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Humic Acid Reduces the Available Cadmium, Copper, Lead, and Zinc in Soil and Their Uptake by Tobacco

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Abstract: Tobacco (*Nicotiana tabacum* L.) is a crop that is able to accumulate metals. In this study, humic acid was selected as a Cd, Cu, Pb, and Zn passivator, and added to calcareous field soil in amounts of 6.4, 10.3, and 14.8 kg·ha^{-1}. Its impact on the soil fractions of the metals in the soil was extracted by the Community Bureau of Reference (BCR) sequential extraction method, and their accumulation of the metals in tobacco leaves was investigated. Application of 14.8 kg·ha^{-1} humic acid decreased the DTPA-extracted concentrations of Pb, Cd, Zn, and Cu by 39%, 37%, 29%, and 18%, respectively, as compared with untreated soil. The fractions of Cd, Pb, Cu, and Zn in soil were extracted by the BCR sequential extraction method, and the relationship between the difference metal fractions in the soil and the metal contents in the plant materials were analyzed. The exchangeable fractions of Cd, Pb, Cu, and Zn and the reducible fractions of Pb and Cu are the main bioavailable fractions. Additionally, the reducible fractions of Cd and Zn, the oxidizable fractions of Pb, Cu, and Zn, and all residual fractions of metals were nonbioavailable fractions in the soil. The soils were treated with humic acid (HA) to shift bioavailable metals to stable phases that were less bioavailable. The available Cd, Pb, Cu, and Zn were strongly retained in the soil after the application of humic acid, which decreased the uptake in tobacco in the upper, middle, and lower leaves.

Keywords: humic acid application; metal; bioavailable metal fraction; tobacco leaf

1. Introduction

Metal pollution in soils is a serious environmental problem with potentially harmful consequences for agriculture and human health. Modern agricultural practices and industrial activities have resulted in Cd, Cu, Pb, and Zn accumulation in soils [1]. Metals occur in soil naturally and their concentrations can differ due to the soil type. [2]. Research has shown that the accumulation of Cu, Cd, Zn, Hg, and Pb, and especially Cd in soils were enhanced by geological background and carbonate rock weathering [3,4]. The long-term use of phosphate fertilizers that contain these metals and the application of contaminated sewage sludge can further enrich agricultural soils with metals [5]. Because these trace elements can be found at relatively high concentrations in crops, their presence in plants is of concern for humans [6]. Hence, there is a need to either remove them from the environment or to avoid their uptake by crop plants that are consumed by humans.

Tobacco has the potential to accumulate Cd and Pb from contaminated soil [7]. Excessive Cd taken up by tobacco inhibits the absorption of N, P, and K, reducing the biomass of shoot and root [8], while Pb mainly influences seed germination and photosynthesis [9]. In contrast, Cu and Zn, which are essential mineral elements for plant growth, are crucial for maintaining chloroplast structure, enzyme activity, tobacco aroma and growth [10]. Research has shown that soil with 2.0 to 4.8 mg/kg Zn benefits tobacco growth, making it possible to increase production and quality, and accelerate the uptake of nutrients [11]. However, tobacco can be poisoned by high concentrations (>9.8 mg/kg) of Zn in the soil [12,13]. It is known that heavy metals in tobacco get into the human body from the smoke that gets through the lungs of people who smoke. Indeed, tobacco is considered a major source of toxic metals in human blood and various organs [14]. Unfortunately, heavy metals in tobacco have not aroused widespread public awareness, and administration related to the content of heavy metals in tobacco is still lacking.

Total metal concentrations alone cannot evaluate the metal's mobility, bioavailability, and environmental impact sufficiently [15]. The Community Bureau of Reference (BCR) sequential extraction method is widely used to determine the binding forms and the mobilities of metals in soils. Furthermore, diethylene triamine pentaacetate acid (DTPA) extracted metals have been reported to have better correlations between soil metal content and crop tissue content [16,17]. In general, Pb, Cd, and Zn are preferentially taken up by tobacco due to their potential bioavailability [18,19]. In comparison to the total metal concentrations, the available or exchangeable and reducible fractions are highly correlated [20]. Some studies have shown that the available, exchangeable, and reducible fractions mainly determine the mobility and bioavailability [21] or phytotoxicity [22] of, for example, Cd, Cu, Pb, and Zn in soils. Passivation methods, by transfer metals to stable phases, have been widely used in the remediation of metal-contaminated soils [23]. Various methods and techniques have been used for passivation of Cd, Pb, Cu, and Zn, but the application of humic acid (HA) is one of the most promising [24,25]. HA has a high complexation capacity for these metals as it contains carboxylic and phenolic groups as complexation sites, which can reduce the bioavailability and mobility of metals [26–29]. Therefore, quantifying the available, exchangeable, and reducible fractions after HA application is necessary to assess metal mobility.

The application of humic acid to stabilize (i.e., immobilize) metal ions, especially cations, in soil has been widely used [21,24,25]. HA amendments have been proven to be effective to improve soil functions, and especially to enhance the ability of anti-acidification and fertility [24,30,31], allowing for the establishment of a plant cover. However, most of these experiments were conducted in the laboratory, and the effects of HA on heavy metal uptake by tobacco in the field have not yet been evaluated.

In this study, HA was applied in a field experiment with tobacco, and the available and chemical fraction contents of Cu, Zn, Cd, and Pb and their accumulation in different parts of tobacco leaves were determined. The aims of this study were as follows: (1) To illustrate the passivation effects of HA at different dosages in a field experiment and(2) to determine the effects of HA-treated soil on the uptake of these metals into tobacco leaves. The findings could help reducing the risk of metal contamination in soil and improve the quality of tobacco.

2. Materials and Methods

2.1. Soil, Plant, and Humic Acid Characterization

The field experiment was carried out in Luo Cheng County which is located in the northern part of the Zhuang Nationality Autonomous Region, Guangxi. The soil was classified as a typical calcareous soil. Its total Cd content is significantly higher than that given in the Chinese soil environmental quality standards, while the total Pb, Cu, and Zn content are lower than the standard value. The basic soil characteristics are shown in Table 1.

Table 1. Soil characteristics and Chinese soil environmental quality standards (total concentration in mg·kg^{-1}).

Item	pH	Organic Matter Content	Available N	Available P	Available K	Total Content				DTPA Extracted			
						Cd	Pb	Cu	Zn	Cd	Pb	Cu	Zn
Soil	7.8	21	109	12	141	3.82	21.31	36.02	146.69	0.73	1.42	3.38	4.46
Chinese standard	>7.5	–	–	–	–	0.6	170	100	300	–	–	–	–

–, the value was not defined in Chinese soil environmental quality standards (no. GB15618-2018).

Tobacco seeds (variety K326, Guangxi Nanning Tobacco Co., China) were germinated in pots filled with vermiculite in a greenhouse. Seedlings were transferred into the field 28 days after germination.

HA was extracted from lignite (provided by Guangxi Nanning Ruihong Energy Technology Co., China) [32], following a modified International Humic Substances Society procedure. Briefly, air-dried coal was extracted with a solution of 0.1 mol L^{-1} $Na_4P_2O_7$ and 0.1 mol·L^{-1} KOH using a coal-to-extractant ratio of 1:10 (w:v) by mechanical shaking at room temperature. Then, the mixtures were allowed to stand for 24 h to separate the solid residue by sedimentation and the supernatant natural deposition. The extraction was repeated three more times. The combined supernatants were acidified to a pH of 1 with 6 mol L^{-1} HCl, allowed to stand for 24 h to permit coagulation of HA, and then natural deposited for 12 h. The precipitated HA was purified by dissolution in an extraction solution, allowed to stand for 12 h to deposit the residue completely, and acidified again to a pH of 1 with 6 mol·L^{-1} HCl. The suspensions were allowed to stand for 24 h at 20 ± 2 °C, and the sediments were recovered. The purification steps were repeated three more times together. The precipitated HAs were, then, recovered with distilled water and dialyzed. When free of Cl^- ($AgNO_3$ test), the HAs were recovered and air-dried for 24 h at 20 ± 2 °C.

2.2. Experimental Design

Each of the four experimental areas was 46.8 m^2 (13.0 m × 3.6 m), and each treatment was replicated on four plots in a randomized block design. In each plot, there were three rows 1.2 m apart, with 26 tobacco plants per row spaced in each row 0.5 m apart, for a total of 78 tobacco plants per plot. For separation between the plots of different treatments, three rows of tobacco were used. The four treatments were as follows: one control without HA addition (Control), and three treatments with total amounts of dry HA of 6.4 kg·ha^{-1} (HA6.4), 10.3 kg·ha^{-1} (HA10.3), and 14.8 kg·ha^{-1} (HA14.8) by application of an aqueous suspension of HA (dry HA dissolved in 100 L water) to the soil near the plants root, 24% of the total amount at day 30, 38% at day 45, and 38% at day 64 after transplantation. The ambient temperature in the tobacco growing area was approximately 25/19 °C (day/night), the photoperiod was approximately 15 h per day, and the relative humidity was 75%. Prior to tobacco planting, basic fertilizer was applied at 30 kg·ha^{-1} N, 100 kg·ha^{-1} P_2O_5, and 260 kg·ha^{-1} K_2O. Tobacco growth management was based on the cultural practices recommended by the Tobacco Institute in the Zhuang Nationality Autonomous Region, Guangxi.

2.3. Sampling and Analysis

In the spring before fertilization and in the fall after tobacco harvest, soil samples consisting of five subsamples of 1 kg were taken from the layer from 0 to 20 cm of each experimental plot, and composite samples of treatment were prepared for each plot by mixing the subsamples. The composite samples were air-dried for 7 days, removed of plant residue and stones, crushed, and passed through a 2 mm sieve for available metals analyses and through a 0.15 mm sieve for total metals analyses. The soil samples were used for determinations of the pH (soil to carbon dioxide-free water = 1:2.5, w/v, UB-10, Denver Instrument, U.S.), available N (1.0 mol L^{-1} NaOH and H_3BO_3), available P (0.5 mol L^{-1} $NaHCO_3$), and available K (1.0 mol L^{-1} NH_4OAc) [33]. For total metals analyses, 0.2000 g of soil was digested by 8 mL of HNO_3/HCl (1/3, v/v). The available metals contents were extracted from 5.00 g of soil with 20 mL of an aqueous solution of diethylene triamine pentaacetatic acid (DTPA) (Sinopharm Chemical Reagent Co., Shanghai, China) [34]. The soil samples were air-dried and ground to pass through a 60-mesh sieve, and then the modified BCR sequential extraction method (European Communities Bureau of Reference) was applied to measure different fractions of Cd, Pb, Cu, and Zn in the soil as follows [25,35]:

Exchangeable fraction of metals The air-dried soil samples (2 g dry weight) were extracted with 20 mL of 1 mol L^{-1} $MgCl_2$ (pH = 7.0) in Teflon centrifuge tubes for 1 h at 25 ± 1 °C with continuous agitation. The suspension was then centrifuged (5000 g for 5 min). The supernatant liquid was filtered through 0.45 μm filter paper and stored at 4 °C prior to analysis.

Reducible fraction The residue from the exchangeable fraction was extracted with 20 mL of 0.04 mol·L^{-1} $NH_2OH·HCl$ (solvent is HAc solution, Hac: water = 1:4) with a pH of 1.5 for 6 h at 25 ± 1 °C with continuous agitation. The suspension was then centrifuged and filtered as in the first step.

Oxidizable fraction The residue from the reducible fraction was extracted with 20 mL of 30% H_2O_2 (pH = 2.0, with 1M HNO_3) for 2 h at 85 ± 2 °C with occasional agitation and an additional 20 mL of 3.2 mol L^{-1} NH_4Ac for 16 h at 85 ± 2 °C with continuous agitation. The suspension was centrifuged and filtered as described in the first step.

Residual fraction of metals The residue from the oxidizable fraction was removed and microwave-digested with 8 mL of HNO_3, 2 mL of $HClO_4$, and 2 mL of HF.

The flowers were removed when the tobacco was mature. The leaves were divided into upper, middle and lower leaves according to the different developmental stages of each plant from top to bottom at maturity. The lower leaves were the 1st to 6th leaves, the middle leaves were the 7th to 12th leaves, and the upper leaves were the 13th to 19th leaves. The leaves were collected by hand, tips and veins were removed after washing with deionized water, and the leaves were dried at 65 °C for 48 h in an oven before being grounded with a crusher. The plant materials (0.5000 g) were microwave-digested by 8 mL HNO_3/H_2O_2 (4/1, v/v) in a microwave digester (Mars 6, CEM, US) at 220 °C for 30 min until a clear solution about 1 to 2mL was obtained. The concentrations of Cu, Zn, and Pb were determined by flame atomic absorption spectroscopy (ZEEnit 700P, Analytik Jena, Jena, Germany). The content of Cd was analyzed using graphite furnace atomic absorption spectrometry (ZEEnit 700P, Analytik Jena, Jena, Germany). During the procedure, digestion blanks and certified reference materials (GBW10044, GSB-22) were included for quality control.

2.4. Data Processing

The data were analyzed using analysis of variance (ANOVA). Duncan's multiple range tests were used to detect differences between means for the fixed effects at the probability levels of $p < 0.05$ and $p < 0.01$ (IBM SPSS Statistics 19.0). The figures were drawn with Origin Pro 8.0.

3. Results

3.1. DTPA Extracted Cd, Pb, Cu, and Zn in the Soil

The contents of the DTPA-extracted metals contents were significantly decreased with HA treatment ($p < 0.05$, Figure 1) as compared with the CK treatment. The HA10.8-amended soil presented lower concentrations of DTPA-extracted metals than the other treated soils, with DTPA-extracted Cd, Pb, Cu, and Zn contents reduced by 37%, 39%, 18%, and 29%, respectively, as compared with those in CK. The reduction of the DTPA-extracted metal fraction was more significant for Cd and Pb than for Cu and Zn.

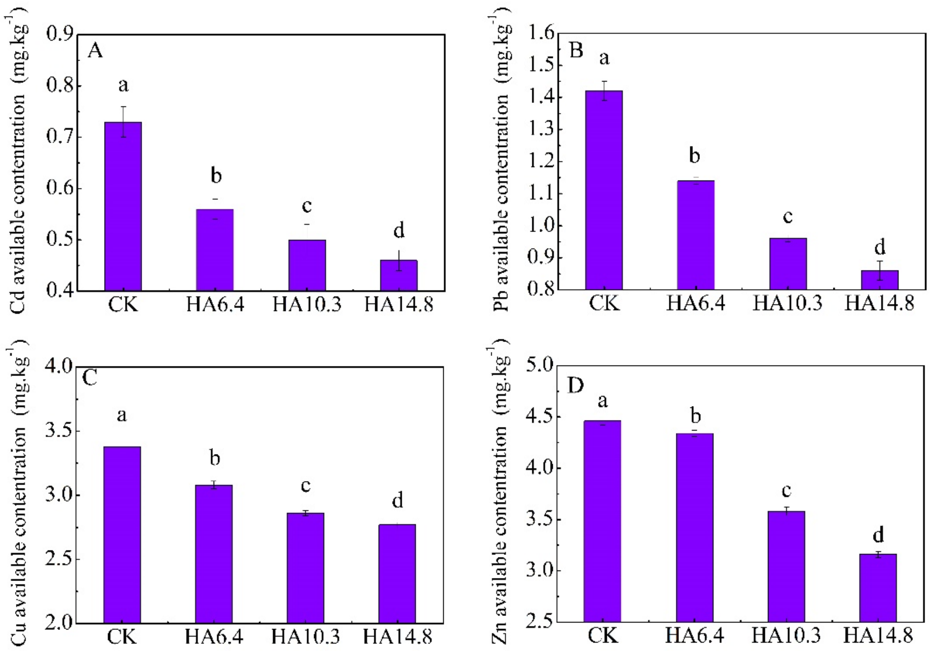

Figure 1. The DTPA-extracted Cd (A), Pb (B), Cu (C), and Zn (D) in soil after harvest of tobacco. Different lowercase letters in the same column indicate significant differences at $p < 0.05$ according to Duncan's test. Bars represent standard errors ($n = 4$).

3.2. Chemical Fractions of Cd, Pb, Cu, and Zn in the Soil

The modified BCR sequential extraction was applied to determine the metal fractions in the soil. The RES Cd, Pb, Cu, and Zn constituted the largest proportion of the total content, which exceeded 40%, and Zn reached 65% (Figure 2). The fates and behaviors of the Cd, Pb, Cu, and Zn fractions were different with the HA treatments. With increasing amounts of HA, the concentrations of exchangeable fractions of Cd, Pb, Cu, and Zn, and the reduction fraction of Pb and Cu decreased with a corresponding increase of the other fractions. The oxidizable fraction is mainly the chelates of organics and metals [25], and in this study, it was clearly observed that the oxidizable fractions of Pb, Cu, and Zn increased with increasing HA; however, there was no significant change in the fraction of the oxidizable Cd. It is worth noting that the RESs of Cd, Cu, and Zn with HA treatment showed no significant difference as compared with the CK treatment. Overall, the soils treated with HA showed shifts of the soil metals away from exchangeable fractions to forms that are stable phases and less bioavailable.

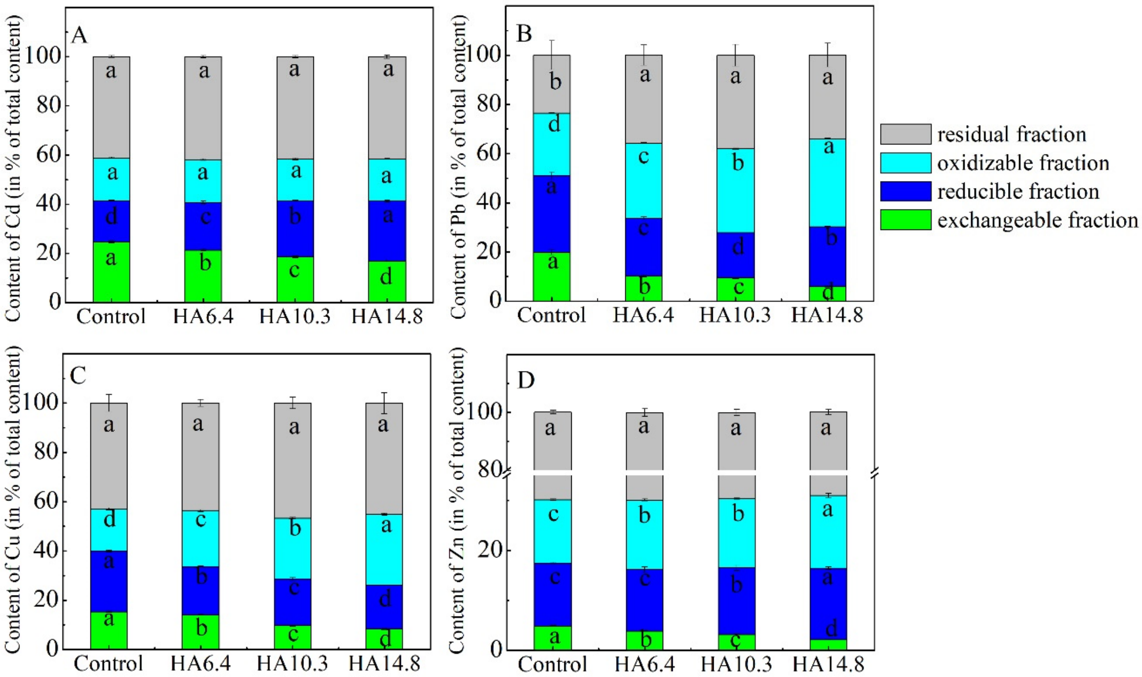

Figure 2. The chemical fractions of Cd (A), Pb (B), Cu (C), and Zn (D) in soil after harvest of tobacco. Data are means ± SD (n = 4) and bars represent standard errors. Lowercase letters represent significant differences at the 0.05 level between different treatments at the same fraction.

3.3. Metal Transport into Leaves of Tobacco of Different Ages.

Tobacco has the ability to accumulate metals and transport them from the roots into the shoots. The contents of metals in soil and tobacco leaves were analyzed. Here "accumulation" means the concentration of a metal in the plant is higher than that in the soil. Figure 3 shows that tobacco had an accumulation effect for Cd, while it had only an uptake effect for Pb, Cu, and Zn. The soils treated with HA received a dramatic decrease in the Cd, Pb, Cu, and Zn contents in the tobacco leaves ($p < 0.05$, Figure 3), and with the HA dosage increasing, the uptake of metals in the tobacco leaves decreased. It should be noted that in the HA14.8 treatment, the Cd contents in the upper, middle, and lower parts of the leaves were reduced by 32%, 32%, and 33%, and those of Pb were reduced by 50%, 31%, and 25%, respectively. Additionally, the Cu contents were reduced by 15%, 25%, and 29%, and the Zn contents were reduced by 9%, 9%, and 9%, respectively. That is to say, the inhibit effect of Cd and Pb is much stronger than that of Cu and Zn.

The distribution of metals in the tobacco leaves followed certain rules. For Cd, the contents in the leaves exclusively followed the order of lower leaves > upper leaves > middle leaves; for Cu, upper leaves > middle leaves > lower leaves; for Zn, middle leaves > lower leaves > upper leaves. However, the Pb distribution was irregular.

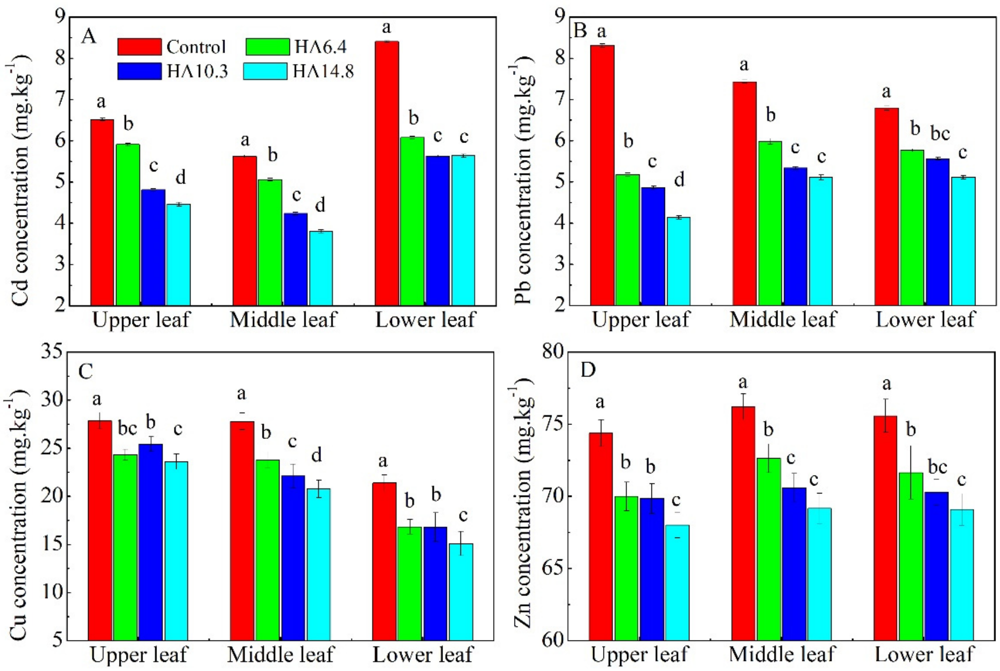

Figure 3. The contents of Cd (A), Pb (B), Cu (C), and Zn (D) in different parts of tobacco leaves. Lowercase letters indicate significant differences among Cd, Pb, Cu, and Zn contents in the same part of the leaves ($p < 0.05$). Bars represent standard errors ($n = 4$).

3.4. Correlations between the Fractions of Metals with the Contents in Tobacco

The DTPA-extracted metals contents showed a positive correlation with the metal contents of tobacco leaves (Table 2). Furthermore, the relationship between the metal fractions and leaf absorption was further analyzed, and the results showed that the Cd, Pb, Cu, and Zn exchangeable fractions and the Pb and Cu reduction fractions presented a significant positive correlation with the enrichment of metals in the leaves ($p < 0.01$, Table 2), while the reduction fractions of Cd and Zn were markedly negatively correlated with leaf uptake. For the oxidizable fractions, there were negative correlations between the Pb, Cu, and Zn contents in leaves and the oxidizable fractions of metals in the soil; in contrast, Cd showed a positive (but not significant) correlation ($p < 0.01$, Table 2). This result is consistent with Figure 2. This finding indicates that the exchangeable fractions of Cd, Pb, Cu, and Zn and the reduction fractions of Pb and Cu were the bioavailable fractions in the soil. Additionally, the reducible fractions of Cd and Zn; the oxidizable fractions of Zn, Pb, and Cu; and all the residual fractions were the nonbioavailable fractions.

Table 2. Pearson correlation coefficients were used to analyze the relationship between the different metal fractions in the soil and the metal content in the plant materials.

Item	C_{leaf}−Cd	C_{leaf}−Pb	C_{leaf}−Cu	C_{leaf}−Zn
DTPA-extraction	0.955 **	0.972 **	0.895 **	0.830 **
Exchangeable fraction	0.985 **	0.993 **	0.754 **	0.928 **
Reducible fraction	−0.979 **	0.807 **	0.921 **	−0.677 **
Oxidizable fraction	0.416 ns	−0.940 **	−0.939 **	−0.876 **
Residual fraction	−0.228 ns	−0.661 **	−0.192 ns	−0.019 ns

F-values for the available fractions of metals were determined by the accumulation and interaction of metals in tobacco; ns, nonsignificant F ratio ($p \leq 0.05$); ** indicates that the correlation is significant at the 0.01 level and indicates a negative correlation.

4. Discussion

The soil of the tobacco growing region has a typical calcareous soil, geological background and the carbonate rock weathering effect releases metals into the soil, especially Cd [3,4], which increases the soil metal concentration and the environmental risk. HA reduces the bioavailability of metals by strong affinity and ability of forming stable chelate with metal ions, with carboxylic groups and phenolic-OH being the dominant binding groups in HA; this effect varies by metals [26–28]. Metals have different affinities for HA based on their stability constants, and Pb (14.8) has a stronger affinity than Cd(7.8), Cu(13.3), and Zn(8.1) [36]. It is worth emphasizing that the binding order of metals to HA in a typical multimetal contaminated soils is Pb > Zn ≈ Cu > Cd [21]. The data processed by the Scatchard method has revealed two binding sites for Cu and Pb and one binding site for Cd and Zn in the sediments; thus, the complexing capacity order was Pb > Cu > Cd [37]. In this study, the reduction of available Pb (Figure 1, removal rate range 20% to 39%) in soil was higher than those of Zn (3% to 29%), Cu (9% to 18%), and Cd (23% to 37%). In addition, metals at low concentrations can be more effectively redistributed than those at high concentrations [38], and the fractions of metals are also critical factors affecting the removal of available metals [21,39]. In this study, the total Cd in the soil was much lower than the amounts of other metals, while its exchangeable fraction content was higher. Conversely, the Pb and Cu exchangeable fractions were less than 20%, and the RES of Cu was up to 65% in the initial soil. Although some studies have shown that Cd has lower affinity for HA than Cu and Zn [26,40], based on the above analysis, the reduction of available Cd (removal rate range was 23% to 37%) was more significant than that of available Zn and Cu (Figure 2 and Table 2).

Using the chemical fraction results instead of the total metal concentrations provides better insight into the distribution and binding forms of metals for a better assessment of their mobility and bioavailability [22]. According to the BCR procedure, metal in the exchangeable fraction easily migrates and transforms in the soil and is accumulated by plants. Metal in the reducible fraction is associated with amorphous Fe and Mn (hydro) oxides, and these oxides strongly sorb metals, but according to soil environmental conditions, they are possibility desorbed. The oxidizable fraction is mainly organic and metal chelate, which has low mobility. Residual metal shows stronger stability and lower leaching potential in soil [21]. Therefore, the exchangeable fraction and reduction fraction are bioavailable, relatively soluble, and easily extracted when soil properties change, and these fractions are taken up by plants, constituting a greater environmental risk than those from OXI and RES [19,41]. The majority of studies have found that the exchangeable fraction and reduction fraction can be transformed by organic amendments and converted into fractions (OXI and RES) scarcely taken up by plants [25,42] because the oxidizable fraction and residual fraction bind with HA forming a chelate. Some complex mechanisms can occur during HA and metal interactions because the HA surface is rich in functional groups (carboxylic and hydroxyl groups) that easily forming complexes with metals. Range analysis (HA additions of 6.4, 10.3, and 14.8 $kg \cdot ha^{-1}$) showed that HA was the major factor that influenced the passivation of Cd, Pb, Cu, and Zn, in all treatments (Figure 1), and the Cu and Pb exchangeable + reducible fractions were bound and rendered unavailable (Figure 2, the oxidizable + residual fraction significantly increased). Combining these results with the Pearson correlation coefficient results (Table 1) indicated that the exchangeable fractions for Cd, Cu, Pb, and Zn and the reduction fractions for Pb and Cu were the main bioavailable metal fractions. Furthermore, the reduction fraction of Cd; oxidizable fractions of Pb, Cu, and Zn; and all the residual fractions were the main nonbioavailable fractions (Table 2, significant correlations of each fraction with the available metals).

The total concentration of metals has been used as an indicator to evaluate soil contamination. However, more and more researchers believe that the available fraction of metals is better when evaluating the metal uptake effect by plants [43]. Tobacco has different responses regarding the accumulation and mobility of Cd, Cu, Pb, and Zn [44,45], and tobacco preferentially accumulates Cd rather than Pb [46]. The distribution of Cd in the tobacco leaves followed the order lower > middle > upper [47]. Wei [48] showed that the Cd content in lower leaves was higher than that in upper and middle leaves, indicated that Cd was more easily accumulated than transferred in mature leaves. The cumulative effect of tobacco with HA treatment was significantly lower than that in the CK treatment, indicating that HA applied to soil could decrease Pb absorption in tobacco. The reason for the high contents of Zn and Cu in the leaves is that these elements are essential nutrients for tobacco plants. However, the growth and quality are negatively affected when Cd and Pb occur in excess in tobacco leaves. The distribution of Cu and Zn in the different parts of the leaves exclusively followed a certain order, but the differences were not distinct (Figure 3). According to the distribution of metals in the tobacco leaves, Zn absorbed more easily in the middle leaves and could be transferred to the lower and upper leaves [49]. In contrast, Cu uptake in tobacco is more likely to occur in immature leaves. Therefore, as compared with those of Cu and Zn, the toxic effects of Cd and Pb on tobacco deserve more attention.

Research showed that China's tobacco plants have a higher concentration of heavy metal [50,51]. Thus, it is of great significance to decrease the heavy metal uptake of tobacco by application of HA. This study indicates that HA can be applied to soil contaminated by heavy metals, significantly reducing the bioavailability of heavy metals and inhibiting the migration of metals in the soil-plant system. However, Cu and Zn are essential mineral elements for tobacco growth, and application of HA inhibits their uptake by tobacco, as previous studies observed [52]. Due to the limited complexing capacity of HA [26], the accumulation capacity of tobacco toward different metals varies. When soil has a high content of heavy metals (or has a high effective state content), tobacco still has a certain risk of Cd and Pb accumulation.

5. Conclusions

The addition of HA significantly reduced the available metal contents in the soil. The available Cd, Pb, Cu, and Zn was inactivated by maximum percentages (HA14.8 kg·ha^{-1}) of 37%, 39%, 18%, and 29%, respectively. The bioavailable fractions were transformed into nonbioavailable fractions more significantly upon applying HA. Tobacco had an accumulation effect toward Cd, whereas it had only an absorption effect toward Pb, Cu, and Zn. HA distinctly decreased the uptake of toxic elements such as Cd and Pb in tobacco leaves. It is worth noting that HA treatment also inhibited the uptake of mineral elements such as Cu and Zn into tobacco leaves. The metal contents in the upper, middle, and lower leaves were significantly reduced with HA treatment. However, the concentration of Cd in the modified soil far exceeded the standard limit of the Chinese environmental quality standard for soil (0.6 mg·kg^{-1}, GB15618-2018), which means that this polluted soil would still not be suitable for agricultural use, even after the addition of HA.

Author Contributions: Conceptualization, Q.R. and C.Z.; methodology, H.H.; project administration, K.Z.; software, K.Z.; supervision, X.N.; validation, C.Z.; writing—original draft, Q.R.; writing—review and editing, C.L. All authors have read and agreed to the published version of the manuscript.

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