

# Accumulation and availability of copper in citrus grove soils as affected by fungicide application

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## Abstract

**Purpose** Copper is a trace element of environmental concern. Repeated applications of Cu-containing fungicides have resulted in a large scale of Cu contamination in agricultural soils. However, limited information is available regarding Cu accumulation and availability in soils under citrus production in the Indian River Area, South Florida, which has received increasing amounts of Cu fungicides to control canker and other diseases. The purpose of this study was to investigate Cu transformation, availability, and mobility in soils as affected by external Cu loading and soil properties.

**Materials and methods** Soil samples ( $n=18$ ) were collected from representative commercial citrus groves in the Indian River area. These samples were characterized to understand the accumulation, availability to plant, and leaching loss, as influenced by soil properties, agricultural practices, and Cu fungicide inputs.

**Results and discussion** Significant accumulation and increased availability and mobility of Cu occurred in the soils with increasing citrus production period. Soil acidification enhanced Cu leaching in soil profiles. Available Cu estimated by Mehlich-3 or  $\text{NH}_4\text{OAc}$  extraction was highly correlated with total recoverable Cu, whereas the relation-

ship between  $\text{CaCl}_2$ -extractable Cu and total recoverable Cu was affected by soil pH, with a greater slope of the relationship curve at soil  $\text{pH}<6.5$ . Vertical movement of Cu in a soil profile was more pronounced at  $\text{pH}<6.5$ . A large portion of soil Cu was present as organically bound, ranging from 43% to 62%, which was significantly correlated with both recoverable Cu and Mehlich-3-extractable Cu ( $P<0.001$ ).

**Conclusions** Significant accumulation of Cu occurred in citrus grove soils due to anthropogenic inputs, and the availability and mobility of Cu in the soils were related to Cu accumulation and soil pH. Copper migration from Cu-contaminated soils to the environment was reduced when soil pH was 6.5 or higher.

**Keywords** Accumulation · Availability · Citrus grove · Copper · Copper fungicide

## 1 Introduction

Copper is an essential trace element for plant, animal, and human but is potentially toxic at an excessive level (Alloway 1995; Fageria 2001). Copper contamination in soil and water is on the rise worldwide due to anthropogenic inputs. Copper is widely used in agriculture as fungicides to enhance crop production and control fungus diseases in vines, citrus, coffee, and other fruit plants (Epstein and Bassein 2001; He et al. 2005). The application of Cu-based fungicides can be traced back to the end of the nineteenth century (Millardet 1885). Presently, a large portion of fungicides containing Cu such as  $\text{CuSO}_4$  and  $\text{Cu}(\text{OH})_2$  are still being used. As a result, substantial amounts of Cu have been accumulated in the soils, with total Cu as high as several thousand milligrams per

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kilogram (Thrupp 1991; Delusia et al. 1996; Besnard et al. 2001; Msaky et al. 2002; Pietrzak and McPhail 2004; Mirlean et al. 2007; Wightwick et al. 2008).

The maximum allowable Cu concentration in agricultural soils proposed in the USA is 100 mg kg<sup>-1</sup> according to Kabata-Pendias and Pendias (2001). Florida is well-known for its citrus production. In the last two decades, increased amounts of Cu-containing fungicides have been used to control canker diseases, especially for grapefruit trees (Timmer et al. 1998; Stover et al. 2002). As a result, Cu has significantly accumulated in the soils, accelerating transport to the environment, particularly in low-pH soils (Zhang et al. 2003a). In sandy acidic soil, Cu concentrations at >100 mg kg<sup>-1</sup> caused phytotoxicity to citrus plants and subsequently decreased their productivity (Alva 1993). Copper accumulation has been reported to reduce microbial biomass and diversity in these soils (Zhou et al. 2011). Transport of Cu from land to surface waters through surface runoff and/or leaching has been identified as an environmental issue, since Cu concentration as high as 300 mg kg<sup>-1</sup> was measured in the sediment of Saint Lucie River in the citrus growing area (Zhang et al. 2003c). Aquatic organisms are reported to be more susceptible to Cu contamination than terrestrial plants (Eisler 1998). Therefore, it is important to monitor the status of Cu accumulation in soils under citrus and other horticultural crop production system.

The availability of Cu accumulated in citrus groves can be defined as its ability to be transported from the soil to the plant roots. This is dependent on plant species and soils properties (Juste 1988). In soil, Cu is subject to various chemical reactions including sorption on colloidal surfaces, complexed with humic substances and precipitation as sparingly soluble compounds (Alloway 1995; Sauve et al. 1997). Cu content in citrus root decreased with increasing soil pH in an acidic sandy soil receiving long-term Cu-containing chemicals application (Alva et al. 1993). The bioavailability of Cu is lower in soils with a higher cation exchange capacity or organic matter content (Gupt and Aten 1993). Root Cu content can be considered as an indicator of Cu availability (Hinsinger 2001). However, this procedure is time-consuming and difficult to separate the Cu adsorbed onto root surface, which may lead to overestimation of the root Cu concentration (Alva et al. 1993). Alternatively, several one-step extraction methods were frequently used to estimate available Cu in soil, including Mehlich-3, CaCl<sub>2</sub>, NH<sub>4</sub>OAc, and HCl (Zhang et al. 2003a; Yu et al. 2002; Brun et al. 2001). These procedures allow defining pools of extractable metals in particular chemical conditions and thus estimate the availability indirectly (Nirel and Morel 1990). Sequential extractions are used to predict the chemical fractionation of Cu in soils, which can assist to understand Cu behavior in

soils and subsequently the mobility and availability of Cu to the environment (Nirel and Morel 1990; Pietrzak and McPhail 2004; He et al. 2006).

Though research has been conducted on the transformation and availability of Cu in soils, limited information is available on sandy soils under citrus production. The objectives of this study were to (1) estimate and compare Cu availability in soils using various extraction procedures and (2) understand the processes and soil properties that control Cu availability and mobility in the sandy soils under citrus production.

## 2 Materials and methods

### 2.1 Soil collection and characterization

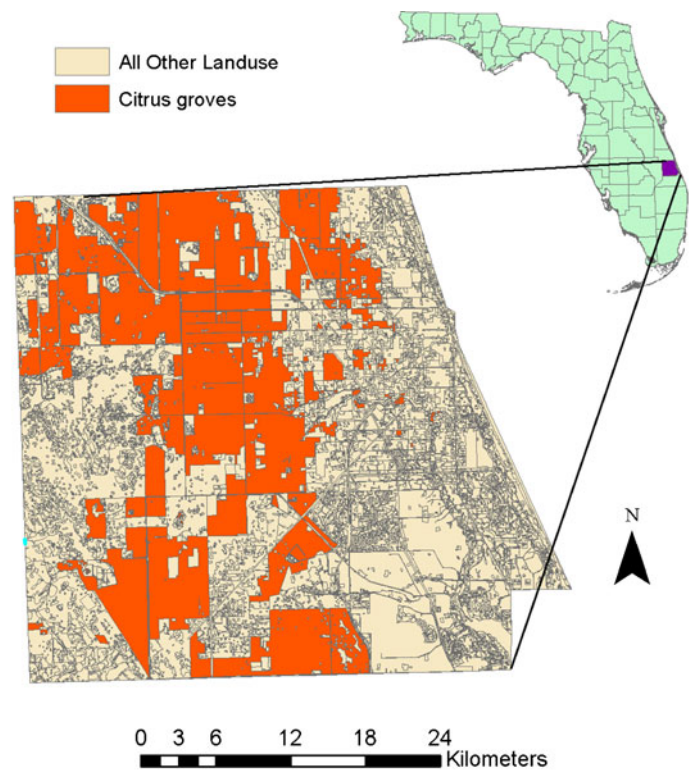
Soils (nine Alfisols and nine Spodosols) were collected from representative commercial citrus groves in the Indian River area, South Florida (Fig. 1). They were selected based on the use of Cu-based fungicides, citrus varieties and planting history, and soil properties. General characteristics of the field sites are presented in Table 1. For each location, triplicate soil samples were randomly collected at 0–15 cm depth, with three cores (0.5 kg per core) being combined to make a composite sample. In order to study the vertical movement of Cu, profile soil samples were collected at 0–5, 5–15, 15–30 cm, and then every 30 cm increment up to 120 cm depth. A total of six representative profile samples were collected. The Spodosol had a typical soil profile of A, E, B (Bt and Bh), and C horizons whereas the Alfisol had a weak “E” horizon under surface followed by an argillic B horizon.

The soil samples were air-dried, ground, and passed through a 2-mm sieve prior to physical and chemical analyses. Soil pH (1:1 soil/water ratio) and electrical conductivity (1:2 soil/water ratio) were measured in deionized water using a pH/ion/conductivity meter (Denver Instrument, Co.). Total soil organic C was determined by combustion using a C/N analyzer (Vario MAX CN Macro Elemental Analyzer; Elemental Analysensystem GmbH, Hanau, Germany). Soil texture and cation exchange capacity (CEC) data were obtained from local soil survey (United States Department of Agriculture, Soil Conservation Service 1980).

### 2.2 Soil Cu analysis and fractionation

Total recoverable Cu in the soils was determined following EPA method 3050B. In brief, 1.0 g of soil was digested with repeated additions of nitric acid (HNO<sub>3</sub>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Labile Cu in the soils was estimated by three extraction methods: (a)

**Fig. 1** Location of the study area



Land uses: Southwest Florida Water Management District: 1:12000 (2004-2005) (2004)  
 County boundaries: U.S. Census Bureau/Tiger Mapping Service: 1:100,000 (1990)

0.01 M CaCl<sub>2</sub> (1:10 ratio of soil/0.01 M CaCl<sub>2</sub>), 60-min reaction time (Zhang et al. 2003a), extracting water-

**Table 1** General properties of the soils

No.	Citrus variety	Cu use history	Soil orders	Soil texture
1	Red GF	1988	Alfisol	Sand
2	NAV	1988	Alfisol	Sand
3	Red GF	1973	Alfisol	Sand
4	Red GF	1966	Alfisol	Sand
5	NAV	1982	Alfisol	Sand
6	VAL	1970	Alfisol	Loamy sand
7	VAL	1970	Alfisol	Loamy sand
8	Red GF	2005	Spodosol	Sand
9	Red GF	1973	Spodosol	Sand
10	Red GF	1994	Spodosol	Sand
11	Red GF	1975	Spodosol	Sand
12	White GF	1975	Alfisol	Sand
13	VAL	1975	Alfisol	Loamy sand
14	White GF	1989	Spodosol	Sand
15	Murcott	1989	Spodosol	Sand
16	Red GF	1989	Spodosol	Sand
17	White GF	1955	Spodosol	Sand
18	Murcott	1959	Spodosol	Sand

GF grapefruit, VAL Valencia orange

soluble and readily exchangeable Cu; (b) 1 M ammonium acetate (NH<sub>4</sub>OAc; 1:4 ratio of soil/1 M NH<sub>4</sub>OAc solution), 60-min reaction time (Reed and Martens 1996), including water-soluble, exchangeable, and acid-soluble Cu; and (c) Mehlich-3 (1:10 ratio of soil/Mehlich-3 solution (0.2 M CH<sub>3</sub>COOH+0.25 M NH<sub>4</sub>NO<sub>3</sub>+0.015 M NH<sub>4</sub>F+0.013 M HNO<sub>3</sub>+0.001 M EDTA, pH 2.0)), 5-min reaction time (Mehlich 1984), including water-soluble, exchangeable, part of CaCO<sub>3</sub>- and organically bound Cu. At the end of extractions, the soil suspensions were centrifuged at 7,500×g for 30 min, and the supernatant was passed through a 0.45-μm membrane filter before being analyzed for Cu concentrations.

To understand the relationship between chemical fractions and the availability of Cu, soil Cu was fractionated into five different fractions following the modified procedure of Amacher (1996). Soil samples (2.0 g) were sequentially extracted with 20 mL of 0.1 M Mg(NO<sub>3</sub>)<sub>2</sub>, 20 mL of 1 M NaOAc, 40 mL of 0.1 M Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>, and 40 mL of 0.2 M ammonium oxalate+0.2 M oxalic acid, and 0.1 M ascorbic acid (pH 3) for the water-soluble and exchangeable, acid-soluble, organically bound, and oxide-bound Cu fractions, respectively. After each extraction, the suspension was centrifuged at 7,500×g for 30 min, and the supernatant was filtered through a 0.45-μm membrane filter. The soil residues were rinsed three times with 5 mL

of ethanol and evaporated to dryness before next extraction. Residual Cu was calculated by subtracting the sum of the four fractions from their total recoverable soil concentration. There were three replicates per soil sample for all the soil extractions.

Copper concentrations in the extracts and digested solution were determined using an inductively coupled plasma optical emission spectrometer (ICP-OES; Ultima, JY Horiba Group, Edison, NJ, USA) following EPA method 200.7. The National Environmental Laboratory Accreditation Conference 2003 standards were followed for quality assurance and quality control of chemical analyses with one blank, duplicate, spike, and standard reference material every 20 samples. For Cu analysis with the ICP-OES, the detection limit was  $0.8 \mu\text{g L}^{-1}$  with a recovery of 95–105%.

### 2.3 Data analysis

All data were analyzed using SAS program (version 8.2, SAS Institute, 2004). Correlation analyses were conducted between total recoverable Cu, extractable Cu concentrations, and different Cu fractions. Citrus soil Cu concentrations among the sites were subjected to a one-way analysis of variance. Tukey's test was performed to determine significant Cu

differences among the soils. Stepwise regression analysis was also used to calculate the relationships between total recoverable Cu and extractable Cu concentration. Statistical significance is accepted at  $\alpha=0.05$ .

## 3 Results and discussion

### 3.1 Copper accumulation in relation to citrus planting history

The dominant soils in the Indian River area under commercial citrus production are Spodosols and Alfisols. The soils are mostly sandy with sand content above 85%, acidic to neutral, with low organic matter content, and low buffering capacity (Zhang et al. 2003b). Surface soil pH varied from 3.8 to 7.3, with an average of 5.5. Total organic carbon content ranged from 3.3 to  $11.1 \text{ g kg}^{-1}$  (Table 2). According to soil survey (United States Department of Agriculture, Soil Conservation Service 1980) CEC of the studied soils ranged from 1.7 to  $11 \text{ cmol kg}^{-1}$ , with an average of  $5.81 \text{ cmol kg}^{-1}$ . All the 18 tested soils were sandy with sand >85% and clay <10%. The texture was similar in the surface layer between Alfisol and Spodosol soils.

**Table 2** Selected chemical properties and Cu concentration of the soils (mean±SE)

No.	pH (H <sub>2</sub> O)	EC ( $\mu\text{S cm}^{-1}$ )	Total org. C ( $\text{g kg}^{-1}$ )	Cu (mg kg)			
				Total recoverable	Mehlich-3 extractable	NH <sub>4</sub> OAc extractable	CaCl <sub>2</sub> extractable
1	5.0±0.0b	129±9a	3.3±2.0a	43.2±19.4cd	20.8±7.9b	1.4±0.5bc	0.9±0.0d
2	5.3±0.5b	155±67ab	7.8±1.4b	96.5±11.1e	30.5±4.1b	2.7±0.4c	1.5±0.5d
3	5.8±0.6b	142±78ab	5.3±1.1ab	102±20.2e	42.9±9.4bc	3.6±1.7c	1.4±0.3d
4	6.7±0.9bc	194±131ab	4.3±0.6a	123±18.6e	60.2±10.5c	2.7±2.3c	0.2±0.1b
5	5.1±0.1b	212±64ab	5.3±0.5a	48.8±11.0cd	26.3±3.0b	2.2±1.5c	0.4±0.2bc
6	7.3±0.7c	118±81ab	7.2±3.5ab	54.4±1.5d	24.5±7.6b	0.9±0.1bc	0.0±0.0a
7	7.3±0.3c	130±57ab	6.5±1.6ab	32.5±2.3c	14.4±3.0b	0.6±0.1b	0.0±0.0a
8	6.4±1.1bc	107±44ab	5.3±0.5ab	4.74±0.02a	2.3±0.1a	0.2±0.1a	0.0±0.0a
9	4.8±0.2b	268±155ab	8.6±1.2b	70.1±6.7de	44.8±4.8bc	1.2±0.6bc	1.0±0.1d
10	3.9±0.1a	194±16b	7.2±1.8ab	44.7±26.5cd	18.0±5.0b	1.1±0.1bc	1.0±0.2d
11	4.9±0.2b	129±28a	11.1±0.5c	67.5±3.5de	40.3±12.1bc	0.6±0.1b	0.6±0.1c
12	7.3±0.6c	113±61ab	5.6±2.4ab	228±97.7f	119±13.8d	10.9±2.1d	0.3±0.1b
13	6.0±0.7bc	224±28b	6.5±0.8ab	60.4±19.5de	41.5±15.0b	0.9±0.3bc	0.8±0.0c
14	4.4±1.5ab	164±9ab	3.4±0.4a	27.8±19.0bc	19.1±12.7b	0.6±0.2b	0.1±0.0b
15	4.6±0.1b	278±7b	4.2±1.1a	19.3±1.1b	13.9±0.4b	0.5±0.1b	0.1±0.1b
16	3.9±0.3a	195±35ab	6.2±3.0ab	39.0±6.0cd	24.6±1.6b	1.0±0.2bc	1.0±0.3d
17	4.4±0.4ab	147±7.0ab	5.3±0.7ab	63.4±7.9de	36.7±20.1bc	1.2±1.0abc	0.5±0.2bc
18	4.2±0.9ab	105±14a	7.6±0.1b	41.5±12.8cd	20.0±3.5b	0.6±0.3b	0.4±0.1bc

Means followed by the same letter within a column are not significantly different at  $p<0.05$

Total recoverable Cu included all the Cu in the soil except for those bound in the mineral crystal structure and therefore is generally lower than total Cu concentration. Total recoverable Cu concentrations in the soils ranged from 4.74 to 228 mg kg<sup>-1</sup> (see Table 2), which were higher than the background concentration (3.7 mg kg<sup>-1</sup>) in Florida soils (Ma et al. 1997). Copper accumulation in the soils was related to citrus planting history or inputs of Cu mainly through use of fungicides since Cu loading from other sources such as fertilizers and manures was limited. The lowest total recoverable Cu (4.74 mg kg<sup>-1</sup>) was measured in the soil from the youngest (6 years old) citrus grove, whereas the highest total recoverable Cu (228 mg kg<sup>-1</sup>) was determined in the soil from an old grove (35 years old; see Table 2). Copper accumulation in the soils from the 4-year-old grove increased by 27% compared to 60-fold in the soil from the 33-year-old grove. Among the tested soils, three (soils 3, 4, 12) exceeded the maximum allowable concentration of Cu (100 mg kg<sup>-1</sup>) for agricultural soils proposed in the USA (Kabata-Pendias and Pendias 2001). The results from a previous study in the same area indicated that Cu accumulation has significantly reduced soil microbial biomass and microbial community diversity in these soils (Zhou et al. 2011). Copper accumulation in the soils also accelerated soil acidification as external Cu loading enhanced the replacement of exchangeable H<sup>+</sup>/Al<sup>3+</sup> on clay minerals and oxides, thus reducing soil pH. The growers were recommended to regularly apply lime to increase soil pH resulting from the acidification from N fertilization and leaching base cations under subtropical climate (He et al. 1999). However, soil acidification by increased Cu inputs was not accounted for.

In addition to cropping history, citrus variety may affect Cu accumulation in the soil. In general, grapefruit production system received more Cu in fungicides than orange production system as grapefruit trees are more susceptible to fungal diseases such as canker and melanose. Among the different products, Cu-containing fungicides have been considered the most effective (Timmer et al. 2006). One single application of Cu-based fungicides may introduce 3 to 5 kg Cu ha<sup>-1</sup>. Furthermore, three to six applications per year would correspond to an annual load of approximately 10–30 kg Cu ha<sup>-1</sup>. Soil # 8, 9, 10, and 11 were all planted with red grapefruit and belonged to the same soil type with similar management practices, and therefore, their significant difference in total recoverable Cu concentration can be attributed to citrus production duration. Correlation analysis showed that total recoverable Cu was positively related to soil pH but had no correlation with EC or organic matter (data not shown). A simple linear regression with citrus production time indicated that total recoverable Cu in the soil was, on average, increased by

1.9 mg kg<sup>-1</sup> year<sup>-1</sup> or 4.3 kg Cu ha<sup>-1</sup> year<sup>-1</sup>. Considering that fruit harvest removes only a small amount of Cu from the soil and the increase in storage capacity of a mature tree for Cu is limited, the majority of the applied Cu may have been lost into surface runoff water or deeper soil layers.

### 3.2 Effects of pH on downward movement of copper in soils

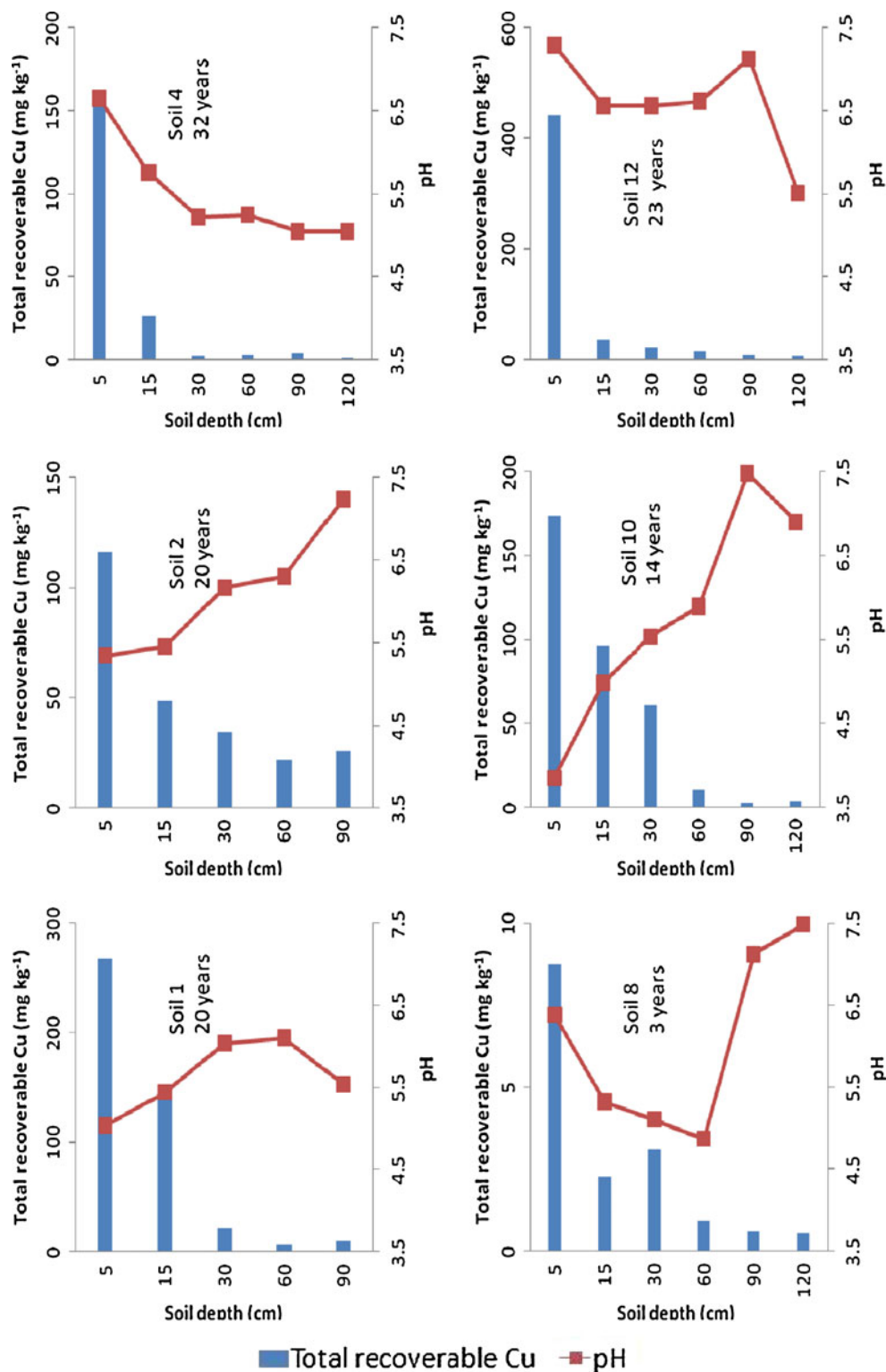
Based on the soil properties and Cu content, six soils were selected for Cu vertical distribution study. Soil pH affected the downward movement of Cu (Fig. 2). Total recoverable Cu decreased more rapidly with depth in relatively high-pH soils (soils 4 and 12) than low-pH soils (soil 2). Copper concentration up to 441 mg kg<sup>-1</sup> was measured in the top 5 cm of soil 12, but the downward movement of Cu along soil profile was minimal and Cu concentration decreased markedly with soil depth, whereas soil 2 had lower Cu concentration in the top 5 cm but Cu had been significantly leached down the soil profile, accumulating occurred even at the 90-cm depth.

These results suggest that soil pH played a critical role in Cu downward migration, as these soils had similar properties except for pH (United States Department of Agriculture, Soil Conservation Service (USDA-SCS) 1980; see Tables 1 and 2). When soils had pH > 6.5 such as soils 4 and 12, most Cu was concentrated in the top 5-cm soil layer with a minimal amount being leached downward even after over 30 years' cultivation of citrus (see Fig. 2). In these soils, Cu downward migration along soil profiles was restricted and subsequently pollution to groundwater was minimal. However, low pH-enhanced Cu leaching losses. When a soil had pH below 6.5, such as soils 1 (pH 5.03) and 10 (pH 3.85), downward movement of Cu was significant, with >50 mg kg<sup>-1</sup> of total recoverable Cu being measured at the 15–30-cm soil layer in soil 10 (Fig. 3). Due to high temperature and humidity in South Florida, organic materials such as plant litters were quickly mineralized in sandy soils, as evidenced by low organic C content in the surface soils. Copper downward movement along large roots was limited. In the Indian River area, since the water table was close to surface during the rainy season (June–November), 90% of the citrus roots are within the surface 40 cm (Zhang et al. 1996). Therefore, Cu was leached down soil profile due to the low retention capacity of the soils.

### 3.3 Copper availability as affected by Cu accumulation and soil pH

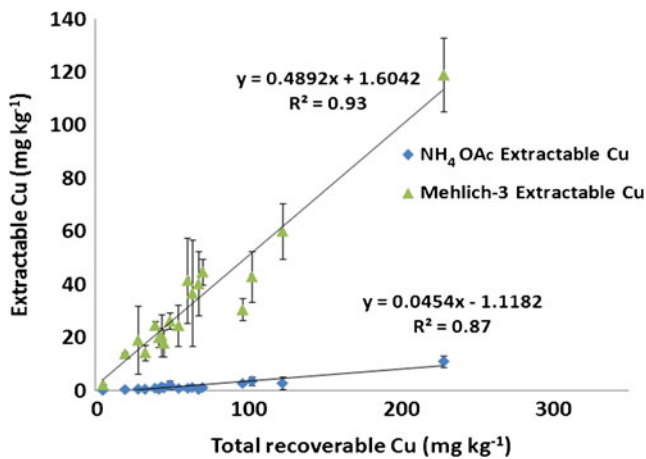
Soil available Cu as estimated by Mehlich-3 ranged from 2.31 to 119 mg kg<sup>-1</sup>, with an average of 31.3 mg kg<sup>-1</sup>,

**Fig. 2** Distribution of Cu along soil profiles



while  $\text{NH}_4\text{OAc}$ -extractable Cu ranged from 0.20 to 10.9  $\text{mg kg}^{-1}$  and  $\text{CaCl}_2$ -extractable Cu were 0.01–1.50  $\text{mg kg}^{-1}$  (see Table 2). The soil available Cu extracted by the three methods was correlated among themselves and with total recoverable Cu. Except for  $\text{CaCl}_2$ -extractable Cu, a significant correlation occurred for all pairs ( $r=0.89$ – $0.94$ ,

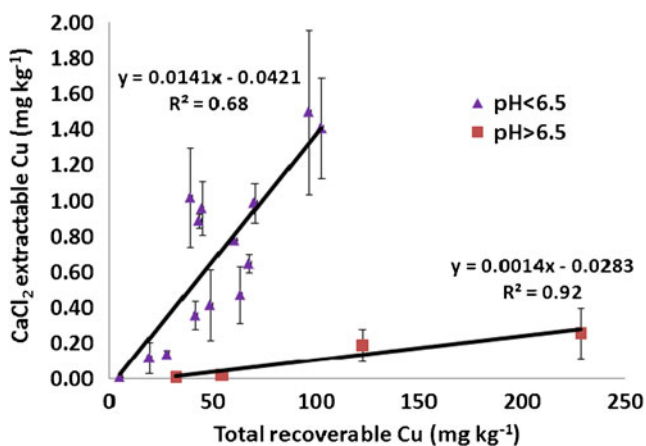
$p<0.001$ ; data not shown). Based on the slope of Mehlich-3- or  $\text{NH}_4\text{OAc}$ -extractable Cu to total recoverable Cu (see Fig. 3), of the total recoverable Cu, 48.9% was extractable by Mehlich-3 and 4.5% by  $\text{NH}_4\text{OAc}$ , indicating that Cu in these soils was highly mobile. When the  $\text{CaCl}_2$ -extractable Cu was grouped based on soil pH, it was highly correlated



**Fig. 3** Relationship between Mehlich-3-extractable and  $\text{NH}_4\text{OAc}$ -extractable Cu and total recoverable Cu concentration in soils. Vertical bars represent standard errors

with total recoverable Cu. The regression line slope was greater at  $\text{pH} < 6.5$  ( $b_1 = 0.014$ ) than  $\text{pH} > 6.5$  ( $b_1 = 0.0014$ ; Fig. 4). These results indicate that water-soluble Cu in soil was probably controlled by pH, decreasing with increasing soil pH.

Total recoverable Cu alone may not provide adequate information regarding Cu phytoavailability in soils as only a small portion of the total recoverable Cu is available to plant or subjected to leaching. Plants influence Cu availability, especially in the rhizosphere. For example, root activity can change rhizosphere pH (Neumann and Romheld 2002); root exudation can lead to the mobilization of soil organic matter, which complexes and solubilizes Cu (Dessureault-Rompré et al. 2008); and plants can transform Cu into metallic nanoparticles near the roots with evidence of assistance by fungi to reduce Cu toxicity (Manceau et al. 2008). Various extraction methods have been used to estimate available Cu in soils. Mehlich-3 procedure is commonly used to measure labile Cu in soil, which



**Fig. 4** Relationship between  $\text{CaCl}_2$ -extractable Cu and total recoverable concentration Cu in soils. Vertical bars represent standard errors

includes water-soluble, exchangeable, and a variable portion of soil Cu being bound to organic matter, oxides, or acid-soluble fraction (Mehlich 1984). These fractions of Cu are readily dissolved in the mild acidic/chelation (containing EDTA) conditions like the rhizosphere, particularly when N fertilizer is from ammonium, which induces acidification. Calcium chloride ( $\text{CaCl}_2$ ) is an analog of soil solution and extracts mainly water-soluble plus a part of exchangeable Cu that can be readily taken up by the roots. The  $\text{NH}_4\text{OAc}$  extraction is intermediate between these two procedures.

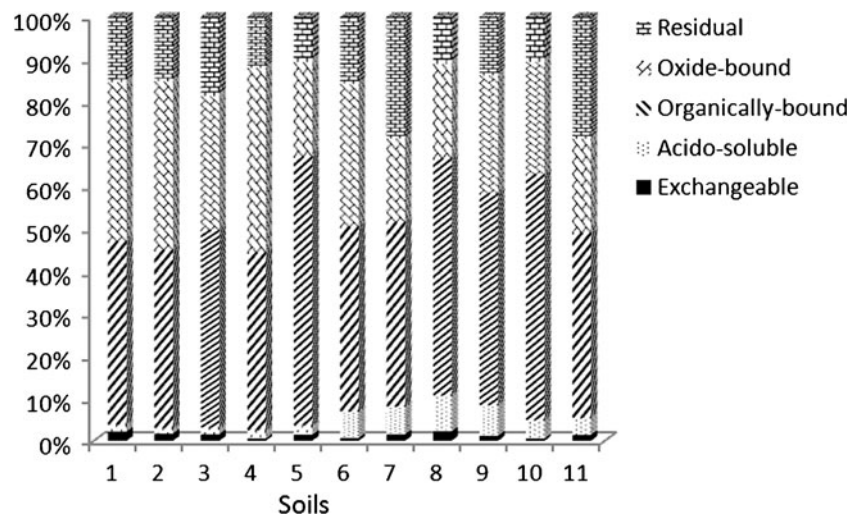
In this study, Mehlich-3- and  $\text{NH}_4\text{OAc}$ -extractable Cu were closely correlated with the total recoverable Cu. However, the correlation between  $\text{CaCl}_2$ -extractable and total recoverable Cu was affected by soil pH. Brun et al. (1998) recommended 0.1 M  $\text{CaCl}_2$  extraction as the most suitable for the determination of Cu availability to plants in acid-neutral soils. Zhang et al. (2003a) studied the losses of dissolved Cu in runoff in Florida sandy soils under commercial citrus production. They reported that mean dissolved Cu concentration in surface runoff water from agricultural fields was significantly correlated with 0.01 M  $\text{CaCl}_2$ -extractable Cu. The results from this study together with those from previous reports indicate that  $\text{CaCl}_2$ -extractable Cu may be an improved indicator of Cu availability in soil as it reflects the effects of both total Cu and pH in sandy soils with low to medium OM content.

### 3.4 Soil Cu fractionation and availability

Sequential fractionation has proven useful in distinguishing Cu association with different soil fractions (Ma and Rao 1997). The Cu fractions in the surface soil decreased in the order of organic bound (48.2%) > oxide bound (29.8%) > residual (16.5%) > acid soluble (4.2%) > exchangeable (1.3%; Fig. 5). The dominance of organic-bound Cu in soil Cu (from 43% to 62%) indicates its role in controlling the fate and transport of Cu in soils. In addition, oxide-bound Cu was also significantly correlated with total recoverable Cu and accounted for a third of the total recoverable Cu in the soils.

To understand the availability of soil Cu, Mehlich-3-extractable Cu was correlated with various chemical Cu fractions. Mehlich-3-extractable Cu was significantly correlated with organic-bound Cu fraction ( $r = 0.959$ ,  $p < 0.001$ ). In addition, Mehlich-3-extractable Cu was also highly correlated with acid-soluble and oxide-bound Cu but less with exchangeable Cu, which may be attributed to a large portion of Cu in the two fractions being associated with the labile Cu pools in the sandy soils. In comparison,  $\text{NH}_4\text{OAc}$  or  $\text{CaCl}_2$  method extracted a smaller amount of Cu than the Mehlich-3 procedure (see Table 2), but the  $\text{CaCl}_2$ -extractable Cu was more correlated with water-

**Fig. 5** Copper fractions as a percentage of total recoverable in soils



soluble and exchangeable Cu than the other fractions ( $r=0.828$ ,  $p<0.01$ ). This agrees with the mild nature of the reagent, implying that this part of Cu was more readily leached into groundwater or surface waters.

There is no significant difference in Cu fractions among the tested soils, indicating that further conversion of Cu from available to less available fractions (aging) takes longer than citrus cultivation history. Our results indicate that in the sandy soils under citrus production, Cu is mainly bound to organic materials, oxides, and clay minerals (residual). Approximately 50% of the soil Cu was tied with organic matter, which is unexpected in sandy soils with low organic matter but consistent with other reports on acidic soils (Flores-Veles et al. 1996; Fernandez-Calvino et al. 2009). Similar results were also obtained by Strawn and Baker (2008) who examined Cu speciation in a contaminated agricultural soil using advanced X-ray spectroscopy. This may be attributed to the formation of inner-sphere complexes of Cu with humic acids (Komarek et al. 2010), which are very stable. The other possible reasons include low concentration of clay and oxides in the sandy soils. Similarly, Cu can be specifically adsorbed on the surfaces of Fe, Al, and Mn oxides, thereby Cu being also concentrated in the oxide fraction. Generally speaking, the water-soluble and exchangeable Cu is readily bioavailable whereas the acid-soluble, oxide-, and organic matter-bound fractions are potentially bioavailable since they are sensitive to environmental changes such as decomposition of organic matter, redox potential, and pH and can be mobilized under changing chemical environment (Shuman 1991; Alva et al. 2000).

Total recoverable Cu in some of the tested soils exceeded or approached the maximum allowable Cu level ( $100 \text{ mg kg}^{-1}$ ) for agricultural soils (Kabata-Pendias and Pendias 2001). Due to low organic matter and clay content, Cu in these sandy soils was highly bioavailable and mobile,

as evidenced by the high percentage of Mehlich-3-extractable Cu from the total recoverable Cu (48.9% on average, see Fig. 3). Reduced microbial biomass and microbial community diversity were reported in the high Cu soils (Zhou et al. 2011). Therefore, best management practices need to be developed to remediate Cu-contaminated soils. Various amendments have been shown to decrease Cu leachability and bioavailability via chemical processes (Delas 1963; Bolan and Duraisamy 2003; Kumpiene et al. 2006; Bes and Mench 2008; Fan et al. 2011). Since copper solubility in these soils is strongly pH dependent, adequate application of liming materials coupled with reducing Cu inputs should be given priority consideration to minimize the negative impacts of Cu on soil quality and the environment

#### 4 Conclusions

The repeated uses of Cu-based fungicides have caused significant accumulation of Cu in the soils under citrus production. The availability of Cu as estimated by chemical extraction was positively correlated with total recoverable Cu and negatively to soil pH. However, the downward migration of Cu along soil profiles was mainly controlled by soil pH in the sandy soils with low organic matter content. Among the commonly used soil tests,  $\text{CaCl}_2$ -extractable Cu appeared to be an adequate indicator of Cu availability in the sandy soils as it reflected the effects of both Cu accumulation and soil pH change. Most of the Cu in the soils was associated with organic matter and oxides fractions. The organic- and oxide-bound Cu were highly correlated with extractable Cu and were therefore potentially subjected to leaching loss. The relatively higher clay content in the subsoil can enhance Cu sorption, but Cu may transport from land to surface water through surface runoff.



These findings merit attention in the development of best management practices to reduce the adverse impacts from application of Cu-based fungicides in the citrus production regions.

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