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Stabilization of Lead and Copper in a Contaminated Typic Torripsament Soil Using Humic Substances

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Potassium humate (KH) has been known as an effective metal stabilizer. Thus, contaminated soils amended with KH can be implied in horizontal expansion of forage maize to diminish the shortage in summer forage crops in Egypt. The implications of KH on the bioavailability, uptake and accumulation of lead (Pb) and copper (Cu) in maize plants grown on a contaminated soil are investigated. A pot experiment is conducted using a typic Torripsament soil naturally contaminated with Pb and Cu and treated with KH at rates of 0.0, 0.5, 1.0, and 2.0 g kg^{-1} soil. The KH applications decrease the diethylene triamine pentaacetic acid and water-extractable Pb and Cu significantly and accordingly decrease their contents in maize shoots and roots compared with control treatment. The highest KH rate of 2.0 g kg^{-1} soil lower Pb concentration in maize shoots below the safe limit for animal feeding (5 mg kg^{-1}). On the other hand, the KH applications could not achieve a significant reduction of bioavailable Cu and the contents in maize remained far away from the safe limit (4–15 mg kg^{-1}). These findings prove the success of KH in restricting Pb transfer to food chain via maize uptake, while further work with higher application rates is recommended for Cu.

1. Introduction

The factors affecting plant uptake of potentially toxic metals from metalliferous soils are important to the remediation of polluted areas. Environmental contamination with trace elements has become a global phenomenon that has negative implications on soil, water, air, and biosphere.[1] The severity is derived from their potential toxicity, abundant sources, resistance to degradation process, and accumulative behavior.[2] In Egypt, one of the most important sources of contaminants in soil is industrial activities that lead to emissions of considerable amounts of metal particulates which are transported and accumulated in soil ecosystems.[3] Lead (Pb) and copper (Cu) are abundant toxicants in soils and have adverse effects on all intermediates of the food chain.[4] High levels of Pb inhibit seed germination, prohibit root and stem elongation and leaf expansion, induce abnormal morphology, and affect photosynthesis adversely. Although Cu is an essential micro-nutrient, excessive levels cause disturbance of metabolic pathways and degeneration of important cellular components which finally leads to cell death.[5]

A number of plant species known as "hyperaccumulators" can grow on contaminated soils and accumulate metals 0.01–1% of the dry weight in the aerial parts, without showing toxicity symptoms. Among species, maize (*Zea mays* L.) can accumulate more than 1000 mg kg^{-1} Pb (0.1% dry weight).[6] Moreover, even under low or high Pb levels in soils, maize plants can uptake, translocate, and accumulate it in the shoots.[4] Unlikely, maize roots can accumulate higher amounts of Cu than the above ground parts.[7] Thus, high levels of Pb in soils lead to bioaccumulation in the food chain, causing animal and human health risks. Furthermore, as metals, unlike organic contaminants, do not undergo chemical or microbial degradation,[6] maize grown on a Cu-contaminated soil would pose potential risks.

One of the most common scenarios for soil remediation is chemical stabilization, an in-situ cost-effective technology that decreases metals bioavailability in soils.[8] Organic additives such as humic substances are able to bind metals, reducing their mobile fraction in soils.[7] Thus, successful trials on using such materials in impeding toxic elements in contaminated soils have been reported by numerous researchers, for example, Janoš et al.,[9] Zhuo et al.,[10] and Burlakovs et al.[11] This treatment would be more effective in soils of low sorption capacity such as sandy soils.

In this context, the current work is a trial for the exploitation of a loamy sand soil naturally contaminated with Pb and Cu for a

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safe production of forage maize, the main summer forage crop in Egypt. The implications of amending such soil with potassium humate (KH) on Pb and Cu bioavailability as well as their uptake, accumulation, and translocation within forage maize plants were evaluated.

2. Experimental Section

2.1. Soil

A typic Torripsament soil[12] was collected from the surface layer (0–30 cm) of an arable field adjacent to the industrial zone in Abu-Zaabal District, Qalubiya Governorate, Egypt (31°21′21.86″E and 30°15′58.01″N). A background soil sample was collected from a nearby control area. Soil collected from each site was thoroughly mixed, air-dried, and stored at room temperature. About 2 kg of the contaminated as well as background soil was sieved using a 2-mm mesh and subjected to laboratory analysis to determine initial physicochemical properties whose results are shown in **Table 1**. For the remediation experiment, the contaminated soil was passed through a 5-mm diameter sieve to be used for the experimental work.

2.2. Potassium Humate

The KH used in the current work was obtained from Techno Gene (Giza, Egypt). It is imported from China and available as a black colored granulated soil conditioner with a moisture content <15% and solubility of about 99%. Elemental analysis of the used KH is shown in **Table 2**.

2.3. Experimental Work

During summer 2016, a greenhouse pot experiment was conducted in the Faculty of Agriculture, Benha University, Egypt, in a randomized complete block design with three replicates. PVC closed pots of 16 cm diameter × 24 cm depth were uniformly packed with 5 kg soil. KH was added at rates of 0.0, 0.5, 1.0, or 2.0 g kg^{-1} soil. On 1 September 2016, ten seeds of maize (Zea mays L. 30 K8 cv) were seeded in each pot and seedlings were thinned to five plants per pot after germination. Just after thinning, the pots were supplied with the optimum doses of mineral N, P, and K fertilizers (120, 13, 20 mg N, P, and K, respectively). Soil moisture content was kept around 70% of the field capacity through applying tap water two times a week.

2.4. Analyses

2.4.1. Soil Analysis

The particle size distribution was determined using the pipette method as outlined by Gee and Dani.[13] Soil pH was determined

Table 1. Characteristics of the soil used for the experimental work.

Parameter	Study soil	Background soil	ANC[c]
Particle size distribution, %			
Coarse sand	16.43	15.32	–
Fine sand	67.18	65.13	–
Silt	7.91	6.54	–
Clay	8.48	13.01	–
Soil texture	Loamy sand	Loamy sand	–
pH[a]	7.40	7.33	–
EC[b], dS m^{-1}	0.81	0.72	–
Organic matter, g kg^{-1}	9.21	8.62	–
Calcium carbonate, g kg^{-1}	11.23	11.95	–
CEC, cmolc kg^{-1} soil	8.37	7.94	–
Total trace element content, mg kg^{-1}			
As	1.61	1.22	6.83
Cd	0.21	0.11	0.41
Cr	3.77	1.36	59.50
Co	0.13	0.01	11.30
Cu	311.52	10.13	38.90
Pb	942.27	7.44	27.00
Ni	9.52	4.16	29.00
Zn	26.31	33.54	70.00

[a] Soil/water suspension, 1:2.5.
[b] Soil paste extract.
[c] ANC, average natural content of trace elements.[6]

Table 2. Properties of the KH.

Parameter	Value
Solubility, %	99.22
Moisture content, %	14.35
pH (1:1 w/v)	8.71
EC (1:1 w/v), dS m^{-1}	9.75
C, %	42.61
H, %	3.51
O, %	33.42
N, %	3.86
S, %	0.97
K, %	8.43
P, %	0.78
Fe, %	5.86
Humic acid, %	8.17
Fulvic acid, %	2.46
Ash, %	22.62
As, mg kg^{-1}	0.001
Cd, mg kg^{-1}	0.007
Cu, mg kg^{-1}	22.13
Cr, mg kg^{-1}	0.009
Co, mg kg^{-1}	0.003
Mn, mg kg^{-1}	14.13
Ni, mg kg^{-1}	0.008
Pb, mg kg^{-1}	1.13
Zn, mg kg^{-1}	9.32

in a soil/water suspension, 1:2.5.[14] Soil electrical conductivity (EC) was determined in soil paste extract.[15] The Walkely–Blake method, as described by Nelson and Sommers,[16] was used to determine total soil organic matter. Total calcium carbonate was determined using Collin's calcimeter.[17] Cation exchange capacity (CEC) was determined according to Sumner and Miller.[18] The total forms of trace elements were extracted according to US EPA[19] method 3052. Microwave-assisted acid digestion using HNO_3, HF, and HCl. Deionized water was used for extracting soluble Pb and Cu, while the available Pb and Cu were extracted with di-ethylene tri-amine penta-acetic acid (DTPA) as outlined by Lindsay and Norvell.[20] Trace elements were measured by inductively coupled plasma-optical emission spectrophotometry (ICP-OES, Optima 5300 DV/107, Perkin Elmer, USA).

2.4.2. Potassium Humate Analysis

KH (50 g) was dissolved in distilled water (50 mL) and thereafter, the pH and EC were measured in the solution. Moisture content of the KH was determined gravimetrically. Ash content was determined by dry combustion using a muffle oven heated at 650 °C for 24 h. The total carbon, hydrogen, oxygen and nitrogen contents were measured by a CNHOS element analyzer (ElementorVario EL III, Elementar Analysen Systeme, Germany). Total forms of P, K, and trace elements were extracted according to the US EPA method 3052. The concentrations were measured in the digested samples using ICP-OES (PERKIN ELMER, OPTIMA 5300 DV/107, USA) for K and trace elements and by spectrophotometry (Spectronic 20 D, USA). Extraction and purification of humic and fulvic acids were executed following the procedure outlined by Swift[21] using 0.1 M NaOH extract acidified to pH 2 with concentrated HCl. Thereafter, the concentrations were measured by UV–Vis spectroscopy (Cary 50 UV–Vis spectrophotometer, Varian, USA) within two wavelengths of 465 and 665 nm.

2.4.3. Plant Analysis

At the end of the experiment (60 days after sowing), the chlorophyll content index (CCI) of the uppermost leaves was measured using a chlorophyll content meter (CCM-200 Plus), stem diameter, and plant height were also recorded. The whole maize plants were harvested, cleaned, weighted, and washed using tap water and deionized water, before being divided into shoots and roots. After drying for 2 days at 70 °C, the shoots and roots dry weights were recorded. Dry plant materials (0.2 g) were digested in a mixture of concentrated sulfuric and perchloric acids[22] then filtered through Whatman No. 41 filter papers before measuring Pb and Cu by ICP-OES.

2.4.4. Toxic Metal Accumulation and Translocation in Plant Tissues

The bioaccumulation factor (BAF) was calculated in the current study for roots and shoots as outlined by Zhuang et al.[23] according to the following equation:

$$\text{BAF} = \text{Metal total content in plant tissue } (\text{mgkg}^{-1}) / \text{DTPA-extracted form in soil } (\text{mgkg}^{-1}) \tag{1}$$

Moreover, the translocation factor (TF) was calculated according to Kidd et al.[24]

$$\text{TF} = \text{Total metal content in shoots } (\text{mgkg}^{-1}) / \text{Total metals content in roots } (\text{mgkg}^{-1}) \tag{2}$$

2.4.5. Statistical Analysis

Data were subjected to one-way ANOVA using the IBM SPSS statistics (ver. 19.0). Tukey's test was used to evaluate the significant difference among treatments ($p < 0.05$). Pearson's correlation coefficients ($p < 0.05$ and 0.01) were calculated for various parameters.

2.5. Chemicals

All chemicals used in this work were of analytical grade (Merck, Germany).

3. Results and Discussions

3.1. Toxic Elements in Soil

As shown in Table 1, the concentrations of As, Cd, Cr, Co, Ni, and Zn in the tested soil remained within the safe limit, since they did not surpass the average natural content suggested by Kabata-Pendias.[6] On the other hand, the concentrations of Pb and Cu were about 127- and 31-fold higher, respectively, than the corresponding values of the background soils (7.44 mg kg^{-1} for Pb and 10.13 for Cu mg kg^{-1}). Moreover, their concentrations were about 35- and 8-fold higher than the average natural level.[6] This demonstrates that the industrial activities resulted in a significant accumulation of Pb and Cu in the studied soil.

3.2. Effects of KH on Soil Chemical Properties and Pb and Cu Availability

The soil chemical properties at the end of the experimental period (60 days) are shown in **Table 3**. The applied KH rates did not significantly affect soil pH ($p < 0.05$), except for the highest application rate of 2.0 g kg^{-1}, which induced a significant increase in soil pH (pH 7.67) compared with the control treatment (pH 7.41). Such increase is related mainly to high pH value of the KH (8.71) and to low acid neutralizing capacity of sandy soils due to the low content of soil colloids; organic matter and clay fractions.[25]

Table 3. Chemical properties of the soil in response to KH application.

KH, g kg^{-1}	pH	EC, dS m^{-1}	SOM, g kg^{-1}	DTPA-extractable, mg kg^{-1}		Water-extractable, μg kg^{-1}	
				Pb	Cu	Pb	Cu
0.0	7.41 b	0.50 a	9.27 a	22.67 a	9.55 a	195.47 a	351.77 a
0.5	7.57 b	0.54 a	10.81 ba	7.63 b	6.53 b	170.11 ba	307.34 ba
1.0	7.61 b	0.58 a	11.46 b	6.91 b	5.95 b	156.92 ba	279.52 b
2.0	7.67 a	0.63 a	11.85 b	5.33 b	4.99 b	146.83 b	261.65 b

Means followed by different letters within a column are significantly different at $p < 0.05$.

Likewise, the different KH application rates did not have significant effects on the EC, while a slight increase in soil organic matter (SOM) content was observed. The applied rates of 1.0 and 2.0 g KH kg^{-1} soil increased the SOM by 23.6% and 27.8% compared with control treatment, respectively; however, soils amended with 0.50 g KH kg^{-1} showed insignificant increase of SOM. The increased SOM content could be attributed to the organic portion of KH compound, which is about 70–80% of the organic fraction in soil.[26]

The DTPA extractable Pb and Cu showed a gradual reduction in response to KH additions; and the corresponding reductions were 66.4%, 69.5%, and 76.5% for Pb and 31.6%, 37.7%, and 47.7% for Cu in soils that received 0.5, 1.0, and 2.0 g KH kg^{-1}, respectively. The addition of 2.0 g KH kg^{-1} decreased the water extractable Pb by 24.9% compared with the control treatment, whereas the Pb level in the soils that received 0.5 and 1.0 g KH kg^{-1} was not significantly affected. The rates of 1.0 and 2.0 g KH kg^{-1} resulted in reductions of water extractable Cu by 20.5% and 25.7%, respectively, when compared with the control; however, insignificant effect was recorded in soils treated with 0.5 g KH kg^{-1}. This result is in agreement with those obtained by Janoš et al.[9] who showed that application of KH at a rate of 10 g kg^{-1} to a contaminated loamy soil with a TOC of 17.80 g kg^{-1} resulted in a significant immobilization of Pb and Cu. This effect could be attributed to the modification in soil chemical properties, particularly pH; the key factor determining metals availability in contaminated soils.[27]

Humic substances can bind metals forming metal-humate "stable" complexes due to colloidal characters and the presence of surface functional groups.[28] Moreover, pH rise enhanced metal binding capacity due to the decreases in H^{+} ions that reduce completion with metal ions, and to the increased negative surface potential of KH.[29,30] Hence, the free metal ions decrease, and thus the immobile fractions dominate, limiting metals bioavailability.[31,32] The superiority of KH in immobilizing Pb rather than Cu related to the higher stability constant of Pb-humate complex than that of Cu, which finally promotes larger stabilization of Pb [33].

3.3. Effects of KH on Maize Growth Parameters

The results in **Table 4** indicate that the different rates of KH introduced to the soil did not significantly ($p < 0.05$) affect maize growth parameters, that is, fresh weight, plant height, stem diameter and roots dry weight. This goes beyond the fact that maize plants adapt defense mechanisms enable copping with metals toxicity.[34,35] On the other hand, the highest KH rate caused a significant increase of both chlorophyll content (CCI values) and shoots dry weight. The corresponding increases over the control were 35.0% and 31.4%, respectively. Such stimulatory effects resulted from the hormone-like activity; the main mechanism for stimulating plant growth.[36] Moreover, the KH contains nutrients as well as humic and fulvic acids, that have positive effects on the dry matter content and the mineral composition of maize plants.[37]

3.4. Effects of KH on Pb and Cu Content and Uptake in Maize Shoots and Roots

The results in **Figure 1** indicate that treating the soil with KH resulted in a significant decrease in Pb content in both shoots and roots with slight differences among the applied rates. The highest KH rate caused a 35.1% decrease in Pb content in the shoots, while the application rates of 1.0 and 2.0 g kg^{-1} decreased

Table 4. Effect of KH rates on plant growth parameters.

KH, g kg^{-1}	CCI	Plant height, cm	Stem diameter, cm	Fresh weight g kg^{-1}	Dry weight, g kg^{-1}	
					Shoots	Roots
0.0	3.77 a	61.64 a	7.56 a	36.20 a	3.82 bc	1.69 a
0.5	4.15 ba	64.74 a	8.78 a	36.60 a	4.69 ba	1.62 a
1.0	4.60 ba	62.12 a	8.11 a	37.20 a	4.85 ba	1.42 a
2.0	5.09 cb	62.87 a	8.39 a	37.60 a	5.02 ba	4.53 a

Means followed by different letters within a column are significantly different at $p < 0.05$.

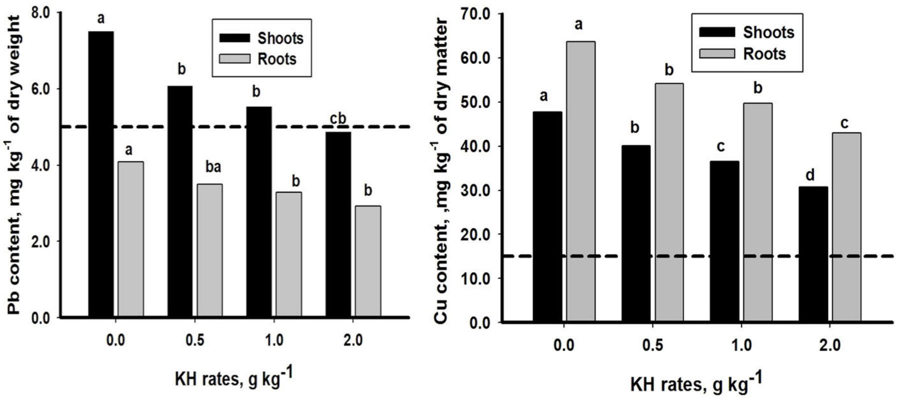

Figure 1. Effect of KH rates on Pb and Cu content in maize plants (dash line indicates reference level in shoots).

the Pb content of the roots by 19.8% and 28.4%, respectively. The Cu reduction in shoots was 16.1%, 23.6%, and 35.6% for soils amended with 0.5, 1.0, and 2.0 g KH kg^{-1} soil, respectively. For roots, the highest KH rate resulted in a 32.5% decrease, while insignificant differences occurred between the applied rates of 0.5 and 1.0 g kg^{-1}. The reductions of Pb and Cu contents in shoots and roots related to the reductions of the bioavailable forms; the DTPA and water extractable forms.[38]

Regarding metals uptake (**Figure 2**), only Pb uptake by shoots decreased significantly due to KH application with slight differences among the applied rates. This may be a result of the relatively higher reductions in Pb availability rather than Cu. The highest rate reduced uptake by 15.2% compared with the control treatment, while the applied rates of 0.5 and 1.0 g KH kg^{-1} appeared had no significant effect on Pb uptake by shoots. For roots, no significant differences in Pb or Cu uptake were observed, since the root biomass did not show significant differences after KH application.

3.5. Correlations Among KH Rates, Soil Properties, and Plant Growth Parameters

As shown in **Table 5**, soil pH and shoots dry weight showed positive significant correlations with the applied KH rates ($p < 0.01$). Negative significant correlations ($p < 0.01$) were observed between KH rates and each of the DTPA and both water extractable Pb and Cu, and shoots and roots contents of Pb and Cu. Positive significant correlations were established ($p < 0.01$) between each of the DTPA-extracted Pb and Cu and each of shoots and roots contents of Pb and Cu, respectively. Significant positive correlations ($p < 0.01$) were found between the water extracted Pb and Cu from the soil and Pb and Cu content of the shoots; however, insignificant correlation was found between the water extracted Pb and roots content of Pb, while significant correlation ($p < 0.01$) was found between the water extracted Cu and roots content of Cu. Furthermore, concentrations of Pb and Cu in shoots correlated positively and significantly ($p < 0.01$) with their contents in roots. For shoots dry weight, it correlated negatively and significantly ($p < 0.01$) with each of the DTPA extracted Pb and Cu and the water extracted Cu, while insignificantly correlated with the water extracted Pb.

3.6. Effect of KH on Bioaccumulation and Translocation of Pb and Cu Within Maize Shoots and Roots

The Pb BAF for shoots and roots (**Table 6**) increased due to KH application with slight differences among the applied rates. This resulted from the concurrent decrease in the extractability of

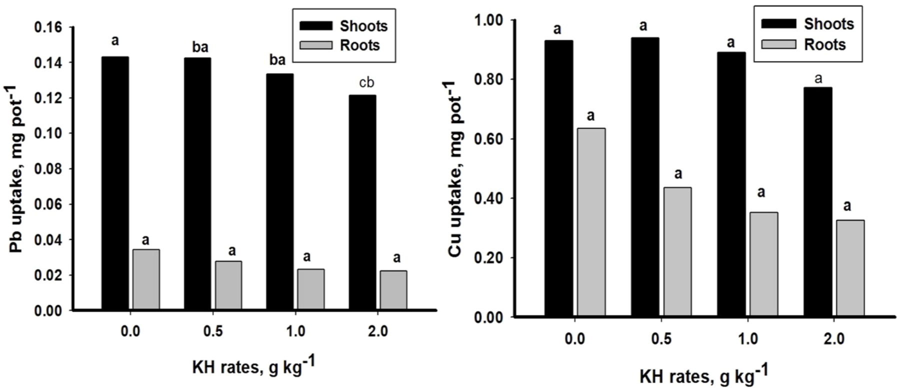

Figure 2. Effect of KH rates on Pb and Cu uptake by maize plants.

Table 5. Pearson's correlation coefficient among soil and plant parameters.

	KH	Soil pH	DTPA-extracted Pb	Water-extracted Pb	DTPA-extracted Cu	Water-extracted Cu	Shoots, dry weight	Shoots, Pb content	Roots, Pb content	Shoots, Cu content	Roots, Cu content
KH											
Soil pH	0.87**										
DTPA-extracted Pb	−0.84**	−0.91**									
Water-extracted Pb	−0.76**	−0.70*	0.71**								
DTPA-extracted Cu	−0.89**	−0.88**	0.90**	0.79**							
Water-extracted Cu	−0.83**	−0.89**	0.81**	0.73**	0.88**						
Shoots, dry weight	0.71**	0.87**	−0.76**	−0.53	−0.78**	−0.77**					
Shoots, Pb content	−0.91**	−0.88**	0.85**	0.72**	0.93**	0.83**	−0.86**				
Roots, Pb content	−0.88**	−0.78**	0.83**	0.47	0.79**	0.69*	−0.71**	0.84**			
Shoots, Cu content	−0.98**	−0.90**	0.89**	0.72**	0.91**	0.84**	−0.72**	0.90**	0.90**		
Roots, Cu content	−0.97**	−0.83**	0.88**	0.73**	0.87**	0.75**	−0.64*	0.88**	0.91**	0.97**	

$^{*}p < 0.05$.
$^{**}p < 0.01$.

DTPA-Pb in soil. On the other hand, the KH rates did not significantly affect the Cu BAF for shoots and roots, nor did they affect the Pb and Cu root-shoot TF. The results of Pb TF revealed that the presence of high concentrations of extractable Pb induced an increase in the amount of Pb transferred to shoots. These findings prove maize efficiency in transferring Pb into the harvestable parts, and thus maize could be a good candidate for Pb removal from contaminated soils.[39,40] Contradictory, maize roots tended to sequester Cu with the presence of highly extractable Cu. Such a finding confirms those obtained by Kumar et al.[41] and Guo et al.[42] who reported that Cu translocation to the upper parts of plants is slowed down by a large accumulation of Cu in roots. Hence, maize could not be recommended for clean-up Cu in contaminated soils, as it retained in roots, but then roots would be decomposed, releasing Cu-contaminants to the soil system. It is worth to mention that forage maize grown on Pb-contaminated soil that received 2.0 kg KH kg^{-1} could be used safely in animal feeding. This is because the level of Pb in shoots did not exceed 5 mg Pb kg^{-1} that is known as the acceptable safety limit of Pb in complete feeding stuff proposed by the European Commission.[43] Also, none of the applied rates of KH used in the current work could be effective in achieving such a purpose in Cu-contaminated ones. Even with the highest application rate of KH, Cu content in maize shoots reduced to 30.78 mg kg^{-1}. Such a level was far away from the safe range of 4–15 mg Cu kg^{-1}.[44] Therefore, further trials are recommended to examine higher KH application rates.

Table 6. Bioaccumulation and translocation of Pb and Cu within maize plants.

	Pb BAF		Cu BAF		TF	
KH, g kg^{-1}	Shoots	Roots	Shoots	Roots	Pb	Cu
0.0	0.33 b	0.18 b	5.06 a	6.76 a	1.83 a	0.75 a
0.5	0.79 a	0.46 a	6.17 a	8.34 a	1.76 a	0.74 a
1.0	0.80 a	0.48 a	6.17 a	8.37 a	1.69 a	0.72 a
2.0	0.93 a	0.56 a	6.18 a	8.65 a	1.66 a	0.71 a

Means followed by different letters within a column are significantly different at $p < 0.05$.

4. Conclusion

Soil applications of KH induced a pH rise that increased the adsorption of Pb and Cu that enhanced humate binding capacity, thereby free metal ions concentration decreased. As a result, the DTPA- and water-extractable forms of Pb and Cu were reduced, and thus their contents in maize tissues were decreased. The KH was more effective for Pb immobilization rather than Cu, and thus it resulted in greater reduction in Pb content in maize tissues. Maize could be classified as a Pb excluder, while it is a Cu stabilizer. Forage maize grown on Pb-contaminated soil amended with 2.0 g KH kg^{-1} could be used safely for animal feeding. However, higher application rates would be applied in soil contaminated with Cu. Generally, the study demonstrated a practical possibility of applying KH for the in-situ remediation of contaminated soil. The application would provide two benefits: 1) an efficient stabilizer for Pb and Cu that mitigate their disruptive effects on soil groundwater ecosystems and 2) minimizing Pb in forage maize and prevent its accumulation in the food chain.

Abbreviations

BAF, bioaccumulation factor; CCI, chlorophyll content index; CEC, cation exchange capacity; DTPA, diethylene triamine pentaacetic acid; EC, electrical conductivity; ICP-OES, inductively coupled plasma-optical emission spectrophotometry; KH, potassium humate; SOM, soil organic matter; TF, translocation factor.

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Conflict of Interest

The authors have declared no conflict of interest.

Keywords

bioavailability, contamination, forage maize, immobilization, potentially toxic metals

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