Bull. Environ. Contam. Toxicol. (2003) 71:1265–1271

DOI: 10.1007/s00128-003-8523-8

Bulletin of Environmental Contamination and Toxicology

Accumulation of Copper and Cadmium in Small and Large Nile Tilapia *Oreochromis niloticus*

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Received: 7 May 2002/Accepted: 3 July 2003

Most heavy metals occur naturally in aquatic environments in trace amounts. Heavy metals are increasingly being released to the environment as a result of industrialization. Heavy metals are usually divided into two groups; the first includes Fe, Co, Zn, Cu which are essential for biological processes; the second includes Cd, Hg, Pb which is made up of metals with no established biological function.

Copper can be found as a trace metal in nearly all waters. However anthropogenic sources such as pollution from industry, agriculture and mining, may produce environmental concentrations that cause toxic effects in aquatic animals (Brungs et al.,1972; Erdem and Kargın, 1992). Cadmium ranks among the most toxic metals in the aquatic system, and it is a particular problem because it is highly toxic with a long biological half–life, and its toxicity is also cumulative at least in invertebrata and fish (Larson et al., 1985; Heath, 1987). Several effects of exposure to sublethal concentrations of copper and cadmium have been reported in fish. These include decreased survival, growth, and reproduction (Buckley et al., 1982; Hilmy et al., 1985).

The Nile tilapia, *O. niloticus* is an important food fish in many countries and is increasingly in many important in aquaculture. Relatively little information exists on the influence of body size on accumulation of metals in fish. It is a suitable model to use to study the accumulation of heavy metal in different tissues of fish.

Body size, which is closely related to fish growth, age and metabolism, has been shown to attribute most of the variations of heavy metal content in fish (Moriarty et al., 1984). Most of the variation in heavy metal concentrations in aquatic animals is dependent on body size (Schuhmacher et al., 1992). Several researchers have proposed that body size may influence the accumulation of heavy metals in invertebrata and fish (Schuhmacher et al., 1992; Hornung et al., 1993; Mastala et al., 1993; Swaileh and Adelung, 1995; Chong and Wang, 2001).

In the present study, copper and cadmium concentrations were determined in organs and tissues from *O. niloticus* of two size groups.

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MATERIALS AND METHODS

Two size groups of Nile tilapia, *O. niloticus,* were obtained from culturing pools and allowed to acclimate to conditions in the laboratory for one month, at 25 ± 1°C, which was also the temperature of the experimental conditions. The animals were fed with an artificial fish food. After this period, two size groups were separated as follows: the first group contained fishes of length 10-11 cm and the second group contained fishes of length 17-19 cm. Both weight and length were measured for each fish in each group.

A total 10 aquariums sized 40X100X40 cm in height were divided in two groups of 5 aquariums each. These were filled with 100 L. of test solutions or tap water. (The tap water used for the experiments had a pH 8.0 ± 0.2, dissolved oxygen 7.3 ± 0.4 mg/l, total hardness of 196.4 ± 2.3, and total alkalinity of 291.5 ± 2.6 $CaCO_3$ mg/L). Six fish were put in each aquarium. Four aquariums of the first group contained 0.1 and 1.0 mg Cu/L ($CuSO_4$ $5H_2O$) and 0.1 and 1.0 mg Cd/L ($CdSO_4$ $8H_2O$) solutions and four aquaria of the second group contained 0.1 and 1.0 mg Cu/L and 0.1 and 1.0 mg Cd/L solutions. The fifth aquarium of each group was used as a control. The test media were changed every two days to replenish either the Cu or Cd.

At the end of the 30 day exposure period, surviving six fish from each aquarium were removed and their liver, gill and muscle tissues were dissected separately. Tissues were dried at 105°C for 48 hours and digested with concentrated nitric acid and concentrated perchloric acid (2 : 1 v/v) at120°C for three hours (Liang et al., 1999). After dilution the volume was made up to 5 ml with distilled water and copper and cadmium concentrations of tissues were measured by atomic absorption spectrophotometry (Perkin Elmer AS 3100). The data were analyzed using both Correlation Coefficient and Student-Newman Keul's Test (SNK) to determine any differences among tissues and the effects of body size on copper and cadmium concentrations.

RESULTS AND DISCUSSION

The concentrations of copper and cadmium are expressed μg/g dry wt. in muscle, liver and gill of *O. niloticus* and given in tables 1 and 2, together with their means and standard error. Values shown with different letters indicate significant statistical differences $P< 0.01$ level.

In all tissues, accumulation of copper and cadmium showed a significant increase with increasing concentrations of copper and cadmium in the medium at the end of 30 days of exposure period. A tenfold increase in concentration caused 1.5-2 fold increase in tissue accumulation.

In both exposure concentrations, copper always being accumulated at highest levels in the tissue. Contrarily, cadmium accumulation was at lower levels at the

Table 1. Concentration of copper (μg Cu/g dw) in two size groups of *Oreochromis niloticus* after exposure for 30 days.

	Body length (cm) Range	Body weight (g) Range	Liver	Gill	Muscle
			$\overline{X} \pm S\overline{x}$ *	$\overline{X} \pm S\overline{x}$ *	$\overline{X} \pm S\overline{x}$ *
Control	10-11	18-19	11.24 ± 1.06 **ax**	6.29 ± 0.89 **ay**	1.48 ± 0.13 **az**
	17-19	85-90	12.31 ± 1.21 **ax**	7.02 ± 1.10 **ay**	1.49 ± 0.12 **az**
0.1 ppm Cu	10-11	18-19	497.3 ± 3.12 **ax**	16.15 ± 1.12 **ay**	4.23 ± 0.52 **az**
	17-19	85-90	589.5 ± 4.18 **bx**	27.52 ± 1.14 **by**	4.54 ± 0.65 **az**
1.0 ppm Cu	10-11	18-19	522.1 ± 2.89 **ax**	30.87 ± 1.84 **ay**	6.05 ± 0.73 **az**
	17-19	85-90	618.6 ± 4.71 **bx**	40.67 ± 1.45 **by**	6.20 ± 0.89 **az**

* = SNK : Letters x, y and z show differences among tissues; a and b between body size. Data shown with different letters are statistically significant at the P<0.01 level. $\overline{X} \pm S\overline{x}$: Mean ± Standard Error of the mean

Table 2. Concentration of cadmium (μg Cd/g dw) in two size groups of *Oreochromis niloticus* after exposure for 30 days.

	Body length (cm) Range	Body weight (g) Range	Liver	Gill	Muscle
			$\overline{X} \pm S\overline{x}$ *	$\overline{X} \pm S\overline{x}$ *	$\overline{X} \pm S\overline{x}$ *
Control	10-11	18-19	ND	ND	ND
	17-19	85-90	ND	ND	ND
0.1 ppm Cd	10-11	18-19	112.5 ± 2.17 **ax**	22.34 ± 1.32 **ay**	3.63 ± 0.52 **az**
	17-19	85-90	114.5 ± 2.14 **ax**	23.18 ± 1.24 **ay**	2.02 ± 0.25 **bz**
1.0 ppm Cd	10-11	18-19	276.1 ± 3.29 **ax**	31.42 ± 1.86 **ay**	4.23 ± 0.85 **az**
	17-19	85-90	274.9 ± 3.11 **ax**	32.26 ± 1.45 **ay**	2.50 ± 0.24 **bz**

* = SNK : Letters x, y and z show differences among tissues; a and b between body size. Data shown with different letters are statistically significant at the P<0.01 level. $\overline{X} \pm S\overline{x}$: Mean ± Standard Error of the mean ND : Not Detectable

end of 30 days exposure. Copper was probably stored in the tissue to form metallothionein and metalloenzymes complex species through the disulphide group present in the protein (McCarter and Roch, 1984; Schuhmacher, 1992). Copper granules in the cellular organelles may also be sequestered in the mitochondria of Trout, Plaice, Cod and Pike (Zaba and Harrs, 1978).

After 30 days of exposure to 0.1 or 1.0 mg Cu/L or 0.1 or 1.0 mg Cd/L, all fish survived. Anderson and Spear (1980) found that rainbow trout LC_{50} for copper did not change with body size.

In all concentrations tested, the distribution pattern of both copper and cadmium follows order; liver>gill>muscle. Studies carried out with various aquatic species have shown that the liver is the prime organ for metal accumulation and also plays an important role in storage, redistribution, detoxificiation or transformation of metals (Heath, 1987; Erdem and Kargın, 1992; Evans et al., 1993). The livers in fish have been considered suitable as bioindicator organs for contaminants. This is possibly attributed to the tendency of liver to accumulate pollutants of various kinds at higher levels from their environment (Galindo et al., 1986). The specific metabolic process and enzyme catalysis reactions taking place in liver involving Cu and Cd may also account for this behavior (Jaffar and Pervais, 1989). Fish exposed to cadmium and copper synthesize metal binding in the liver (Noel-Lambot et al., 1978). Thus, increased liver concentrations of copper and cadmium may be related to elevated metal-binding protein synthesis. In fish, the gills are responsible for the water flow and are exposed to a large aquatic environment, therefore, high levels of metals accumulate first in the gill tissues by absorption and adsorption (Heath, 1987). Muscle generally accumulates the lowest concentrations of metals during exposure for 30 day. Fresh water fish muscle is not considered as a metal accumulator (Legorbru et al., 1988; Kargın and Erdem, 1991).

Metal accumulation in tissues of fish is dependent upon environmental factors such as temperature, size of fish, age of fish and the processes of biotransformation and excretion (Heath, 1987; Carru et al., 1996; Kargın, 1996; Zhou et al., 2001). Body size has been shown to be critical factor in influencing metal concentration in fish (Cross et al., 1973; Vinikour et al., 1980; Law and Singh, 1991; Batty et al., 1996; Liang et al., 1999; Zhou et al., 2001).

The accumulation behavior of Cu and Cd in the liver, gill and muscle with size was examined in term of correlation coefficient (r). In liver of *O. niloticus,* good correlation coefficients in both 0.1 and 1.0 ppm concentrations for Cu (r=0.903 and r=0.901) were found, while in both concentrations of Cd no correlation was found with fish size, respectively. Similar correlations were found in the livers of *Lethrinus lentjan* (Al-Yousuf et al., 2000) and *Anguilla anguilla* (Batty et al., 1996). In the present study, liver accumulation of copper increased with the increase of body size in both media, possibly linked with the growth cycle of *O. niloticus*, for the synthesis of protein and enzymes.

A positive relationship was found between the body size and concentrations of Cu in the gill in *O. niloticus*. In gills in both 0.1 ppm and 1.0 ppm concentrations, copper (r=0.877 and r=0.612) levels increased with increasing fish size while in both concentrations of Cd, levels did not change with body size. Anderson and Spear (1980) found in the gills highly significant correlation between body size and copper concentrations in rainbow trout. In both media, copper accumulation in gill of fish increased with increasing body size.

Copper accumulation in muscle of *O. niloticus* was not significantly correlated with body size, while a negative correlation was found between the concentrations of cadmium in the muscle and body size. In both exposure concentrations, muscle of small fish was found to contain more cadmium than large ones. In this study, concentrations of Cd decreased significantly with body size in muscle of *O. niloticus* but concentrations of Cu remained constant in both media. Cross et al. (1973) indicated that the significant decrease in metal concentrations with body size in muscle of fish could be due to compositional changes in muscle, a decreased intake of metals in the diet of older fish, or dilution by growth.

The relationship between metal accumulation and body size appears to differ depending on the species, metals and tissues studied. Law and Singh (1991) found that an increase in the concentration of Cu with increasing body size of *Plotosus anguillaris*, while Arnac and Lassus (1984) found that a decrease in the concentration of Cu with increasing body size of *Osmerus mordax* and Vinikour et al (1980) found Cu concentrations to be independent of *Rutilus rutilus* size. Our result show that Cu has a positive relationship with size while Cd has a negative or independent relationship with size of *O. niloticus*. The difference in metal content between small and large animals may be due to differences in their metabolic activity and thus metal metabolism (Williamson, 1980).

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