Chemosphere 76 (2009) 407–414

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Exposure routes of copper: Short term effects on survival, weight, and uptake in Florida apple snails (*Pomacea paludosa*)

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ARTICLE INFO

Article history:
Received 29 November 2008
Received in revised form 17 February 2009
Accepted 2 March 2009
Available online 11 April 2009

Keywords:
Soil exposure
Water exposure
Copper bioavailability
Copper uptake
Pomacea paludosa
Comprehensive Everglades Restoration Plan (CERP)

ABSTRACT

The uptake and effects (survival, weight) of copper (Cu) on Florida apple snails (*Pomacea paludosa*) via exposures to copper-enriched agricultural soil–water and water-only treatments were investigated. Soils were collected from citrus sites in south Florida and flooded with laboratory freshwater for 14 d. Neonate apple snails (⩽96-h-old) were then exposed to either Cu from a soil-overlying water (i.e., flooded agricultural soils) treatment or overlying water-only (i.e., equilibrated overlying water produced from 14 d flooding of agriculture soils) treatment for 14 d under standard laboratory conditions. Survival, weight (dry, wet), and whole body Cu uptake were measured. Copper exposure via soil–water exposures resulted in higher mortality and whole body Cu uptake than water-only exposures, indicating Cu uptake from soils. However, snail wet and dry weights were higher in soil–water treatments than in water-only treatments. Micronutrients from soils may be consumed by snails increasing weights. Survival, apple snail dry weight, and whole body Cu concentrations were significantly correlated with soil and water Cu concentrations in soil–water treatments. Survival was significantly correlated with the concentration of $Cu(CO_3)_2^{2-}$ in water-only treatments. This suggests that $Cu(CO_3)_2^{2-}$ is toxic to apple snails. Whole body Cu concentrations were higher in surviving snails than dead snails, suggesting that apple snails have the ability to detoxify accumulated Cu (e.g., through metallothionein induction, granules).

1. Introduction

Copper (Cu) has been used in Florida citrus agriculture for many decades. This has resulted in elevated Cu concentrations in South Florida ecosystems (Leslie, 1990; Alva et al., 1995; USDA, 2006). According to soil Cu measurements conducted by the South Florida Water Management District (SFWMD) from 2001 to 2006, Cu concentrations in soils were as high as 1200 mg kg^{-1} in St. Lucie County, 924 mg kg^{-1} in Dade County, 406 mg kg^{-1} in Hendry County, 180 mg kg^{-1} in Martin County, 110 mg kg^{-1} in Palm Beach County, 72 mg kg^{-1} in Highlands County and 47 mg kg^{-1} in Broward County (SFWMD, 2001–2006). The implementation of the Comprehensive Everglades Restoration Plan (CERP) under the Water Resources Development Act of 2000 requires acquisition of thousands of acres of land in these counties for maintaining hydrologic buffer areas and for the creation of storm water treatment areas, water storage reservoirs and wetlands (Everglades National Park, 2001).

As a key species in the Everglades ecosystems, Florida apple snails (*Pomacea paludosa*) play an important role in the ecosystem since it is the main 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). Under the CERP, flooding of these Cu-contaminated soils will result in Cu exposures to Florida apple snails from soil/sediment and overlying water. A recent study conducted by Hoang et al. (2008a) found that dissolved Cu concentrations in over-lying water (i.e., water on top of soils) collected from flooded agriculture soils originating from the above counties were as high as 300 μg L^{-1}, depending on initial soil Cu concentrations. Hoang et al. (2008a,b) also demonstrated that Florida apple snails can be exposed to Cu in flooded agriculture soils collected from Martin and Palm Beach (FL) counties. Finally, Frakes et al. (2008) reported that tissue Cu concentrations in field-collected Florida apple snails were correlated with sediment (i.e., flooded agriculture soils in Martin County, FL) Cu concentrations. However, there is no investigation on the effects of Cu from exposure to flooded field-collected agriculture soils versus Cu effects from exposure to overlying water-only following flooding of these soils.

Because copper can enter freshwater snails through various routes including water and soils (Laskowski and Hopkin, 1996; Gomot and Pihan, 1997; Gomot-de Vaufleury and Pihan, 2002; Heng et al., 2004; Notten et al., 2005), the present study characterizes the effects of Cu from exposure to a soil-overlying water system (i.e., flooded field-collected citrus agriculture soils) and from

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doi:10.1016/j.chemosphere.2009.03.004

exposure to overlying water-only (i.e., equilibrated overlying water produced from 14 d flooding of agriculture soils) on the survival, weight (wet and dry), and accumulation of Cu in Florida apple snails (*Pomacea paludosa*).

2. Materials and methods

2.1. Pomacea paludosa culture

Pomacea paludosa was cultured under flow-through conditions outdoors at the Ecotoxicology and Risk Assessment Laboratory on the Biscayne Bay Campus of Florida International University, North Miami, FL, USA. Water for culturing and toxicity studies was carbon-filtered and UV-sterilized freshwater. Water hardness, alkalinity, and pH of the fresh water were 60, 45 mg L^{-1} as $CaCO_3$, and 7.8, respectively. Snails were fed romaine lettuce daily with a background Cu concentration of 2.4 mg kg^{-1} dw. Temperature of freshwater ranged from 25 to 30 °C. Snail eggs were collected and hatched indoors at 25 ± 1 °C with a 16 h light: 8 h dark photoperiod. Neonate snails were incubated in laboratory freshwater for a 4-d period prior to toxicity testing.

2.2. Soil–water exposure study

Soils were collected from agriculture sites ($n = 9$), including citrus, in four counties (Dade ($n = 1$), Palm Beach ($n = 1$), Martin ($n = 2$), St. Lucie ($n = 5$)) of south Florida. At each of the nine sites, soils were collected from three locations, 2 m apart between each location. At each location, 30 L of soil were collected at 6 cm depth from the surface and transported to the laboratory. In the laboratory, soils collected from the three locations at each site were composited before conducting the toxicity studies. Physical and chemical characteristics of these soils were published in our earlier study (Hoang et al., 2008a). Soil Cu concentrations were published in Hoang et al. (2008a) and are also presented in Table 1.

To determine the effect of Cu exposures via soil–water and water-only routes to Florida apple snails, 6 L of field-collected soils including a control soil (Table 1) were randomly distributed to 18-L glass tanks. Tanks were subsequently flooded with 12-L of carbon-filtered, UV-sterilized laboratory freshwater to create a volume ratio of 2 (water):1 (soil). Based on our earlier study, Cu desorption from soil to water reached equilibrium after 14 d of flooding (Hoang et al., 2008a). Tanks were held under static conditions in the laboratory for 14 d with a temperature of 25 ± 1 °C and 16 h light: 8 h dark photoperiod. On day 14 of flooding, 10 neonate apple snails (⩽96-h-old) were placed in each of three replicate tanks and held for 14 d toxicity tests under static conditions at a temperature of 25 ± 1 °C and 16 h light:8 h dark photoperiod. At test initiation, snails were also collected for background tissue Cu analysis and determination of wet weight and dry weight. During the 14 d of exposure, snails were fed 4 g of fresh romaine lettuce with a background Cu concentration of 2.4 mg kg^{-1} dw, every 2 d. Overlying water (i.e., water on top of soils) was aerated during exposure to maintain sufficient dissolved oxygen concentration for snails.

Snail survival, water temperature, and dissolved oxygen concentration (DO) were measured daily. The average measured DO and temperature of the overlying water of the ten soils ranged from 7.6 ± 0.5 to 8.1 ± 0.3 mg L^{-1} and 24.4 ± 0.2 to 25.3 ± 0.1 °C, respectively. Water Cu, cations, anions, ammonia, and dissolved organic carbon (DOC) concentrations, hardness, alkalinity, and pH were measured three times per week during 14 d of exposure. Total ammonia concentrations of the overlying water ranged from 0.1 to 0.9 mg L^{-1}. Water samples for Cu and DOC analysis were filtered through 0.45-μm Gelman Nylon Mesh® before analysis. Dead snails were removed daily. Dead snails and surviving snails at test termination (14-d exposure) were collected and rinsed three times with 0.01 M ethylenediamine tetraacetic acetate (EDTA) solution, followed by deionized water (DI) to wash bound Cu and soils adsorbed on snail shell surfaces and stored at −20 °C for tissue Cu analysis.

To measure weights, snails (including shell) from each replicate were wrapped with Kimwipe paper to adsorb condensed moisture on the snail shell surface and then weighed on a Mettler Toledo Scale (Samela Inc., Northbrook, IL, USA) to determine the wet weight. Weighed snails were dried at 80 °C for 24 h in a Muffle Furnace Oven (Fisher Scientific, Fairlawn, NJ, USA), cooled down to laboratory temperature (25 °C) in a Nalgene™ Desiccator (Nalgene Company, Rochester, YN, USA), and reweighed to determine the dry weight of snails. To determine whole body Cu uptake, dry snails were digested with HNO_3 using method 3050B (US EPA, 1996) for Cu analysis.

Copper and other cations (Ca^{2+}, Mg^{2+}, K^+, and Na^+) concentrations were analyzed by using inductively coupled plasma atomic emission spectrometry (ICP-AES) (Perkin–Elmer Corporation, Toronto, Canada). Anion (Cl^-, SO_4^{2-}, NO_3^-, PO_4^{3-}) concentrations were analyzed by ion chromatography (IC) (Sunnyvale, CA, USA). Hardness and alkalinity were measured by titrating with 0.01 M EDTA solution and 0.02 N H_2SO_4, respectively. DO and pH were measured

Table 1
Soil copper and water chemistry of the tests and effect of copper on survival of *Pomacea paludosa*.[a]

Exposure Route	Soil	Soil–Cu (mg kg^{-1})	Water–Cu (μg L^{-1})	Hardness (mg L^{-1} $CaCO_3$)	Alkalinity (mg L^{-1} $CaCO_3$)	pH	DOC (mg L^{-1})	Survival (%)
Soil and water	Control	8 ± 2	6 ± 3	120 ± 11	118 ± 14	8.1 ± 0.3	29	100 ± 0
	Agler	90 ± 5	62 ± 10	136 ± 6	114 ± 8	8.1 ± 0.2	19	80 ± 0
	Aquacalma	234 ± 28	276 ± 119	115 ± 4	94 ± 31	8.0 ± 0.2	28	5 ± 7*
	Arcco	93 ± 2	56 ± 15	106 ± 3	90 ± 3	8.3 ± 0.9	31	85 ± 21
	Birdsall	210 ± 5	231 ± 33	86 ± 8	64 ± 6	7.9 ± 0.3	38	0 ± 0*
	Equus	5 ± 2	10 ± 1	164 ± 23	100 ± 0	7.9 ± 0.3	28	100 ± 0
	L31N Buffer	185 ± 12	56 ± 5	164 ± 11	130 ± 25	8.2 ± 0.2	22	100 ± 0
	McArthur	223 ± 15	129 ± 28	140 ± 11	124 ± 17	8.0 ± 0.4	22	0 ± 0*
	Sunrise Boys-A	31 ± 5	29 ± 6	106 ± 3	90 ± 3	8.1 ± 0.2	18	100 ± 0
	Sunrise Boys-B	175 ± 45	225 ± 97	140 ± 57	108 ± 62	8.1 ± 0.5	20	50 ± 0*
Water only	Control	8 ± 2	6 ± 3	115 ± 8	100 ± 26	7.5 ± 0.4	27	100 ± 0
	Agler	90 ± 5	62 ± 10	125 ± 19	109 ± 15	8.1 ± 0.2	19	100 ± 0
	Aquacalma	234 ± 28	175 ± 28	90 ± 15	78 ± 12	7.9 ± 0.5	28	87 ± 6
	Arcco	93 ± 2	56 ± 15	95 ± 16	76 ± 17	7.9 ± 0.2	21	100 ± 0
	Birdsall	210 ± 5	122 ± 28	72 ± 37	66 ± 8	8.1 ± 0.2	30	100 ± 0
	McArthur	223 ± 15	136 ± 7	178 ± 23	154 ± 16	8.4 ± 0.1	20	23 ± 40*
	Sunrise Boys-B	175 ± 45	132 ± 21	129 ± 11	119 ± 19	8.3 ± 0.2	16	100 ± 0

[a] Exposure duration was 14 d.
* Significant difference from control, Sunrise Boys-A: north field; Sunrise Boys-B: south field.

with a YSI Meter (YSI Inc., Yellow Springs, OH, USA) and Accumet Meter (Fisher Scientific, Fairlawn, NJ, USA), respectively. DOC concentrations were measured with a Shimadzu TOC-5000 (Shimadzu Scientific Instruments, Columbia, MD, USA).

2.3. Water-only exposure study

Soils collected from six of the nine agricultural sites and the control soil were flooded with laboratory freshwater for 14 d under the same conditions as the conditions described in the soil–water exposure study. On day 14 of flooding, overlying water from two replicate tanks of each soil was collected, mixed, and aerated for 24 h before conducting 14 d toxicity tests. Based on our evaluation of the effect of Cu in the soil–water exposure study, the effect of Cu from the water-only exposure study was determined using overlying water of control soils and soils collected from Agler, Aquacalma, Arcco, Birdsall, McArthur, and Sunrise Boys-B sites.

Toxicity tests (14 d) were also used to determine the effect of desorbed Cu in the selected overlying waters to Florida apple snails. Ten neonate apple snails were exposed to 700 mL of overlying water in 1-L polypropylene beakers under the same temperature and light conditions as described in the soil–water exposure study. Three replicates for each treatment were used. Water in each test beaker was aerated continuously during 14 d of exposure. Test water was renewed every 2 d. Concentrations of Cu, cations, anions, and DOC, hardness, alkalinity, pH, and DOC in test water were measured at test initiation, water renewal, and at test termination. Results of cation and anion analysis were used to determine Cu speciation in the overlying water with the Visual Minteq Model (http://www.lwr.kth.se/English/OurSoftware/vminteq). Snail survival, DO, and temperature were measured daily. The average measured DO and temperature of test water ranged from 7.8 ± 0.4 to 8.3 ± 0.9 mg L^{-1} and 25.1 ± 0.1 to 25.3 ± 0.2 °C, respectively. Snail weight, whole body Cu concentrations, and water chemistry of test water were measured as described in the soil–water exposure study.

2.4. Data analysis

Multiple treatment comparisons with controls were conducted using Dunnett-test (SAS Institute Inc., Cary, NC, USA). Data were subjected to determinations of normality and heterogeneity and were log-transformed if assumptions were not met. As such, data for soil–water exposures were log-transformed to meet these assumptions. The linear contrast procedure using SAS was used for statistical analysis of data without standard deviation [e.g., Aquacalma (Figs. 2a and 3a), McArthur (Fig. 3b)]. Pearson correlations were conducted using SPSS (SPSS Inc., Chicago, IL, USA). All figures were constructed with Microsoft Excel 2003 (Microsoft, Redmond, WA, USA). An effect with a p value <0.05 was considered significant.

3. Results

Results of daily mortality are presented in Fig. 1. The highest mortality occurred in treatments (i.e., McArthur, Aquacalma, Birdsall) with the highest soil and overlying water Cu concentrations. Mortality also occurred earlier in soil–water treatments than in water-only treatments. For both soil–water and water-only treatments, mortality increased with exposure time. Cumulative mortality to day 14, as a result of soil–water treatments (Fig. 1a) (i.e., Aquacalma, Birdsall, McArthur, and Sunrise Boys-B) was significantly higher compared to control mortality (Table 1). Water-only treatment from the McArthur site resulted in significantly higher mortality than control mortality (Fig. 1b).

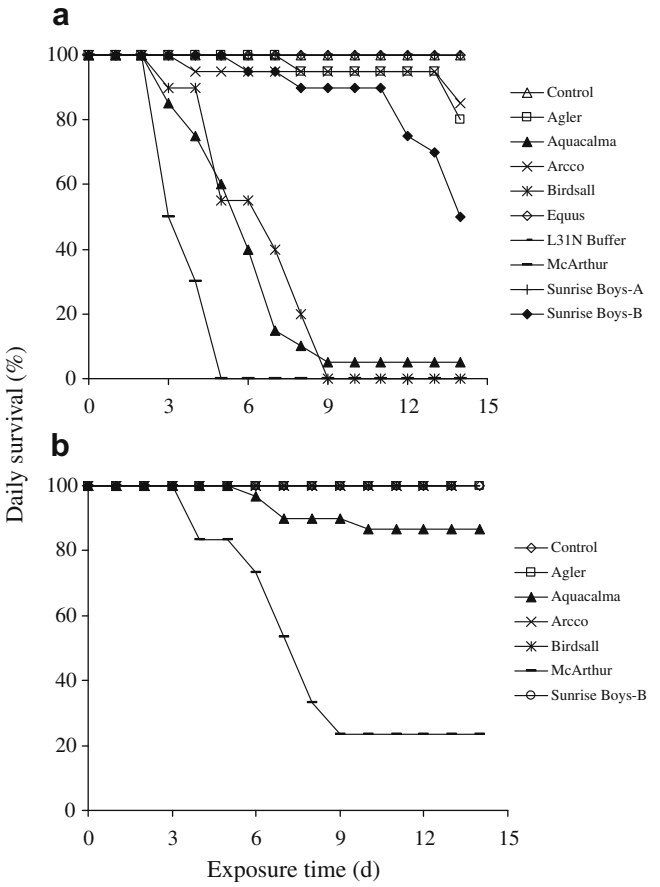

Fig. 1. Effect of copper on survival of *Pomacea paludosa* (a: soil and water exposures; b: water-only exposure).

Copper concentrations in the soils and overlying water ranged from 5 to 234 mg kg^{-1} dw and 6 to 276 μg L^{-1}, respectively (Table 1). Hardness, alkalinity, DOC, and pH of the overlying water ranged from 72 to 178 mg L^{-1} as $CaCO_3$, 64 to 154 mg L^{-1} as $CaCO_3$, 16 to 38 mg L^{-1}, and 7.5 to 8.4, respectively (Table 1).

Results of whole body concentrations Cu in apple snails are illustrated in Fig. 2. In general, Cu tissue concentrations in the snails from exposure treatments were significantly higher than the Cu concentrations in the snails from control treatment. Copper concentrations were significantly higher in surviving snails than in dead snails from the same treatment. Although Cu concentrations in the overlying water of soil–water and water-only treatments were not significantly different, soil–water treatments resulted in higher whole body Cu concentrations than in water-only treatments.

The effect of Cu on apple snail weight is illustrated in Fig. 3. The average wet and dry weights of surviving snails exposed to Cu via soil–water treatments ranged from 44 ± 0 to 190 ± 20 mg $snail^{-1}$ and from 10 ± 0 to 55 ± 4 mg $snail^{-1}$, respectively (Fig. 3a). Copper from soil–water Aquacalma and Sunrise Boys-B treatments significantly reduced the weight of apple snails compared to the control treatment (Fig. 3a).

The average wet and dry weights of surviving snails exposed to Cu via water-only treatments ranged from 36 ± 0 to 70 ± 6 mg $snail^{-1}$ and 10 ± 0 to 19 ± 1 mg $snail^{-1}$, respectively (Fig. 3b). Copper from water-only treatments of Aquacalma and McArthur treatments significantly reduced the weight of surviving apple snails compared to the control treatment (Fig. 3b). For both soil–water and water-only treatments, the average wet weight of surviving snails was about 3.7-fold (on average) higher than their average dry weight. The dry weight of surviving apple snails ex-

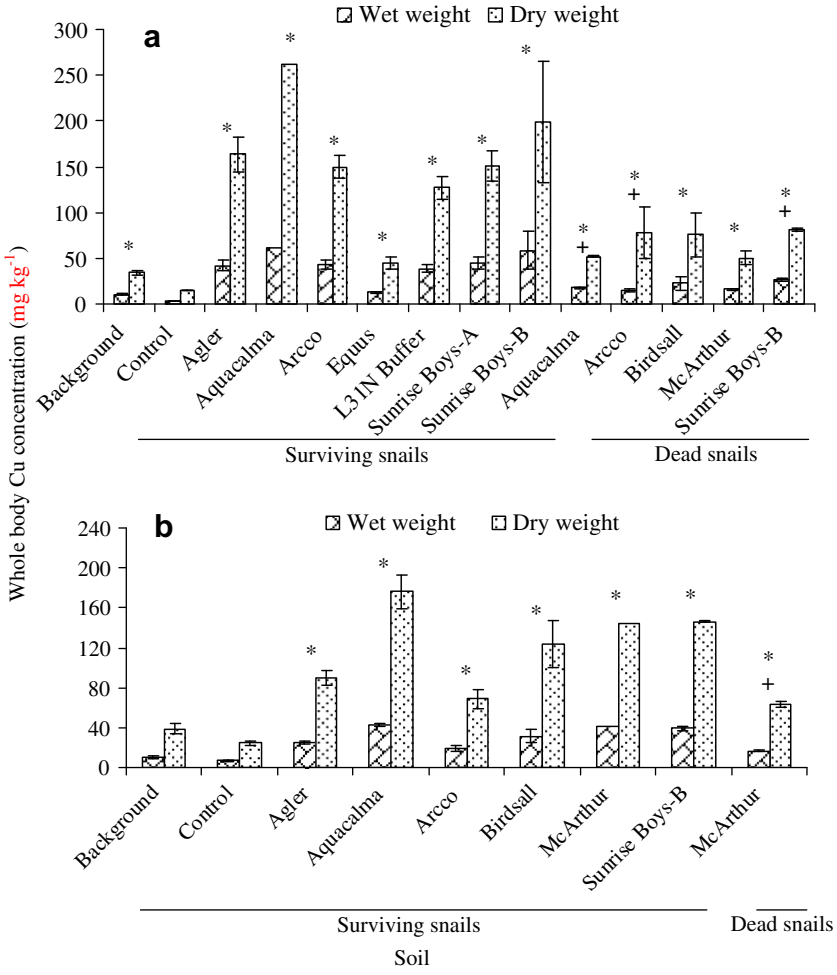

Fig. 2. Copper uptake by *Pomacea paludosa* (a: uptake from soil–water; b: uptake from water-only; error bars represent standard deviations; No standard deviation indicates 100% mortality in other replicates; asterisk represents significant difference from control in both wet and dry weight; cross symbols represent significant difference between dead and surviving snails from the same treatment in both dry and wet weight; Sunrise Boys-A: north field; Sunrise Boys-B: south field).

posed to soil–water treatments was about 2.7-fold higher than the dry weight of apple snails exposed to water-only.

Results of Cu speciation analysis for water-only treatments were used to determine the correlations between biological endpoints and Cu species. For soil–water treatments, Cu in the overlying water did not totally represent Cu exposure. Therefore, Cu speciation was not determined for soil–water treatments. Results of the correlation analysis for the soil–water exposure study and the water-only exposure study are shown in Tables 2 and 3, respectively. When snails are exposed to Cu via soil–water exposure, survival, dry weight, and tissue Cu concentrations were significantly correlated with total Cu concentrations in the soil and overlying water (Table 2). However, water-only treatments resulted in no significant correlation between total water Cu concentration and survival and tissue Cu concentrations (Table 3). Survival was significantly correlated with $Cu(CO_3)_2^{2-}$, suggesting that $Cu(CO_3)_2^{2-}$ would be bioavailable to apple snails. Survival was also significantly correlated with the interactions of free Cu and $CuCO_3$ (as Cu–$CuCO_3$) and $Cu(CO_3)_2^{2-}$ (as Cu–$Cu(CO_3)_2^{2-}$).

4. Discussion

4.1. Dependence of copper uptake on routes of exposure

The present study found that whole body Cu concentration of juvenile apple snails was significantly correlated with soil and water Cu concentrations. Apple snails accumulated more Cu from soil–water than from water-only treatments. This suggests that apple snails accumulate Cu from soil (-sediment)/water systems. This is supported by Hoang et al. (2008b) who reported a similar result for adult apple snails. In addition, Frakes et al. (2008) reported that the tissue Cu concentrations of apple snails from the same agricultural areas where we collected soil in the present study (Sunrise Boys and Arcco) were correlated with soils Cu concentrations.

Gomot-de Vaufleury and Pihan (2002) demonstrated that Cr can enter snails via dermal contact. Coeurdassier et al. (2002) also reported that snails significantly accumulated Cd via soil-dermal contact. During exposure in the present study, movement of the snails occurred at the soil–water interface and the snails occasionally burrowed under the soil surface. Most gastropod species ingest soil and/or sediment (Thorp and Covich, 2001). Hence, the possibility of Cu uptake by apple snails via soil ingestion and/or dermal contact would provide an explanation for the difference between whole body Cu concentrations in the snails exposed to Cu via soil–water versus water-only treatments. Heng et al. (2004) also demonstrated that sediment ingestion is the main route of Cu and Zn uptake by freshwater snails (*Turritella* sp.).

The tissue Cu concentrations of the snails collected from Sunrise Boys (205 mg kg^{-1} dw) and Arcco (299 mg kg^{-1} dw) areas reported by Frakes et al. (2008) were higher than the whole body Cu concentrations found in the present study (146–199 and 149 mg kg^{-1} dw for Sunrise Boys-A/B and Arcco, respectively). Snails in the field

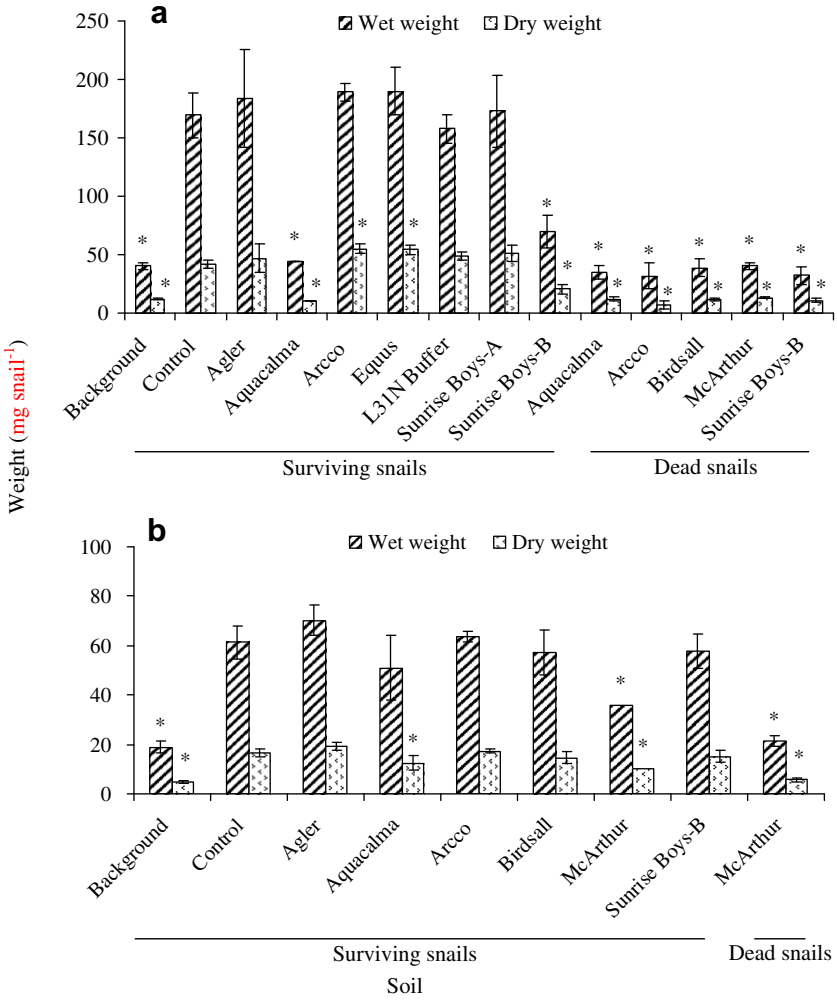

Fig. 3. Effect of copper on weight of *Pomacea paludosa* (a: exposed to Cu from soils and flooded water; b: exposed to Cu from flooded water-only; error bars represent standard deviations; No standard deviation indicates 100% mortality in other replicates; asterisk represents significant difference from control; Sunrise Boys-A: north field; Sunrise Boys-B: south field).

Table 2
Pearson correlations between exposure media copper and biological endpoints for soil and water exposure study.[a]

	Soil–Cu	Water–Cu	Dry weight	Tissue–Cu	Survival
Soil–Cu	1				
Water–Cu	0.827 (0.003)	1			
Dry weight	−0.708 (0.049)	−0.932 (0.001)	1		
Tissue–Cu	0.811 (0.015)	0.839 (0.009)	−0.646 (0.084)	1	
Survival	−0.824 (0.003)	−0.858 (0.001)	0.911 (0.002)	−0.813 (0.014)	1

[a] Tissue–Cu data were in dry weight of surviving snails. Numbers in parentheses are significant values.

accumulate Cu from various routes (e.g., water, sediment, and diet) (Notten et al., 2005). The snails in the field study conducted by Frakes et al. (2008) were adult snails, while juvenile snails were used in the present study. Long-term accumulation in the field results in higher body Cu burden compared to the laboratory studies presented here. Frakes et al. (2008) also demonstrated the correlation between snail tissue Cu concentration and Cu concentration in vascular plants, one of the main food sources in the diet of apple snails in the environment.

In the present study, we analyzed Cu in whole body of snails, that included shells, and the shell Cu concentration was less than 5 mg kg^{-1} dw (Hoang et al., 2008b) and accounted for less than 5% of the soft tissue Cu concentration (Hoang et al., 2008b; Laskowski and Hopkin, 1996). The differences of the analysis methods, snail age, and field versus laboratory condition exposures explains the difference of the snail tissue Cu concentrations found by Frakes et al. (2008) and in the present study.

4.2. Copper uptake related to survival, weight, and bioavailability

Toxicant accumulation can cause biological and physiological effects to animals. The present study found that when exposing to Cu from soil–water treatments, survival of apple snails was negatively correlated with soil, water, and whole body Cu concentrations (Table 2). This indicates that snail survival decreased with increasing soil, water, and whole body Cu concentrations. These results are in agreement with the literature (De Oliveira-Filho et al., 2004; Hoang et al., 2008a). However, survival of the snails exposed

Table 3
Pearson correlations between copper species and biological endpoints for water exposure study.[a]

	Total-Cu	Cu^{2+}	$CuCO_3$	$Cu(CO_3)_2^{2-}$	Cu–$CuCO_3$	Cu–$Cu(CO_3)_2^{2-}$	$CuHCO_3^+$	$CuOH^+$	$Cu(OH)_2$	Cu-DOC	Dry weight	Tissue–Cu	Survival
Dry weight	−0.762 (0.047)	−0.087 (0.852)	−0.903 (0.005)	−0.776 (0.040)	−0.756 (0.049)	−0.716 (0.070)	−0.610 (0.146)	−0.779 (0.039)	−0.787 (0.036)	−0.727 (0.064)	1		
Tissue–Cu	0.992 (0.000)	0.079 (0.866)	0.835 (0.019)	0.417 (0.352)	0.456 (0.304)	0.322 (0.481)	0.708 (0.075)	0.898 (0.006)	0.804 (0.029)	0.970 (0.000)	−0.720 (0.068)	1	
Survival	−0.385 (0.394)	−0.151 (0.747)	−0.714 (0.072)	−0.989 (0.000)	−0.934 (0.002)	−0.979 (0.000)	−0.230 (0.619)	−0.405 (0.368)	−0.606 (0.149)	−0.374 (0.408)	0.792 (0.034)	−0.382 (0.398)	1

[a] Tissue–Cu data were in dry weight of surviving snails. Cu speciation was predicted by using Visual Minteq Model. Numbers in parentheses are significant values.

to Cu via water-only treatments was not significantly correlated with total water Cu concentration and whole body Cu concentration. This indicates that total Cu in the overlying water was not toxic. It has been assumed that free Cu (Cu^{2+}) and $CuOH^+$ are the two toxic Cu forms to organisms (Pagenkopf et al., 1974; Pagenkopf, 1983; Santore et al., 2001). However, the measured concentration of Cu^{2+} in the overlying water from flooding of contaminated soils was equal to or less than the free Cu concentration in the overlying water from flooding of control soil (0.036 μg L^{-1}). This concentration of free Cu had no effect on apple snails (Rogevich et al., 2008). In addition, survival was not significantly correlated with either free Cu or $CuOH^+$ (Table 3). Concentrations of other toxic metals (e.g., As, Zn, and Cd) and pesticides in these soils were well below detection limits and/or water quality criteria (Hoang et al., 2008a). Therefore, mortality would be produced by other Cu species rather than free Cu and $CuOH^+$. Among the Cu species, survival was significantly correlated with $Cu(CO_3)_2^{2-}$ and $CuCO_3$. The predicted concentrations of $Cu(CO_3)_2^{2-}$ and $CuCO_3$ were $\geqslant$1 order magnitude higher than the measured concentration of free Cu. These results suggest that $Cu(CO_3)_2^{2-}$ and $CuCO_3$ would be bioavailable to apple snails. This suggestion is supported by Rogevich et al. (2008) who demonstrated the potential toxicity of $CuCO_3$ to apple snails. De Schamphelaere and Janssen (2002) also suggested the bioavailability of $CuCO_3$ to invertebrate species. Interestingly, free Cu alone was not significantly correlated with survival. However, when combining free Cu with either $CuCO_3$ or $Cu(CO_3)_2^{2-}$ as a potential interactive species, survival was significantly correlated with these interactive Cu species. This suggests that free Cu may contribute to the mortality of apple snails.

The potential toxicity of Cu carbonate to apple snails may be explained by the carbonate content in the snails. The carbonate requirement for snails is more than for fish because snails require it for shell development. We found that the biomass of apple snail shells accounted for up to 70% (in dw, unpublished data) of total body biomass. In addition, the carbonate content accounted for 38% of total shell biomass (unpublished data). Copper may enter snails as Cu carbonate. After entering snails, Cu carbonate may be disassociated through biological and chemical reactions. Carbonate would be available for shell development and Cu would be accumulated in soft tissue. This is supported by Laskowski and Hopkin (1996) and Hoang et al. (2008b) who found that Cu in snail shells was less than 5% of total body Cu. In addition, we found that most of the carbonate content in apple snails was located in the shells. Soft tissue of apple snails contained <1% of total carbonate in the snails (unpublished data).

With soil–water treatments, although survival was correlated with tissue Cu concentrations of surviving snails, the tissue Cu concentrations were lower in dead snails than surviving snails (Fig. 2). This indicates that the toxic effect was not produced by total accumulated Cu in the snails. Apple snails might have a detoxification mechanism. It has been demonstrated that snails can develop granules and induce metallothionein to sequester metals and transform the accumulated metals to unavailable metal forms (Howard et al., 1981; Brown, 1982; George et al., 1982; Recio et al., 1988; Roesijadi, 1992; Dallinger and Berger, 1993, 1997; Simkiss and Taylor, 1994; Gibbs et al., 1998; Leung et al., 2003). Recently, Desouky (2006) demonstrated the development of granules containing P and S in the digestive gland of pond snails exposed to Al, Cd, and Zn. Our earlier work suggested the development of P and S granules in Florida apple snails exposed to Cu (Hoang and Rand, submitted for publication). This would explain the higher Cu concentrations in surviving versus dead snails.

With water-only treatments, survival was not significantly correlated with tissue Cu concentrations of surviving snails (Table 3). Similar to soil–water exposure, the toxic effect was not produced

by total accumulated Cu in the snails. The tissue Cu concentration was not significantly correlated with the concentrations of Cu species that were significantly correlated with survival $Cu(CO_3)_2^{2-}$, Cu–$CuCO_3$, Cu–$Cu(CO_3)_2^{2-}$. However, the tissue Cu concentration was significantly correlated with the concentrations of Cu species that were not significantly correlated with survival (total Cu, $CuCO_3$, $CuOH^+$, $Cu(OH)_2$, Cu-DOC). This suggests that total Cu, $CuCO_3$, $CuOH^+$, $Cu(OH)_2$, Cu-DOC were bioavailable for snail uptake; however, they did not produce toxic effects. These results convey the message- "Cu bioavailability" should be cautiously interpreted.

For both soil–water and water-only treatments, Florida apple snail's dry weight was negatively correlated with total soil and water Cu concentrations. This indicates the effect of Cu on apple snail weight. Hoang et al. (2008a) also demonstrated the effect of Cu on the growth of Florida apple snails. The results of the effect of Cu on apple snail weight found in the present study is also supported by Real et al. (2003) who reported the effect of Cu on the growth rate of snails (*Stagnicola vulnerata*). Gomot-de Vaufleury and Pihan (2000) and Geffard et al. (2007) demonstrated the effects of metal contaminated sediment (Cu, Zn, Cd, Pb) on metal accumulation and the growth of *Helix aspersa* and Oyster larvae (*Crassostrea gigas*), respectively.

The effect of Cu on apple snail weight may be due to the inhibition of Cu on metabolic rate and uptake of essential minerals. MacInnes and Thurberg (1973) reported that Cu reduced metabolic rate (oxygen consumption rate) of mud snails. De Schamphelaere et al. (2008) also suggested that the growth of snails was affected by Co due to the inhibition of Co on metabolic rate. During exposure in the present study, we observed that the aperture of exposed snails was mostly closed and the movement of exposed snails was less than that of control snails. In addition, Hoang and Rand (submitted for publication) indicated that Cu inhibited K, Na, Mg, and Ca uptake by apple snails. This provides an explanation for the effect of Cu on apple snail weight in the present study because these elements are the major micronutrients for growth.

Tissue Cu concentration was not significantly correlated with dry weight. The accumulated Cu did not appear to affect apple snail dry weight. The detoxification mechanism in snails (e.g., sequestering Cu to granules, metallothionein) may explain this result. Although exposing apple snails to Cu via soil–water treatments resulted in higher mortality and whole body Cu concentrations than exposing apple snails to Cu via water-only treatments, the apple snails grew better in soil–water treatments than in water-only treatments. Minerals from soils likely provide a richer micronutrient environment for snails to grow.

5. Conclusions and implications

The present study demonstrated that Cu from field-collected agricultural soils in south Florida affected survival and weight of Florida apple snails regardless of soil–water and water-only exposure routes. The present study also suggested that Cu carbonate may be toxic to Florida apple snails. Copper bioavailability should also be interpreted cautiously. A Cu species can be bioavailable for uptake; however, it may not produce toxic effects. Exposure to Cu via soil–water exposure resulted in higher whole body Cu concentrations and mortality than did water-only exposure. Whole body Cu concentrations were higher in surviving snails than dead snails. High Cu accumulation in Florida apple snail tissue indicates the potential of Cu transfer from soils to Florida apple snails and higher trophic levels which feed on snails.

Acknowledgement

This is SERC contribution number 417.

References

Alva, A.K., Graham, J.H., Anderson, C.A., 1995. Soil-pH and copper effects on young Hamlin Orange Trees. Soil Sci. Soc. Am. J. 59, 481–487.

Brown, B.E., 1982. The form and function of metal-containing granules in invertebrate tissues. Biol. Rev. 57, 621–667.

Coeurdassier, M., Gomot-de Vaufleury, A., Lovy, C., Badot, P.M., 2002. Is the cadmium uptake from soil important in bioaccumulation and toxic effects for snails. Ecotox. Environ. Safe. 53, 425–431.

Dallinger, R., Berger, B., 1993. Function of metallothionein in terrestrial gastropods. Sci. Total Environ. 141, 607–615.

Dallinger, R., Berger, B., 1997. Metallothionein in snail Cd and Cu metabolism. Nature 388, 237–238.

De Oliveira-Filho, E.C., Lopes, R.M., Paumgartten, F.J.R., 2004. Comparative study on the susceptibility of freshwater species to copper-based pesticides. Chemosphere 56, 369–374.

De Schamphelaere, K.A.C., Janssen, C.R., 2002. A biotic ligand model predicting acute copper toxicity for *Daphnia magna*: the effects of calcium, magnesium, sodium, potassium, and pH. Environ. Sci. Technol. 36, 48–54.

De Schamphelaere, K.A.C., Koene, J.M., Heijerick, D.G., Janssen, C.R., 2008. Reduction of growth and haemolymph Ca levels in the freshwater snail *Lymnaea stagnalis* chronically exposed to cobalt. Ecotox. Environ. Safe. 71, 65–70.

Desouky, M.M.A., 2006. Tissue distribution and subcellular localization of trace metals in pond snail *Lymnacea stagnalis* with special reference to the role of lysosomal granules in metal sequestration. Aquat. Toxicol. 77, 143–152.

Everglades National Park, 2001. Everglades National Park Strategic Plan 2001–2005, National Parks Service, US Department of the Interior, Homestead, FL, pp. 1–39.

Frakes, R.A., Bargar, T.A., Boughner, E.A., 2008. Sediment copper bioavailability to freshwater snails in South Florida: risk implications for the Everglade snail kite (*Rostrhamus sociabilis plumbeus*). Ecotoxicology 17, 598–604.

Geffard, A., Geffard, O., Amiard, J.C., His, E., Amiard-Triquet, C., 2007. Bioaccumulation of metals in sediment elutriates and their effects on growth, condition index, and metallothionein contents in oyster larvae. Arch. Environ. Contam. Toxicol. 53, 57–65.

George, S.G., Coombs, T.L., Pirie, B.J.S., 1982. Characterization of metal-containing granules from the kidney of the common mussel, *Mytilus edulis*. Biochem. Biophys. Acta 16, 61–67.

Gibbs, P.E., Nott, J.A., Nicolaidou, A., Bebianno, M.J., 1998. The composition of phosphate granules in the digestive glands of marine prosobranch gastropods: variation in relation to taxonomy. J. Moll. Stud. 64, 423–433.

Gomot, A., Pihan, F., 1997. Comparison of the bioaccumulation capacities of copper and zinc in two snail subspecies (*Helix*). Ecotox. Environ. Safe. 38, 85–94.

Gomot-de Vaufleury, A., Pihan, F., 2000. Growing snails used as sentinels to evaluate terrestrial environment contamination by trace elements. Chemosphere 40, 275–284.

Gomot-de Vaufleury, A., Pihan, F., 2002. Methods for toxicity assessment of contaminated soil by oral or dermal uptake in land snails: metal bioavailability and bioaccumulation. Environ. Toxicol. Chem. 21, 820–827.

Heng, L.Y., Mokhtar, M.B., Rusin, S., 2004. The bioaccumulation of trace essential metals by the freshwater snail, *Turritella* sp. found in the rivers of Borneo East Malaysia. J. Biol. Sci. 4, 441–444.

Hoang, T.C., Rogevich, E.C., Rand, G.M., Gardinali, P.G., Frakes, R.A., Bargar, T.A., 2008a. Copper desorption in flooded agricultural soils and toxicity to the Florida apple snail (*Pomacea paludosa*): implications in everglades restoration. Envion. Pollut. 154, 338–347.

Hoang, T.C., Rogevich, E.C., Rand, G.M., 2008b. Copper uptake and depuration by juvenile and adult Florida apple snails (*Pomacea paludosa*). Ecotoxicology 17, 605–615.

Hoang, T.C., Rand, G.M., submitted for publication. Inhibition of copper on whole body uptake concentrations of osmotic ions in Florida apple snails (*Pomacea paludosa*): mechanisms of toxicity and detoxification. Aquat. Toxicol.

Howard, B., Mitchell, P.C.H., Ritchie, A., Simkiss, K., Taylor, M.G., 1981. The composition of intracellular granules from the metal-accumulating cells of the snail *Helix aspersa*. Biochem. J. 194, 507–511.

Laskowski, R., Hopkin, S.P., 1996. Accumulation of Zn, Cu, Pb and Cd in the garden snail (*Helix aspersa*): implications for predators. Environ. Pollut. 91, 289–297.

Leslie, A.J., 1990. Aquatic Use of Copper-Based Herbicides in Florida. Florida Department of Natural Resources Bureau of Aquatic Plant Management, Tallahassee, FL, USA.

Leung, K.M., Ibrahim, H., Dewhurst, R.E., Morley, N.G., Crane, M., Lewis, J.W., 2003. Concentrations of metallothionein-like proteins and heavy metals in freshwater snail, *Lymnacea stagnalis* exposed to different levels of waterborne cadmium. Bull. Environ. Contam. Toxicol. 71, 1084–1090.

MacInnes, J.R., Thurberg, F.P., 1973. Effects of metals on the behaviour and oxygen consumption of the mud snail. Mar. Pollut. Bull. 4, 185–186.

Notten, M.J.M., Oosthoek, A.J.P., Rozema, J., Aerts, R., 2005. Heavy metal concentrations in a soil–plant–snail food chain along a terrestrial soil pollution gradient. Environ. Pollut. 138, 178–190.

Pagenkopf, G.K., Russo, R.C., Thurston, R.V., 1974. Effects of complexation on toxicity of copper to fishes. J. Fish. Res. Board Canada 31, 462–465.

Pagenkopf, G.K., 1983. Gill surface interaction model for trace metal toxicity to fishes: role of complexation, pH, and water hardness. Environ. Sci. Technol. 17, 342–347.

Real, M., Munoz, I., Guasch, Navarro, E., Sabater, S., 2003. The effect of copper exposure on a simple aquatic food chain. Aquat. Toxicol. 63, 283–291.

Recio, A., Marigonmez, J.A., Angulo, E., Moya, J., 1988. Zinc treatment of the digestive gland of the slug, *Arion ater* L. 1. Cellular distribution of zinc and cadmium. Bull. Environ. Contam. Toxicol. 41, 858–864.

Roesijadi, G., 1992. Metallothioneins in metal regulation and toxicity in aquatic animals. Aquat. Toxicol. 22, 81–114.

Rogevich, E.C., Hoang, T.C., Rand, G.M., 2008. Influence of water quality and organism age on copper toxicity to Florida apple snail (*Pomacea paludosa*). Arch. Environ. Contam. Toxicol. 54, 690–696.

Santore, R.C., DiToro, D.M., Paquin, P.R., Allen, H.E., Meyer, J.S., 2001. Biotic ligand model of the acute toxicity of metals. 2. Application to acute copper toxicity in freshwater fish and *Daphnia*. Environ. Toxicol. Chem. 20, 2397–2402.

Sharfstein, B., Steinman, A.D., 2001. Growth and survival of the Florida apple snail (*Pomacea paludosa*) fed 3 naturally occurring macrophyte assemblages. J. North Am. Bentholog. Soc. 20, 84–95.

Simkiss, K., Taylor, M.G., 1994. Calcium magnesium phosphate granules: atomic simulations explaining cell death. J. Exp. Biol. 190, 131–139.

South Florida Water Management District, 2001–2006. Reports submitted to South Florida Water Management District for Phase I/II Environmental Site Assessments, South Florida Water Management District, West Palm Beach, FL.

Thorp, J.H., Covich, A.P., 2001. Ecology and Classification of North American Freshwater Invertebrates, second ed. Academic Press, New York, NY, USA. pp. 297–329.

US Department of Agriculture, 2006. Agricultural Chemical Usage Summary, 2005 Fruit Summary, National Agricultural Statistical Service, Washington, DC.