Copper Release, Speciation, and Toxicity Following Multiple Floodings of Copper Enriched Agriculture Soils: Implications in Everglades Restoration

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Abstract This study characterizes the effects of water–soil flooding volume ratio and flooding time on copper (Cu) desorption and toxicity following multiple floodings of field-collected soils from agricultural sites acquired under the Comprehensive Everglades Restoration Plan (CERP) in south Florida. Soils from four field sites were flooded with three water–soil ratios (2, 4, and 6 [water] to 1 [soil]) and held for 14 days to characterize the effects of volume ratio and flooding duration on Cu desorption (volume ratio and flooding duration study). Desorption of Cu was also characterized by flooding soils four times from seven field sites with a volume ratio of 2 (water) to 1 (soil) (multiple flooding study). Acute toxicity tests were also conducted using overlying waters from the first flooding event to characterize the effects of Cu on the survival of fathead minnows (*Pimephales promelas*), cladocerans (*Daphnia magna*), amphipods (*Hyalella azteca*), midges (*Chironomus tentans*), duckweed (*Lemna minor*), and Florida apple snails (*Pomacea paludosa*). Acute tests were also conducted with *D. magna* exposed to overlying water from the second and third flooding events. Results indicate that dissolved Cu concentrations in overlying water increased with flooding duration and decreased with volume ratio. In the multiple flooding study, initial Cu concentrations in soils ranged from 5 to 223 mg/kg (dw) and were similar to Cu concentration after four flooding events, indicating retention of Cu in soils. Copper desorption was dependent on soil Cu content and soil characteristics. Total Cu concentration in overlying water (Cu_w) was a function of dissolved organic carbon (DOC), alkalinity, and soil Cu concentration (Cu_s): $\log(Cu_w) = 1.2909 + 0.0279\ (DOC) + 0.0026\ (Cu_s) - 0.0038$ (alkalinity). The model was validated and highly predictive. Most of the desorbed Cu in the water column complexed with organic matter in the soils and accounted for 99% of the total dissolved Cu. Although total dissolved Cu concentrations in overlying water did not significantly decrease with number of flooding events, concentrations of free Cu^{2+} increased with the number of

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flooding events, due to a decrease in DOC concentrations. The fraction of bioavailable Cu species (Cu^{2+}, $CuOH^+$, $CuCO_3$) was also less than 1% of the total Cu. Overlying water from the first flooding event was only acutely toxic to the Florida apple snail from one site. However, overlying water from the third flooding of six out of seven soils was acutely toxic to *D. magna*. The decrease in DOC concentrations and increase in bioavailable Cu^{2+} species may explain the changes in acute toxicity to *D. magna*. Results of this study reveal potential for high Cu bioavailability (Cu^{2+}) and toxicity to aquatic biota overtime in inundated agricultural lands acquired under the CERP.

Keywords Copper release · Speciation · Bioavailability · Toxicity · Flooding · Soils · Everglades restoration

1 Introduction

Copper (Cu) has been used in Florida citrus agriculture as an algaecide, fungicide and soil amendment for over 50 years (Alva et al. 1995). In 2005, according to the US 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 (USDA 2005). Under implementation of the Comprehensive Everglades Restoration Plan (CERP), thousands of acres of citrus agriculture soils will be flooded for maintaining hydrologic buffer areas and for the creation of storm water treatment areas, water storage reservoirs, and wetlands in South Florida. Reports on these soils indicate Cu concentrations as high as 1,200 mg/kg, dw (SFWMD 2001–2006). Our earlier study found high Cu concentrations in overlying water as a result of Cu desorption from a single flooding of citrus agricultural soils (Hoang et al. 2008a). Desorbed Cu from flooded soils also adversely affected the survival and growth of the Florida apple snail, an important food resource for many species, including the federally endangered Everglades snail kite (Hoang et al. 2008a, b).

In water, Cu can occur bound to particulate and/or colloidal matter or as different dissolved species (Eisler 1998). Copper bioavailability in water is influenced by environmental factors such as pH, alkalinity, hardness,

dissolved organic carbon (DOC), and salinity (Arnold 2005; De Schamphelaere et al. 2003; Erickson et al. 1996; Gensemer et al. 2002; Hall et al. 2008; Nriagu 1979; Santore et al. 2001). Free cupric ion (Cu^{2+}) and Cu hydroxide ($CuOH^+$) are the two most bioavailable forms to aquatic organisms (Pagenkopf et al. 1974, 1983, Santore et al. 2001). Recent studies suggest that Cu carbonate ($CuCO_3$) is also bioavailable to invertebrate species (De Schamphelaere and Janssen 2002; Rogevich et al. 2008). Agriculture soils usually contain DOC, carbonate salts, and other minerals which will also complex with Cu and influence Cu speciation and bioavailability (He et al. 2006). Monitoring studies have shown that the quality of receiving waters has been reduced as a result of increased Cu loads in surface runoff from agriculture (Moore et al. 1998; Zhang et al. 2003). It should be noted that in the Clean Water Act, Section 303 (D) special list of impaired waters, the U.S.EPA (Office of Water) ranks metals as the principle reason for water bodies having impaired water quality and places Cu, out of ten metals, in the top tier for producing such changes (Reiley 2007).

South Florida receives between 40 and 65 inches in rainfall annually, mostly during the wet season (i.e., June through October) and with a flat topography it is susceptible to multiple floodings events with uncertain water–soil volume ratios. For example, rainfall measurements in two large citrus farming counties (St. Lucie and Martin) in south Florida indicate a monthly rainfall range of 5 to 270 mm, resulting in varying water–soil volume ratios (Zhang et al. 2003). Citrus agricultural lands, inundated under the CERP, will thus be subjected to multiple rainfall events and various soil-water volume ratios.

This study characterizes the effect of water–soil volume ratio, flooding duration (hereafter referred to as the volume ratio and flooding duration study) and multiple flooding events (hereafter referred to as the multiple flooding study) on Cu desorption and the bioavailability of Cu in flooded water (overlying water) from south Florida citrus agricultural soils. A model was formulated to predict dissolved Cu concentrations in water following desorption from flooded agricultural soils. This study also evaluates the acute toxicity of overlying waters to fathead minnows (*Pimephales promelas*), daphnids (*Daphnia magna*), amphipods (*Hyalella azteca*), midges (*Chironomus tentans*), duckweed (*Lemna minor*), and Florida apple snails (*Pomacea paludosa*).

2 Materials and Methods

2.1 Volume Ratio and Flooding Duration Study

Soils were collected from agriculture sites ($n=11$ 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, soil was collected at three locations, 2 m apart, to a depth of 6 cm from the surface. In the laboratory, soils were composited. Physical and chemical characteristics of soils are published in our earlier study (Hoang et al. 2008a). Soil Cu concentrations (Cu_s) are presented in Table 1 and Fig. 2. Pesticide (organochlorine and organophosphate) and metal (Cd, Pb, As, Hg, and Zn) concentrations were at negligible levels.

To determine the effect of water–soil flooding volume ratio and flooding duration on Cu desorption, 0.15 L of soil from four out of the eleven sites were chosen to represent four different soil textures [Aquacalma-A (Sandy), Birdsall (loamy sand), L31N Buffer (silty loam), Agler (sandy loam)]. Each soil was flooded with 0.3, 0.6, and 0.9 L of laboratory freshwater (carbon-filtered and UV-sterilized city water) in 1 L glass vessels to obtain volume ratios of 2, 4, and 6 (water) to 1 (soil), respectively. Vessels were held under static conditions for 14 days. Three replicates were used for each water/soil ratio. During
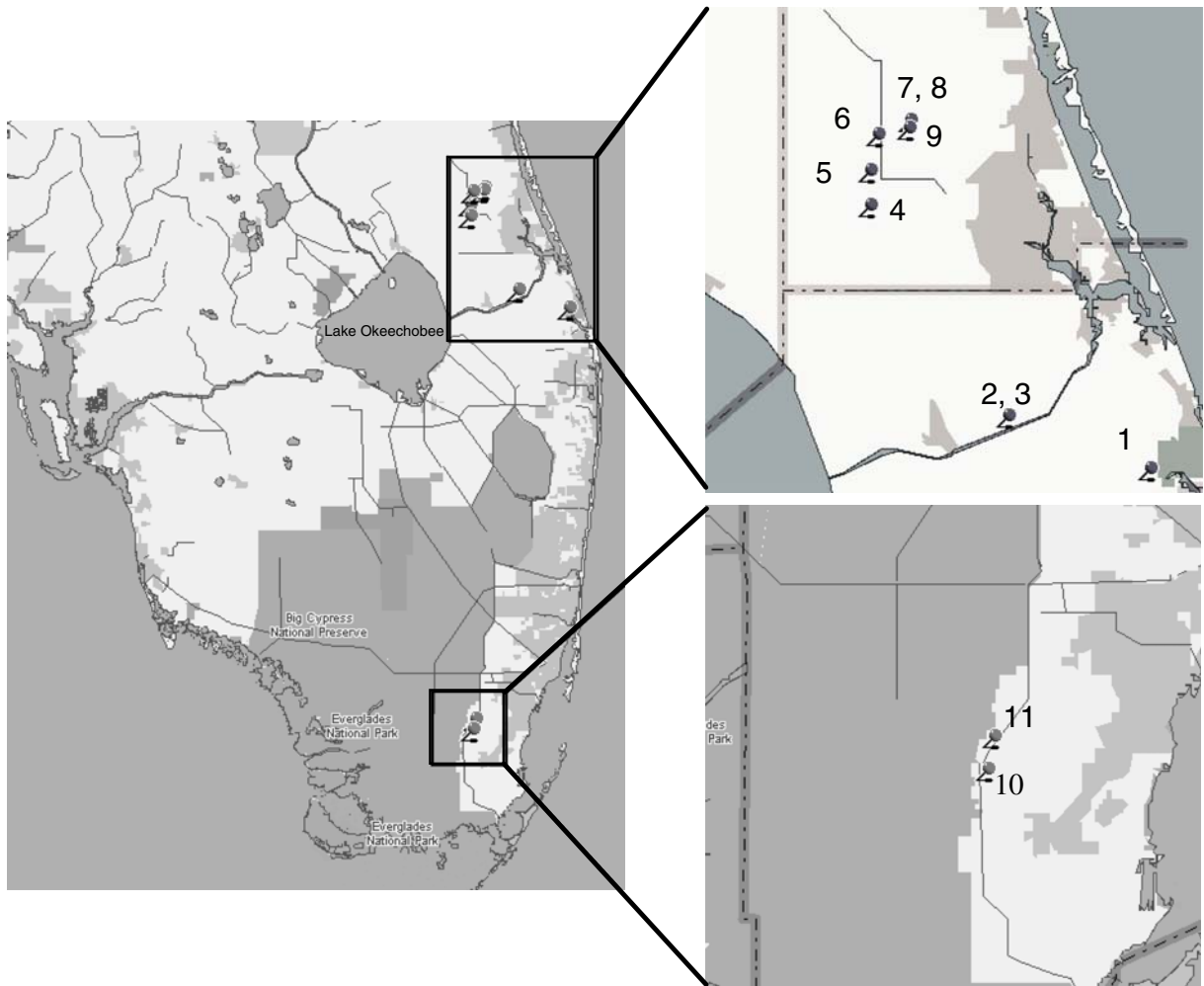
Fig. 1 Soil sampling sites in South Florida: *1* Sunrise Boys, *2* Aquacalma-A (south field), *3* Aquacalma-B (north field), *4* Agler, *5* McArthur, *6* Arcco, *7* Equus-A (west field), *8* Equus-B (east field), *9* Birdsall, *10* Rodriguez Sanchez, *11* L31N Buffer

Table 1 Copper concentrations in soil initially and after four 14-day flooding events

Site	Soil texture	Soil Cu concentration (mg/Kg, dw)		Total Cu in soils (mg)	Total Cu released[a] (mg)
		Original	After fourth flooding		
Agler	SL	117±9	90±5	591	1.95 (0.33)
Arcco	SL	66±20	93±2	324	1.67 (0.51)
Birdsall	LS	210±45	220±13	300	12.74 (4.24)
McArthur	LS	223±15	247±4	1,282	4.18 (0.33)
Control	StL	8±2	6±2	6	0.03 (0.53)
L31N Buffer	StL	185±12	202±27	1,050	1.97 (0.19)
Equus-A	S	5±2	11±2	31	0.50 (1.58)
Sunrise Boys	S	31±5	39±5	239	1.14 (0.48)

Total Cu in soils was calculated by multiplying soil Cu concentrations with total soil used

SL sandy loam, *LS* loamy sand, *StL* silty loam, S sandy

[a] Numbers in parentheses are total Cu released in percent of the total soil Cu

the 14 days, overlying water (i.e., water on top of soils) samples from each replicate were collected on days 1, 4, 7, and 14 for dissolved Cu analysis.

2.2 Multiple Flooding Study

To determine Cu desorption from multiple flooding events, soils from seven sites (Agler, Arcco, Birdsall, McArthur, L31N Buffer, Equus-A, Sunrise Boys) representing four different soil textures (sandy, sandy loam, loamy sand, silty loam) and a control soil were flooded and dried four times. A water–soil volume ratio of 2 (water) to 1 (soil) was chosen for this study based on monthly average rainfall in St. Lucie and Martin Counties, South Florida (approximately 126 mm; Zhang et al. 2003). Six liters of soil from each of the seven sites and a control soil were randomly distributed to 18-L glass tanks, with two replicates per site. Tanks were subsequently flooded with 12 L of laboratory freshwater and held for 14 days. Overlying water was then removed and soil was allowed to air dry for 14 days. Flooding (14 days)/drying (14 days) events were repeated four times for each soil. During the first three flooding events, overlying water samples from each replicate were collected on days 1, 4, 7, and 14, and for the fourth flooding event on days 1, 7, and 14, to measure dissolved Cu, free Cu (Cu^{2+}) and DOC concentrations. On day 14 of the first flooding, water was also collected for anion analysis. Water samples for dissolved Cu, DOC, and anions were filtered through 0.45-μm Gelman Nylon Mesh®. Total water hardness, alkalinity, and pH were measured at each

water sampling day. Soils were then air-dried for 14 days at 25±1°C prior to the next flooding event. Soil Cu concentrations were re-measured after the fourth flooding using the method published by Hoang et al. (2008a). On day 14, all overlying water from each replicate soil–water treatment was collected and composited to conduct acute toxicity tests.

A regression model was developed (as described in data analysis) to predict dissolved Cu concentrations from flooded soils based on results of the multiple flooding study. Results of the first flooding of Aquacalma-A, Aquacalma-B, Rodriguez Sanchez and Equus-B soils were used to validate the model.

Free Cu^{+2} was analyzed with a copper selective electrode using a buffering method (Steenbergen et al. 2005). The buffering method allows measurement of free Cu^{+2} concentrations as low as 10^{-14} M Cu. Dissolved Cu was analyzed using inductively coupled plasma atomic emission spectrometry (ICPAES; Perkin Elmer Corporation, Toronto, Canada). Concentrations of DOC were measured using a Shimadzu Total Carbon Analyzer (model OC-5050A; Shimadzu Scientific Instruments, Columbia, MD, USA). Anions were measured with Dionex DX500 ion chromatography (IC) (Dionex Corporation, Sunnyvale, CA, USA). Hardness and alkalinity were measured by titrating with 0.01 M ethylenediamine tetraacetic acetate (EDTA) solution and 0.02 N H_2SO_4, respectively. Concentrations of DO and pH were measured with a YSI Meter (YSI Inc., Yellow Springs, OH, USA) and an Accumet Meter (Fisher Scientific, Fairlawn, NJ, USA), respectively. Results of chem-

istry of day 14 overlying water for the first flooding event were used to determine the chemical speciation of Cu for each soil using the Visual Minteq Model (http://www.lwr.kth.se/English/Our Software/vminteq).

2.3 Toxicity Study

Overlying water (day 14) collected from the first flooding event was used to conduct toxicity tests with *D. magna*, *H. azteca*, *P. promelas*, *C. tentans*, *L. minor*, and *P. paludosa*. The overlying water from all replicates of each flooded soil treatment was composited and aerated for 24 h prior to test initiation. All toxicity tests were conducted based on US EPA Standard Methods (US EPA 2002). Test conditions for each species are described in Table 2. All tests were conducted in polypropylene beakers under standard laboratory conditions (25±1°C, 16 h darkness/8 h light). Water hardness, alkalinity, pH, dissolved oxygen (DO), and ammonia were measured at test initiation and termination for all organisms. Mortality was monitored daily. Water samples were collected at test initiation and termination for analyses of free Cu, dissolved Cu, and DOC. Sampling procedures and analyses were described in the fate study.

To evaluate the toxicity of Cu in overlying water from later flooding events, two additional toxicity tests were conducted with *D. magna* using overlying water (day 14) from the second and the third flooding events. *D. magna* was chosen for these two tests because they represent the most sensitive species to Cu in the present study based on acute toxicity test

results from the species sensitivity distribution for Cu (Schuler et al. 2008). Test procedures for the *D. magna* tests were similar to the procedures described above.

2.4 Data Analysis

The effects of water–soil volume ratio and flooding duration on Cu desorption were analyzed using two-way ANOVA analysis. The correlations between soil and water Cu concentrations, water quality parameters, and number of flooding events were determined using the Pearson correlation analysis. Multiple regression analysis was also conducted to determine the relationship of dissolved Cu concentrations in overlying water from the four flooding events and water quality parameters (alkalinity, pH, hardness, DOC) and soil Cu concentrations. Data were log transformed to meet the assumptions of normality and homogeneity of variance before conducting multiple regression analysis.

Principal component and factors analysis (PCA) was conducted for all soils, using the varimax rotation method to characterize desorption of Cu forms (e.g., Cu^{2+}, Cu-DOC, $CuCO_3$, etc.) and other factors in soils (alkalinity, hardness, pH, DOC). Survival data were analyzed using multiple treatment comparisons with Dunnetts-test. An effect with a p value < 0.05 was considered significant. Data from control and Equus-A soils (low Cu concentrations) were not used for the multiple regression and PCA analyses.

All analyses were conducted using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) and SPSS (SPSS Inc. Chicago, IL, USA).

Table 2 Toxicity test conditions

Test conditions	Daphnia magna	Hyalella azteca	Pimephales promelas	Chironomus tentans	Lemna minor	Pomacea paludosa
Replicates	5	3	3	10	4	3
Organisms/rep	10	10	10	1	12	10
Organism age	≤24-h-old	7 to 14-day-old	≤ 24-h-old	10-day-old	Adult	≤96-h-old
Test water (mL)	40	100	200	25	150	700
Test duration (h)	72, 96	96	96	96	96	336
Feeding[a]	No	Yes	Yes	Yes	Yes	Yes
Renewal[b]	No	No	Yes	No	Yes	Yes

[a] *Hyalella azteca*: fed 0.5 mL YCT at 0 and 48 h from test initiation; *Pimephales promelas*: fed 0.2 mL brine shrimp at <2 h to renewal (48 h); *Chironomus tentans*: fed 0.25 mL Aquatox® flake flood slurry at 0 and 48 h from test initiation; *Lemna minor*: fed 0.15 mL nutrient solutions (A, B, C) before placing fronds in; *Pomacea paludosa*: fed 0.5 g romaine lettuce every 3 days

[b] *Pimephales promelas*: at 48 h from test initiation; *Lemna minor*: daily; *Pomacea paludosa*: every 3 days
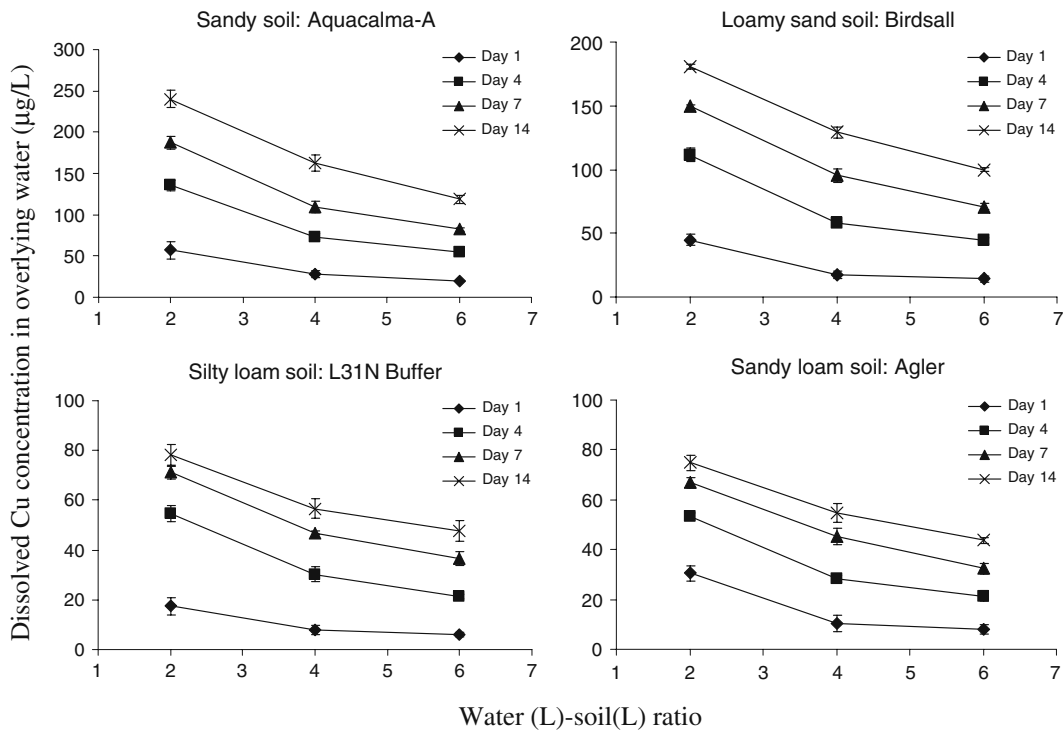
Fig. 2 Dependence of copper desorption on water–soil flooding volume ratio [Initial soil copper concentrations (dw) are 204 mg/kg (Aquacalma-A), 210 mg/kg (Birdsall), 185 mg/kg (L31N Buffer), and 117 mg/kg (Agler). *Error bars* represent standard deviations ($n=3$)]

3 Results

### 3.1 Volume Ratio and Flooding Duration Study

Results of the volume ratio and flooding duration study are illustrated in Fig. 2. In general, dissolved Cu concentrations in overlying water significantly increased with flooding duration and decreased with volume ratio. At each time point, Cu concentrations in overlying water were higher at the lower volume ratio. At each volume ratio, Cu concentrations in overlying water increased with flooding time. There was also a significant effect on Cu desorption as a result of the interaction of water–soil volume ratio and flooding duration.

3.2 Multiple Flooding Study

Results of the multiple flooding study are shown in Fig. 3 and Table 1. Measured concentrations of total dissolved Cu, free Cu^{2+} and water quality parameters of overlying water on day 14 from each of the four flooding events are shown in Table 3.

In general, soils with higher Cu concentrations resulted in higher dissolved Cu concentrations in overlying water. Similar desorption patterns were found following each of the four flooding events; dissolved Cu concentrations in overlying water increased with flooding duration. Dissolved Cu concentrations in overlying water at day 14 were similar for flooding events one through four (Figs. 3 and 4). However, free Cu^{2+} significantly increased with number of flooding events while DOC concentrations significantly decreased with number of flooding events (Fig. 4). After the fourth flooding event, total Cu release from the soils was less than 5% of the initial soil Cu content (Table 1).

Results of Pearson correlation analysis are shown in Table 4. Total dissolved Cu concentrations in water were significantly correlated with alkalinity, DOC, soil Cu concentrations, and pH. Free Cu^{2+} concentrations were significantly correlated with hardness and flooding number. Hardness was significantly correlated with alkalinity and flooding number. Alkalinity was significantly correlated with pH. DOC concentrations were significantly correlated with pH, flooding number, and soil Cu concentration.
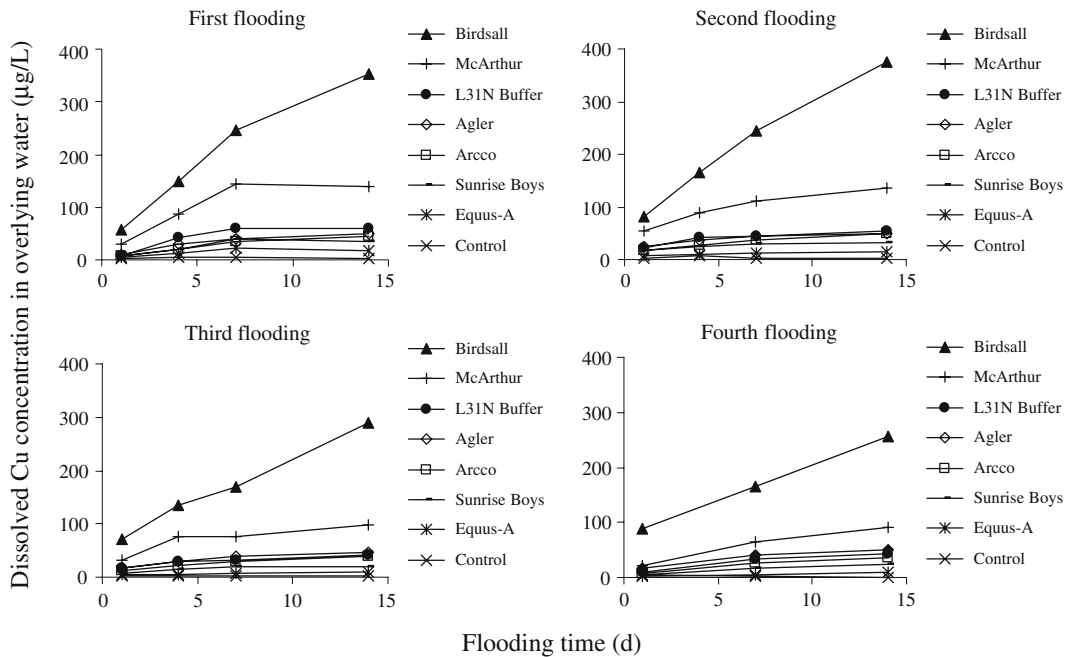
Fig. 3 Copper desorption from soils following four floodings

Results of the PCA are shown in Table 5. There were four components characterizing desorption of Cu and other constituents from the soils. Component 1 had high loading factors for Cu (0.862) and DOC (0.951), characterizing desorption of total dissolved Cu and DOC. Component 2 had high loading factors for alkalinity (0.698) and pH (0.916), characterizing the desorption of carbonate. Component 3 had a high loading factor for hardness (0.922), characterizing desorption of calcium and magnesium. Component 4 had a high loading factor for Cu^{2+} (0.975), characterizing desorption of free Cu^{2+}.

The total dissolved Cu concentration in overlying water (Cu_w) was a function of the DOC concentration in water, water alkalinity, and soil Cu concentration (Cu_s): $\log (Cu_w) = 1.2909 + 0.0279 (DOC) + 0.0026 (Cu_s) - 0.0038$ (alkalinity). The variation of DOC, alkalinity, and soil Cu concentration explained 92% of the variation of Cu concentration in overlying water ($R^2 = 0.92$). Results of model validation are shown in Fig. 5. In general, the measured and predicted total dissolved Cu concentrations were highly correlated ($R^2 = 0.81$) (Fig. 5).

Results of Cu speciation in overlying water after the first flooding characterized by the Visual Minteq model are illustrated in Fig. 6. The copper and organic matter complex (Cu-DOC) was the dominant species and accounted for 99% of total dissolved Cu in the overlying water. Other Cu species were less than 1% of total dissolved Cu.

3.3 Toxicity Study

Results of toxicity studies for overlying water collected from the first flooding event are illustrated in Fig. 7. There was no significant effect of Cu on survival of *P. promelas*, *D. magna*, *H. azteca*, *C. tentans* or *L. minor*. In one soil (McArthur), the Florida apple snail had significantly lower survival than in the control and other treatments. Figure 8 shows the effects of exposure of overlying water collected from the second and third flooding events on the survival *D. magna* neonates after 72- and 96-h. The survival (%) of *D. magna* was not significantly affected by exposure to overlying water from the first or second flooding event (64–100% survival), but was significantly affected by exposure to overlying water from the third flooding event (14–54% survival) in six out of the seven soils. Water hardness, alkalinity, DOC, and pH at the beginning and the end of each toxicity test were not significantly different. Measured values of water quality parameters are shown in Table 3. Dissolved oxygen and ammonia concentrations for all tests were 6.0 ± 1 and 0.5 ± 0.2 mg/L, respectively.

Table 3 Water quality parameters of overlying water after flooding for 14 days

Flooding	Soil	Total dissolved Cu (μg/L)	Free Cu^{2+} (μg/L)	Hardness (mg/L CaCO$_3$)	Alkalinity (mg/L CaCO$_3$)	pH	DOC (mg/L)	Cl$^-$ (mg/L)	CO$_3^=$ (mg/L)	SO$_4^=$ (mg/L)	NO$_3^-$ (mg/L)
1	Control	2	0.022	144	140	8.23	78.8	89	33	12	0
1	Equus-A	14	0.028	184	180	8.32	50.9	60	51	9	1
1	Agler	48	0.016	172	124	8.31	23.5	68	36	35	30
1	Arcco	41	0.023	156	104	7.75	24.3	141	35	33	8
1	McArthur	142	0.014	184	132	8.17	25.3	122	38	20	8
1	Birdsall	364	0.025	152	16	6.46	34.6	252	2	96	50
1	Sunrise Boys	41	0.029	108	104	7.98	16.5	69	28	8	10
1	L31N Buffer	59	0.035	204	208	8.43	30.4	100	57	19	8
2	Control	5	0.045	112	136	7.72	38.0	–	–	–	–
2	Equus-A	10	0.020	200	196	7.69	42.4	–	–	–	–
2	Agler	51	0.034	120	120	7.93	23.1	–	–	–	–
2	Arcco	48	0.049	140	136	7.83	24.3	–	–	–	–
2	McArthur	137	0.065	180	136	7.82	24.2	–	–	–	–
2	Birdsall	427	0.030	76	76	7.48	33.6	–	–	–	–
2	Sunrise Boys	34	0.038	100	100	7.84	18.5	–	–	–	–
2	L31N Buffer	54	0.021	180	176	7.79	24.1	–	–	–	–
3	Control	4	0.031	108	124	7.74	36.8	–	–	–	–
3	Equus-A	9	0.026	168	160	7.57	23.2	–	–	–	–
3	Agler	46	0.042	140	136	7.70	18.6	–	–	–	–
3	Arcco	38	0.032	140	124	7.84	19.6	–	–	–	–
3	McArthur	99	0.048	120	128	7.85	23.0	–	–	–	–
3	Birdsall	290	0.056	88	84	7.70	28.1	–	–	–	–
3	Sunrise Boys	21	0.055	104	96	7.74	17.9	–	–	–	–
3	L31N Buffer	41	0.037	148	168	7.75	21.0	–	–	–	–
4	Control	4	0.037	104	112	7.86	26.6	–	–	–	–
4	Equus-A	10	0.036	192	112	7.46	29.3	–	–	–	–
4	Agler	49	0.070	136	116	8.07	19.2	–	–	–	–
4	Arcco	35	0.077	116	168	7.82	21.1	–	–	–	–
4	McArthur	90	0.112	108	112	8.28	19.5	–	–	–	–
4	Birdsall	256	0.202	88	88	7.51	30.2	–	–	–	–
4	Sunrise Boys	25	0.056	100	116	8.06	15.6	–	–	–	–
4	L31N Buffer	43	0.044	144	152	8.15	19.9	–	–	–	–

(–) not measured

4 Discussion

4.1 Volume Ratio and Flooding Duration Study: Dependence of Copper Desorption on Duration, Concentration, and Soil Characteristics

Results of the volume ratio and flooding duration study indicated that Cu desorption from soils was dependent on flooding time and water–soil volume ratio. During a 14 day flooding period, dissolved Cu concentrations in water increased as flooding duration increased and as water–soil volume ratio decreased. He et al. (2006) reported similar results for Cu and Zn desorption. Stemmer et al. (1990) investigated the influence of

sediment volume and surface area (SA) on selenium-spiked sediment toxicity to *D. magna* and found a decrease in sediment-to-water ratio from 1:4 to 1:8 and increased SA decreased survival of test organisms.

The current study also confirmed that copper desorption is dependent on soil characteristics, as previously reported by Gasser et al. (1994); Zhang et al. (2003); Ponizovsky et al. (2006) and Hoang et al. (2008a). For example, Agler soil had lower soil Cu concentrations than L31N Buffer soil; however, overlying water Cu concentrations were similar in both soil-water systems (Fig. 2). The higher cation exchange capacity (CEC=35 meq/100 g) and lower sand composition (19%) in L31N Buffer soil compared

Fig. 4 Dependence of copper and DOC desorption on number of floodings. (Total dissolved Cu, free Cu, and DOC concentrations were quantified on day 14 of flooding)
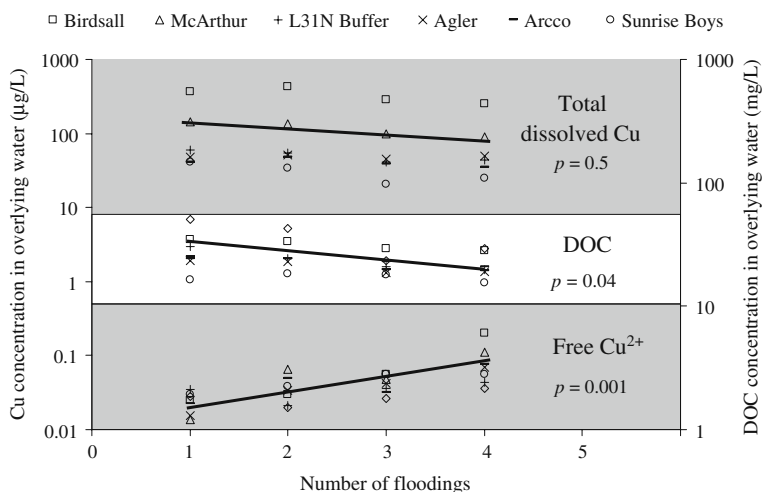
to Agler soil (CEC=30 meq/100 g and % sand=72%) may explain the difference in Cu desorption in these two soils (Hoang et al. 2008a).

4.2 Multiple Flooding Study: Copper Speciation and Bioavailability

Copper in natural soils can complex with organic matter (e.g., humic and fulvic acids) and inorganic ligands (e.g., carbonate, mineral oxides; Nriagu 1979). These Cu complexes can desorb into water and may change depending on water quality (e.g., pH). The present study found that the total dissolved Cu concentration did not significantly decrease with the number of flooding events. In contrast, free Cu^{2+} concentration significantly increased with the number of flooding events. This may be due to the decrease in DOC concentration with increasing flooding events, resulting in decreased concentrations of Cu-DOC.

Pearson correlation analysis indicated that total dissolved Cu concentrations were significantly correlated with DOC concentrations and up to 99% of the total dissolved Cu was complexed with DOC. This may be a result of desorption of Cu-DOC from the soils and/or complexation of Cu and DOC in the overlying water. The significant correlations between total dissolved Cu and DOC in water and soil Cu and DOC suggest that most of Cu in the soils was complexed with DOC.

Results of PCA indicated that desorbed Cu was classified as total dissolved Cu and DOC in component 1 and free Cu in component 4. This suggests that

desorbed Cu was in both organic complexes (Cu-DOC) and free (Cu^{2+}) forms. However, concentrations of Cu^{2+} were minor as compared with those of Cu-DOC. Copper bicarbonate ($CuHCO_3^+$) and copper carbonate ($CuCO_3$) did not contribute to desorbed Cu, as indicated by the low loading factor of total dissolved Cu concentration in component 2, which characterized the desorption of carbonate. These data are similar to findings by Sajwan et al. (2006), who also demonstrated that the fraction of Cu present in carbonate form in soils collected from citrus groves in Lake Alfred, Florida and Savannah, Georgia were small compared to total Cu. Sajwan et al. (2006) reported that the fraction of organic Cu and exchangeable Cu (free Cu^{2+}) in soils from Florida citrus groves and Savannah (Georgia) soils ranged from 32% to 59% and 1% to 4% of total Cu, respectively. McBride and Bouldin (1984) found that up to 99.5% of total Cu in soils was in an organically complexed form. He et al. (2006) also reported up to 70% of total dissolved Cu released from Florida soils was in organic complexes. The fraction of organic Cu complexes varies, depending on soil organic matter content. Saeki et al. (2002) reported that the fraction of organic Cu complexes ranged from 1% to 56% of total Cu in soils that had soluble organic carbon from 1 to 102 mg/L.

Desorption of Cu is also dependent on water pH. The negative correlation between total Cu concentration and pH indicates that Cu desorption decreased with increasing pH. Essington (2004) reported that surface enhanced metal hydrolysis at mineral surfaces

Table 4 Results of Pearson correlation analysis between total water Cu and soil Cu concentrations, water quality parameters, and number of flooding event

Factor	Total dissolved water Cu	Free Cu^{2+}	Hardness	Alkalinity	DOC	pH	Soil Cu	Number flooding event
Total dissolved water Cu	1							
Free Cu^{2+}	0.170 (0.427)	1						
Hardness	−0.293 (0.165)	−0.439 (0.032)	1					
Alkalinity	−0.629 (0.001)	−0.126 (0.558)	0.528 (0.008)	1				
DOC	0.815 (0.000)	−0.012 (0.956)	0.155 (0.471)	−0.282 (0.182)	1			
pH	−0.636 (0.001)	−0.042 (0.846)	0.230 (0.279)	0.679 (0.000)	−0.490 (0.015)	1		
Soil Cu	0.594 (0.002)	0.195 (0.361)	0.226 (0.287)	0.001 (0.995)	0.625 (0.000)	−0.142 (0.509)	1	
Number flooding event	−0.136 (0.528)	0.629 (0.001)	−0.503 (0.012)	0.091 (0.673)	−0.423 (0.040)	0.113 (0.598)	0.000 (1.000)	1

Numbers in parentheses are significance values (p value), $N=24$, Pearson correlation

Table 5 Results of principal component and factor analysis (PCA)

|	Component			
	1	2	3	4
Total water Cu	0.862	−0.366	−0.204	0.080
Free Cu	0.036	−0.006	−0.291	0.975
Hardness	0.043	0.138	0.922	−0.276
Alkalinity	−0.252	0.698	0.572	0.033
DOC	0.951	−0.203	0.168	−0.011
pH	−0.325	0.916	0.065	−0.031

Rotation component matrix. Varimax with Kaiser Normalization
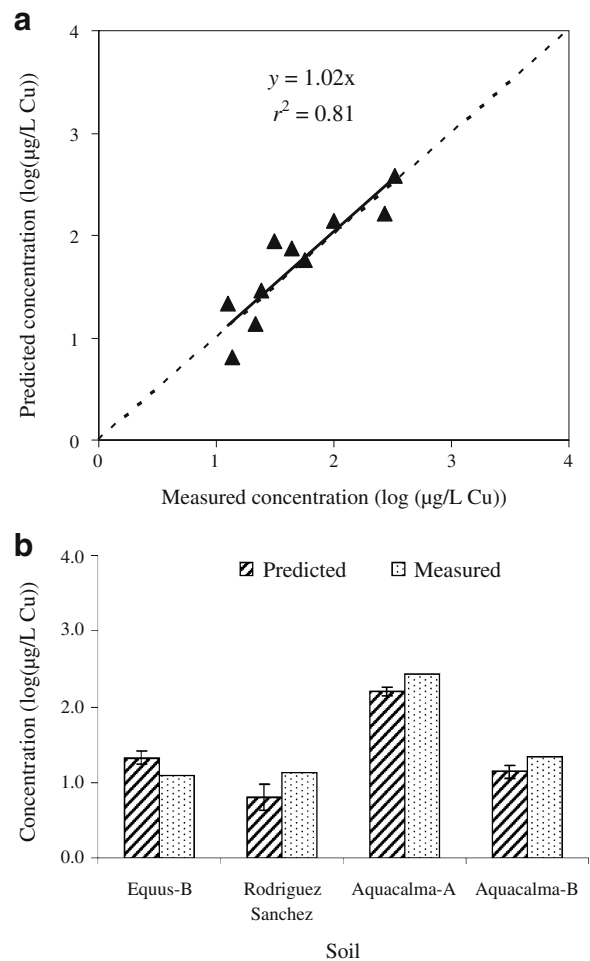
Fig. 5 Model validation. **a** Model predicted versus measured dissolved Cu concentrations in overlying water, **b** error bars are 95% confidence intervals for model predicted concentrations

Fig. 6 Distribution of Cu speciation in overlying water of the first flooding
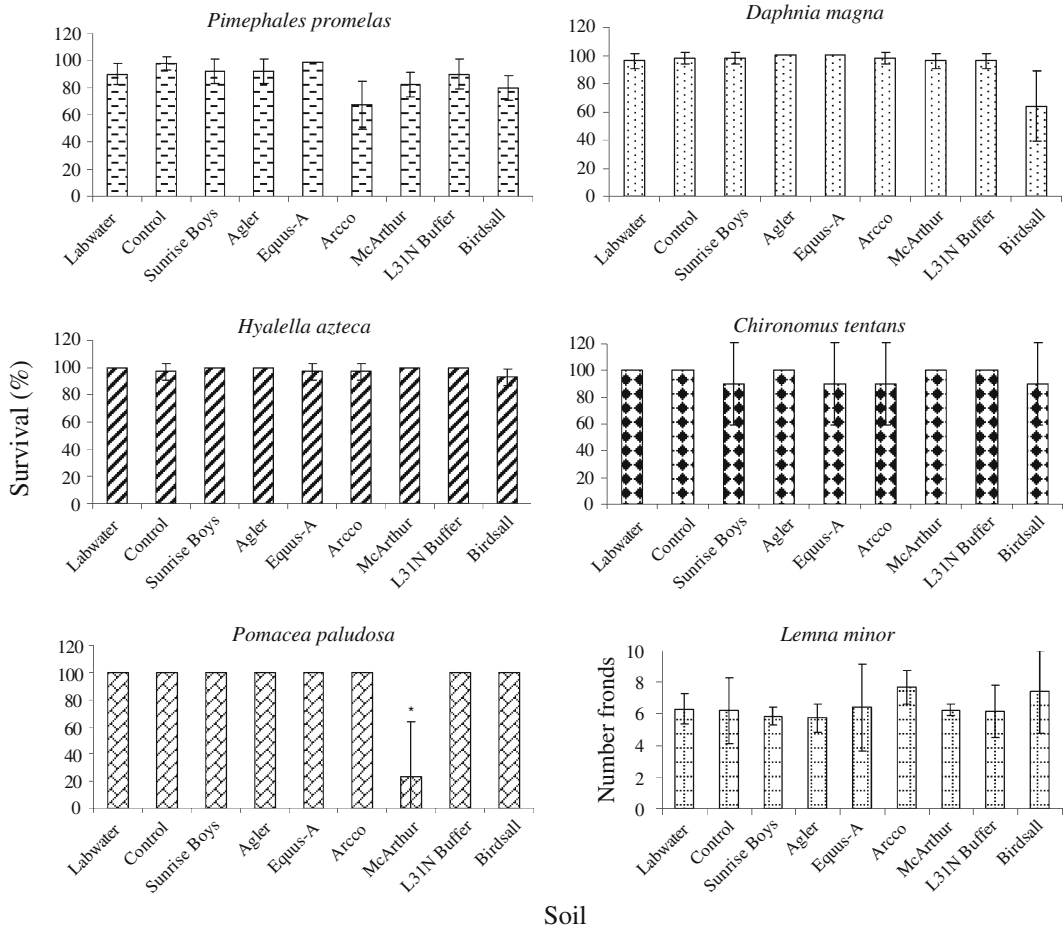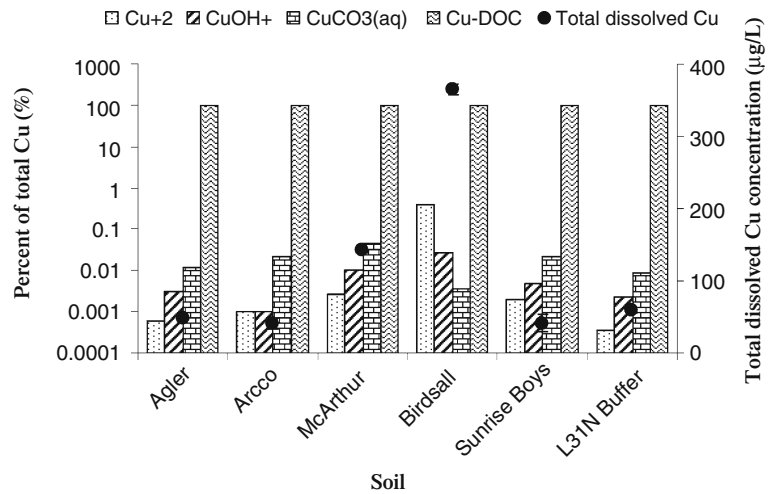
Fig. 7 Effect of desorbed Cu from soils on survival of aquatic organisms (Labwater is laboratory freshwater. Others are soil flooded waters. *Asterisk* Significant difference from Labwater, control and others)
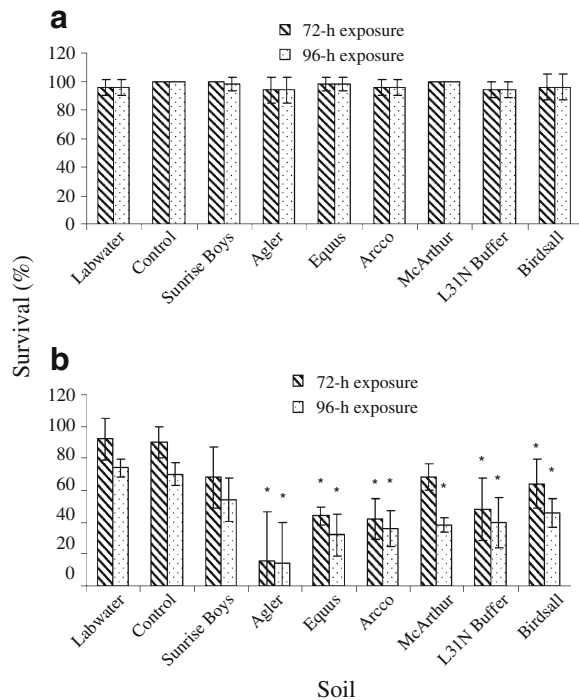
Fig. 8 Effect of desorbed Cu from soils on survival of
Daphnia magna (Labwater was laboratory freshwater. Others
were overlying water. **a** Second flooding event, **b** third flooding
event, *asterisk* significant difference from control)

(e.g., SiO_2, kaolinite, gibbsite, etc.) results in in-
creased metal adsorption and decreased water pH.
This may explain the inverse relation between Cu
desorption and pH in the present study.

Results of the PCA and Visual Minteq model
prediction suggested that copper bicarbonate and
copper carbonate complexes did not contribute to Cu
desorption; however, our empirical model indicated
that total Cu concentration was inversely related to
alkalinity. Most of the Cu in the soils was complexed
with organic matter; therefore, desorption of Cu
would be confounded by desorption of organic matter.
In the natural environment, carboxylic acid is the
most important functional group of organic matter
(Thurman 1986a). The dissociation of carboxyl
groups results in organic matter with a negative
charge. The presence of carbonate and bicarbonate
in overlying water first increased the ionic strength of
overlying water and second creates a negative charge
repulsion between Cu–organic matter and carbonate
and bicarbonate. This would decrease desorption and
solubility of Cu–organic matter complexes. The effect
of alkalinity on desorption of Cu–organic matter

hence, may be called "salting out" effect (Thurman
1986b; Schwarzenbach et al. 1993). This may explain
the inverse relation between total Cu concentration
and alkalinity in the present study. Similarly, the
presence of Ca^{2+} and Mg^{2+} in overlying water may
decrease desorption of Cu^{2+}. The "salting out" effect
may explain the negative relationship between hard-
ness and Cu^{2+}. The relationship between total Cu and
DOC concentrations in the model can be explained by
desorption of Cu–organic matter complexes.

Results of the PCA (component 1) also support the
relationship between total Cu and DOC concentrations
in the model. Among the three significant variables,
DOC alone accounted for 68% of the total desorbed Cu
concentration. Soil Cu concentrations and alkalinity
accounted for 24% of the total desorbed Cu. The
dependence of desorbed Cu concentrations on soil Cu
and organic matter concentrations in the present study
is in agreement with the literature (Sauve et al. 2000;
Impellitteri et al. 2003; He et al. 2004).

The relationship between the measured and model
predicted total Cu concentrations in the overlying
water yielded a slope of 1.02 and an intercept not
significantly different from zero. This indicates that
the model is accurate in its prediction. Results of the
model validation also indicate that the model is highly
predictive. The predicted concentrations were within
20% of the measured concentrations. Using this
model, the total desorbed Cu concentration in
overlying water can be predicted based on soil Cu
concentration, DOC, and alkalinity.

Among the aqueous Cu species, Cu^{2+} and $CuOH^+$
are considered bioavailable to aquatic organisms in
acute exposures (Pagenkopf et al. 1974; Pagenkopf
1983; Santore et al. 2001). Recently, $CuCO_3$ was also
suggested to be bioavailable to invertebrate species (De
Schamphelaere and Janssen 2002). Rogevich et al.
(2008) indicate that $CuCO_3$ may be bioavailable to the
Florida apple snail. Results of the present study
indicated that the fraction of bioavailable Cu species
in the overlying water of the first flooding were less
than 1% of total Cu. The measured Cu^{2+} concentrations
in the overlying water from the first flooding were less
than 0.06 μg/L Cu and were similar to Cu^{2+} concen-
tration (0.07 μg/L) in our laboratory freshwater, which
contained a total dissolved Cu concentration of 4 μg/L
and DOC=0.4 mg/L. The low fraction of bioavailable
Cu in the overlying water of the first flooding explains
the insignificant effect of Cu on survival of the tested

organisms. This is in agreement with the literature (Erickson et al. 1996; Santore et al. 2001; Sciera et al. 2004; Ryan et al. 2004; DeSchamphelaere et al. 2003; DeSchamphelaere and Janssen 2004).

5 Conclusions and Implications in Everglades Restoration

Reports on agricultural properties acquired under the CERP show the presence of Cu in soils with concentrations as high as 1,200 mg/kg (dw). The present study showed that once inundated by rainwater events, Cu will desorb from these flooded agricultural soils [in soil (sediment)–water systems] and overlying water will contain dissolved Cu concentrations which will increase with flooding duration and decrease with soil/water volume ratios. In addition, concentration of the free ion (Cu^{2+}), a readily bioavailable and toxic species, increases with number of flooding events due to a decrease in DOC concentrations. The latter was supported by the significant decrease in survival in the acute toxicity tests to *Daphnia magna* neonates, as a result of exposure to overlying waters from flooding one (64–100%) to flooding three (14–54% survival). Furthermore, although there is a significant increase in the concentration of Cu^{2+} in overlying water, total soil Cu concentrations were similar between the initial measurement (before flooding) and after flooding four, which was four months in duration. This indicates that Cu will be retained in agricultural soils.

Recruitment of organisms into these newly managed systems may initially lead to diverse communities which ultimately will be exposed to Cu from several potential environmental sources-via water, sediment and food. At first, these systems will serve as a reservoir for long-term Cu exposures and low-level chronic uptake with few implications for acute exposures because bioavailable, free Cu^{2+} concentrations will be limited. However, over time these systems will present a challenge to organisms as a result of acute exposures because an increase in natural rainfall events will eventually lower DOC concentrations with concomitant increases in the bioavailable Cu^{2+}, the toxic form. Therefore, as these systems age they will produce high potential for acute and chronic risks to aquatic organisms. Risk will be especially high for planktonic herbivores and herbivorous fish since they will be exposed directly from water and indirectly from consuming phytoplankton and periphyton; two trophic groups which have a high propensity to bioconcentrate Cu. Since Cu will remain in sediment for long periods of time, both infaunal and epibenthic species will also be at risk because of their short life cycles.

The long-term persistence of copper in these managed systems and consequent biological effects as a result of such exposures must be considered in the future acquisition of agricultural lands under the CERP. Based on the results of copper studies with flooded agricultural soils and freshwater organisms from this laboratory, it appears that, in some cases, the creation of these wetland systems under the CERP may not be consistent with the goal of improving the functional quality of habitats or increasing the abundance and diversity of native plant and animal species in south Florida. Therefore, with respect to the remedial actions and environmental management decisions taken on agricultural lands acquired under the CERP, the "precautionary principle" should be applied. In essence, although cause-and-effect relationships and scientific evidence are not completely established for the biological consequences of flooded, copper-contaminated agricultural soils, we have an obligation to adopt a conservative approach and try to avoid increasing risks to the ecosystem that we are trying to restore.

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