Bull. Environ. Contam. Toxicol. (1999) 63:797—804

Bulletin of Environmental Contamination and Toxicology

Toxicity and Differential Tissue Accumulation of Copper in the Tropical Freshwater Fish, *Prochilodus scrofa* (Prochilodontidae)

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Received: 2 July 1999/Accepted: 16 September 1999

Copper levels in natural unpolluted waters is as low as 0.5 to 1 µg L^{-1} (Moore and Ramamoorthy, 1984). However, industrial development has contributed to a continuous increase of copper in the aquatic environment. The toxic effect of copper on fish varies from one species to another and is strongly influenced by hardness, alkalinity, pH, temperature and dissolved oxygen in the water. Brazilian freshwaters are usually soft and ion-poor, having low buffer capability. Although copper limits for the protection of aquatic life (20 µgCu.L^{-1}) recommended by the U.S. EPA (1984) have been adopted by the Brazilian Environmental Bureau, copper levels in Brazilian rivers have increased. In the Mogi-Guacu river, located in the state of Sao Paulo (49°W; 23° S), Brazil, copper has varied from 5 to 50 µg L^{-1} which may cause serious risk to aquatic life. However, no investigation on fish contamination has so far been made.

This study determines the toxicity of copper in water (LC_{50}) for an important commercial autochthon river species, curimbatá *Prochilodus scrofa* and the differentiated accumulation of copper in fish tissues was examined after acute sublethal copper exposure.

MATERIALS AND METHODS

Juvenile *P. scrofa* (W = 15 - 25 g; L = 10 - 15 cm) were taken from the Hydrobiology and Aquaculture Station at the Furnas Hydroelectric Power Plant in Furnas, MG, Brazil at least one month prior to the experiments and maintained at 25 ± 1°C in tanks with a continuous dechlorinated water flow and aeration. The laboratory photoperiod was 12D:12L. Fish were fed with balanced fish food for this species provided by the Aquaculture Research and Training Center - CEPTA/IBAMA. Feeding was suspended 24 h before the experiments began.

The experimental design consisted of six nominal copper levels (16, 20, 25, 32, 40 and 51 µgCu.L^{-1}) in static systems with continuous aeration, constant temperature (25 ± 1 °C), pH (7,3 ± 0.1) and total hardness

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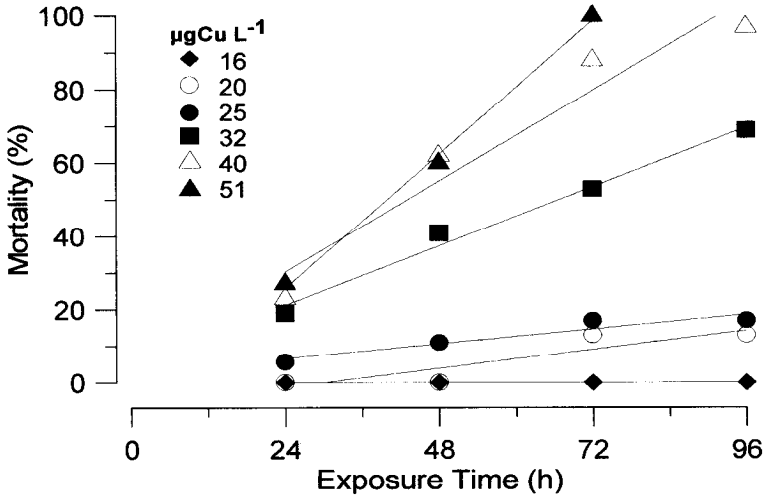

Figure 1. Mortality percentage of *P. scrofa* after 24, 48, 72 and 96 h exposure to different copper concentrations in water (µgCu.L^{-1}).

($CaCO_3$= 24 mg L^{-1}) for 96 h. Temperature, pH, hardness and copper levels were the same as the mean values found in the water of the Mogi Guacu river in Pirassununga, SP(CETESB 1989-1996). The copper agent was $CuS0_4.5H_20$ and the copper concentration in the water was determined at the beginning and end of each experiment by means of Atomic Absorption Spectrophotometry.

Experiments, with two replications, (30 fish in each copper concentration and control) were carried out in a 200-liter glass aquarium containing 10 fish each, never exceeding 1 g,fish.L^{-1}. No copper was put into the aquarium containing the control fish. After the introduction of copper into the water, the dead fish in each aquarium were removed and counted every 4 h and the behavior of the fish was observed during the experiments. The 96-h LC_{50} were calculated using the Trimmed Spearman-Karber method and the LC_{50} Program JSpear test (Hamilton et al., 1977) with 95% confidence limits.

Copper accumulation was investigated in fish exposed for 96 h to copper concentrations below (0, 20, 25 µgCu.L^{-1}) and equal 96-h LC^{50} (29 µgCu.L^{-1}). After exposure, the survivors were removed and anaesthetized with 0.01% benzocaine. Blood samples were taken from the caudal vein, centrifuged and the plasma was removed for copper analysis. Tissue samples were taken from the gill, liver, kidney, white muscle and intestine, washed in distilled water, weighed and dried at 60 °C until they reached a constant weight. Both plasma and tissue were digested in ultra pure

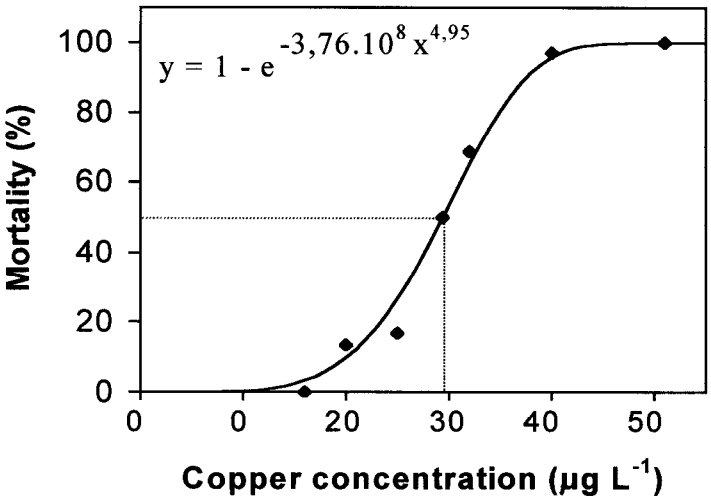

Figure 2. Mortality percentage curve of *P. scrofa* in response to copper concentration (µgCu.L^{-1}) showing the 96-h LC_{50}.

H_2SO_4 and H_2O (1:1) at 60°C and copper analyses were made using an Atomic Absorption Spectrophotometer.

The Kruskal-Wallis non-parametric test was used to determine the levels of significance of the copper accumulation data, and Dunn's multiple comparison test with a 95% significance level was applied for comparison of the ranks when significant differences occurred.

RESULTS AND DISCUSSION

The results of static bioassays are shown in Figures 1 and 2. Characteristic behavior of contact with the toxicant, such as an increase in motor activity and mucus production described by Marek (1991), was observed in *P. scrofa* immediately after copper was added to the water.

Fish mortality increased with increased concentrations of copper in the water and with exposure time (Fig. 1). However, the mortality of fish exposed to 20 and 25µgCu.L^{-1} did not show statistical significance ($p > 0.05$). Neither the controls nor the fish exposed to 16 µgCu.L^{-1} died during the experiments but all the *P.scrofa* exposed to 51 µgCu.L^{-1} died after 72 h of exposure. The 96-h LC_{50} of copper for *P. scrofa* were calculated at 29 ± 3 µgCu.L^{-1} and a sigmoid curve described the relationship between copper concentration and mortality (Fig. 2). The acute toxicity test does not show

Table 1. LC_{50} for copper in *P. scrofa* and several teleost species. The hardness, pH and temperature of experimental water conditions are given.

Species	W (g)	Hardness ($mgCaCO_3 L^{-}$)	pH	Temp. (ºC)	96-h LC_{50} ($\mu g\ L^{-1}$)	References
Salvelinus fontinalis	-	45.5	7.5	12	900	McKim and Benoit 1971
Salmo clarki	4,2	205,0	7.7	13.7	367	Chakoumakos et al. 1979
	3.2	70,0	8.5	13.7	186	
	9.7	18.0	8.0	13.7	36.8	
Pimephales promelas	1-2	360.0	7.5	25.0	1020	Pickering and Henderson 1966
	1-2	20.0	7.5	25.0	22	
Lepomis macrochirus	1-2	20.0	7.5	25.0	660	Pickering and Henderson 1966
Carassius auratus	1-2	20.0	7.5	25.0	36	Pickering and Henderson 1966
Poecilia reticulatus	0.15	20.0	7.5	25.0	36	Pickering and Henderson 1966
Oncorhynchus mykiss	2.88	25.0	4.7	14.5-17.5	66	Cusimano et al. 1986
	2.88	25.0	5.7	14.5-17.5	4.2	
	2.88	25.0	7.0	14.5-17.5	2.8	
Prochilodus scrofa	20	24.0	7.3	25.0	29	Present study

the effects of long-term exposure such as the effects on the growth rate and reproduction. It does however, provide important data about copper toxicity in soft and ion-poor waters and thus contributes to the development of water quality criteria for environmental monitoring.

Table 1 shows the 96-h LC_{50} for several teleost species and the physical and chemical characteristics of the water used in the experiments. The 96-h LC_{50} estimated for *P. scrofa* were similar to those found for *P. promelas, C. auratus* and *P. reticulatus* in water with similar hardness, pH and temperature (Pickering and Henderson 1966). Due to the copper complex with calcium carbonate and, consequently, the reduction of the availability of copper ion in water, water hardness reduces copper toxicity, as was found for *P. promelas* (Pickering and Henderson 1966) and *S. clarki* (Chakoumakos et al. 1979) (Table 1). *P. scrofa* seems to be more sensitive to copper than *P. promelas* and *S. clarki,* considering that copper toxicity is related to fish size. Small fish are more sensitive to copper (Chakoumakos et al. 1979) and high mortality rates were observed in *P. scrofa* exposed to copper (20 $\mu gCu.L^{-1}$) during the early stages of development (Takasusuki and Fernandes, *unpublished data).*

Accumulation of copper in *P. scrofa* after exposure from 0 to 29 $\mu gCu\ L^{-1}$ for 96 h was progressive and significantly higher than in the control fish, in the liver, intestine, kidneys and gills of fish exposed to 25 and 29 $\mu gCu.L^{-1}$ (Fig. 3).

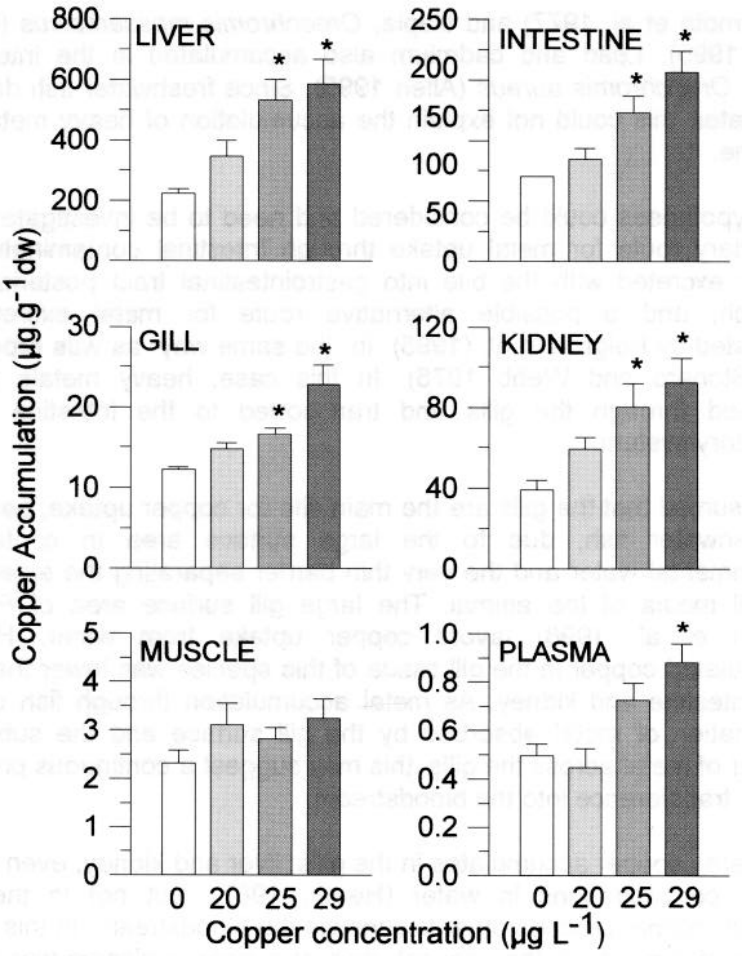

Figure 3. Accumulated copper in tissues and plasma of *P. scrofa* during exposure to different copper concentrations in water. The values are the mean ± ED. (*) indicate significant differences from the controls (0 µgCu L^{-1}).

No significant difference was found in copper accumulated in white muscle (Fig. 3). Copper accumulation in these organs has been reported in several fish species exposed to copper in water or in contaminated food (Yamamoto et al 1977, Handy, 1993, Pelgrom et al. 1995).

Copper accumulation was greatest in the liver when compared to other tissues, followed by the intestine and kidney. The liver and kidneys are known to play an important role in the detoxification and excretion of toxicants, which explains the high accumulation of copper in these organs. However, accumulated copper in the intestine, such as was found in *P. scrofa,* was reported in two other species, carp *Cyprinus carpio*

(Yamamoto et al. 1977) and tilapia, *Oreochromis mossambicus* (Pelgrom et al. 1995). Lead and cadmium also accumulated in the intestine of tilapia, *Oreochromis aureus* (Allen 1995). Since freshwater fish drink very little water, this could not explain the accumulation of heavy metal in the intestine.

Two hypotheses could be considered and need to be investigated, i.e., a secondary route for metal uptake through intestinal contamination from copper excreted with the bile into gastrointestinal tract posterior to the stomach, and a possible alternative route for metal excretion, as suggested by Pelgron et al. (1995) in the same way as was reported for rats (Stonard and Webb 1976). In this case, heavy metals may be absorbed through the gills and transported to the intestine via the circulatory system.

It is assumed that the gills are the main site for copper uptake, particularly in freshwater fish, due to the large surface area in contact with environmental water and the very thin barrier separating the external and internal media of the animal. The large gill surface area of *P. scrofa* (Mazon et al. 1998) favors copper uptake from water. However, accumulated copper in the gill tissue of this species was lower than in the liver, intestine and kidney. As metal accumulation through fish gills is a combination of metal absorbed by the gill surface and the subsequent transfer of metal across the gills, this may suggest a continuous process of copper transference into the bloodstream.

In general, copper accumulates in the gills, liver and kidney, even with low copper concentrations in water (Heath, 1987), but not in the blood, although copper reaches other tissues via the bloodstream. In this study, a significant increase in the concentration of copper in plasma was found in *P. scrofa* exposed to 29 $\mu gCu.L^{-1}$ (LC_{50} for this species) (Fig. 3) which may indicate a relationship between copper concentrations in water and the tolerance limit of the fish. It is accepted that metal uptake through the gills and, less intensively, through the body surface, is transported to the liver via the bloodstream, metabolized and then excreted through the bile. Increased levels of metals in water lead to the production of metal-binding proteins such as metallothioneins, which are stored in the hepatocytes bound to copper. Metal excess bind to the a-globulin in the liver, producing ceruloplasmin and is excreted through the kidney. When exposure to very high copper levels occurs and the liver's capacity to remove copper is exceeded, the more toxic types of copper (Cu^{2+}) may be transported through the bloodstream to other organs.

In this context, increased amounts of copper in the blood, such as found in *P. scrofa,* may reflect copper concentrations in the environment that exceed the fish's tolerance limit, as pointed out by Banerjee and

Homechaudhuri (1990) and Allen (1995) suggesting that increased amounts of metals in the plasma may be a strong indication of metal toxicity stress in the aquatic environment.

To summarize, these results indicate that *P. scrofa,* a representative fish species of Southeastern Brazil, can be a useful vertebrate bio-indicator organism of copper contamination in water. This species is highly sensitive to copper and its blood can be used to detect copper contamination. Accumulation of copper in tissues occurs at low copper concentrations, such as the limits for aquatic life protection (20 $\mu gCu.L^{-1}$) recommended by U.S. EPA (1984) and, although no statistical significance was found at this copper level, histopathological tissue changes were observed in *P. scrofa* (Mazon 1997).

Acknowledgments. This study was supported by grants from the Brazilian financing institutions FAPESP and CNPq. The authors thank the Hydrobiology and Aquaculture Station of the Furnas Hydroeletric Powerplant, Furnas, Minas Gerais, Brazil for supplying the fish and Jovino A. Souza of the Chemistry Department, Federal University of Sao Carlos, for the spectrophotometric atomic analyses. A.F.Mazon thanks CAPES for the award of scholarships.

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