, particularly for the removal of Cd, which was mainly due to the enough reaction time to activate heavy metals in soil (Guo et al., 2018a). Most of these soil washing studies were carried out in the ways of laboratory washing by stirring and shaking (Alberto et al., 2015). However, the shaking or stirring operations are generally not suitable for the remediation of large-scale farmland soil. The *in-situ* remediation methods for effective removal of heavy metals from alkaline farmland soil are urgently needed to ensure safe agricultural production in these soils.

The pot experiment is similar to *in-situ* soil washing operation, which is an important soil washing way to put soil washing technology into practice for field application (Hu et al., 2014; Zhai et al., 2018). Our previous research primarily focused on the acid soil and neutral soil remediation by the multiple washing operation using MC (the molar ratio of citric acid, EDTA, and KCl = 10:2:3). In addition, Hu et al. (2014) reported that the removal percentages of Cd, Cu, Pb, and Zn via column leaching experiment (50 mM EDTA) were 90%, 88%, 90%, and 67%, respectively. Nevertheless, further studies on the soil washing experiment using the pot experiment are needed to carry out to explore the removal efficiencies of heavy metals from soil under the condition of "*in-situ*" washing operation.

Besides removal efficiencies of heavy metals from alkaline farmland soil, another important consideration is soil productivity and food safety. Many studies have reported that EDTA is difficult to be degraded in the soil environment, which has negative role in soil function, soil nutrients, growth of plant, and agricultural production (Gluhar et al. 2020; Zhao et al. 2019; Jelusic et al. 2014; Kaurin et al. 2020; Guo et al. 2020a). Therefore, the mixed-chelators (MC) of EDTA and environment-friendly agents have aroused widespread interest in the remediation of heavy metals-contaminated soil in recent years for keeping the removal efficiencies of heavy metals and alleviating the negative effect of chelator on the soil function and properties (Peng et al. 2020; Thinh et al. 2020; Wang et al. 2020a). Recently, we also developed a kind of the mixed-chelators (the mixture of EDTA, GLDA, and citric acid with their molar ratio of 1:1:3), which obviously reduced the contents of Pb, Cu and Zn by 34.5%, 24.6% and 10.7% from an alkaline soil, respectively (Guo et al. 2018b). Additionally, the mixed-chelator significantly decreased the negative effects of washing agents on soil properties and enzyme activities when compared with the EDTA did (Guo et al. 2018a). Similar results were also reported by several other studies (Wang et al., 2018a; Xue et al., 2019; Hazrati et al., 2020). Although some studies have reported that various mixed-chelators can effectively remove heavy metals from the acid and alkaline farmland soil (Wei et al., 2016; Guo et al., 2018a; Begum et al., 2020). However, the effect of the mixed-chelator of EDTA, GLDA, and citric acid on the removal efficiencies of heavy metals from an alkaline soil after multiple washing via pot experiment and the strategies of agricultural reuse after soil washing are still not fully developed and understood.

The primary objectives of this study were (1) to investigate the effects of the mixed-chelator of EDTA, GLDA, and citric acid on the removal efficiencies of heavy metals from an alkaline farmland soil using multiple washing operation under the static pot experiment condition; (2) to examine the key step of effective removal of heavy metals by the mixed-chelator and to explore its mechanism; (3) to examine the changes in soil physico-chemical properties and crop productivity and safety after soil washing. The results of this study will be helpful for effectively remediating alkaline farmland soil contaminated with heavy metals and proposing the strategy of agricultural reuse in safe way after washing.

2 Materials And Methods

2.1 Soil, washing agent, and plant

The surface soil sample (0-20 cm, 18.4% of CaCO_3) was collected from a derelict farmland around a metal smelter in Zhuzhou City (China, Hunan Province). Wiping out the plant roots and other debris in soil sample. The soil sample was air-dried at room temperature. The soil was grounded and passed through different sieves (2-mm and 0.149-mm) for subsequent experiments and analysis.

The mixed chelator (MC) was the mixture of EDTA, GLDA, and citric acid (molar ratio of 1:1:3). The pH value of each chelator solution was adjusted to 3.50 using the diluted NaOH and HNO_3 solution. The Ethylenediaminetetraacetic acid (EDTA) and citric acid (analytical reagents grade) were purchased from Sinopharm Chemical Reagent Co., Ltd. The N, N-bis(carboxymethyl)glutamic acid (GLDA, relative molecular mass: 351.1, solid content: > 47%, and density: $1.400 \text{ g}\cdot\text{cm}^{-3}$) was purchased from Akzo Nobel Chemicals Co., Ltd.

Z. mays (Yunshi-5) and Chinese cabbage were selected as representative crops to investigate the effects of soil washing on plant growth and the uptakes of heavy metals by shoots and roots.

2.2 Experimental design

The soil washing experiment was conducted in plastic pots (diameter $\times$ height = 9.5 cm $\times$ 12.5 cm). From the bottom to top, each pot was filled in a nylon mesh (200 mesh), quartz sand, and the air-dried soil (1.1 kg). The soil was kept with the field moisture by deionized water for one week. A plastic pot as washing devices was equipped over the plastic pot with soil. The flow velocity of washing agents was $1 \text{ mL}\cdot\text{min}^{-1}$. The trapping device for leachate collection was placed under the plastic pot with soil. The design of washing process is shown in Table 1. The 1st, 2nd and 5th washing used EDTA or MC solution ($0.1 \text{ mol}\cdot\text{L}^{-1}$, 0.55 L), while the 3rd, 4th, 6th and 7th washing used deionized water (0.55 L). The control treatment was washed by deionized water (0.55 L) throughout the experiment. Each treatment was set up in triplicate.

After soil washing, the soil amendment was applied into soil to improve the physiochemical properties. According to our previous studies, the combination of inorganic and organic amendments (0.2% of CaCO_3 and 2% of chicken manure) was thoroughly mixed with the washed soil because this amendment was favorable for the growth of plant (Guo et al. 2019; Yang 2019). The amended-soils were used to plant three-seasons crops. The first-season crop was Chinese cabbage. Ten Chinese cabbage seeds were sown in each pot. Two weeks later, just three young Chinese cabbages were retained. The each pot with Chinese cabbages was watered by deionized water every day (50-100 mL) to keep at 60%-70% of soil water holding capacity. After seven weeks, the aboveground (shoots) and underground (roots) parts of Chinese cabbage were harvested. The second- and third-season crop was *Z. mays*. Five seeds of *Z. mays* for the second-season crop were sown in each pot after harvesting Chinese cabbage. Two weeks later, just three *Z. mays* were retained. The each pot with *Z. mays* was watered by deionized water every day (50-100 mL) to maintain at 60%-70% of soil water holding capacity. After a month, *Z. mays* were harvested. The plant method of the third-season crop (*Z. mays*) was the same as the second-season crop.

2.3 Collection and analysis of soil and plant samples

The shoots and roots of plants were thoroughly washed by the deionized water. The fresh weight of shoots and roots were weighted after being wiped. After that, the plant samples were dried for 30 min (105°C), and then dried to constant weights at 65°C for 72 h. The shoots and roots of plants were grounded, and then stored in a polyethylene bag for further analysis. The soil samples were destructively collected, and then air-dried at room temperature. The dried soil samples were grounded to pass through a 0.85-mm sieves for further analysis. The soil properties (pH, organic matter, cation exchange capacity, total and available nitrogen/phosphorus/potassium) were analyzed according to the methods in the book of Analytical Methods of Soil Agrochemistry (Lu 2000).

The total concentrations of heavy metals (Cd, Pb, Cu, and Zn) in soil samples were determined by atomic absorption spectrometry (AAS, Hitachi Z-2300, Japan) after digestion with HF-HNO₃-HClO₄. The speciation of heavy metals in soil samples was sequentially extracted according to Tessier et al. (1979). The available concentrations of heavy metals in soil were assessed by chemical extraction method using 1M ammonium acetate solution (Wang et al. 2018b). The plant samples (0.5 g aboveground part, or 0.1 g underground part) were digested with HNO₃/H₂O₂ (5:2 v/v) (Hu et al. 2014). After digestion, the solution was filtered through 0.45-μm filters and diluted with deionized water. The concentrations of heavy metals in plant were determined by AAS (Wang et al. 2018b).

2.4 Statistical analysis

The statistical analysis of data was performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). The data was presented as mean values ± standard deviation (SD). Duncan's test at a significance level of $p \leq 0.05$ was used to test the differences between mean values.

3 Results

3.1 The changes in soil properties after washing

Soil washing significantly changed the soil physicochemical properties (Table 2). Soil pH increased 1.5 units after washing when compared with the control ($p < 0.05$). The CEC in soil after washing by MC significantly decreased by 5.5% and 8.3% when compared with the control and EDTA treatment, respectively ($p < 0.05$). In contrast, the EDTA treatment did not evidently change the CEC in soil when compared with the control. The nutrient such as total nitrogen, total and available potassium did not change obviously between soils that being washed with different washing agents. However, the organic matter, total phosphorus, available nitrogen and phosphorus in soil significantly increased after washing by MC. As compared with the control ($p < 0.05$), organic matter, available phosphorus and nitrogen in soil after being washed by MC increased by 5.1%, 43.4% and 65.9%, respectively. As compared with EDTA treatment, total phosphorus and available nitrogen in soil after being washed by MC significantly increased by 42.9% and 55.6%, respectively ($p < 0.05$). These results demonstrated that MC had more positive effect on soil physicochemical properties and soil nutrient than EDTA did.

3.2 The removal efficiencies of heavy metals from the alkaline soil by multiple washing operation

The removal efficiencies of heavy metals from alkaline soil were dependent on washing times (Fig. 1). The high removal efficiencies of heavy metals by MC were obtained at the first, second and fifth washing operations. The highest removal efficiencies of heavy metals by MC (8.8%-23.8%) was obtained at the second washing operation, which was similar to those by EDTA. There was significant difference between the removal efficiencies of all heavy metals by EDTA and MC after the two-step washing. As shown in Fig. 2, the total removal percentages of Cd, Pb and Cu from soil by MC after the seven times washing were distinctly lower than those in EDTA washed soil ($p < 0.05$). The total removal percentages of Zn from soil by MC after the seven times washing were similar to those by EDTA ($p > 0.05$). The removal efficiencies of Pb from soil by MC were evidently higher than other heavy metals.

3.3 The fraction distribution of heavy metals in the alkaline soil after washing

Soil washing significantly changed the fraction distribution of heavy metals in soil (Fig. 3). As compared with the control, each fraction of heavy metals in soil after being washed by MC was significantly decreased. For example, the carbonate-bound and Fe-Mn oxides-bound heavy metals decreased by 10.3%-68.2% and 47.7%-60.3%, respectively. The organic matter-bound Cu decreased by 25%. The exchangeable fraction of Cd significantly decreased, while the exchangeable fraction of Pb, Cu and Zn slightly increased.

The proportion of each Cd fraction in soil after being washed by MC was comparable to that in the soil after being washed by EDTA ($p > 0.05$). The carbonate-bound heavy metals in soil after being washed by MC were lower than those in soil after being washed by EDTA, particularly for the carbonate-bound Pb. Additionally, the proportion of residual fraction of Cu and Zn in soil by after being washed by MC were lower than those from the EDTA treatment. The heavy metals in soil after being washed were more stable than those in the original soil. Similar to EDTA, MC also effectively removed the active fractions of Cd and Zn from the alkaline soil.

3.4 Plant growth and uptake of heavy metals by plant in the washed soil after amendment

3.4.1 Plant growth

Table 3 lists the changes in biomass weight of Chinese cabbage and *Z. may* in soil after being washed by different types of washing agents. The biomass of Chinese cabbage, *Z. may* (batch 1), and *Z. may* (batch 2) in soil after being washed by MC was similar to that in soil after being washed by EDTA and the control ($p > 0.05$). Nevertheless, the biomass weight of *Z. may* in the second batch was 2-3 times of that in the first batch.

3.4.2 The uptakes of heavy metals by plants

The concentrations of heavy metals in the shoots and roots of crops are listed in Table 3. According to Chinese Maximum Levels of Contaminants in Foods (GB 2762-2017), the concentrations of Cd and Pb in food should not exceed 0.2 and 0.3 mg·kg⁻¹, respectively. As shown in Table 3, the concentrations of Cd, Pb, Cu and Zn in Chinese cabbages in soil being washed by MC and EDTA were higher than those in the control. Especially, the concentrations of Cd and Pb in shoots (edible parts) of Chinese cabbage were 1.51-1.78 mg·kg⁻¹ and 0.62-0.74 mg·kg⁻¹, respectively, which exceeded the threshold of aforementioned standard. The concentrations of Cd, Pb and Cu in shoots of Chinese cabbages from the MC treatment were comparable to those from the EDTA treatment ($p < 0.05$). The concentrations of Cd, Pb, Cu and Zn in roots significantly increased by 433.7%, 478.5%, 29.2% and 72.7% from the MC treatment as compared with those from the control, respectively ($p < 0.05$). However, the concentrations of Pb and Cu in roots of Chinese cabbages from the MC treatment were lower than those from the EDTA treatment, while the opposite was true for the other heavy metals.

The concentrations of Cd and Zn in roots and shoots of *Z. may* (batch1) from the MC treatment were obviously lower than those from the control ($p < 0.05$). When compared with the control, The concentrations of Cd and Zn in roots of *Z. may* (batch1) decreased by 71% and 71%, in shoots of *Z. may* (batch1) decreased by 52% and 41%, respectively. In addition, the concentration of Cu in the shoots of *Z. may* (batch1) from soil after being washed by MC were 2.8 times of those from the control. The Pb and Cu in all parts of *Z. may* (batch1) from MC treatment were similar to those from the EDTA treatment ($p > 0.05$). When compared with the control, the concentrations of Cd and Pb in the roots of *Z. may* (batch 2) in the MC treatment significantly decreased ($p < 0.05$). When compared with the EDTA treatment, the concentrations of Pb in the roots and shoots of *Z. may* (batch 2) from the MC treatment were decreased by 49% and 57%, respectively.

3.4.3 Factors influencing on the uptakes of heavy metals by plants

The different washing treatments had different effects on the availability of heavy metals in the alkaline soil (Fig. 4). For the MC treatment after harvest, the available heavy metals in soil first decreased, and then kept stable, which were higher than those in the soil from control. After the three-seasons planting, MC agent more effectively decreased the availability of Cd, Pb and Zn in soil than EDTA treatment did ($p < 0.05$).

4 Discussion

4.1 Effect of soil washing on soil properties

The increase in soil pH was mainly due to the residual of chelators in soil after washing operations (Zupanc et al. 2014a). The increases in available nitrogen and organic matter in soil were due to the introduction of nitrogen and carbon elements from the washing agents (Zupanc et al. 2014b). After washing, phosphorus in minerals could be dissolved and transformed to the form of available one (Guo et al. 2018b), which resulted in the increase in available phosphorus in soil. Furthermore, the citric acid could dissolve and transform insoluble minerals by proton dissociation, carboxyl coordination exchange, and reduction reaction to release nutrient elements, which contributed to the increase in availability of nutrients (Xiao et al. 2019). The little change of CEC in soil after being washed with EDTA was mainly due to the high introduction of Na^+ into soil. The lower CEC in soil after being washed by MC than that by EDTA and control treatment was most likely due to the less introduction of Na^+ and non-selective extraction of ions by MC (Chen et al. 2021). As compared with the results from our previous studies on alkaline soil properties with MC by stirring (soil-solution ratio of 1:2) (Guo et al. 2018a), the changes of soil properties and nutrients were relatively small here. The pot experiment after multiple washing operations extremely alleviated the change of alkaline soil properties.

4.2 Effect of washing times on the removal efficiencies of heavy metals from the alkaline soil

Among seven times washing, the relative high removal efficiencies of heavy metals by MC occurred at the first, second and fifth washing operations. The highest removal efficiencies of heavy metals occurred at the second washing, which was most likely attributed to the desorption of heavy metals from soil particles after the first washing (Yang et al 2016). Additionally, the competitive cations (e.g., Ca^{2+} , Mg^{2+} , Na^+ , K^+ , Fe^{2+}) in the soil after first washing decreased, leading to the decrease in competition of above cations for the binding sites on the components of MC during the second washing (Polettini et al 2007; Finzgar et al 2007). Therefore, to effectively improve the removal efficiencies of heavy metals in the alkaline soil, it is necessary to activate heavy metals by multiple washing operations. The lower removal efficiencies of heavy metals from soil at the 3rd-7th washing operations than those at the 2nd washing operation was most likely due to the following reasons: (1) the washing with deionized water only removed the residual chelates and weakly adsorbed chelates in soil (Jing et al. 2017); (2) there was the decrease in heavy metal contents and redistribution of heavy metal fractions after each washing (Xiao et al. 2019); (3) after the 5th washing, organic matter bound and residual heavy metals in soil were activated and leached, resulting in a relatively dynamic equilibrium in the subsequent washing process.

The types of heavy metals played important role in their removal efficiencies, which was mainly due to their different complexing ability with chelators and different fraction distribution in the alkaline soil. For example, the stability constants of heavy metals with EDTA were 16.46 for Cd, 18.04 for Pb, 18.80 for Cu, and 16.50 for Zn (Jiang et al. 2003). The molecular of EDTA has several carboxyl functional groups, which can effectively activate the heavy metals that bounded on soil particles (Klik et al. 2021). The stability constants of citric acid and GLDA with Cd, Cu and Zn were 3.50 and 9.10, 5.90 and 10.50, and 6.21 and 10.00, respectively (Jiang et al. 2003). Evidently, the components of GLDA and citric acid in MC had relatively weaker complexing ability with heavy metals than EDTA did (Jean-Soro et al. 2012; Thinh et al. 2020). Therefore, the decrease in the amount of EDTA in MC resulted in the relatively lower removal efficiencies of heavy metals from the alkaline soil by MC than the EDTA treatment. The higher removal efficiencies of Pb and Cu from the alkaline soil by MC than other heavy metals was mainly due to the higher complexing ability of Pb and Cu with the components in MC than those of other heavy metals. Although the high content of Zn in the original soil and the strong covalently bonding between Zn and soil particle (Thinh et al. 2020), the MC had similar removal efficiency of Zn to the EDTA did after several times washing. This finding was most likely due to the easy leaching of Zn-EDTA complexes from soil after several times washing (Qi et al. 2019). Meanwhile, the citric acid and GLDA in MC was also effective for the removal of Zn from the alkaline soil.

As compared with the results from our previous studies on removal of heavy metals from acid soil by MC under pot washing condition (Guo et al. 2020b), the removal efficiencies of heavy metals from the alkaline soil by MC were higher than those from the acid soil. The finding indicates that multiple washing operation using MC agent was an effective way for effective removal of heavy metals from alkaline soil because of enough reaction time and washing ability for activating heavy metals in such type of soil.

4.3 Effect of soil washing on the fraction distribution of heavy metals in the alkaline soil

The stable fraction of heavy metals in the alkaline soil after being washed by MC increased (Fig. 3). The decrease in carbonate-bound and Fe-Mn oxides-bound heavy metals after being washed by MC was most likely due to that these fractions of heavy metals were sensitive to soil pH and easily being washed out by acidic agents (Zhao 2019). The percentages of residual Cu in soil were higher than those of organic matter-bound fraction of Cu, which resulted in a low removal efficiency of Cu during washing. The crystal structure of residual fraction of heavy metals was hardly destroyed by EDTA, GLDA and citric acid agents because the heavy metals were tightly trapped in the mineral crystal structure (Xiao et al. 2019). The effective removal of the active Cd and Zn in the alkaline soil by MC contributed to the decrease in environmental risk and bioavailability of these heavy metals (Wang et al. 2018a). Nevertheless, a portion of stable heavy metals were activated after soil washing operations. The ligand bridges in soil between mineral surface and metal cations were formed after washing, which caused the re-adsorption of heavy metal-chelate complexes on the mineral surface (Chen et al. 2016; Guo et al. 2018a). It is consistent with the increase in the exchangeable fraction of Pb, Cu and Zn in soil after being washed by MC. Moreover, the organic matter-bound Cu in the alkaline soil was activated by MC, which was most likely due to soil organic matter was washed during washing process (Liu et al. 2013).

The fraction distribution of heavy metals was different in the soils that being washed by MC and EDTA. (Fig. 3). Particularly, the more carbonate-bound Pb, Cu and Zn in the alkaline soil from MC treatment than those from the EDTA treatment was mainly due to the easily extraction of carbonate-bound heavy metals by EDTA (Chen et al. 2015). Moreover, the lower proportions of residual Cu and Zn in the alkaline soil from MC treatment than those from EDTA treatments led to the increase in Fe-Mn oxides bounded Zn and exchangeable Cu. Furthermore, the changes in fraction of heavy metals were related to various factors, especially the types of heavy metals, soil properties, and inorganic and organic colloid (Guo et al. 2020b).

4.4 Effect of the washed soil after amendment on plant growth and plant uptakes of heavy metals

When compared with the soil from control and EDTA treatment, the soil from MC treatment did not have evidently negative effect on the plant growth (Table 3). The biomass weight of *Z. may* in the second batch was greater than that in the first batch, which was primarily due to the different available contents of heavy metals in soil (Guo et al. 2018b).

The heavy metals in the alkaline soil were activated after soil washing operations, particularly being washed by EDTA and GLDA (Wei et al. 2015), resulting in the easy uptakes of heavy metals by Chinese cabbage and *Z. mays* (Chaturvedi et al. 2019). The Chinese cabbage is a kind of leafy crops that has the higher transpiration rate than *Z. mays*, resulting in the easier transportation of heavy metals from soil to shoots of Chinese cabbage than *Z. mays* (Cheng et al. 2021). Additionally, the toxic effect of washing agents in soil can also promote phytoextraction of heavy metals. The EDTA had toxic effects on the plants because EDTA could enhance the destruction of plasma membrane and physiologic barrier of plant roots (Wang et al. 2021). With the increase of membrane effusion, the hazardous substances easily transport from roots to shoots through transpiration (Zhou et al. 2021). The GLDA and citric acid had a weakly negative influence on plasma membrane of plant, which benefited for GLDA-metal complexes absorbed by plant (Wu et al. 2015). Therefore, there were high levels of heavy metals in roots of Chinese cabbages from the MC

treatment, especially Pb and Cu. Simultaneously, the concentrations of Cd and Pb in the shoots (edible parts) of Chinese cabbage were exceeded the threshold of Chinese Maximum Levels of Contaminants in Foods (GB 2762-2017).

For the MC and EDTA treatments, the uptakes of heavy metals in the two batches of *Z. mays* had evident differences. On the one hand, it needed time for the inorganic and organic amendments to effectively improve the soil physiochemical properties (Spana et al. 2019; Yang et al. 2019). On the other hand, the plant uptakes of heavy metals were also related to the changes in proportion of available heavy metals in soil and the application of amendment (Guo et al. 2020a). After the soil being washed by MC, the concentrations of Cd and Pb in the shoots of *Z. mays* (two batch) were still below the threshold of the Standards of Feeds of China (GB 13078-2017) ($\text{Cd} < 1 \text{ mg}\cdot\text{kg}^{-1}$, $\text{Pb} < 30 \text{ mg}\cdot\text{kg}^{-1}$). Therefore, the *Z. mays* (batch1 and batch2) from the soil after being washed by MC was safe for the use as feed production.

For the three-season crops, the concentrations of Cu and Zn in the shoots and roots of plants from both MC and EDTA treatment generally increased, which was mainly due to that the essential trace elements for crop growth could transport them into plants by the transport proteins on membrane surface (Liu et al. 2020). However, the non-essential elements (Cd, Pb) could be uptake by plant through binding with carboxyl uronic acid (Chen et al. 2021). The concentrations of heavy metals in roots were obviously higher than those in shoots was mainly attributed to that the compounds from the binding between heavy metals and ligand of root secretion were adsorbed and immobilized on the negatively charged cell wall of roots (Wei et al. 2015; Etesami et al. 2018; Yadav et al. 2018). Furthermore, the roots of *Z. mays* could effectively block the transport of heavy metals to its shoots, which could further explain the reason why the lower concentrations of heavy metals in shoots of *Z. mays* than those in shoots of Chinese cabbage (Cheng et al. 2021).

During planting, the lower available heavy metals in soil from MC treatment was most likely due to that the inhibition of acid secretion from plant roots by citric acid in MC, resulting in less activated heavy metals in MC-treated soil than those in other treatments (Li et al. 2021). Therefore, the MC washing agent could effectively remediate the alkaline soil that was contaminated by heavy metals and benefited for the agricultural reuse of soil.

The correlations between the available heavy metals in soil and the contents of heavy metals in plants are showed in Fig. 5. There were positive correlations between the available Cd, Pb, Cu and Zn in soil before planting and heavy metals in roots and shoots of Chinese cabbage ($p < 0.05$). There were significant positive correlations between available Pb in soil before planting *Z. mays* (batch 1) and Pb in roots and shoots of *Z. mays* (batch 1) ($p < 0.01$). The available Cu and Zn in soil before planting *Z. mays* (batch 2) positively correlated with Cu and Zn in the roots and shoots of *Z. mays* (batch 2) ($p < 0.01$). These results showed that the available heavy metals in soil were easily transported from soil into the roots with plant growing and then to shoots, which improved the uptakes of heavy metals by plant, particularly by the roots (Shahid et al. 2012). Additionally, the uptakes of heavy metals by plants were also related to the soil properties, plant physiological characteristics, and the bioavailability of heavy metals (Chen et al. 2021). Significantly negative correlations between the available Zn in soil and the uptake of Cd in roots and shoots of *Z. mays* ($p < 0.05$) further demonstrated the occurrence of competition for transfer protein between Cd and Zn (Cai et al. 2019).

5 Conclusions

This study investigated the influence of multiple washing operations on the removal efficiencies of heavy metals from an alkaline soil and the reuse strategy of the washed soil for safe agricultural production. According to the results, the removal percentages of Cd, Pb, Cu and Zn from the alkaline soil by MC were similar to those by EDTA. The highest

removal efficiencies of heavy metals from all the treatments were obtained in the second washing. The multiple washing operations by pot experiment alleviated the change of physicochemical properties of the alkaline soil when compared our previous studies where used the combination of washing and shaking/stirring. The washing agents enhanced the transformation of relatively stable fractions of heavy metals to the unstable ones, which was not conducive to the agricultural reuse. After application of amendment, the soil washing operations had no obviously inhibition of the plant growth. Although the uptakes of heavy metals by shoots of Chinese cabbage had no significant difference when compared with the control, the heavy metals in the shoots of *Z. mays* (two batch) notably decreased and meet to the Hygienical Standard for Feeds of China (GB 13078 – 2017). When compared with the soil from EDTA treatment, the available heavy metals in the soil from MC treatment were significantly decreased after three-seasons harvests. Therefore, the multiple washing operation by MC is recommended for remediating alkaline soil that is heavily contaminated by heavy metals.

Declarations

Authors contributions Xiaofang Guo: Methodology, Original draft preparation, and funding, Yu Gao: Data analysis, graphic production, and writing, Wei Han: Experiment and data analysis, Guohui Zhao: Experiment and data analysis, Guixiang Zhang: Supervision, writing, reviewing and editing, Dandan Liu: Reviewing and editing

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Data Availability All data are true and valid and can be available.

Conflicts of interests The authors declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

Ethics approval I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously.

Consent to participate All the authors listed consent to participate

Consent for publication All the authors listed have approved the manuscript that is enclosed.

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Tables

Table 1 The design of washing times

Treatments	Soil washing times						
	first	second	third	fourth	fifth	sixth	seventh
Control treatment	DW ^a	DW	DW	DW	DW	DW	DW
MC treatment	MC ^b	MC	DW	DW	MC	DW	DW
EDTA treatment	EDTA ^c	EDTA	DW	DW	EDTA	DW	DW

a DW, Deionized water (0.55 L)

b MC, Mixture of chelators (0.55 L)

c EDTA, (0.55 L)

Table 2 The basic physiochemical properties and heavy metal contents in different soils

Parameter	Original soil	Control soil	MC-washed	EDTA-washed
pH	8.36±0.01 a	8.56±0.04 a	9.94±0.18 b	9.71±0.20 b
Organic matter (g·kg ⁻¹)	31.3±0.0 a	31.6±0.8 a	33.2±0.3 b	34.8±1.1 b
Total nitrogen (g kg ⁻¹)	3.78±0.69 a	3.46±0.30 a	4.38±0.45 a	3.92±0.60 a
Total phosphorus (g kg ⁻¹)	0.66±0.00 b	0.61±0.01b	0.60±0.01 b	0.42±0.36 a
Total potassium (g kg ⁻¹)	19.6±3.3 b	15.0±1.3 a	13.8±1.6 a	14.4±4.2 a
Available nitrogen (mg kg ⁻¹)	78.8±0.0 a	78.8±4.6 a	130.7±9.6 b	84.0±9.9 a
Available phosphorus (mg kg ⁻¹)	17.6±0.0 a	16.6±0.5 a	23.8±1.5 b	24.1±1.0 b
Available potassium (mg kg ⁻¹)	203.1±0.0 b	181.0±6.9 a	173.2±6.0 a	177.4±6.3 a
Cation exchange capacity (cmol kg ⁻¹)	42.9±0.6 b	43.4±1.3 bc	41.0±0.6 a	44.7±1.0 c
Total Cd (mg·kg ⁻¹)	19.2±0.3 a	20.6±0.6 a	11.7±1.4 b	8.9±0.8 c
Total Pb (mg·kg ⁻¹)	373±8 a	293±8 a	172±9 b	108±9 b
Total Cu (mg·kg ⁻¹)	67.6±1.2 a	70.9±2.4 a	60.2±1.4 b	53.6±8.7 c
Total Zn (mg·kg ⁻¹)	970±13 a	964±22 a	621±1 b	597±24 b

Note: The values in the table are mean ± Standard deviation (n = 3), according to Duncan's multiple range test ($p < 0.05$), the means in the same line followed by the same letter were not significantly different.

Table 3 Yields (g·pot⁻¹) of plants and heavy metal contents (mg·kg⁻¹) in plants in the soils from different treatments.

Parameters		Aboveground			Underground		
		Control	MC	EDTA	Control	MC	EDTA
Chinese cabbage	Fw	17.05±2.93a	19.32±0.96a	19.23±1.64a	0.26±0.08a	0.22±0.07a	0.24±0.09a
	Cd	1.49±0.07a	1.78±0.37a	1.51±0.52a	2.52±0.50a	13.45±3.80b	1.10±0.22a
	Pb	0.45±0.08a	0.62±0.07b	0.74±0.13b	3.54±1.44a	20.48±0.67b	53.15±5.36c
	Cu	5.34±0.51a	5.35±0.48a	6.01±2.79a	25.44±4.64a	32.87±1.29 b	47.98±1.84c
	Zn	19.13±2.22b	18.93±2.37b	13.31±3.83a	76.14±8.55a	131.51±14.91b	62.34±15.34a
<i>Z. mays</i> (batch1)	Dw	0.45±0.13a	0.48±0.06a	0.41±0.13a	0.07±0.02a	0.08±0.01a	0.07±0.06a
	Cd	0.86±0.08b	0.41±0.00a	0.38±0.09a	5.67±0.38c	1.63±0.05b	1.02±0.35a
	Pb	0.25±0.13a	0.70±0.34a	1.12±1.38a	0.25±0.13a	0.70±0.34a	1.12±1.38a
	Cu	1.62±0.05a	1.49±0.24a	1.62±0.15a	4.30±1.11a	3.99±0.70a	4.23±0.70a
	Zn	23.84±0.84c	14.10±2.21a	18.40±0.20b	46.63±5.17b	13.53±7.51a	6.58±0.53a
<i>Z. mays</i> (batch2)	Dw	1.34±0.14a	1.09±0.35a	0.98±0.54a	0.37±0.09a	0.24±0.16a	0.16±0.17a
	Cd	0.36±0.03a	0.26±0.04a	0.29±0.13a	1.57±0.18c	0.77±0.15b	0.32±0.21a
	Pb	0.14±0.02a	0.44±0.01ab	1.03±0.83c	2.64±0.65b	1.08±0.24a	2.10±0.38b
	Cu	0.84±0.09a	1.58±0.09c	1.25±0.15b	3.96±0.84a	2.37±0.48a	7.47±6.74a
	Zn	14.79±0.35a	14.23±2.56a	16.27±7.77a	6.31±1.87a	36.17±10.86b	9.26±7.23a

Note: FW, Fresh weight; DW, Dry weight.

Figures

Fig. 1

Figure 1

Effect of different treatments on the removal efficiencies of heavy metals in the contaminated soil.

Fig. 2

Figure 2

The total removal efficiencies of heavy metals from the contaminated soil by washing.

Fig. 3

Figure 3

Effect of different treatments on the changes in fractions of heavy metals in soil (OS: Original soil; CK: Control treatment; MC: MC treatment; EDTA: EDTA treatment)

Fig. 4

Figure 4

Effect of different treatments and washing times on NH_4Ac -extracted heavy metals in soil.

Figure 5

Correlations between the NH_4Ac -extracted heavy metals and the uptake of heavy metals by plants (A, Chinese cabbage; B, batch 1 *Z. mays*; C, batch 2 *Z. mays*) .

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