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Copper Bioaccumulation and Depuration by Red Swamp Crayfish, *Procambarus clarkii*

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Environmental contamination of copper occurs from two major sources: weathering and land-drainage. Mine production of Cu was estimated to be 307 million metric tons sometime ago (Nriagu et al 1979). A considerable amount of copper is contributed by the effluents of metal finishing industries to sewage sludge. Organic wastes of industrial and agricultural origin enter aquatic ecosystems and Cu may exist in ionic form or be complexed to organic and inorganic ligands. Generally, crustaceans accumulate some metals (including Cu) directly proportional to the increase in bioavailability from water and food-chains (Rainbow et al 1990). Many decapod crustaceans can regulate their tissue and body burdens of heavy metals effectively (Rainbow & White 1989). Red swamp crayfish of Louisiana, Procambarus clarkii occur naturally in road-side ditches, bayous, swamps, marshes and the Atchafalaya Basin, and support a multi-million dollar industry. Approximately 60% are collected naturally and 40% from commercial ponds (Huner and Barr 1984). Since humans are the primary consumers of crayfish, heavy metals in natural waters present a great potential for bioaccumulation and biomagnification through any simple food-chain.

Most reports have focused on metal residues from field-collected crayfish (Dickson et al 1980; Madigosky et al 1991). We have previously reported that cadmium, lead and arsenic exposed crayfish accumulate these metals rapidly (Naqvi & Howell 1991; Naqvi et al 1990). Two major objectives of this study were: (1) the extent of Cu accumulation in sublethally exposed crayfish and, (2) possible depuration by different tissues.

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MATERIALS & METHODS

Adult crayfish (8-10 cm) were obtained from Ben Hur Experiment Station, Louisiana State University which is not accessible to the general public and is kept free of possible environmental contamination by humans. Crayfish were acclimatized in the laboratory conditions for 168 hrs. They were kept in several wooden-vats (1 meter X 2.5 m) supplied with 4 cm depth aged tap water, which causes low mortalities in controls and requires no aeration (Naqvi et al 1990). Dissolved oxygen and temperature were measured by a YSI electrode type oxygen meter (Model 541). Water hardness and pH are known to affect the toxicity of some pesticides, therefore the hardness was measured by a commercial kit (Lab-Aids, Inc) and pH by a pH meter.

Test solutions were prepared by diluting a 1% copper sulfate stock solution in distilled water to desired concentrations. In order to determine sublethal concentration of Cu (for crayfish), they were exposed to 0, 1, 5, and 10 mg/L copper sulfate solutions for 1 wk in aquaria (70 X 125 X 20 cm), filled to a 4 cm depth for 96 hrs. Ten crayfish per aquarium were exposed to copper sulfate (replicated thrice) and the LC50 value was determined by an IBM probit analysis program. Mortalities were recorded daily and water temperature, hardness and pH recorded at the beginning of each test.

Based on the LC50 for copper sulfate, 5 mg/L was considered as a sublethal level. Two hundred and forty two crayfish were exposed to 5 mg/L test solution of which 192 were used during this study. Additional 50 were exposed simultaneously to 5 mg/L copper sulfate for possible replacements if any mortality occurred during experimentation. Copper accumulation and depuration were determined by exposing 64 crayfish to 5 mg/L copper sulfate solution for 8 wks in groups of 8/aquarium.

Every two weeks, 8 crayfish were removed, washed with water and wrapped in aluminum foil prior to freezing. At the end of 8 wks (accumulation study) the remaining 32 were transferred to aged tap water to be used for depuration study. Similarly, 8 crayfish were removed from the water every 2 wks, washed and frozen. Both ‘accumulation’ and ‘depuration’ experiments were repeated thrice. All control crayfish were kept in aged

tap water throughout the study period and the same number removed during accumulation and depuration. Test solutions were changed each week. Crayfish were fed 'Top - Choice'® dogfood (54 g), once a week. Test solutions and food samples were analyzed for the presence of Cu by EPA approved atomic absorption spectrophotometric technique.

After complete thawing, each crayfish was separated into 3 regions (abdomen, exoskeleton, gills). Samples were digested by (3:1) concentrated nitric/perchloric acids under a special perchloric acid hood at 80-100°C until yellow-orange vapors turned to white color. All samples were analyzed on a Perkin-Elmer (Model 3030) AAS unit equipped with a built-in computer for calibration of standards and sample quantitation. Standard were purchased from a Chemical Supply vendor. Analyses were done at State Feed & Fertilizer Laboratory at Louisiana State University campus.

Means and standard deviations were calculated by a hand-held computer. Whole-body residues were computed by combining mean residue levels of each region. Multiple comparisons between two pairs were conducted by Tukey-type test.

RESULTS & DISCUSSION

The bioassay parameters for water were as follows: temperature 25±3°C, dissolved oxygen 5.5-6.5 mg/L, pH 7-7.8 and total hardness 30.32 mg/L. The solubility of copper sulfate in water is less than 100 mg/L (Lange's Handbook of Chemistry 1992). The actual amount of Cu in test solutions (5 mg/L) was 1.97 mg/L as determined by the AAS technique. Crayfish mortalities (mean) during the 96 hr bioassays were: 6% in 1 mg/L, 33% in 5 mg/L , 66% in 10 mg/L and 2% in control. Therefore, 5 mg/L was considered suitable for this study.

Only 5 crayfish died during the 3rd wk and two during the 6th wk of exposure which were replaced by previously exposed crayfish. Cu-accumulation by various tissues of crayfish in 8 wks is given in Table 1. All data presented in this table are 'mean' for triplicate samples. During the first 2 wks of exposure the maximum accumulation occurred in exoskeleton (41.5 mg/L) followed by gills (24.8 mg/L) and abdomen (21.8 mg/L). Tukey's multiple comparison test showed significant

difference between gills and abdomen, gills and exoskeleton ($P<0.05$) but not between exoskeleton and abdomen ($P>0.05$). Cu- accumulation was time-dependent, e.g., compared with the amount present at the end of 2nd wk, 35% accumulated after 4 wks, 57 % after 6 wks and 60% after 8 wks. Control crayfish maintained in aged tap water did not contain detectable Cu residues after 8 wks.

Table 1. Cu (mg/L) accumulation in crayfish (Procambarus clarkii) (N=96) exposed to 5 mg/L copper sulfate for 8 wks. Mean and standard deviations (SD) are given in parentheses.

Wk	Abdomen	Exoskeleton	Gills
2	21.80	41.47	24.77
	(0.70)	(1.34)	(0.62)
4	33.55	53.00	34.32
	(0.65)	(0.53)	(0.93)
% INCREASE*	35.00	22.00	28.00
6	50.53	65.12	84.25
	(1.17)	(0.53)	(0.90)
%INCREASE*	57.00	36.00	71.00
8	54.42	83.02	87.45
	(0.92)	(0.62)	(0.13)
%INCREASE*	60.00	50.00	72.00

* Compared with 2nd wk Cu accumulation.

Pattern of accumulation was gills>exoskeleton>abdomen; 87,83 and 54 mg/L Cu respectively, after 8 wks. Thirtynine percent Cu accumulated in gills (whole-body) followed by exoskeleton (37%) and abdomen (24%) at the end of 8 wks. In a similar study, Nott (1991) reported the highest amount of Cu accumulation in gills of marine crustaceans and concluded that it is readily absorbed by gills and transported to other organs via hemolymph. Its presence in exoskeleton of crayfish could have a survival value as a possible elimination mechanism through molting.

Different amounts of Cu in various tissues were also reported by Martin (1973), Roldan and Shivers (1987) which were due to the general accumulation pattern for several decapods. Carbonell and Tarzona (1994) concluded that different tissues of aquatic animals provide and/or synthesize non-exchangeable binding sites resulting in different accumulation during long exposures such as in the present study. In our earlier work (Naqvi et al 1990) this crayfish (Procambarus clarkii) accumulated only 2 mg/L arsenic after 8 wk exposure to 5 mg/L monosodium methanearsonate herbicide. In the present study 54-87 mg/L Cu accumulated in the same period. Perhaps crayfish utilize some Cu in hemocyanin for oxygen transport and are unaffected by higher amounts.

A considerable amount of Cu depuration (loss) occurred within the first 2 wks when crayfish were transferred from continuous exposure for 8 wks to uncontaminated freshwater (Table 2).

Table 2. Cu (mg/L) depuration in crayfish (Procambarus clarkii) (N=96) for 8 wks(initial exposure 5 mg/L copper sulfate). Mean and standard deviations (SD) are given in parentheses.

Wk	Abdomen	Exoskeleton	Gills
2	18.97	22.02	24.05
	(1.15)	(0.97)	(0.62)
%DECREASE*	65.00	73.00	72.00
4	14.76	13.88	21.27
	(0.80)	(0.71)	(1.31)
%DECREASE*	73.00	83.00	76.00
6	11.30	11.97	20.82
	(0.84)	(0.53)	(0.71)
%DECREASE*	79.00	86.00	76.00
8	8.71	11.80	19.10
	(0.47)	(0.55)	(1.04)
%DECREASE*	84.00	86.00	78.00

* Compared with 8th wk Cu accumulation given in Table 1

Depuration of Cu was time-dependent until the end of the experimental period. Copper in exoskeleton depurated from 83 in the 8th wk of accumulation to 22 mg/L at the end of 2nd wk of depuration period (73% decrease), gills from 87 to 24 (72% decrease) and abdomen from 54 to 19 (65%). We experienced similar rapid depuration of As, Cd and Pb during the first 2 wks of deputation in the same animal (Naqvi et al 1990; Naqvi and Howell 1993). Depuration of Cu in marine prawn, Penaeus monodon and brine shrimp, Artemia franciscana has been reported to be slow (Vogt and Quinitio 1994; Blust et al 1986). The latter investigators explained that more than 98% of Cu was transferred into the olive layer inside the cells of brine shrimp, forming high stability complexes and rendering the depuration process difficult and slow.

Based on our present work and earlier studies we conclude that crayfish has a great potential for rapid accumulation and depuration of Cu in fresh waters. if these animals from a contaminated area are consumed in large quantities they could cause adverse health consequences. We concur with Rainbow and White (1989) that certain decapods (amphipods, barnacles and crayfish) are not suitable for long-term monitoring of heavy metal contamination due to their rapid depuration capabilities. We feel that P. clarkii can however, be used satisfactorily for testing metal bioavailability of polluted fresh waters but not for long-term monitoring programs. We invite further research on this subject.

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