



eCOMMONS

Loyola University Chicago
Loyola eCommons

Institute of Environmental Sustainability:
Faculty Publications and Other Works

Faculty Publications


8-2014

Effects of Contaminated St. Lucie River Saltwater Sediments on an Amphipod (*Ampelisca abdita*) and a Hard-Shell Clam (*Mercenaria mercenaria*)

Tham C. Hoang
Loyola University Chicago, thoang@luc.edu

Gary M. Rand
Florida International University

Follow this and additional works at: https://ecommons.luc.edu/ies_facpubs

 Part of the [Environmental Sciences Commons](#), and the [Pharmacology, Toxicology and Environmental Health Commons](#)

Author Manuscript

This is a pre-publication author manuscript of the final, published article.

Recommended Citation

Hoang, Tham C. and Rand, Gary M.. Effects of Contaminated St. Lucie River Saltwater Sediments on an Amphipod (*Ampelisca abdita*) and a Hard-Shell Clam (*Mercenaria mercenaria*). *Archives of Environmental Contamination and Toxicology*, 67, 2: 224-233, 2014. Retrieved from Loyola eCommons, Institute of Environmental Sustainability: Faculty Publications and Other Works, <http://dx.doi.org/10.1007/s00244-014-0029-3>

This Article is brought to you for free and open access by the Faculty Publications at Loyola eCommons. It has been accepted for inclusion in Institute of Environmental Sustainability: Faculty Publications and Other Works by an authorized administrator of Loyola eCommons. For more information, please contact ecommons@luc.edu.



This work is licensed under a [Creative Commons Attribution-NonCommercial-No Derivative Works 3.0 License](#).
© Springer Science+Business Media New York 2014

1 Effects of St. Lucie River (FL) saltwater sediments on the amphipod (*Ampelisca abdita*) and the hard
2 shell clam (*Mercenaria mercenaria*)

3

4 Tham C. Hoang^{1,2}, Gary M. Rand^{1*}

5 ¹Florida International University, Southeast Environmental Research Center, Earth & Environment
6 Department, North Miami, Florida, USA

7 ²Present address: Institute of Environmental Sustainability,
8 Loyola University Chicago, Chicago, Illinois, USA

9

10 *Corresponding author

11 Dr. Gary M. Rand

12 Ecotoxicology and Risk Assessment Laboratory

13 Southeast Environmental Research Center

14 Florida International University

15 North Miami Beach, FL

16 Email: randg@fiu.edu

17

18 **Abstract**

19 The St. Lucie estuary ecosystem in South Florida has been noted to be contaminated with
20 metals and pesticides. Our earlier studies showed that aquatic organisms, especially benthic species in
21 the St. Lucie estuarine ecosystem are at high risk of copper (Cu) exposures. The objectives of this
22 study are to conduct tests with separate groups of organisms exposed to 7 field-collected sediment
23 samples from the St. Lucie River according to standard procedures to evaluate toxicity and tissue
24 concentrations of Cu and zinc (Zn). Short term and long term whole sediment acute toxicity tests were
25 conducted with *Ampelisca abdita* and *Mercenaria mercenaria*. Analysis of sediment chemical
26 characteristics showed that Cu and Zn are most concern because their concentrations in 86% of the
27 sediments were higher than the threshold effect concentrations for Florida sediment quality assessment
28 and the NOAA SQuiRTsnational Cu sediment quality guidelines. There was no significant effect on
29 survival of the tested organisms. Elevated Cu and Zn concentrations in the test organisms were found.
30 Dry weight of the tested organisms was inversely related to Cu and Zn concentrations in sediments and
31 organisms. The effects on organism weight and Cu and Zn uptake raise a concern about the organism
32 population dynamics of the ecosystem because benthic organisms are primary food sources in the St.
33 Lucie system and are continuously exposed to the Cu and Zn contaminated sediments for their life
34 cycle. The present study also indicates that Cu and Zn exposures via sediment ingestion were more
35 important than pore water exposure.

36

37 **Key words:** Cu uptake, Cu-contaminated sediment, St. Lucie River sediments, *Ampelisca abdita*,
38 *Mercenaria mercenaria*

39 **Introduction**

40 The St. Lucie Estuary (SLE) watershed is composed of five major drainage basins and several
41 smaller basins in the northern portion of St. Lucie County, Florida. It contains the most concentrated
42 citrus agriculture acreage in South Florida. The SLE is located on the Martin/St. Lucie County line. The
43 inner SLE is comprised of the North Fork and the South Fork of the St. Lucie River (SLR) and has a
44 total surface area of 6.4 square miles. The two forks converge to form a single middle estuary with a
45 surface area of 4.7 square miles. The middle estuary extends east from approximately 5 miles until it
46 meets the Indian River Lagoon, which opens to the Atlantic Ocean at the St. Lucie Inlet. A heavier
47 concentration of citrus agriculture (~60%) land use potentially affects the drainage basins into the North
48 Fork compared to the South Fork (~45%) of the SLR. In 1972, the Florida Trustees recognized the
49 ecological importance of the North Fork of the St. Lucie River by designating it an Aquatic
50 Preserve/Outstanding Florida Water.

51 Copper has a long history of use in agriculture (e.g., citrus groves) as a fungicide and fertilizer in
52 south Florida (Alva et al. 1995). In the early 1900's copper containing fertilizers for citrus groves
53 accounted for as much as 34 kg Cu/ha annually and fungicidal sprays contributed an additional 10kg
54 Cu/ha annually. Surface soils (0-15 cm) for mature citrus groves contained as much as 540kg Cu/ha.
55 Increased levels of copper in South Florida soils have been a result of repeated applications of copper
56 over several decades to agricultural areas and soil copper concentrations increase proportional age of
57 citrus production (Reuther and Smith, 1952). According to the U.S. Department of Agriculture in 2005,
58 over 500,000 kg of copper (as copper hydroxide, copper sulfate or basic copper) were applied to
59 grapefruit, orange, tangelo, tangerine and temple crops on 259,563 ha in Florida (USDA 2006). These
60 quantities do not reflect use of Cu on other citrus crops or the use of copper sulfate and chelated Cu
61 formulations (e.g., Cutrine-Plus, Komeen, etc.) as algacides-herbicides, which are permitted by the

62 Florida Department of Environmental Protection (FDEP) for control of nuisance planktonic and
63 filamentous algal and vascular plants (Leslie, 1990). Note that Cu also does leach from boats into both
64 fresh- and salt-water (e.g., harbors and marinas) because it is a component of antifouling paints. More
65 recently, statewide pesticide usage data (based on total lbs a.i. applied) compiled by the Florida
66 Department of Agriculture and Consumer Services (FDACS 2010) from 2007-2009 for 14 crops and 169
67 active ingredients (a.i.) ranked copper hydroxide (1,176,500 lbs. a.i. applied) number 5 out of 10
68 pesticides and the most applied fungicide (number 1 out of 10 fungicides).

69 A comparison of aqueous Cu concentrations in agriculture and non-agriculture watersheds shows
70 higher concentrations in runoff where agriculture was practiced compared to runoff near non-agriculture
71 land (Dietrich et al., 2001). Copper loads in surface runoff are related to total Cu in soils, soil
72 properties, metal characteristics and environmental factors, especially in sandy soils (He et al., 2006).
73 Enrichment of Cu in runoff will adversely affect receiving surface water quality (Moore et al., 1998).
74 As a result of the use of copper in agriculture in St. Lucie County, it appears that concentrations of
75 copper (and other contaminants, such as Zn) from drift and/or from surface runoff of contaminated soils
76 (or soil erosion) may also produce exposures that adversely affect saltwater benthic communities, when
77 the Cu-contaminated soils reach and become incorporated as part of the sediments of the St. Lucie River
78 system. Sediment chemistry data indicate that Florida coastal sediments in several areas are
79 contaminated with metals (Long and Morgan 1990, Delfino et al. 1991, FDEP 1994), especially Cu
80 (Haunert 1988, Trefry and Trocine 2011, Trocine and Trefry 1993, 1996).

81 Our early laboratory results indicate that copper-contaminated Florida agricultural soils that are
82 flooded likely promote the release of Cu from soils producing adverse effects on freshwater organisms
83 (Hoang et al. 2008a, 2009a, b). In addition, we showed high potential ecological risks to aquatic species
84 as a result of Cu exposures in sediment and water and high probability of exceedences of the Florida

85 Department of Environmental Protection Sediment Quality Assessment Guideline values for the
86 Threshold Effect Concentration and the Probable Effect Concentration (FDEP SQAGs TECs and PECs)
87 for Cu (FDEP 2003) in the St. Lucie River (Schuler et al. 2008). More recently, Carriger and Rand 2013
88 (in press) also showed high ecological risks of Cu in this system to aquatic organisms. The objectives of
89 the present study are to conduct whole sediment toxicity studies with the clam (*Mercenaria mercenaria*)
90 and the benthic amphipod (*Ampelisca abdita*) exposed to field-collected sediment samples from the St.
91 Lucie River to evaluate uptake (bioconcentration) and toxicity of Cu and Zn.

92

93 **Materials and Methods**

94 Sediments used in the present study (n=7; 6 test and 1 reference site) were collected by the
95 National Oceanic and Atmospheric Administration (NOAA) from the St. Lucie River, south Florida,
96 USA and transferred to the Ecotoxicology and Risk Assessment Laboratory (ERAL) of Florida
97 International University on April 5-7, 2011 for toxicity testing (Figure 1). ERAL is a NELAC-
98 accredited laboratory facility for fresh- and salt-water toxicity testing. Sediment samples were labeled
99 NOA2581 (reference site in the South Fork of the SLE) along with six test sites as NOA2569,
100 NOA2334, NOA2640, NOA2639, NOA 2333, and NOA2570 (sites in the North Fork of the SLE). Prior
101 to aquatic testing, sediments were physically characterized and background concentrations of metals and
102 organic pollutants were analyzed. The sediments were also analyzed for acid volatile sulfide (AVS) and
103 simultaneously extracted metals (SEM). Using the AVS/SEM ratio, we can predict the bioavailability of
104 metals (Berry et al. 1996).

105 Two separate types of studies were conducted with the 7 field-collected sediment samples to
106 evaluate mortality, growth and accumulation; one study with the tube-dwelling amphipod (*A. abdita*),
107 which is a common standard saltwater benthic test species used for whole-sediment toxicity and

108 bioaccumulation tests and one study with the hard shell clam (*M. mercenaria*) which is a native species
109 in the St. Lucie system. Exposures to the field-collected sediments in both studies were in a flow-
110 through water system to ensure consistent water quality conditions (e.g., low ammonia concentrations).
111 Flow-through systems were calibrated prior to testing to ensure correct water placements in test
112 chambers over each 24-hour time period. Saltwater for the flow-through system was obtained from a
113 saltwater well (with Biscayne Bay water) which was air-sparged, carbon-filtered and UV-sterilized with
114 a salinity of 31ppt and a pH of 8.0-8.5.

115 Toxicity tests with *A. abdita* were 10 days in duration and were conducted according to the
116 methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with
117 marine invertebrates (U.S. EPA. 1994). *A. abdita* were obtained from a commercial supplier. An initial
118 subsample of the *A. abdita* population was used for length and weight measurements and tissue analyses
119 (Cu, Zn) for background data. There were 10 organisms per replicate with 8 replicates per sediment
120 sample site. Organisms (10) were randomly distributed in each test chamber with 350ml of water and
121 150 ml of sediment (8 test chambers /site; 80 organisms exposed/site) in the water bath of the flow-
122 through water system (2 test chamber water volume turnovers/24h). Water quality monitoring for the
123 tests included salinity, ammonia and pH measurements at test initiation and at test termination.
124 Temperature and dissolved oxygen were measured daily. Salinity, ammonia, and pH were measured
125 using a YSI conductivity/salinity meter (YS Inc, Yellow Springs, Ohio, USA), an Accumet® Ammonia
126 Electrode (Fisher Scientific, Pittsburgh, PA, USA), and an Accumet pH meter (Vernon Hills, Illinois,
127 USA), respectively. Water temperature and dissolved oxygen concentrations were measured using a YSI
128 dissolved oxygen meter (YS Inc., Yellow Springs, Ohio, USA). Mortality of *A. abdita* was measured at
129 test termination along with growth (dry weight and length) of surviving organisms. The quality criterion
130 for control survival was 80%. Tissue concentrations (whole body) of Cu and Zn were measured at test

131 initiation and again at test termination. To increase the detection limits for Cu and Zn, surviving
132 organisms from all replicates were combined for each treatment and digested with HNO₃ acid using a
133 Hotblock and based on the U.S. EPA Method 3050B (U.S. EPA 1996a) for tissue Cu and Zn analyses.
134 Analysis of Cu, Zn, and other minerals were conducted with an inductively coupled plasma
135 spectrometer (Thermo Scientific Inc. 5225 Verona Road, Madison, WI 53711).

136 In addition, a 28-day bioconcentration study was conducted similarly to U.S. EPA Ecological
137 Effects Test Guidelines (U.S. EPA, 1996b) except that sediment was the source of the contaminants (not
138 water exposure). The hard shell clam (*M. mercenaria*), an economically important native species in the
139 SLR system was the test species and only a 28-day uptake phase (without a depuration phase) was used.
140 *M. mercenaria* juveniles were obtained from a commercial supplier. An initial subsample of the *M.*
141 *mercenaria* juvenile population was used for weight measurements and tissue analyses of Cu and zinc.
142 There were 50 organisms per replicate with 2 replicates per sediment sample site used. Organisms (50)
143 were randomly distributed to an 18L test chamber with 12L of water and 3-4 cm of sediment (2 test
144 chambers /site; 100 organisms exposed/site) in a flow-through water system (4 tank water volume
145 turnovers/24h). Water quality monitoring for the test included salinity, conductivity, and pH
146 measurements for the first 3 days, daily and again at the end of the test. Temperature and dissolved
147 oxygen were measured daily. Mortality was measured on days 3, 7, 14, 21 and 28. Tissue samples (n=4)
148 were also collected and measured on days 3, 7, 14, 21 and 28 of surviving organisms for Cu and Zn.
149 The quality criterion for control survival was 80%. Overlying and porewater samples were also
150 collected when the tissue samples were collected for analyses of Cu, Zn and dissolved organic carbon
151 (DOC). Porewater was collected by centrifuging the sediments at 2500g and 4° for 30 minutes (Ankley
152 et al. 1991). The samples were filtered with 0.45 µm filters prior to analysis. DOC was analyzed with a
153 Shimadzu TOC-5000 (Shimadzu Scientific Instruments, Columbia, MD, USA). Measurement of water

154 quality and tissue Cu and Zn concentrations were conducted as described for the *A. abdita* test above.
155 Digestion of sediment, clam, and amphipod samples for analysis of Cu and Zn were conducted at
156 Loyola University Chicago.

157 At the end of the study, survival, dry weights, Cu and Zn tissue concentrations were analyzed to
158 determine whether there were statistically significant ($p < 0.05$) treatment-related effects (responses) of
159 the test substance. The ANOVA F-test was used to test the null hypothesis; that the effects of all
160 sediments including the reference sediment are the same. Tissue Cu and Zn concentrations at the end of
161 the tests or at time intervals during the test (*M. mercenaria* test only) were compared with initial Cu and
162 Zn concentrations (background) using Dunnett's procedure. Multiple correlations between dry weight,
163 tissue Cu and Zn concentrations, sediment Cu and Zn concentrations were conducted to determine
164 cause-effects (response) relationship. All statistical analysis was conducted using SAS (version 9.2).

165

166 **Results and discussion**

167 ***Sediment characteristics and chemistry***

168 Characteristics of the sediments are shown in Table 1. In general, the cation exchange capacity
169 was high which suggests that the sediments have high potential to retain metals. Results of AVS and
170 total SEM are shown in Table 1. AVS for sediments NOA2333, NOA2334, NOA2569 and NOA2570
171 were below the detection limits. The total SEM/AVS ratios for these sediments were estimated based on
172 the detection limits of AVS for those samples. The ratio of total SEM/AVS for all sediments was greater
173 than 1 which suggests that metals in the sediments are more bioavailable to benthic organisms (Ankley
174 et al. 1996, McGrath et al. 2002).

175 Concentrations of metals and minerals in the sediments from the sites are shown in Table 2a.

176 Concentrations of the metals and minerals varied from site to site. Among the toxic metals, Cu and Zn

177 were of most concern because concentrations of both metals exceeded (with Cu 6 out of 7 sediments;
178 with Zn 5 out of 7 sediments) the sediment quality assessment guideline (SQAGs) threshold effect
179 levels (TELs) for coastal and marine waters set by the Florida Department of Environmental Protection
180 (FDEP) for Cu (18.7 mg/kg) and Zn (124 mg/kg, dw) (MacDonald et al.1996). Cu concentrations of the
181 6 sediments also exceeded the informal quick screening marine sediment value (effect range low (ERL)
182 concentration = 34 mg/kg) used for the NOAA SQuiRTs (Buchman, 1999; Long et al. 1995). Zn
183 concentrations were equal to or greater than the ERL (150 mg/kg) for 3 sediments. Neither copper nor
184 zinc concentrations exceeded the NOAA SQuiRTs ERM (effects range median) values which are
185 representative of concentrations above which effects frequently occur (Cu ERM = 270 mg/kg; Zn ERM
186 = 410 mg/kg). Concentrations of the other toxic metals (e.g., As, Cd, Cr, Ni, Pb) in the sediments were
187 not detected or were less than the FDEP TEC SQAGs and the NOAA SQuiRTs ERLs. In a sediment
188 survey in 1982 from the St. Lucie Estuary (SLE) the mean concentration of Cu and Zn were 41
189 (maximum: 229 mg/kg) and 67 (maximum: 235 mg/kg) mg/kg, respectively (Hauert, 1988). Metal
190 concentrations were related to particle size and organic content; as the quantity of clay- and silt-sized
191 particles increased the concentrations of these metals increased. The sediment in the central part of the
192 North Fork of the SLE had the highest concentrations of organic material with the highest
193 concentrations of metals. In 1992, a sediment survey in the Indian River Lagoon, system also showed
194 high concentrations of Cu above background in sediments and clams (Trocine and Trefry, 1993, 1996).
195 In a follow-up sediment survey in 2006-2007, the mean concentrations of Cu (44mg/kg; maximum: 162
196 mg/kg) and Zn (95 mg/kg; maximum: 231 mg/kg) increased from the 1992 survey (Trefry and Trocine,
197 2011).

198 Minimal concentrations of chlorinated organic pollutants were detected in the sediments (7)
199 from the sites except for DDT metabolites (e.g., p,p-DDD) (Table 2b). The total concentrations of DDTs
200 in 3 out of the 7 sediments were higher than the NOAA SQuiRTs ERL concentration (1.58 mg/kg, dw).

201 At the end of the *M. mercenaria* bioconcentration study, sediments samples were collected for
202 Cu and Zn analysis. In general, Zn and Cu concentrations at the beginning (day 0) and the end (day 28)
203 of the study were not significantly different except for NOA 2581 and 2639, Cu and Zn concentrations
204 at the end of the study appeared to be higher than those at the beginning (Table 3). This result indicates
205 that Cu and Zn did not desorb to the overlying water. The high percent of silt, clay, and organic matter
206 in the sediments explains why little Cu and Zn release occur during the study. Zn and Cu concentrations
207 in the sediments were also significantly correlated, revealing that Zn and Cu would come from the same
208 source.

209

210 ***Water quality and chemistry***

211 Water quality conditions for both studies were within U.S. EPA test guideline requirements. For
212 the *A. abdita* test, the 10-d average temperature, DO, pH, and salinity of the overlying water during the
213 test were $21 \pm 1^\circ \text{C}$, $7.1 \pm 1.2 \text{ mg/L}$, 8.26 ± 0.07 , and $30 \pm 1 \text{ ppt}$, respectively. Ammonia concentration
214 ranged from 0.4 to 1 mg/L which were less than the U.S. EPA criteria at a pH of 8.26 (3.4 mg/L).

215 Concentrations of dissolved Cu in the overlying and pore water were at the background level ($6 \mu\text{g/L}$ Cu
216 in saltwater used for testing). Concentration of dissolved Zn in the overlying and pore waters ranged
217 from the background level ($6 \mu\text{g/L}$ Zn) to $33 \mu\text{g/L}$ Zn. These results may be explained by the high
218 percent of silt, clay, and organic matter in the sediments, resulting in negligible desorption of Cu and Zn
219 from the sediments to water. These results also suggest low bioavailability of Cu and Zn in pore water.
220 Concentrations of dissolved organic matter in the overlying water were low ($< 5 \text{ mg/L}$). For the *M.*

221 *mercenaria* study, the 28-d average temperature, DO, pH, and salinity of the overlying water were $24 \pm$
222 0.1°C , $6.6 \pm 0.2 \text{ mg/L}$, 8.24 ± 0.11 , and $30 \pm 1 \text{ ppt}$, respectively. Similar to the *A. abdita* study,
223 concentrations of dissolved Cu and Zn in the overlying and pore water were at the background levels,
224 suggesting low Cu and Zn bioavailability. Concentrations of DOM in the overlying water were also low
225 ($< 4 \text{ mg/L}$).

226

227 ***Effects on survival, Cu and Zn uptake, and growth***

228 Since concentrations of Cu and Zn exceeded both the Florida sediment quality guidelines and the
229 NOAA SQUIRTs and the other toxic metals (e.g., As, Cd, Cr, Ni, Pb) were below these numerical
230 values, the discussion in this section considers only Cu and Zn. Results of organism survival are shown
231 in Table 4. Mortality of *A. abdita* and *M. mercenaria* ranged from 14% (NOA2333) to 28% (NOA2581)
232 and 0% (NOA2639, NOA2570) to 4% (NOA2569), respectively. In general, there was no significant
233 difference between mortality of the tested organisms for the field-collected reference and contaminated
234 sediments. Although the results of SEM and AVS indicate metal bioavailability, the high organic matter
235 content in the sediments most likely decreased Cu and Zn bioavailability and toxicity. No mortality was
236 reported in a similar study conducted by Rule (1985) with *M. mercenaria* and sediments collected from
237 the Port of Hampton Roads, Virginia which had similar total sediment concentration of Zn, Pb, Ni, and
238 Cu ($3 \mu\text{mol/g}$) compared to the present study.

239 Concentrations of Cu and Zn in *M. mercenaria* tissue ranged from 9 (background) to 35 mg/kg
240 dw and 102 (background) to 271 mg/kg dw, respectively (Table 5). In general, Cu and Zn
241 concentrations in *M. mercenaria* tissue were higher on days 3 through 28 than the background
242 concentrations (day 0). In addition, sediment Cu and Zn concentrations were positively correlated with
243 tissue Cu and Zn concentrations (Table 9). These results indicate that *M. mercenaria* accumulated Zn

244 and Cu from the sediments. As discussed above, results of the sediment chemistry (e.g., total SEM/AVS
245 > 1) indicate metal bioavailability. This might explain the Cu and Zn accumulation in *M. mercenaria*.
246 Rule (1985) also found that *M. mercenaria* accumulated Zn from the sediments which had a similar Zn
247 sediment concentration to the present study. However, Zn accumulation by *M. mercenaria* in the Rule
248 (1985) study was approximately half the Zn accumulation in the present study but Zn bioavailability in
249 the present study was higher than in the Rule (1985) study.

250 Cu and Zn concentrations in *M. mercenaria* tissue in the present study did not increase over time.
251 This is in contrast with our earlier studies with freshwater Florida apple snails (*Pomacea paludosa*)
252 where apple snails accumulated Cu from sediment overtime (Hoang et al. 2008b, Hoang et al. 2011). Cu
253 and Zn concentrations in *M. mercenaria* shell were also below the detection limits but in general,
254 negligible Cu and Zn concentrations were detected in apple snail shells as well (Hoang et al. 2008b).

255 Zn and Cu concentrations in *A. abdita* varied from site to site and ranged from 111 (NOA2569) to
256 355 mg/kg dw (NOA2581) and 65 (background) to 364 mg/kg dw (NOA2570), respectively (Table 6).
257 In general, Cu concentrations in *A. abdita* were higher at the end of the study than at the beginning of
258 the study for all sites. There was a positive correlation between the *A. abdita* tissue Cu concentration
259 and sediment Cu concentration (Table 10). Results of the present study indicate that *A. abdita*
260 accumulated Cu from the sediments during the 10 day study. Results of the sediment chemistry suggest
261 Cu bioavailability and therefore explain the Cu accumulation in *A. abdita*. The final tissue results
262 obtained for *A. abdita* in the present study were similar to the tissue Cu concentrations found in our
263 earlier 10 day study with the freshwater benthic amphipod, *Hyalella azteca* exposed to Cu-contaminated
264 soils from citrus agricultural sites near the St. Lucie watershed (Hoang et al. 2009b). Hoang et al. 2009b
265 showed that Cu tissue (whole body) concentrations ranged from 128 to 294mg/kg after 10 days
266 exposure when initial Cu soil concentrations ranged from 5-234 mg/kg from these citrus agricultural

267 sites. Note that in this *A. abdita* study, Cu tissue concentrations were up to 6 times the initial Cu tissue
268 concentrations following only 10 days of exposure and similar to the response of the freshwater
269 amphipod, *H. azteca* following 10 day exposures. Furthermore, the tissue results for both freshwater and
270 saltwater benthic species, following exposures to Cu in sediment, raise some interesting issues for
271 burrowing and tube dwelling in faunal benthic species which have habitats in close contact with
272 sediment (pore water) for part or most of their life cycle. The influence on Cu uptake in these benthic
273 species on upper trophic level diets and species has not been extensively investigated.

274 Also note that accumulated metal within and between invertebrate taxa, vary considerably, even
275 in the absence of anthropogenic pollution (Rainbow, 2002). For trace metals like zinc and copper, which
276 play essential roles in metabolism of most invertebrate, the quantity necessary to perform these
277 functions may also vary widely within and between invertebrate taxa. For aquatic organisms used in
278 toxicity tests, it is also critical to know the background holding, culture and water quality conditions
279 because if test organisms are obtained from different aquaculture sources the trace metal concentrations
280 in their tissues and organs may be different and will obviously reflect prior water quality and diet they
281 were exposed to. Background tissue concentrations (day 0) of zinc (Table 6) for *A. abdita* were as high
282 as concentrations at the end of the 10d treatment and therefore precluded any comparisons of zinc tissue
283 concentrations. In addition, to being cautious when obtaining organisms from aquaculture sources for
284 aquatic toxicity testing, the use of field-collected species requires even greater awareness for use in
285 toxicity testing especially for the evaluation of hazards and risks.

286 The dry weight of *M. mercenaria* and *A. abdita* are shown in Tables 7 and 8, respectively. Dry
287 weight of *M. mercenaria* shell, tissue, and whole body from days 0-28 ranged from 194 to 355
288 mg/organism, 5 to 8 mg/organism, and 198 to 552 mg/organism, respectively. In general, dry weight
289 was not affected by sediment exposure up to day 21. However, there was a slight negative correlation

290 between *M. mercenaria* tissue weight and Cu and Zn concentrations in the sediments at the end of the
291 study (day 28) (Table 9). This suggests that Cu and Zn concentrations in the sediments may have started
292 to produce an effect on *M. mercenaria* growth (as measured by dry weight) after 28 days exposure and
293 that in the experimental design the uptake (exposure) phase was too short and it should have been
294 extended. Clams that live in these sediments will be continuously exposed to Cu and Zn for most of
295 their life cycle in the in the St. Lucie system, with little time for depuration and recovery, hence the
296 effect might be greater. Results of the present study thus raise concern about the population dynamics of
297 bivalves in the St. Lucie ecosystem.

298 The St. Lucie estuarine ecosystem has been documented as a Cu-contaminated system for decades
299 (Long and Morgan 1990, Delfino et al. 1991, FDEP 1994). The cause of Cu contamination is likely due
300 to Cu release from the sandy soils of nearby citrus agriculture farms through surface runoff. Cu will
301 continuously be used in citrus agriculture, with a long season of application thus increasing the Cu load
302 and release from soils into runoff water, adding more Cu into the St. Lucie estuary and the Everglades
303 ecosystems (Hoang et al. 2008a, Hoang et al. 2009a). This is also based on an exposure analyses of
304 copper concentrations in water and sediment in south Florida aquatic systems for over 15 years from
305 1990-2008, which shows high Cu concentrations in aquatic systems and potential risks to mollusks in
306 the North and South forks of the SLR and in the SLE (Carriger and Rand, in press).

307 Dry weight of *A. abdita* was negatively correlated with tissue Cu concentration (Table 10). This
308 suggests that tissue Cu concentrations affected *A. abdita*'s growth. Since water concentrations of Cu
309 and Zn were at typical background levels, the effects of Cu and Zn on *M. mercenaria* tissue weight and
310 *A. abdita* weight would be due to exposure via sediment ingestion. Furthermore, tissue Cu and Zn
311 concentrations were correlated with both sediment Cu and Zn concentrations, suggesting that Cu and Zn
312 simultaneously entered the organisms. Ingestion of Cu- and Zn-bound to organic matter in sediments

313 was thus a major exposure route. Several studies have demonstrated that metal exposure to clams and
314 amphipods via food and sediment ingestion was more important than pore water exposure (Eriksson and
315 Sundelin 2002, Labreche et al. 2002, Forbes et al. 1998).

316

317 **Summary and conclusions**

318 The present study showed that sediments collected from the St. Lucie estuarine system contained
319 Cu and Zn concentrations that exceeded both the Florida State sediment quality criteria and NOAA
320 SQuiRTs sediment values. The total concentration of DDTs in 3 out of the 7 sediments was also higher
321 than the NOAA SQuiRTs sediment values. *M. mercenaria* and *A. abdita* exposed to the St. Lucie
322 sediments resulted in Cu accumulation in *A. abdita* and Cu and Zn accumulation in *M. mercenaria*. The
323 present study also indicated that Cu and Zn exposures via sediment ingestion were most likely an
324 important route of exposure. However, there was no effect of the contaminated sediments on organism
325 survival.

326 Elevated Cu and Zn concentrations in the tissues and the effects on the weight of both *M.*
327 *mercenaria* and *A. abdita* raise concerns for the long-term viability of invertebrate populations, for
328 higher trophic organisms in the St. Lucie estuarine ecosystem and the population dynamics of the
329 ecosystem because these are only two organisms that are primary food resources in the St. Lucie system
330 which are exposed to the contaminated sediments for either part or their entire life cycles. The
331 significance of these results can only be fully realized when studies are conducted with other organisms
332 exposed to a greater number field-collected sediments from a larger number of sediment sites. These
333 studies gain in importance in lieu of the continued input of these metals into the environment.

334

335

336 **Acknowledgement**

337 We would like to thank Dr. Joan Browder-National Oceanic and Atmospheric Administration
338 (Miami, FL) for funding this research under contract WC133F-09-CQ-0006-“Ecological Studies of
339 Trophic Web Species and Potential Harmful Materials found in the St. Lucie Estuarine System” with
340 Florida International University. We are grateful to Indra Chacin Lares, Abraham Smith and Brandi
341 Echols in the Ecotoxicology and Risk Assessment Laboratory of Florida International University for
342 their assistance with conducting the study. This publication is SERC contribution # and ISE contribution
343 #.

344

345 **References**

- 346 Alva, A.K., Graham, J.H., Anderson, C.A. 1995. Soil-pH and copper effects on young Hamlin Orange
347 Trees. *Soil Science Society of America Journal* 59: 481-487
- 348 Ankley, G.T. 1991. Predicting the toxicity of bulk sediments to aquatic organisms with aqueous test
349 fractions: Pore water vs. Elutriate. *Environ. Toxtcol. Chem.* 10:1359-1366
- 350 Ankley, G.T., Di Toro, D.M., Hansen, D.J., Berry, W.J., 1996. Technical basis and proposal for deriving
351 sediment quality criteria for metals. *Environ. Toxtcol. Chem.* 15: 2056-2066.
- 352 Berry, W.J., Hansen, D.J., Mahony J.D., Robson, D.L., DiToro, D.M., Shipley B.P., Rogers, B.P.,
353 Corbin J.M., Boothman, W.S. 1996. Predicting the toxicity of metal-spiked laboratory sediments
354 using acid-volatile sulfide and interstitial water normalizations. *Environ. Toxtcol. Chem.* 15:2067–
355 2079.
- 356 Buchman, M.F. 1999. NOAA screening quick reference tables, NOAA HAZMAT report 99-1, Seattle
357 WA, coastal protection and restoration division, National Oceanic and Atmospheric Administration,
358 12 pages.

359 Carriger, J.F., Rand, G.M. Copper in saltwater ecosystems of South Florida: Use of probabilistic
360 aquatic risk assessment methodology and application of kriging methods to determine potential
361 systems at risk. *In press*.

362 Delfino, J.J., Coates, L.A., Davis, W.M., Garcia, K.L., Jacobs, M.W., Marincic, K.J., Signorella, L.L.
363 1991. Toxic Pollutants in Discharges, Ambient Waters, and Bottom Sediments, Volume I and II,
364 submitted to Florida Department of Environmental Regulation, Tallahassee, FL. University of
365 Florida; Gainesville, FL.

366 Dietrich, A.M., Gallagher, D.L., Klawiter, K.A. 2001. Inputs of copper-based crop protectants to coastal
367 creeks from pasticulture runoff. *J Amer Wat Resour Assoc* 37: 281-293.

368 Eriksson Wiklund, A., Sundelin, B. 2002. Bioavailability of metals to the amphipod *Monoporeia affinis*:
369 interactions with authigenic sulfides in urban brackish-water and freshwater sediments. *Environ.*
370 *Toxicol. Chem.* 21: 1219-1228.

371 Florida Department of Agriculture and Consumer Services (FDACS). 2010. Summary of Agricultural
372 Pesticide Use in Florida: 2007-2009, October 2010. Accessed May 25, 2011.
373 http://www.flaes.org/pdf/PUI_narrative_2010.pdf.

374 Florida Department of Environmental Protection (FDEP).1994. Florida Coastal Sediment Contaminants
375 Atlas, Tallahassee, FL: Office of the Secretary. 112 pp.

376 Florida Department of Environmental Protection (FDEP). 2003. Development and evaluation of
377 numerical sediment quality assessment guidelines for Florida inland waters. Report by MacDonald,
378 Environmental Sciences Ltd. Technical Report.

379 Forbes, T.L., Forbes, V.E., Giessing, A., Hansen, R., Kure. L.K. 1998. Relative role of pore water
380 versus ingested sediment in Bioavailability of organic contaminants in marine sediments. *Environ.*
381 *Toxicol. Chem.*17:2453–2462.

382 Haurert, D.E. 1988. Sediment characteristics and toxic substances in the St. Lucie estuary, Florida.
383 Technical publication 88-10. Environmental sciences Division, resource Planning Department,
384 South Florida Water Management District. 42pp.

385 He, Z.L., Zhang, M., Yang, X.E., Stoffella, P.J., 2006. Release behavior of copper and zinc from sandy
386 soils. *Soil Science Society of America Journal* 70, 1699-1707.

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

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

392 Hoang, T.C., Schuler, L.J., Rogevich, E.C., Bachman, P.M., Rand, G.M., Frakes, R.A. 2009a. Copper
393 Release, Speciation, and Toxicity Following Multiple Floodings of Copper Enriched Agriculture
394 Soils: Implications in Everglades Restoration. *Wat. Air. Soil Pollut.*199, 79-93.

395 Hoang, T.C., Schuler, L.J., Rand, G.M. 2009b. Effects of Copper in Flooded Florida Agricultural Soils
396 on *Hyalella azteca*. *Arch. Environ. Contam. Toxicol.* 56: 459-467.

397 Hoang, T.C., Pryor, R.L., Rand, G.M., Frakes, R.A. 2011. Bioaccumulation and toxicity of copper in
398 outdoor freshwater microcosms. *Ecotoxicol. Environ. Saf.* 74: 1011-1020.

399 Labrech, T.M.C., Dietrich, A.M., Gallagher D.L., Shepherd, N. 2002. Copper toxicity to larval
400 *Mercenaria mercenaria* (hard clam). *Environ. Toxicol. Chem.* 21:760-761.

401 Leslie, A.J., 1990. Aquatic Use of Copper-based Herbicides in Florida. Bureau of Aquatic Plant
402 Management, Florida Department of Natural Resources, Tallahassee, FL. 1e14.

403 Long, E.R., Morgan, L.G. 1990. The Potential for Biological Effects of Sediment-sorbed Contaminants
404 Tested in the National Status and Trends Program, Seattle, WA: National Oceanic and Atmospheric
405 Administration.

406 Long, E. R., MacDonald, D.D., Smith, S.L., Calder, F.D. 1995. Incidence of adverse biological effects
407 within ranges of chemical concentrations in marine and estuarine sediments. *Environ. Manag.* 19:
408 81-97.

409 MacDonald, D.D., Carr R.S., Calder, F.D., Long, E.R., Ingersoll, C.G. 1996. Development and
410 evaluation of sediment quality guidelines for Florida coastal waters. *Ecotoxicology* 5:253-278.

411 McGrath, J.A., Paquin, P.R., Di Toro, D.M. 2002. Use of the SEM and AVS approach in predicting
412 metal toxicity in sediments. HydroQual, Inc., Mahwah, NJ (USA). Published by the International
413 Council on Mining and Metals, ICMM.

414 Moore, P.A., Jr., T.C. Daniel, T.C., Gilmour, J.T., Shreve, B.R., Edwards, D.R., Wood, B.H. 1998.
415 Decreasing metal runoff from poultry litter with aluminum sulfate. *J. Environ. Qual.* 27:92-99.

416 Rainbow, P.S. 2002. Trace metal concentrations in aquatic invertebrates: why and so what? *Environ.*
417 *Pollut.* 497-507.

418 Reuther, W., Smith P.F. 1952. Iron chlorosis in Florida citrus soils in relation to certain soil
419 constituents. *Proc. Fla. State Hort. Soc.* 65: 62-69.

420 Rule, J.H. 1985. Chemical extractions of heavy metals in sediments as related to metal uptake by grass
421 shrimp (*Palaemonetes pugio*) and clam (*Mercenaria mercenaria*). *Arch. Environ. Contam. Toxicol.*
422 14: 749-757.

423 Schuler, L.J., Hoang, T.C., Rand, G.M. 2008. Aquatic risk assessment of copper in freshwater and
424 saltwater ecosystems of South Florida. *Ecotoxicology*, 17:642-659

425 Trefry, J.H., Trocine, R.P. 2011. Metals in sediments and clams from the Indian River Lagoon, Florida:
426 2006-7 versus 1992. *Florida Scient. 74: 43-62.*

427 Trocine, R.P., Trefry, J.H. 1993. Toxic substances survey for the Indian River Lagoon system. Final
428 report to St Johns River Water Management District. Available from St Johns River Water
429 Management District, Palatka, Fl.

430 Trocine, R.P., Trefry, J.H. 1996. Metal concentrations in sediment, water and clams from the Indian
431 River Lagoon, Florida. *Mar. Poll. Bull. 32: 754-759.*

432 U.S. Department of Agriculture. 2006. Agricultural chemical usage summary, 2005 fruit summary;
433 National Agricultural Statistical Service, Washington, DC.

434 U.S. Environmental Protection Agency. 1994. Methods for Assessing the Toxicity of Sediment-
435 associated Contaminants with Estuarine and Marine Amphipods EPA- 600/R-94/025. Office of
436 Research and Development U.S. Environmental Protection Agency, Narragansett, Rhode Island
437 02882.

438 U.S. Environmental Protection Agency. 1996a. Acid digestion of sediment, sludges and soils: EPA
439 method 3050B, SW-846 manual, Washington, DC

440 U.S. Environmental Protection Agency. 1996b. Ecological Effects Test Guidelines. Office of
441 Prevention, Pesticides and Toxic Substances, EPA 712-C-96-129, OPPTS 850.1730, Fish BCF.
442 Washington, DC.