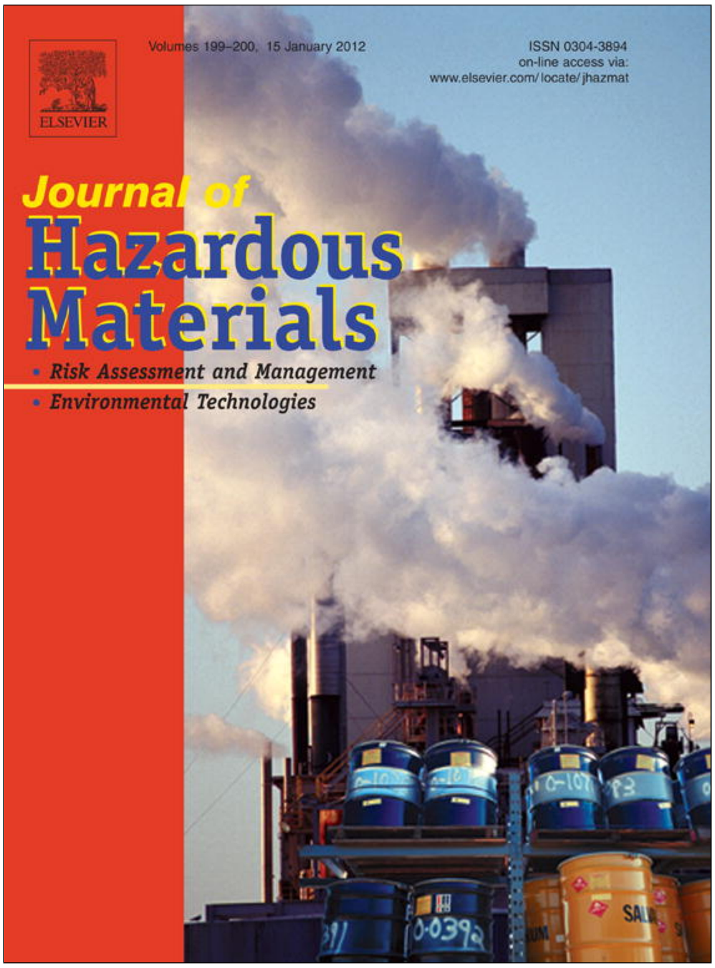

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Calcium water treatment residue reduces copper phytotoxicity in contaminated sandy soils

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ABSTRACT

Calcium water treatment residue (Ca-WTR), an industrial by-product, was found to be effective in decreasing Cu availability in contaminated soils and transport to the environment. In this study, a greenhouse study was conducted to test the ability of Ca-WTR to reduce the toxicity and uptake of Cu by ryegrass (*Lolium perenne* L.) and lettuce (*Lactuca sativa* L.) as indicator crop plants in Cu-contaminated sandy soils. Eight weeks growing period was observed in Alfisol and Spodosol amended with different levels of Ca-WTR (5–100 g kg^{-1} soil). Plant biomass yields increased with WTR application rates at the low levels (5–20 g kg^{-1} for Alfisol, pH 5.45 and 5–50 g kg^{-1} for Spodosol, pH 4.66), and decreased at the high levels (>20 g kg^{-1} for Alfisol and >50 g kg^{-1} for Spodosol). The maximum growth of ryegrass with Ca-WTR was 133% and 149% of the control (without Ca-WTR) for the original Alfisol and Spodosol (without spiked Cu), respectively, while the corresponding values for lettuce was 145% and 206%. Copper concentrations in ryegrass shoots decreased significantly with increasing Ca-WTR application rates. For lettuce, Cu concentration decreased only at high Ca-WTR rates (>50 g kg^{-1}). In addition, ryegrass had a greater potential for Cu uptake and translocation than lettuce in both soils.

1. Introduction

Copper is an essential trace element to plant growth but when present in excess amount, Cu is toxic to plants by disrupting photosynthesis and a wide range of enzymatic activities [1]. The intensive use of Cu-based fungicides in agriculture and horticulture poses a great threat to the environment and ecosystem functions due to its widespread nature. Since the end of 19th century, Cu compounds such as Bordeaux mixture ($CuSO_4 + Ca(OH)_2$), have been introduced to protect fruits and vegetables against fungus diseases [2]. Due to repeated application and its relatively low mobility, Cu has been substantially accumulated in agricultural soils, with total Cu up to several hundred to several thousand ppm [3–8]. The maximum allowable Cu concentration in agricultural soils proposed in the USA is 100 mg kg^{-1} according to Kabata-Pendias and Pendias [1].

Copper contamination in agricultural soils resulting from fungicide application reduces soil quality for crop growth [9]. The Cu accumulated in the soil above a threshold can result in phytotoxicity, depending on both plant species and soil properties. The general expressions of Cu toxicity are stunted root growth and leaf chlorosis [10]. Jarvis [11] found that dry weight of perennial ryegrass roots decreased from 4.5 g at 0 mg Cu kg^{-1} to 2.4 g at 953 mg kg^{-1} Cu. Studies conducted by Gharbi et al. [12] showed Cu being inhibitory to root elongation of lettuce and spinach in the concentration from 250 to 1000 mg Cu kg^{-1}. Chlorosis is the common symptom, and has been observed in cabbage [13], young Hamlin orange trees [14] and three Brassica genotypes [15], attributed to Cu toxicity.

Only a small fraction of the total Cu present in the soil is available for plant uptake. Although most Cu-based fungicides are highly water soluble, Cu can be strongly bound, adsorbed, or precipitated to soil particles when it is applied to soil [4,7,8]. These processes are, in turn, influenced by soil factors such as pH, organic matter content, redox potential, and composition of clay minerals [16,17]. The leaching potential of Cu, though low in soils, could be high in sandy soils. The phytotoxicity of Cu depends on soil pH given the same level of total concentration [18,19]. Generally, Cu has higher phytotoxicity in acidic soils with a low cation exchange capacity than in slightly alkaline conditions. Fine texture soils with high concentrations of organic matter, carbonates, clay, and oxides can have a higher holding capacity for Cu. Li et al. [20] studied a wide range of soils with different properties and climate characteristics and concluded that soil pH, organic carbon (OC) content, and cation

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Table 1
Selected physical and chemical properties of tested soils and calcium type water treatment residue (Ca-WTR).

Property		Alfisol	Spodosol	Ca-WTR
pH	(H_2O)	5.45	4.66	9.05
	(KCl)	4.48	3.5	8.69
EC	($\mu S\,cm^{-1}$)	140	151	659
Total C	($g\,kg^{-1}$)	3.29	9.24	112
Total N	($g\,kg^{-1}$)	0.29	0.69	0.19
Organic matter	($g\,kg^{-1}$)	5.7	15.9	–
CEC	($cmol_c\,kg^{-1}$)	4.6	11.0	–
KCl extractable N	($mg\,kg^{-1}$)			
$NO_3{}^-$		7.0	8.9	–
$NH_4{}^+$		4.7	11.1	–
Particle distribution	($g\,kg^{-1}$)			
Sandy		945	909	12
Silt		43	51	136
Clay		12	40	852

exchange capacity (CEC) can explain over 80% of the variance in Cu toxicity in soils from Asia and Europe.

When determining potential risks associated with soil contamination, total Cu in soil is often a poor measure. Attempts have been made to measure a fraction of the total amount that is readily available to plant or subjected to leaching. In general, Cu availability in soil can be estimated using chemical extraction procedures. Most extracting solutions contain multiple reagents including chemically aggressive strong acids, reducing and oxidizing agents, metal chelators and dilute salts, e.g., Mehlich-1, Mehlich-3, EDTA, DTPA, and 0.01 M $CaCl_2$ solution [21–23]. The extracting solution should thus be chosen carefully according to the purpose and specific soil characteristics.

Soil excavation, landfill, and other traditional approaches for remediating metal-contaminated soils, are not economically feasible or environmentally sound at a large scale. Phytoremediation is cost-effective, but requires longer time to accomplish desired results as compared with other approaches. Therefore, chemical approaches are frequently adopted for the remediation of contaminated agricultural soils. Using soil amendment is a sustainable approach to remediate metal-contaminated surface soils in agricultural production systems. The reduction of the mobile metal content in soil solution will minimize metal leaching into the groundwater, or transport to neighboring surface water bodies. Liming materials are perhaps the most common and safe materials for in situ soil remediation [19].

Virtually all water treatment facilities worldwide generate an enormous amount of water treatment residue (WTR) solids for which environmentally friendly end-use options are continually being sought as opposed to their landfilling. A number of studies were conducted to investigate its ability to immobilize phosphorus, but very few focused on the effects of WTR on metals [24–26]. Calcium water treatment residue (Ca-WTR) is a byproduct of water purification processes for removing suspended and dissolved solids, organic matter, and other contaminants. It has a relatively simple composition. From a disposal point of view, Titshall et al. [26] tested the growth of three plants used at the mine for rehabilitation in a soil treated with lime WTR up to 400 $g\,kg^{-1}$, and reported nutrient deficiencies induced by high WTR application rates. In our previous work, treatment with Ca-WTR at low rates was shown to raise soil pH and significantly reduce mobile Cu levels of Cu-contaminated acidic soils in a batch study [23].

In the present study, Ca-WTR was mixed at different proportions: from 5 $g\,kg^{-1}$ to 100 $g\,kg^{-1}$ soil to two representative sandy acidic soils in south Florida. Cu-enriched soils (added with 1000 $mg\,Cu\,kg^{-1}$ soil) were also investigated as a highly contaminated site for comparison. Ryegrass (*Lolium perenne* L.) and lettuce (*Lactuca sativa* L.) were grown on the treated and control soils for

Table 2
Total recoverable soil elements and available nutrients estimated by Mehlich-3 solution.

Soils	Al ($g\,kg^{-1}$)	Ca ($g\,kg^{-1}$)	Fe ($g\,kg^{-1}$)	K ($g\,kg^{-1}$)	Mg ($g\,kg^{-1}$)	Na ($g\,kg^{-1}$)	P	Cu ($mg\,kg^{-1}$)	Ni ($mg\,kg^{-1}$)	Mn ($mg\,kg^{-1}$)	Pb ($mg\,kg^{-1}$)	Zn ($mg\,kg^{-1}$)
Recoverable												
Alfisol	1.87	1.04	1.62	0.06	0.45	0.06	0.22	84.7	0.59	62.5	7.49	59.2
Spodosol	1.02	1.19	1.03	0.05	0.4	0.07	0.18	134	0.87	59.4	5.29	26.9
Available												
Alfisol	0.64	0.64	0.15	0.02	0.32	0.05	0.06	44.7	0.22	42.5	2.49	29.2
Spodosol	0.68	0.85	0.16	0.02	0.33	0.05	0.05	64.1	0.37	29.4	2.29	20.0

Table 3
Effect of Ca-WTR amendment on soil pH and extractable Cu. Letters indicate significant differences between least squares means within a soil at a 95% confidence level (LSD).

Mix	Ca-WTR application rate ($g\,kg^{-1}$)	pH (H_2O)	Mehlich-3 extractable Cu ($mg\,kg^{-1}$)	$CaCl_2$ extractable Cu
Original Alfisol	0	5.45c	39.9a	0.63a
	5	6.38b	35.6ab	0.33b
	10	6.56b	26.3b	0.20c
	20	7.00ab	24.8b	0.17c
	50	7.30a	25.1b	0.20c
Cu enriched Alfisol	0	4.85c	929a	1.01a
	5	5.86bc	853b	0.86ab
	10	6.50b	779c	0.53b
	20	7.07a	758c	0.28c
	50	7.25a	743c	0.29c
Original Spodosol	0	4.66c	68.3a	0.035a
	5	5.90bc	46.4b	0.026ab
	10	6.39b	52.9ab	0.030ab
	50	6.66b	53.2ab	0.020b
	100	7.25a	46.1b	0.011c
Cu enriched Spodosol	0	4.18c	882a	0.82a
	5	5.89b	853ab	0.51b
	10	6.18b	599c	0.29bc
	50	6.69ab	636c	0.17c
	100	6.98a	656c	0.15c

eight weeks. The main objectives of the current study were to determine the effectiveness of Ca-WTR in (a) reducing Cu accumulation in plants and (b) improving plant growth in Cu-contaminated soils.

2. Materials and methods

2.1. Soil samples and Ca-WTR collection

Alfisol and Spodosol are representative soil types under citrus production in South Florida, which received increased amounts of Cu-containing fungicides. Soil samples were collected to a depth of 15 cm from the surface of Riviera fine sand (Loamy, siliceous, active, hyperthermic Arenic Glossaqualfs) and Wabasso sand (sandy, siliceous, hyperthermic alfic alaquods) soil in the Indian River area, South Florida. The soil samples were air-dried and passed through a 2-mm sieve prior to physical and chemical analyses and green house study. The Ca-WTR, a byproduct of drinking water purification containing mainly $CaCO_3$ and minor CaO, was collected from the Fort Pierce Utility Authority facility, Florida. Selected properties of the soils and Ca-WTR are presented in Tables 1 and 2. The Ca-WTR had an effective calcium carbonate (ECC) of 95.4%, similar to high grade lime ($CaCO_3$), but its high pH (9.05) suggests that it may also contain a small amount of CaO. Other properties of the Ca-WTR included: electrical conductivity (EC) 659 $\mu S\,cm^{-1}$, Mehlich-3 extractable Ca 292 $g\,kg^{-1}$, Cu 0.32 $mg\,kg^{-1}$ and total recoverable Cu 0.40 $mg\,kg^{-1}$, indicating that Ca was the major component and contaminants such as heavy metals were minimal in the Ca-WTR.

2.2. Greenhouse experiments

Pot experiments were conducted in a greenhouse with a mean of 10-h sunlight photoperiod. Two levels of Cu for each soil were investigated: with and without being enriched with 1000 $mg\,kg^{-1}$ Cu in form of $Cu(NO_3)_2$. For preparation of Cu-enriched soil, $Cu(NO_3)_2$ dissolved in deionized water was applied to soil at the amount of 1000 mg Cu kg^{-1} by spraying and thoroughly mixing. After 1-week equilibrium, Ca-WTR was applied. According to the properties of the two soils (Spodosol had lower pH and higher buffer capacity), 2 kg (air dried basis) soil amended with Ca-WTR at the rates of 5, 10, 20, 50 $g\,kg^{-1}$ for the Alfisol and 5, 10, 50, 100 $g\,kg^{-1}$ for the Spodosol was weighed and homogenized into a 2-l plastic container. Non-amended soils were carried through the experiment as controls. The soils were incubated for 70 days at room temperature (21–25 °C), with soil moisture being maintained at 70% water holding capacity (WHC) by periodically adding water to compensate for water loss. At the end of the incubation, the soils were transferred to black plastic gardening pots. Additional fertilizers were applied to each pot in the form of liquid NH_4NO_3, KNO_3 and KH_2PO_4 at the rate of 250 $mg\,kg^{-1}$ for N, 200 $mg\,kg^{-1}$ for K and 50 $mg\,kg^{-1}$ for P. The pot experiment was conducted using a randomized complete block experimental design with three replicates for each treatment. After three days equilibrium, water-soaked and sterilized ryegrass seeds were planted at a rate of 100 seeds per pot after one week germination. Similarly, 4 seedlings (30 days old) were grown per pot for lettuce. The lost moisture was supplemented every other day with deionized water by weighing and evaluating the pot.

After 8 weeks of growth, the plant shoots and roots were harvested (cutting the shoots, taking the soil column with the roots from the pot, loosening soil by carefully crushing the column, and washing away any soil particles clinging to the roots), rinsed with tap water followed by deionized water, and oven dried at 70 °C for 72 h. Fresh and dried biomass was recorded. Dried plants were ground using a micro stainless ball mill $\leq$0.4 mm prior to digestion. The soils in the pots were sampled at the end of the experiment, air-dried, and passed through 2-mm sieve prior to analysis of available nutrients, Cu, and related chemical properties.

2.3. Chemical analyses of soil, plant samples and Ca-WTR

The pH and EC of soil and Ca-WTR were measured in deionized water at the soil: water ratio of 1:1 and 1:2, respectively using a pH/ion/conductivity meter (DIM 200, Denver Instrument, Denver, CO, USA). Soil texture was determined with the micropipette method [27]. Total organic carbon (C) and total N was determined using a C/N analyzer (Vario Max, Elemental Analysensystem GmbH, Hanau, Germany). Cation exchange capacity was determined by an ammonium acetate method [28]. Percent organic matter (OM) was calculated by multiplying percent organic C by 1.724. Soil available N (NH_4^+-N and NO_3^--N) was determined by shaking a 2.5 g soil sample in 25 ml 2 M KCl for 1 h. Concentrations of NH_4^+-N and NO_3^--N in the filtrate were analyzed using a discrete autoanalyzer (Easychem plus, Systea Scientific Inc., Italy). Total recoverable Cu in soil was determined following EPA method 3050B. Soil extractable nutrients including Cu were obtained using 0.01 M $CaCl_2$ and Mehlich-3 extraction method (1:10 soil:solution ratio) [29]. Ground plant samples (0.4 g) were digested with 5 ml of concentrated HNO_3 using an A.I. digestion system (A.I. Scientific, Inc., USA). Effective calcium carbonate (ECC) of the Ca-WTR was determined by a titration method [30].

The concentrations of Cu and other elements in soil extracts and digested samples were determined using inductively coupled plasma optical emission spectrometry (ICP-OES, Ultima, J. Y. Horiba, Edison, NJ, USA) following USEPA method 200.7. The NELAC 2003 standards were followed for QA/QC of chemical analyses. QA/QC plan included a blank, a duplicate, a spike and a standard reference material (SRM) analysis every 20 samples with an acceptable recovery of 95–105%.

2.4. Statistical analysis

All treatments were replicated three times. Treatment effects on pH, extractable Cu, plant Cu, and plant biomass were determined by analysis of variance using the Statistical Analysis System software (release 9.1 for Windows; SAS Institute, Cary, NC, USA). Least significant difference (LSD) analysis was conducted to determine the differences among the treatments at the 0.05 probability level. A type I error (α) of 5% was used for all statistical analyses.

3. Results and discussion

3.1. Effects of Ca-WTR amendment on soil pH and extractable Cu

Both soils were acidic, but the Spodosol had lower pH (pH 4.66) than the Alfisol (pH 5.45) (Table 1). The Ca-WTR was alkaline and had a higher electrical conductivity (EC) than the soils. The OM content of the Spodosol was approximately two times higher than that of the Alfisol. Both soils had a high amount of sand (>90%). The Ca-WTR consisted of mainly clay and silt (85.2 and 13.6%, respectively), a minimal amount of organic carbon and non-detectable heavy metals. Higher Cu concentration was measured in the Spodosol due to the long period of fungicide application (Table 2). The Alfisol contained higher concentrations of Zn, Fe and Al than the Spodosol. Other properties of the two soils were similar (Table 2).

Copper loading at 1000 mg kg^{-1} reduced soil pH by 0.60 units for the Alfisol and 0.48 pH units for the Spodosol (Table 3). The mechanisms of pH decrease from external Cu loading or contamination may result from the replacement of exchangeable H^+/Al^{3+} on clay minerals and oxides. Similar results were reported by Yu et al. [31] in acid soils, in which adsorption of one mole of Cu^{2+} resulted in the release of 1.1–2.6 mol of proton (H^+), depending on soil properties such as contents of clay minerals and oxides and on Cu^{2+} adsorption mechanisms.

The application of Ca-WTR significantly increased soil pH from acidic to neutral (Table 3). At 5 g kg^{-1}, soil pH was elevated by 0.93–1.01 units in the Alfisol and 1.24–1.71 units in the Spodosol soil. At 50 g kg^{-1} and 100 g kg^{-1} (equivalent to field application rates of ~89.6 and 224 Mg ha^{-1} incorporated into a soil depth of 15 cm), the soil pHs were increased to 7.25–7.3 in the Alfisol soil and 6.98–7.25 in the Spodosol soil. The initial pH of Spodosol (4.66) was lower than that of the Alfisol (5.45) while the buffer capacity of the former was higher than the latter (Table 1). Therefore, higher rates of Ca-WTR amendments were applied in the Spodosol than in the Alfisol. During the plant growing period, soil pH values had minimal variation, remaining at relatively constant levels for both soils.

Soil extraction with Mehlich-3 and $CaCl_2$ was performed to gain insight into the mobility and bioavailability of Cu in soils. Analysis of variance indicated that the application of Ca-WTR to soil significantly reduced available Cu as estimated by both methods. The extractable Cu generally decreased with increasing Ca-WTR application rate. Mehlich-3 extractable Cu could be reduced by 20–40% whereas 0.01 M $CaCl_2$ extractable Cu could be reduced by as much as 70% as compared to the control for the two Cu levels. $CaCl_2$ is a mild extractant for Cu and it measures the soluble pool of Cu in soil [32]. Mehlich-3 solution is a more aggressive extractant for Cu due to its strongly acidic nature. Chaignon et al. [33] observed significantly higher Cu concentrations in tomato (*Lycopersicon esculentum* Mill.) roots grown on strongly acidic soils as compared to calcareous or mildly acidic soils with the same level of total Cu concentration. Brun et al. [34] recommended the $CaCl_2$ extraction as the most suitable for determining Cu (bio) availability in acid-neutral soils.

These results are consistent with the previous fractionation experiment that Ca-WTR amendment not only had a strong acidity-neutralizing capacity but also converted labile Cu to more stable Cu forms in the amended soils [23]. As the pH of the soil increases, more Cu in the soil solution is adsorbed onto the soil surface due to an increase in surface negative charge [35]. Copper may form inner- and outer-sphere complexes with newly deprotonated functional groups present in soil colloids [36] or may form precipitates at high metal loadings and high pH [21]. It was also possible that less water soluble Cu compounds such as $Cu(OH)_2$ and $CuCO_3$ were formed at the raised soil pH. In addition, exchangeable Al^{3+} and Fe^{3+} were replaced by Ca^{2+} from WTR treatment and precipitated as $Al(OH)_3$ and $Fe(OH)_3$ when soil pH is above 5.5. These newly formed oxides and hydroxides provide additional sites for sorbing Cu. As pH increases, there is an increase in negative surface charge of metal oxides, therefore reducing Cu concentration in soil solution. Moreover, some adsorbed Cu may be also occluded by the newly formed oxides and become inaccessible to chemical extraction, which is referred to as “aging” [37,38].

3.2. Effects of Ca-WTR amendment on plant Cu concentration

Ryegrass was chosen as an indicator plant because of its use as animal fodder and tolerance to low fertility. Lettuce is an important leafy vegetable consumed directly by humans. Copper concentrations in ryegrass shoots decreased significantly with increasing Ca-WTR application rates (Fig. 1). The highest concentrations of Cu occurred in the control for the original Alfisol (without Cu addition). For Cu-enriched Alfisol, treatment with 5 g kg^{-1} Ca-WTR resulted in highest shoot Cu concentration as no plant survived in the Cu-enriched Alfisol without Ca-WTR. The results for Spodosol were similar, except that shoot Cu concentration was higher in Alfisol than Spodosol. However, less Ca-WTR was needed for reducing shoot Cu concentration in Alfisol than Spodosol to below the upper critical toxic level (30–35 mg kg^{-1}, [39]). Davis and Beckett [40] report the upper critical level for lettuce is similar to ryegrass in the same soil situation. For lettuce, shoot Cu concentration of the treatments and the control were all within this range. No significant differences in shoot Cu concentration were found between the two soils (Alfisol and Spodosol) or the two Cu levels (with and without spike of 1000 mg Cu kg^{-1}). Unlike ryegrass, there was no significant difference between low Ca-WTR rate (5–10 g kg^{-1}) treatments and control. Shoot Cu concentration decreased only at the high Ca-WTR rates (>50 g kg^{-1}). Besides, plant Cu concentrations were much lower in lettuce than in ryegrass at the same treatment level. Obviously there were differences in Cu acquisition between these two plant species, but the mechanism was not well understood. Minimal Cu translocation to shoot tissues may be explained by the selective transport mechanism of ions within the plants, which is attributed to its soil–plant barrier to the accumulation of Cu in excess of normal levels [22]. Copper most likely enters roots in dissociated form but is present in root tissues as a complex – attached principally to cell walls due to its affinity for carbonylic, carboxylic, phenolic, and sulfhydryl groups as

Table 4
Correlation coefficients (r) between plant (ryegrass and lettuce) shoot Cu concentration and soil pH and extractable Cu in soil as estimated by Mehlich-3, and 0.01 M $CaCl_2$ extraction procedures.

	$pH_{(H_2O)}$	Mehlich-3 extractable Cu	$CaCl_2$ extractable Cu
Ryegrass tissue Cu concentration	-0.53^{*}	0.59^{**}	0.80^{***}
Lettuce tissue Cu concentration	-0.55^{*}	0.40^{NS}	0.20^{NS}

NS, not significant.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

well as by bonds with N, O, and S atoms [10]. In the same reference, they also summarized other plants in which Cu content in the edible plant parts is not correlated to the level of applied Cu, including corn grain, sugar beet, tomato, onion, bean, carrot, celery. However, more research is needed to investigate this mechanism among different species. Besides, shoot Cu concentration of both ryegrass and lettuce was negatively correlated with soil pH ($P < 0.05$), indicating decreased Cu availability with increasing soil pH (Table 4).

Shoot Cu concentration of ryegrass was closely related to Mehlich-3 extractable Cu ($P < 0.01$) and yielded a higher level of statistical significance to 0.01 M $CaCl_2$ extractable Cu in soil ($P < 0.001$). However, no correlation was found between shoot Cu concentration of lettuce and extractable Cu in the soils (Table 4). McBride et al. [22] reported no relationship between Cu concentration in a clover and $CaCl_2$- or M3-extractable Cu in the soil. Mulchi et al. [41] reported a fairly strong correlation between Mehlich-3 extractable soil Zn and Cu and the concentrations of these metals in tobacco. More researchers recommended dilute $CaCl_2$ as a universal soil extractant for estimating short-term trace metal availability to crop plants. The study of Aten and Gupta [42] also showed that the concentrations of Cu, Zn, Cd, and Pb in plants grown on 35 soils in Switzerland were more correlated with soil extractable Cu obtained with weak extractants (0.1 M $NaNO_3$ and 0.05 M $CaCl_2$).

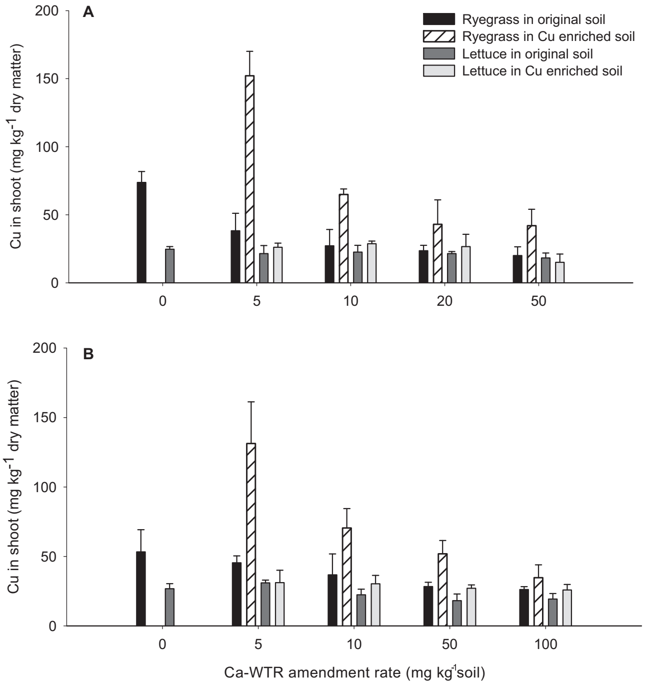

Fig. 1. Relationship between plants (ryegrass and lettuce) shoots Cu concentration and Ca-WTR treatment rates. (A) Alfisol, (B) Spodosol. Vertical bars represent standard errors.

3.3. Effects of Ca-WTR amendment on plant growth/dry matter yields

The overall shoot growth for the two plant species affected by the amendments was very similar. Regression analyses of shoot dry matter yield (DMY) with Ca-WTR application rate had similar quadratic relationships associated with the two soils for both ryegrass and lettuce (Fig. 2).

Overall, plant biomass yields increased with Ca-WTR application rates at the low levels (5–20 g kg^{-1} for Alfisol and 5–50 g kg^{-1} for Spodosol), reached maximum at the moderate level (10–50 g kg^{-1}) and decreased at the higher levels (>20 g kg^{-1} for Alfisol and >50 g kg^{-1} for Spodosol) (Fig. 2). However, the differences in plant yields between the treatments were smaller in original soils than the Cu-enriched soils.

For the original soils, only lettuce grown in the Spodosol had some chlorosis (old leaves turn yellow), but the roots were normal, indicating a slight Cu toxicity. The amendment of Ca-WTR at 10 g kg^{-1} resulted in the maximum growth for ryegrass (133% of the control for Alfisol and 149% of the control for Spodosol), while the maximum yield of lettuce occurred at the Ca-WTR rates of 20 g kg^{-1} and 50 g kg^{-1}, respectively for the Alfisol and Spodosol (145% of the control for Alfisol and 206% of the control for Spodosol). These results indicate that the application of Ca-WTR at proper rates was beneficial to plant growth even in slightly Cu contaminated soils.

Both ryegrass and lettuce grew poorly with minimal biomass yields in the Cu-enriched soils (Fig. 2). The loading of 1000 mg kg^{-1} Cu caused death of most plants and severe Cu toxic effects on plants were indicated by strong chlorosis and necrosis of plant leaves and impairment of root growth as described by Lepp [43]. Chlorosis initially appeared in the older leaves, and moved progressively up to the youngest. The amendment of Ca-WTR significantly improved plant growth with a dramatic increase in biomass yield at the application rate of 5 g kg^{-1}, but the increase became less at higher application rates, and the yield of ryegrass started to decrease at the WTR rate of 20 g kg^{-1} for both soils. The yield of lettuce reached the maximum at the Ca-WTR rate of 50 g kg^{-1} for both soils (Fig. 2).

A negative relationship occurred between the shoot dry matter yields and shoot Cu concentrations for both plant species. The coefficients of correlation (r) were −0.47 and −0.55, respectively for ryegrass and lettuce ($P < 0.05$). This is in agreement with the results of Gharbi et al. [12] regarding plant response to Cu toxicity. The addition of Ca-WTR increased plant biomass and lowered plant Cu concentration as well. Thus the amelioration effect can be categorized as 'safe amelioration' [44].

3.4. Effects of Ca-WTR amendment on Cu uptake and other nutrients

The uptake of Cu by ryegrass decreased with increasing amendment rate for all soils (Table 5). In comparison, Cu uptake by lettuce was less affected by Ca-WTR amendment. The positive effect of Ca-WTR on reduced uptake of heavy metals by plants has generally been explained by an increase in soil pH. Moreover, the Ca-WTR increased soil pH and subsequently influenced the availability of

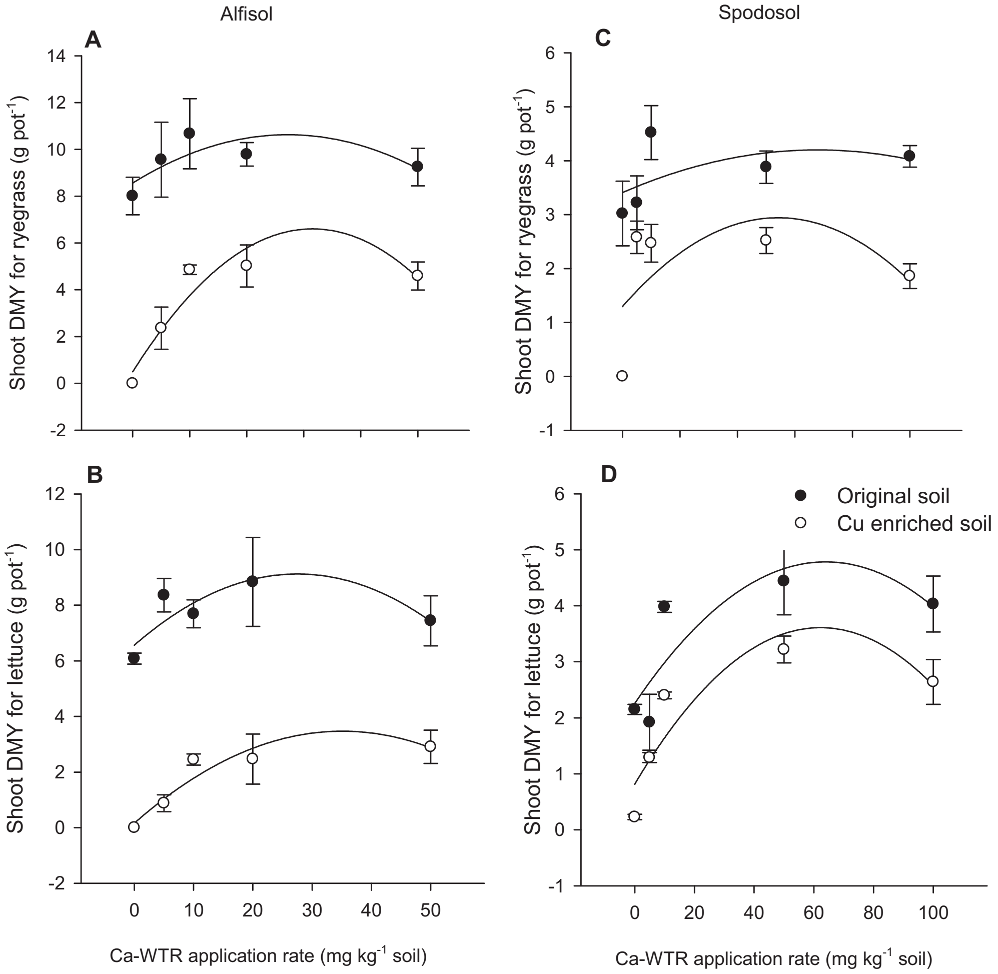

Fig. 2. Relationship between plant shoot dry matter yields after 8 weeks of growth and Ca-WTR treatment rates. (A) Ryegrass in Alfisol, (B) lettuce in Alfisol, (C) ryegrass in Spodosol, (D) lettuce in Spodosol. Vertical bars represent standard errors.

other nutrients, which influenced Cu uptake in turn. According to the Terrestrial Biotic Ligand Model (TBLM), Ca and Mg ions can compete with divalent metals for biotic ligands (e.g., roots) [45]. Alva et al. [46] reported that increased Ca availability in the rooting environment, applied either as $CaCO_3$ or $CaSO_4$, significantly decreased concentrations of Cu in the fibrous roots and therefore ameliorated the effects of Cu phytotoxicity. The Ca-WTR introduced a large amount of Ca ions, which may compete with Cu for plant uptake. A positive relationship occurred between the Ca-WTR application rates and shoots Ca concentrations for both plant species (data not shown).

We also observed that increasing Ca-WTR rates led to a decreased Zn concentration in plant tissue. The concentration of Zn in the Cu-enriched soils at the highest Ca-WTR treatment rates was 6–8 mg kg^{-1} dry matter, which was lower than critical Zn concentration (10 mg kg^{-1}) for normal growth [1]. In most studies, Zn

Table 5
Total Cu uptake in shoots of ryegrass and lettuce determined at the end of 8 weeks of growth. Letters indicate significant differences between least squares means within soil at a 95% confidence level (LSD).

Ca-WTR application rate (g kg^{-1})	Ryegrass (μg pot^{-1})		Lettuce (μg pot^{-1})	
	Original soil	Cu enriched soil	Original soil	Cu enriched soil
	Alfisol			
0	591a	0d	150a	0c
5	366b	359a	179a	23.0b
10	290bc	315a	173a	70.3a
20	230cd	216ab	189a	65.7a
50	185d	156bc	136a	43.8ab
	Spodosol			
0	161a	0d	57.5a	0c
5	146a	339a	59.4a	40.1b
10	166a	174b	89.1a	72.7a
50	110b	131b	80.5a	87.2a
100	107b	64.5c	77.8a	68.3ab

availability is considered to be more sensitive to soil pH change as compared to other micronutrients [47]. And Cu can significantly inhibit the uptake of Zn [10]. This may partly explain the yield reduction at high Ca-WTR rates. The concentrations of other nutrients were in the normal range [10]. Mineral nutrient contents for N, P, K were above 3.0, 0.3, 2.3% of dry weight for plants under all the treatments. For other micronutrients, the contents in plant tissues are in the range of 8–12, 3–5, 6–12, 0.15–0.4, 50–100 mg kg^{-1} dry wt, for Ca, Mg, B, Mo and Mn, respectively. Phosphorus deficiency is often a liming factor, as soluble P can react with Ca to form a series of products of phosphates with decreased solubility (i.e. slightly soluble dicalcium phosphate to very low solubility tricalcium phosphate). Ahmed et al. [48] reported that long-term P deficiency problems were not evident at the field scale, although P concentrations in lawn grass tissue decreased in neutral to alkaline pH in the pot experiment. In the present study, no symptoms of P deficiency (purpling of some leaves) with high WTR application rate were observed. The concentrations of P in plants received different treatments were all in the range of 3–5 g kg^{-1}. This might be attributed to the addition of phosphorus fertilizer at the beginning of cultivation.

4. Conclusions

Ca-WTR is capable of converting labile Cu into more stable forms. Application of Ca-WTR effectively improved plant growth and reduce the uptake of Cu by plants of lettuce and ryegrass in slightly to severely Cu-contaminated soils. Copper concentrations were considerably higher in ryegrass than in lettuce at the same treatment level, suggesting that the latter was less influenced by the level of available Cu. A substantial amount of Ca-WTR is readily available at a low price from local drinking water treatment facilities. Therefore, this material can be regarded as an economically and eco-friendly viable option for copper amelioration in contaminated soils. However, High Ca-WTR application rates may potentially induce Zn deficiency in the plants. Field experiments are needed to demonstrate the effectiveness of Ca-WTR in reducing Cu phytotoxicity under field conditions and to address the questions relating to possible adverse effects on nutrient imbalance and the long-term stability of heavy metal binding.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.11.030.

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