Copper desorption in flooded agricultural soils and toxicity to the Florida apple snail (*Pomacea paludosa*): Implications in Everglades restoration

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Abstract

Copper (Cu) desorption and toxicity to the Florida apple snail were investigated from soils obtained from agricultural sites acquired under the Comprehensive Everglades Restoration Plan. Copper concentrations in 11 flooded soils ranged from 5 to 234 mg/kg on day 0 and from 6.2 to 204 mg/kg on day 28 (steady-state). The steady-state Cu concentration in overlying water ranged from 9.1 to 308.2 μg/L. In a 28-d growth study, high mortality in snails occurred within 9 to 16 d in two of three soil treatments tested. Growth of apple snails over 28 d was affected by Cu in these two treatments. Tissue Cu concentrations by day 14 were 12–23-fold higher in snails exposed to the three soil treatments compared to controls. The endangered Florida snail kite and its main food source, the Florida apple snail, may be at risk from Cu exposure in these managed agricultural soil–water ecosystems.

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Keywords: Copper; Toxicity; Florida apple snail; *Pomacea paludosa*; Everglades; Comprehensive Everglades Restoration Plan (CERP)

1. Introduction

Copper (Cu) has a long history of use in Florida citrus agriculture as fertilizer and fungicides (Alva et al., 1995). In 2005, according to the U.S. Department of Agriculture, 469 350 kg of copper hydroxide and 51 550 kg of copper sulfate or basic copper sulfate were applied to grapefruit, orange, tangelo, tangerine, and temple crops on 259 563 ha in Florida (U.S. Department of Agriculture, 2006). These quantities do not reflect use of Cu on other citrus crops or the use of copper sulfate and chelated Cu formulations (e.g., Cutrine-Plus, Komeen, etc.) as algaecides—herbicides, which are permitted by the Florida Department of Environmental Protection (FDEP) for control of nuisance planktonic and filamentous algal and vascular plants (Leslie, 1990).

Implementation of the Comprehensive Everglades Restoration Plan (CERP) under the Water Resources Development Act of 2000 requires acquisition of thousands of acres of land for maintaining hydrologic buffer areas and for the creation of storm water treatment areas, water storage reservoirs, and wetlands (*Everglades National Park, 2001*). A large portion of these lands is currently or was formerly managed for row crops and citrus fruit orchards with fertilizers and...
pesticides, including Cu. Under the CERP, these soils will be flooded, converting dry aerobic environments to inundated (perennially or intermittently) relatively undisturbed anaerobic sediments which will likely promote the release of Cu from soils. A comparison of aqueous Cu concentrations in agriculture and non-agriculture watersheds showed higher concentrations in runoff where agriculture was practiced compared to runoff near non-agriculture land (Dietrich et al., 2001). Copper loads in surface runoff are related to total Cu in soils, soil properties, metal characteristics, and environmental factors, especially in sandy soils (He et al., 2006). Enrichment of Cu in runoff will adversely affect receiving surface water quality (Moore et al., 1998).

Total soil Cu increases proportionally to the age of citrus production (Reuther and Smith, 1952, 1953). Therefore, although annual contributions are small, Cu concentrations in sandy soils in Florida with long-term citrus production show accumulation and are significantly greater than similar soils with native vegetation (Alva et al., 1993; Zhu and Alva, 1993). Recently, reports on agricultural properties acquired under the CERP documented soil Cu concentrations (in dry weight) as high as 1200 mg/kg in St. Lucie County, 72 mg/kg in Highlands County, and 47 mg/kg in Broward County (SFWMD, 2001–2006). Based on application of a soil–water partition coefficient of 2.5 from the U.S. EPA (U.S. EPA, 2005), these soils would produce a range of 118 (Broward County)–3015 µg/L (St. Lucie County) Cu in surface waters which are significantly higher than the U.S. EPA water quality criteria (13 µg/L) for freshwater organisms (U.S. EPA, 2003) and the 90th centile exposure concentration of 5.8 µg/L for Cu obtained from the exposure distribution of all Cu monitoring data (1990–2006) from freshwater ecosystems in south Florida (Schuler et al., in press).

The Florida apple snail (Pomacea paludosa) is a periphyton-grazing freshwater gastropod and a key species in the Everglades ecosystem. P. paludosa is the sole food source of the federally endangered Florida (formerly Everglades) snail kite (Rostrhamus sociabilis plumbeus) and a prey species for other birds, fish, reptiles, and mammals (Sharfstein and Steinman, 2001). The literature indicates that the gastropod, Busycon canaliculatum, can accumulate and store Cu and use it in the synthesis of hemocyanin (Betzer and Yевич, 1975). The major lethal effects of Cu in gastropod mollusks are disruption of the transporting surface epithelium and osmoregulation and eventual water accumulation in tissues (Cheng, 1979).

Agricultural areas converted to storage basins or wetlands under the CERP will ultimately become habitat for sensitive aquatic receptors like the Florida apple snail. This species is particularly vulnerable to Cu accumulation and toxicity because it spends most of its life cycle submersed, except for the females during oviposition (Turner, 1994; Turner et al., 2001). The snail is therefore in intimate contact with all three routes of Cu exposure: water, sediment, and dietary uptake. The literature indicates that the two major chelated forms of Cu (Komeen and Cutrine-Plus) are acutely toxic (96-h LC50 = 24 and 27 µg/L, respectively) to 1- to 2-old juvenile apple snails and Komeen is acutely toxic (96-h LC50 = 57 µg/L) to adult snails (Wenger et al., 1984).

The following study focuses on the desorption of Cu in field-collected flooded soils from different agricultural sites in south Florida, the development of a model to predict Cu concentrations based upon soil characteristics, the biological effects of desorbed Cu exposures from selected agricultural soils to juvenile apple snails exposed for 28 d and the acute toxicity of Cu in pore versus overlying waters to snails exposed for 96 h.

2. Methods

2.1. Desorption study

Soils were collected from agriculture sites (n = 11), including citrus, in four counties (Dade (n = 2), Palm Beach (n = 1), Martin (n = 2), St. Lucie (n = 6)) of south Florida (Fig. 1). At each of the 11 sites, soils were collected and composited from three locations. In the laboratory, 6 L of soil from each of the 11 sites were randomly distributed to 18 L glass tanks, with two replicates per site. Tanks were subsequently flooded with 12 L of carbon-filtered, UV-sterilized laboratory freshwater (laboratory freshwater) to create a volume ratio of 2:1 (water:soil). Tanks were held under static conditions for 28 d with a temperature of 25 ± 1°C and 16 h light:8 h dark photoperiod.

Soils were physically characterized (pH, CEC, % sand, silt, clay and OC) using method D422 (ASTM) and analyzed for background concentrations of pesticides and metals using methods published by Sericano et al. (1998) and method 3050B (U.S. EPA, 1996), respectively. Pesticide (organochlorine and organophosphate) and metal concentrations (Cd, Pb, As, Hg, and Zn) were at negligible levels. Flooded waters (overlying water) and soil samples were collected on day 0 (initiation), 1, 2, 4, 7, 14, 21, and 28 (termination) for analyses of total and dissolved Cu. Water hardness and alkalinity were also measured at the same time periods. Water temperature, dissolved oxygen (DO) and pH were measured daily. The 28-d average DO, temperature, and pH were 4.16 ± 0.95 mg/L, 24.5 ± 0.01°C, and 7.51 ± 0.25, respectively. Water samples for dissolved Cu were filtered through 0.45-µm Gelman Nylon Mesh® and soil samples were digested using an acid digestion method for Cu (U.S. EPA, 1996). Acid volatile sulfide (AVS) was analyzed for each replicate on day 0, 1, 14, and 28 (U.S. EPA, 1991). Copper and other metals were analyzed using inductively coupled plasma mass spectrometry (ICPMS). Pesticides were analyzed using gas chromatography–mass spectrometry (GC–MS).

Results of quality control (QC) analysis indicated 90 and 80% recovery for Cu and pesticides from reference soils, respectively.

Copper concentrations in soil and overlying water were used to determine the soil–water partition coefficient Kd (L/kg):

\[
K_d = \frac{C_o}{C_w}
\]

where \(C_o\) is the Cu concentration in the solid or soil phase (mg/kg dw), and \(C_w\) is the Cu concentration in the water (or pore water) phase (mg/L). When the average concentration of Cu reached a plateau or steady-state in the overlying water (i.e., when concentration over three successive analyses was within ±20% of each other) the ratio of the average concentration for those days in soil (\(C_o\)) to water (\(C_w\)) was used to determine the \(K_d\) for each of the soils.

The Freundlich isotherm equation was also used to determine the Freundlich constant or distribution coefficient \(K_F\) (L/kg):

\[
C_o = K_F C_w^{1/n}
\]

\(K_F\) and \(n\) are empirical constants dependent on environmental factors; \(1/n\) takes into account non-linearity in the isotherm which occurs at higher chemical concentrations. Data were fit to the linear form of this relationship (Eq. (3)) by using the log transformed equation to determine the value of...
assumes steady-state exists between Cu concentrations in the solid phase and in the pore water.

\[
\log(C_s) = \log(K_F) + \left(\frac{1}{n}\right) \log(C_w)
\]

A plot of the log \(C_s\) against log \(C_w\) yields a straight line. The \(K_F\) is determined from the intercept of the line. Soils were divided into groups based on the USDA system of nomenclature for soil texture which is dependent on mechanical analyses of the distribution of different sizes of mineral particles (% sand, % silt, % clay) for each field-collected soil plus a reference control soil (control). The \(K_F\) was determined for each soil group. Linear equations and correlation coefficients are summarized.

The desorption of Cu from soil to water reaches a plateau when the soil–water system reaches steady-state. Therefore, Michaelis–Menten kinetics was used to describe the release of Cu from soil to water:

\[
C_w = \frac{C_{wsat}}{K_M + t}
\]

where \(C_w\) is the Cu concentration in water at time \(t\) (µg/L), \(C_{wsat}\) is the saturation Cu concentration (steady-state concentration) in water (µg/L), \(t\) is flooding time (day), \(K_M\) is the Michaelis–Menten constant in time (day). \(K_M\) is the time needed for the Cu concentration in water to equal half of the maximum saturation Cu concentration. Data were fit to a linear relationship by rearranging Eq. (4) as follows:

\[
\frac{1}{C_w} = \left(\frac{K_M}{C_{wsat}}\right) \frac{1}{t^+} + \frac{1}{C_{wsat}}
\]

The ratio of \(K_M/C_{wsat}\) is the slope and \(1/C_{wsat}\) is an intercept of the relationship.

### 2.2. Toxicity studies

Three agricultural soils out of the 11 soils used in the desorption study were selected with low (Agler in St. Lucie County), medium (Sunrise Boys in Palm Beach County), and high (Aquacalma-A in Martin County) Cu concentrations in overlying water (i.e., after flooding), including the control soil, to conduct acute and chronic toxicity studies based on U.S. EPA methodology (U.S. EPA, 2002a,b). The control soil had negligible background concentrations of metals and pesticides.

For the chronic toxicity study, 6 L of soil from each of the three sites plus the control soil were randomly distributed into 18 L glass tanks, with three replicates each. Soils were flooded with 12 L of laboratory freshwater and held under flow-through conditions for 28 d, with a water replacement of two tank volumes per 24 h.

*P. paludosa* eggs were collected from Water Conservation Areas 3A and 2B, Dade County, Florida, hatched under laboratory conditions at a temperature of 26 ± 1 °C, 16 h light:8 h dark photoperiod, and fed romaine lettuce. Background Cu concentration in romaine lettuce was 2.4 µg/g dw. Size measurements of snails were conducted based on the method described by Boulding and Hay (1993). Initial measurements of 96-h-old snails (n = 15) were: mean shell length (SL) (4.16 ± 0.25 mm), mean aperture length (AL) (3.89 ± 0.20 mm), mean shell width (SW) (4.35 ± 0.34 mm), and mean dry weight (0.0022 ± 0.0005 g).

Ten juvenile apple snails were transferred to each of the three replicate tanks of each soil type (treatment), including the control soil, to begin the chronic study. Five additional snails were added to each replicate treatment, but were held in a Nytex® chamber in the 18 L glass tanks for tissue analysis (whole body) of Cu on day 14. The initial biomass (day 0) was 0.035 g snail/L.
per tank. Snails were fed romaine lettuce every other day. Overlying water was gently aerated during the 28-d exposure. Overlying water, pore water, and soil samples were collected on days 0, 4, 14, 21, and 28 for Cu (total and dissolved) analysis. Pore water was collected based on methods published by Rausch et al. (2006). Water hardness and alkalinity were measured at each sampling point and ranged from 66.5 ± 4.5 to 73.7 ± 5.4 mg/L (as CaCO₃) and from 61 ± 3.5 to 69 ± 7.1 mg/L (as CaCO₃), respectively.

Survival, DO, temperature, pH, and behavioral observations were monitored daily. The 28-d average measured DO, temperature, and pH were 7.45 ± 0.42 mg/L, 26.05 ± 1.32 °C, and 5.75 ± 0.38, respectively. NH₃ (total) ranged from 0.14 to 3.57 mg/L. NH₃-N on day one and from 0.17 to 3.52 mg/L on day 28. No abnormal behavior of exposed snails compared to control snails was observed. Total body residue analysis of Cu in dead snails was only measured in the lowest exposure soil treatment replicates that contained snail mortality. At day 28, the following parameters were measured on surviving snails: mean shell length, mean aperture length, mean shell width, and mean dry weight (without shell). Tissue was dried at 60 °C in a Muffle Furnace for 24 h and weighed. Snails were collected on day 14 from Nytex chambers (n = 5) and on day 28 from glass tanks (n = 3) of each replicate, homogenized, and digested (U.S. EPA, 1996) for whole body (including shell) tissue Cu analysis.

Pore and overlying waters were collected during week three from each soil treatment in the chronic toxicity study in order to conduct 96-h acute toxicity tests. Three replicates were used for each soil treatment, including an untreated control (laboratory freshwater) with 10, 1-month-old apple snails per replicate. Water was renewed at 48 h. Survival and behavioral observations were made daily. DO, temperature, and pH were monitored at initiation, renewal, and termination. Water hardness and alkalinity were measured for each treatment at the start and the end of the test. Water samples were also collected at 0 h and at 96 h for dissolved Cu and dissolved organic carbon (DOC) analyses. Dissolved organic carbon was analyzed with a Shimadzu TOC-5000 (Shimadzu Scientific Instruments, Columbia, MD, USA).

### 2.3. Data analysis

Data met the assumptions of normality and homogeneity of variance. Linear regression was used for determining Freundlich and Michaelis–Menten constants. Multiple regressions were used for determining the dependence of overlying water Cu concentrations and the Michaelis–Menten constant on soil characteristics using the stepwise procedure. The F-test method was used for single treatment comparison for data from the acute toxicity studies. Multiple treatment comparisons were conducted using Tukey’s procedure for data from the chronic toxicity study. An effect with a p value < 0.05 was considered significant. All statistical analyses were conducted using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

### 3. Results and discussion

#### 3.1. Desorption study

Physical characteristics of soils are shown in Table 1 and Supplementary Information. The citrus soils (Aquacalma-A and -B, Agler, Arcco, Birdsall, Equus-A and -B, Mearthur, and Sunrise Boys) had a high percentage of sand (>70%), while non-citrus soils (Rodriguez Sanchez and L31N Buffer) had a high percentage of silt. Based on the distribution of different sizes of mineral particles used to define soil texture by the USDA, there were three sandy soils (Sunrise Boys, Aquacalma-A, and Equus-A), three loamy sands (Aquacalma-B, Mearthur, and Birdsall), three sandy loams (Equus-B, Agler, and Arcco) and three silt-loams (Rodriguez Sanchez, L31N Buffer, and control soil).

Copper concentrations in soils and water are shown in Fig. 2. Copper concentrations for the agricultural sites at the beginning of the study ranged from 5 ± 0.8 (Equus-A) to 234 ± 28 mg/kg (Aquacalma-A). Copper concentrations in soils decreased slightly with time. He et al. (2006) found that in St. Lucie County, FL, soil samples from citrus groves (n = 10) contained 67.7–400.1 mg/kg Cu and were 10–110-fold higher than undisturbed forest soil samples (n = 3) (1.6–3.6 mg/kg).

Copper concentrations desorbed from soils and reached a steady-state in soils approximately 4–7 d after being flooded (Fig. 2). He et al. (2006) also found that in measuring released Cu from citrus soils (St. Lucie County, FL), soils reached steady-state between 1 and 4 d. The mean of the Cu concentrations in soils from days 14, 21, and 28 ranged from

### Table 1

Soil characteristics and partition coefficients

<table>
<thead>
<tr>
<th>Site</th>
<th>Site name</th>
<th>pH</th>
<th>CEC (meq/100 g)</th>
<th>Sand (%)</th>
<th>Clay (%)</th>
<th>Silt (%)</th>
<th>Organic carbon (%)</th>
<th>Soil texture</th>
<th>Cu in soil (mg/kg dw)</th>
<th>Cu in overlying water (μg/L)</th>
<th>Partition coefficient (Kn) (L/kg)</th>
<th>Freundlich coefficient (Kf) (L/kg)</th>
<th>Kₐ (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sunrise Boys</td>
<td>7.2</td>
<td>10.7</td>
<td>95.5</td>
<td>1.2</td>
<td>3.4</td>
<td>0.92</td>
<td>S</td>
<td>174.4</td>
<td>159.6</td>
<td>3.0</td>
<td>0.22</td>
<td>3.86</td>
</tr>
<tr>
<td>2</td>
<td>Aquacalma-A</td>
<td>6.4</td>
<td>10.7</td>
<td>93.8</td>
<td>2.7</td>
<td>3.5</td>
<td>1.89</td>
<td>S</td>
<td>204.0</td>
<td>274.4</td>
<td>2.9</td>
<td>7.22</td>
<td>1.64</td>
</tr>
<tr>
<td>3</td>
<td>Equus-A</td>
<td>6.4</td>
<td>7.1</td>
<td>87.3</td>
<td>1.7</td>
<td>11.0</td>
<td>1.31</td>
<td>S</td>
<td>62</td>
<td>91</td>
<td>2.8</td>
<td>4.63</td>
<td>1.78</td>
</tr>
<tr>
<td>4</td>
<td>Aquacalma-B</td>
<td>7.6</td>
<td>31.3</td>
<td>84.7</td>
<td>4.7</td>
<td>10.6</td>
<td>1.36</td>
<td>LS</td>
<td>128.8</td>
<td>214</td>
<td>3.8</td>
<td>12.42</td>
<td>1.88</td>
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<tr>
<td>5</td>
<td>Mearthur</td>
<td>7.8</td>
<td>15.9</td>
<td>82.5</td>
<td>4.6</td>
<td>12.9</td>
<td>1.23</td>
<td>LS</td>
<td>191.6</td>
<td>170.2</td>
<td>3.1</td>
<td>1.88</td>
<td>1.78</td>
</tr>
<tr>
<td>6</td>
<td>Birdsall</td>
<td>6.2</td>
<td>7.4</td>
<td>81.6</td>
<td>1.3</td>
<td>17.2</td>
<td>1.97</td>
<td>LS</td>
<td>190.7</td>
<td>308.2</td>
<td>2.8</td>
<td>2.07</td>
<td>1.88</td>
</tr>
<tr>
<td>7</td>
<td>Equus-B</td>
<td>7.5</td>
<td>25.3</td>
<td>82.7</td>
<td>16.5</td>
<td>0.9</td>
<td>2.25</td>
<td>SL</td>
<td>70.3</td>
<td>125</td>
<td>3.8</td>
<td>0.14</td>
<td>4.00</td>
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<tr>
<td>8</td>
<td>Agler</td>
<td>7.7</td>
<td>29.6</td>
<td>72.0</td>
<td>5.3</td>
<td>22.7</td>
<td>1.62</td>
<td>SL</td>
<td>100.2</td>
<td>39.0</td>
<td>3.4</td>
<td>2.83</td>
<td>0.85</td>
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<td>Arcco</td>
<td>6.6</td>
<td>7.6</td>
<td>72.6</td>
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<td>24.2</td>
<td>2.15</td>
<td>SL</td>
<td>64.0</td>
<td>18.9</td>
<td>3.5</td>
<td>0.47</td>
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<td>10</td>
<td>Rodriguez Sanchez</td>
<td>7.9</td>
<td>34.6</td>
<td>25.3</td>
<td>12.1</td>
<td>62.7</td>
<td>15.65</td>
<td>S/L</td>
<td>83.4</td>
<td>13.5</td>
<td>3.8</td>
<td>0.47</td>
<td>0.55</td>
</tr>
<tr>
<td>11</td>
<td>L31N Buffer</td>
<td>7.9</td>
<td>34.7</td>
<td>18.8</td>
<td>2.6</td>
<td>78.6</td>
<td>9.44</td>
<td>SL</td>
<td>183.2</td>
<td>38.0</td>
<td>3.7</td>
<td>5.80</td>
<td>5.80</td>
</tr>
<tr>
<td>12</td>
<td>Control</td>
<td>7.4</td>
<td>9.8</td>
<td>2.1</td>
<td>53</td>
<td>44.9</td>
<td>18.31</td>
<td>S/L</td>
<td>8.1</td>
<td>3.2</td>
<td>3.4</td>
<td>3.3</td>
<td>0.39</td>
</tr>
</tbody>
</table>

a Cation exchange capacity.

b S: sand, LS: loamy sand, SL: sandy loam, StL: silty loam.

c Average of measured concentrations (in dry weight) on days 14, 21, and 28.

d Used for toxicity study.

e Control soil was not used in desorption study but used in 28-d growth study.
6.2 ± 0.7 mg/kg in Equus-A to 204 ± 18.2 mg/kg in Aquacalma-A treatments. Five soils exceeded the Florida DEP preliminary sediment quality assessment guideline (SQAG) threshold effects concentration (TEC) of 31.6 mg/kg and five soils exceeded the probable effects concentration (PEC) of 149 mg/kg (MacDonald et al., 2003).

Dissolved copper concentrations in overlying water from the 11 sites at the beginning of the study (day 1) ranged from 6.1 to 43.7 μg/L, which is up to 3-fold higher than the U.S. EPA numerical freshwater criterion (13 μg/L). Copper concentrations in overlying water were related to soil Cu concentrations. Copper concentrations in overlying water increased with time and reached a steady-state after 14 d (Fig. 2). Therefore, mean Cu concentrations in overlying water and soils on days 14, 21, and 28 were used to determine soil—water partition coefficients ($K_d$). The mean Cu concentrations in overlying water and soils exceeded the probable effects concentration (PEC) of 149 mg/kg (MacDonald et al., 2003).

Soils were grouped into four categories based on soil texture for determining coefficients using the Freundlich isotherm equation. The values of the Freundlich distribution coefficients ($K_F$) ranged from 0.14 to 1.78 (Table 1). Koster et al. (2006) calculated Cu partitioning between solid phase and pore water for different soil sites (Canada, Denmark) using the log transformed Freundlich equation and $K_F$ values ranged from 3.35 ($n = 20$ samples) to 3.76 ($n = 6$). The organic matter content of the latter soils was significantly higher than the sandy soils used in this study and therefore had a higher cation exchange capacity (CEC). Cu concentrations in pore water were also predictive of Cu activities in soils (Koster et al., 2006). There was no correlation between measured free Cu and organic matter (OM) in soils, although OM is assumed to be the major sorbent for Cu ions in the solid phase thus influencing Cu activity in pore water (Ponizovsky et al., 2006).

In this study, multiple regression analysis indicated that overlying water Cu concentrations ($C_w$) were a function of four factors: soil Cu concentrations ($C_s$), the interaction of CEC and $C_s$, potassium (K), and phosphorus (P) (see Supplementary Table for mineral properties of soils). The relationship is described by the following model:

$$C_w = 1.710[C_s] - 0.046(CEC \times C_s) + 1.709[K] - 0.420[P] - 69.436 \quad (R^2 = 0.98, p < 0.02)$$

in which $C_s$ and the interaction of CEC and $C_s$ accounted for 90% and K and P accounted for 8%, respectively, of the total variance in the released Cu amount.

Pedersen et al. (1997) found that desorption of Cu was influenced by soil pH and moisture while Impellitteri et al. (2003) reported that soil pH and % organic matter accounted for about 70% of the variability in Cu partitioning in bioavailable Cu for 40 soils. The present study found that desorption of Cu was related to CEC, K, and P (Eq. (6)). CEC is a function of soil organic matter (when SOM > 20 g/kg) or clay content (when SOM < 20 g/kg) (Essington, 2004). Soils with high CEC retain more Cu. This explains the negative coefficient for the interaction between CEC and soil Cu concentrations in Eq. (6). Soils in this study have low CEC values and therefore a lower capacity to hold Cu. The presence of K likely competes with exchangeable Cu. Therefore, an increase in K concentration would result in releasing more Cu to water. However, in general the effect of K on Cu desorption was minor (account for <8%) compared to the effect of CEC (account for 40%). This is in agreement with the results of He et al. (2006) in which less than 1% of the total released Cu was exchangeable Cu.

Acid volatile sulfide (AVS) concentrations of all soils were below the detection limit. Soils were collected from the top 6 cm and exposed to air; therefore, AVS was not a factor in this study.
The Michaelis–Menten constant \( (K_M) \) for the 11 soils plus the control soil ranged from the fastest release time of 0.55 d (Rodriquez Sanchez) to the slowest release time of 12.42 d (AQUA-B). The equation used to predict \( K_M \) is:

\[
K_M = 0.135\left[ K + 0.293[Mn] + 0.032[Na] - 0.014[P] \right] + 0.016[\% \text{ sand}] - 0.025[\% \text{ carbon}] - 7.016
\]

\( (R^2 = 1, p < 0.00001) \)

K, Mn, Na, P, \% sand, and \% carbon accounted for 100% of the \( K_M \). K alone accounted for 72% of this value.

The value of \( K_M \) reflects the rate of desorption of Cu from soil to water. The positive coefficients of K, Mn, Na, and \% sand in Eq. (7) indicate that increased concentrations of these factors increase \( K_M \) and therefore desorption is slow. The presence of these factors results in formation of Cu with mineral oxides and thus becomes less exchangeable (due to higher molecular weight) compared with free Cu (Nriagu, 1979).

### 3.2. Toxicity studies

Results of measured Cu concentrations in the three agricultural site soils, overlying water, and snail (whole body) tissue are shown in Table 2. Copper concentrations in soils on day 0 ranged from 117 to 234 mg/kg and on day 28 from 97.3 to 209.7 mg/kg. These concentrations are biologically relevant to soil invertebrates because cocoon production is reduced in earthworms at 53–150 mg/kg Cu dw (Spurgeon et al., 1994; Ma, 1984).

Overlying water contained significant concentrations (13.0–37.6 \( \mu \)g/L) of dissolved Cu (time weighted average; TWA) by day 4 based on a U.S. EPA freshwater criterion of 13.0 \( \mu \)g/L. The 28 d TWA overlying water concentrations were 2.7, 8.1, 17.0, and 23.9 \( \mu \)g/L for the control, Agler, Sunrise Boys, and Aquacalma-A soil treatments, respectively. Based on the toxicity literature, the water exposure concentrations from desorbed Cu in the Sunrise Boys and Aquacalma-A soil treatments are high enough to produce adverse effects on freshwater algae, rotifers, mollusks, crustaceans, and fish (Eisler, 1998).

Tissue Cu concentration in control snails did not increase with exposure time. In contrast, by day 14 tissue Cu concentrations of snails from Agler, Sunrise Boys, and Aquacalma-A soil treatments were 12- (158.7 mg/kg), 20- (252.8 mg/kg), and 23-fold (300.6 mg/kg) higher, respectively, than the tissue concentration (12.9 mg/kg) from snails exposed to control soil (Cu soil background: 8.1 mg/kg dw) and background concentration (12.1 mg/kg). In snails that were found dead by day 14 from the Sunrise Boys soil treatment, Cu tissue residues accumulated to 636 ± 5.2 mg/kg Cu dw, which was 2-fold higher than the Cu tissue concentration of live snails in the Sunrise Boys soil treatment. This was also about 2-4, and 49-fold as high as the Cu residue concentrations in live snails on day 14 from Aquacalma-A, Agler, and the control soil treatments, respectively. Day 28 Cu tissue concentrations were similar to day 14 tissue concentrations.

### Table 2

<table>
<thead>
<tr>
<th>Site</th>
<th>Cu in soil (mg/kg dw)</th>
<th>Cu in overlying water ( {\text{( \mu )g/L}} )</th>
<th>Whole body Cu ( {\text{( \mu )g/L}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.1 ± 0.2</td>
<td>8.1 ± 0.2</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td>Agler</td>
<td>117 ± 5.0</td>
<td>123.9 ± 2.5</td>
<td>12.2 ± 0.0</td>
</tr>
<tr>
<td>Sunrise Boys</td>
<td>12.1 ± 0.0</td>
<td>97.1 ± 0.0</td>
<td>12.2 ± 0.0</td>
</tr>
<tr>
<td>Aquacalma-A</td>
<td>175 ± 35</td>
<td>168.8 ± 36.9</td>
<td>12.2 ± 0.0</td>
</tr>
</tbody>
</table>

- *Significant difference from control, \( n = 3 \).
- Data are means ± standard deviation (TWA).
- Data are time weight average values (TWA).
- Not determined; not TWA.
In 2005, the U.S. Fish and Wildlife Service found that live adult apple snails collected in the field at the Sunrise Boys agriculture site had the highest average tissue concentration of Cu (336 mg/kg Cu dw) compared to snail tissue (140 mg/kg Cu dw) from Ten Mile Creek in St. Lucie County, FL (U.S. Fish and Wildlife Service, 2005). The tissue concentration of Cu at the Sunrise Boys site was similar to the average Cu tissue concentration (323.5 mg/kg Cu dw) in this study obtained from live apple snails in the Sunrise Boys treatment after 28-d exposure.

Tissue Cu concentrations found in the present study were related to soil and/or water Cu concentrations. This is in agreement with the results found by Heng et al. (2004) for the freshwater snail (*Turritella* sp.) and Berger and Dallinger (1993) for terrestrial snails (*Arianta arbustorum*). The dependence of tissue Cu concentrations on soil and/or water Cu concentrations suggests that the routes of exposure in the present study were primarily soil (i.e., dermal and ingestion) and water. However, diet is also an important route of Cu accumulation since aquatic plants absorb and adsorb dissolved Cu at high rates and bioconcentration factors for freshwater algae (*Chlorella* sp.) may reach up to 2000 after short-term exposures (14–30 h) (Eisler, 1998). U.S. Fish and Wildlife Service also reported Cu concentrations of 15–113 mg/kg in periphyton in south Florida (U.S. Fish and Wildlife Service, 2005). The literature also indicates that other snail species accumulate Cu from their diet (Laskowski and Hopkin, 1996; Gomot and Pihan, 1997; Dallinger and Wieser, 1984). Therefore, dietary exposure may be an important route for apple snails because aquatic plants, including periphyton, are the main food sources for apple snails.

Results of the 28-d chronic toxicity study with *P. paludosa* are shown in Table 3. Water concentrations of Cu in the Agler, Sunrise Boys, and Aquacalma-A treatments were lower in the toxicity study than in the desorption study because the toxicity study was conducted under flow-through conditions. Snail survival was significantly lower (*p < 0.05*) in the Sunrise Boys and Aquacalma-A treatments compared to the control and Agler treatments, which were both at 100% survival. Mortalities occurred between 9 and 16 d at which time the partitioning of Cu between soil and water reached equilibrium. This was not surprising since the 96-h, 7-d, and 14-d time weighted average water concentrations in Sunrise Boys and Aquacalma-A treatments were similar to the 96-h LC$_{50}$ concentrations (24–27 μg/L) reported in the literature for apple snails (Winger et al., 1984). Furthermore, soil concentrations of Cu on day 14 were up to 126.1 and 184 mg/kg in Sunrise Boys and Aquacalma-A treatments, respectively.

The mean initial total length of apple snails was 4.16 mm (day 0). There was an average 28-d growth rate in apple snails of 2.40 mm/week in the control and 2.34 mm/week in Agler soil treatments with mean final lengths of 13.68 mm (control) and 13.52 mm (Agler). The latter growth rates and length measurements were consistent with those reported by other investigators for *P. paludosa* (P. Darby, personal communication, University of West Florida). Mean shell length, mean aperture length, mean shell width, and mean dry weight of *P. paludosa* exposed to soil and overlying water from the Agler site were also similar to snails exposed to the control soil. A finding of no adverse growth and survival effects in juvenile apple snails exposed to the flooded control and Agler soil treatments is relevant and remarkable since the Cu exposures in water in these two treatments cover the existing range of surface water exposure concentrations (>90%) in south Florida freshwater systems, including the Everglades. However, all growth endpoints of surviving *P. paludosa* exposed to soils and overlying water from the Sunrise Boys and Aquacalma-A treatments were significantly lower than those of control and Agler treatments.

There was no significant difference between survival of *P. paludosa* exposed to overlying and pore waters (OW and PW) although the Cu concentrations in pore water were 5- to 28-fold higher than the Cu concentrations in overlying water (e.g., Agler, OW: 15 μg/L, PW: 77 μg/L; Sunrise Boys, OW: 19 μg/L, PW: 532 μg/L) (Fig. 3a). However, water hardness, alkalinity, and DOC of pore water were higher than those of overlying water (Fig. 3b and c). This may explain the lack of toxicity since an increase in any one of these variables typically decreases the bioavailability of Cu (Hoang et al., 2004; Meyer et al., 1999; Sciera et al., 2004). The high Cu concentrations in pore waters found in this study indicate potential exposure to benthic species.

### 4. Conclusions and implications

Desorption of Cu from agricultural field soils to water does occur after flooding and it is dependent on initial Cu concentrations in soil and the physical and chemical soil characteristics. When flooded, agricultural land will produce Cu exposures in surface water that will likely lead to adverse effects on the survival and growth of the Florida apple snail and other aquatic organisms. The high tissue Cu concentrations found in the present

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival (%)</th>
<th>Total length (mm)</th>
<th>Aperture length (mm)</th>
<th>Shell width (mm)</th>
<th>Dry weight (no shell) (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>13.68 ± 0.32</td>
<td>11.47 ± 0.39</td>
<td>11.98 ± 0.26</td>
<td>0.08 ± 0.003</td>
</tr>
<tr>
<td>Agler</td>
<td>100</td>
<td>13.52 ± 0.52</td>
<td>11.36 ± 0.07</td>
<td>11.81 ± 0.29</td>
<td>0.07 ± 0.007</td>
</tr>
<tr>
<td>SRB*</td>
<td>40 ± 26*</td>
<td>9.72 ± 2.45*</td>
<td>8.40 ± 2.06*</td>
<td>8.83 ± 2.51*</td>
<td>0.03 ± 0.019*</td>
</tr>
<tr>
<td>AQUA-A**</td>
<td>53 ± 35*</td>
<td>9.11 ± 1.29*</td>
<td>8.32 ± 1.29*</td>
<td>4.08 ± 0.87*</td>
<td>0.03 ± 0.010*</td>
</tr>
</tbody>
</table>

*aSignificant from Control and AGLR.

Data are mean ± standard deviation, *n* = 3. Exposure time was 28 days.

b SRB: Sunrise Boys; AQUA-A: Aquacalma-A.
study also reveal potential risk to apple snail predators. The potential risk of Cu to the apple snail may lead to changes in the population dynamics of this species with eventual consequences in the food chain, especially for species that rely on this snail as a prime food source, like the Florida snail kite.

Improving habitat for snail kites and their prey, like the Florida apple snail, is consistent with the CERP goals to improve the functional quality of native habitats and to improve native plant and animal species abundance and diversity (U.S. Army Corps of Engineers, 1999). However, the surface water,
pore water, and soil quality of these managed agricultural soil—water systems, as a result of Cu exposure, may impact population recruitment of apple snails and resulting snail kite habitat suitability. This is therefore not consistent with the goals of the CERP.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.envpol.2007.09.024.

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