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Mineral Composition and Cadmium Accumulation in *Oreochromis mossambicus* Exposed to Waterborne Cadmium

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Exposure of fish to cadmium is known to cause disturbed osmo-ionic regulation (Larsson *et al.* 1981, Giles 1984) and skeletal anomalies (Muramoto 1981). Exposure of the African freshwater cichlid *Oreochromis mossambicus* (tilapia) to sublethal concentrations of waterborne cadmium (10 $\mu g.l^{-1}$) caused significant hypocalcemia on day 2 and 4, followed by a complete recovery on day 14 and 35 (Pratap *et al.* 1989). Cadmium-induced hypocalcemia has been ascribed to the inhibition of calcium uptake mechanisms in the gills of fish (Verbost *et al.* 1987). The branchial Ca^{2+}-ATPase activity accounting for most of the calcium uptake in fish is extremely sensitive to cadmium. Although the mechanism involved in the recovery of cadmium exposed tilapia is not well understood, recovery from hypocalcemia could be achieved by restoration of the uptake rate across the gills, or by mobilization of calcium from the bone. Demineralization of the bone has been shown in carp exposed to cadmium. It was suggested that this mechanism may be involved in the recovery from hypocalcemia (Koyama and Itazawa 1977, Muramoto 1981). Moss (1962) observed mobilization of calcium and phosphate ions from acellular bone of tilapia, while Urasa *et al.* (1985) found selective mobilization of phosphate from the opercular bone of tilapia fed phosphate-free diet. Therefore, this study investigates the mineral composition of the bony tissues such as operculum, scales, vertebrae and tailfin bone periodically during a 35 days exposure to cadmium. The skeletal anomalies observed in fish exposed to cadmium have been attributed to the loss of minerals from the skeletal tissue (Muramoto 1981). In carp exposed to dietary or waterborne cadmium, the calcium and phosphate contents of the bone decreased, while cadmium accumulated in the bone (Koyama and Itazawa 1977, Muramoto 1981). Van Coillie and Rosseau (1974) have also shown that the mineral composition of the scales was related to the inorganic cations in the aquatic environment, with elevated concentrations of trace metals from polluted sites. Likewise, in the Atlantic salmon, brown trout and mummichogs, the zinc concentration of the scales was directly proportional to the zinc levels in the environment (Abdullah *et al.* 1976, O'Grady and Abdullah 1985, Sauer and Watabe 1984). In this study, accumulation of cadmium in the bony tissues was also investigated. The objective was to determine whether cadmium concentration in the bony tissues was invariant or changed with exposure time, and if this parameter could be used to assess the level of cadmium contamination in natural freshwater environments.

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MATERIALS AND METHODS

Laboratory reared stock of freshwater male *Oreochromis mossambicus* of 16.2 ± 4.8 g body weight were used in this study. Fish were maintained in 100L aquaria with continuous aeration and circulating filtered water (360 $l.hr^{-1}$, Eheim pumps 1021) at 28°C on a daily 12 hr photoperiod. Water pH ranged from 7.2 to 7.4. Fish were divided into two groups of which one was exposed to waterborne cadmium and the other served as controls. All fish were acclimatized for two weeks in experimental tanks and fed Tetramin tropical fish food. The aquarium water was changed twice a week. The concentration of electrolytes ($mmol.l^{-1}$) of the water in the aquaria was Ca^{2+} 0.81, Mg^{2+} 0.27, Na^{+} 3.8, K^{+} 0.18, and P_i 0.08. Experimental fish were exposed to 10 $\mu g.Cd.l^{-1}$. Cadmium was administered to the water in the aquaria from a stock solution of 1000 $\mu g.Cd.l^{-1}$ ($Cd(NO_3)_2$, RCB, Bruxelles). The cadmium concentration in all the tanks was monitored daily using a Video 11 Atomic Absorption Spectrophotometer (AAS Thermo Jarell Ash, U.S.A.) and adjusted if necessary. Fish were sampled after 2, 4, 14 and 35 days. The opercular bone, the tailfin bone, vertebrae and the scales from anterior-dorsal part of the body were collected to determine the mineral contents. Also the gills, liver and kidneys were dissected to determine the cadmium concentrations. All bony and soft tissues were dried overnight at 70°C and dry weights determined to the nearest 0.01 mg. The samples were individually digested in teflon beakers with 1:1 mixture of concentrated nitric acid and perchloric acid. The resultant clear solution was diluted with double distilled water. Calcium, magnesium and phosphate concentrations of bony tissues were analysed by Inductively Coupled Plasma Atomic Emission Spectrometer (Plasma IL 200, Thermo Electron, U.S.A.). Sodium and potassium were measured by flame-photometer (Model IV, Autoanalyzer, Technicon). Cadmium was measured in a flameless Video 11 AAS (Thermo Jarrell Ash, USA) at 228.8 nm, fitted with a Furnace Aerosol Sampling Technique with Automatic Calibration (IL FASTAC II™) and Furnace Atomizer Model IL 655. The data were subjected to analysis of variance (ANOVA) and statistical significance set at the 5% level.

RESULTS AND DISCUSSION

During the 35 days exposure of fish to waterborne cadmium, there was no mortality and overt symptoms of cadmium poisoning or other morphological anomalies of the body were not observed. The mean concentration of calcium, phosphate, magnesium, sodium and potassium of the operculum, scales and the tailfin bone are given in table 1. Since none of the control values for 2, 4, 14 and 35 days were significantly different from each other, all the control data were pooled for statistical analysis and expressed as mean ± SD. After 2, 4, 14 and 35 days the mineral composition of the operculum, scales, and tailfin bone (table 1); and the calcium and phosphate content of the vertebrae (table 2), of fish exposed to waterborne cadmium were not significantly different from that of the control group. In the opercular bone, the concentrations of calcium and phosphate were significantly greater ($p<0.001$) than those in the scales, vertebrae and tailfin bone. For each cadmium exposed fish the Ca/PO_4 ratio of the operculum (1.3), scales (1.2), vertebrae (1.4)

Table 1. Mineral composition (mmol.g^{-1} dry weight) of the operculum, scales and tailfin bone of *Oreochromis mossambicus* exposed to 10 µg.Cd.l^{-1} for 2, 4, 14 and 35 days. Values are expressed as means ± SD, N = number of fish.

Tissue	N	Days	Calcium	Phosphate	Magnesium	Sodium	Potassium
	18	0	6.52 ± 0.59	4.98 ± 0.38	0.18 ± 0.01	0.25 ± 0.01	0.03 ± 0.004
Oper-	7	2	6.59 ± 0.43	5.02 ± 0.36	0.17 ± 0.01	0.26 ± 0.02	0.03 ± 0.002
culum	6	4	6.70 ± 0.83	5.05 ± 0.39	0.17 ± 0.01	0.26 ± 0.01	0.02 ± 0.003
	9	14	6.76 ± 0.58	5.23 ± 0.28	0.18 ± 0.01	0.25 ± 0.01	0.02 ± 0.004
	5	35	6.68 ± 0.71	5.07 ± 0.12	0.17 ± 0.01	0.25 ± 0.02	0.03 ± 0.007
	18	0	3.23 ± 0.33	2.70 ± 0.26	0.10 ± 0.01	0.20 ± 0.03	0.04 ± 0.009
	7	2	3.34 ± 0.27	2.70 ± 0.17	0.10 ± 0.01	0.19 ± 0.03	0.04 ± 0.014
Scales	6	4	3.21 ± 0.37	2.61 ± 0.27	0.10 ± 0.01	0.20 ± 0.02	0.03 ± 0.006
	9	14	3.32 ± 0.31	2.76 ± 0.26	0.11 ± 0.02	0.22 ± 0.01	0.03 ± 0.006
	5	35	3.27 ± 0.29	2.71 ± 0.19	0.11 ± 0.01	0.20 ± 0.01	0.04 ± 0.009
	18	0	3.41 ± 0.18	2.83 ± 0.16	0.13 ± 0.01	0.31 ± 0.05	0.12 ± 0.018
	7	2	3.25 ± 0.21	2.52 ± 0.14	0.12 ± 0.01	0.31 ± 0.04	0.12 ± 0.014
Tailfin	6	4	3.33 ± 0.31	2.67 ± 0.29	0.13 ± 0.01	0.30 ± 0.06	0.12 ± 0.019
	9	14	3.52 ± 0.11	2.82 ± 0.09	0.12 ± 0.01	0.31 ± 0.03	0.11 ± 0.019
	5	35	3.42 ± 0.27	2.71 ± 0.23	0.12 ± 0.01	0.31 ± 0.06	0.12 ± 0.018

Table 2. Calcium and Phosphate content (mmol.g.$^{-1}$ dry weight) and Ca/PO_4 ratio in the vertebrae of tilapia (*Oreochromis mossambicus*) exposed to 10 µg.Cd.l^{-1} for 2, 4, 14 and 35 days. Values are expressed as means ± SD, N = number of fish.

Days	N	Calcium	Phosphate	Ca : PO_4
0	18	3.59 ± 0.29	2.44 ± 0.22	1.47
2	7	3.41 ± 0.12	2.31 ± 0.19	1.47
4	6	3.64 ± 0.41	2.49 ± 0.22	1.46
14	9	3.84 ± 0.31	2.62 ± 0.18	1.47
35	5	3.58 ± 0.21	2.56 ± 0.11	1.40

and tailfin bone (1.2) were not significantly different from the ratios of the control group (tables 1 and 2). Fish exposure to waterborne cadmium has been shown to cause osmo-ionic disturbances (Larsson *et al.* 1981, Giles 1984), and this could influence the mineralization of skeletal tissues that serve as a major reservoir of Ca^{2+} and PO_4 ions. In this study, exposure of tilapia to waterborne cadmium had no effect on the mineral composition of the scales, operculum and tailfin bone. This implies that exposure of tilapia to low cadmium concentration (10 µg.l^{-1}) does not cause notable mobilization of minerals from the bone. Thus, it is unlikely that the recovery from cadmium induced hypocalcemia observed in tilapia exposed to the

same concentration (Pratap *et al.* 1989) can be explained by dissolution of calcium from the bone. In similar studies, cadmium induced hypocalcemia was observed in *Girella punctata* exposed to 25, 125, and 250 μg Cd l^{-1} for 4, 8, 16 and 24 weeks (Kuroshima 1987), and flounders exposed to 500 μg.Cd.l^{-1} for 9 weeks (Larsson *et al.* 1981) but both species showed no alteration in the calcium and phosphate content of the skeletal bone. This suggests that no demineralization had occurred. These observations indicate that the bone mineral content is not affected by sublethal concentrations of cadmium, in contrast with some data from the literature (Koyama and Itazawa 177, Muramoto 1981). Bone demineralization and reduced calcium/phosphate ratios have been shown in carp exposed to cadmium. It was therefore suggested that this mechanism may be involved in the recovery of cadmium-induced hypocalcemia (Koyama and Itazawa 1977, Muramoto 1981). The different response of bone to cadmium in carp and tilapia might be due to differences between cellular and acellular bone. Carp has cellular bone whereas the bone of tilapia is acellular. In acellular bone the mobilization of calcium, phosphate or other ions for metabolic purposes, may not occur as readily as in cellular bone (Moss 1965). When operculum fractured tilapia and mummichogs (with acellular bone) and sticklebacks with (cellular bone) were maintained in acalcemic water, only fish with cellular bone were able to mobilize calcium and phosphate ions (Moss 1962). Furthermore, selective mobilization of phosphate from the opercular bone and scales has been shown in tilapia fed phosphate-free diet while in fish fed normal diet the concentration of calcium and phosphate in the skeletal bone remained unchanged (Urasa *et al.* 1985). On the other hand, Flik *et al.* (1986) observed that bone calcium loss occurred in tilapia adapted to low calcium water and Wendelaar Bonga and Dederen (1986) have also shown a significant loss of calcium from the bone of tilapia in acid water. Thus, the possibility of calcium mobilization from the acellular bone cannot be excluded.

The reason that calcium resorption from the bone did not occur in this experiment, in contrast to the literature mentioned above, may be due to the low cadmium concentrations used in this experiment. How then do we explain the previous observation that cadmium induced significant hypocalcemia in tilapia is restored to normal after 4 days (Pratap *et al.* 1989)? It is possible that in a condition of high calcium demand (hypocalcemia), an increase in the branchial Ca^{2+} uptake system provides sufficient supply of calcium for the fish. However, Verbost *et al.* (1987) have shown that Ca^{2+} uptake mechanisms in fish are very sensitive to cadmium, and effectively block the calcium influx from the water.

Another possibility is that the disappearance of hypocalcemia is connected with the reduced need for calcium in fish exposed to cadmium. Results on whole body calcium uptake, Wendelaar Bonga *et al.* (unpublished data) observed that there was no net increase in calcium uptake in the cadmium exposed tilapia, in contrast with the control fish, which showed growth related calcium accumulation. Also the amount of calcium required to restore normal levels of calcium in tilapia (Pratap *et al.* 1989) was small to measure in bone and may not lead to noticeable changes in the bone mineral contents or the fish may not resorb calcium from the bone when exposed to low cadmium concentrations. This probably explains why loss of

calcium in the bony tissues was not observed in this study. The sodium, potassium and magnesium contents of the scales, operculum and tailfin bone were not affected by cadmium.

Cadmium accumulation in the scales was found to increase gradually during the 35 days exposure period. A significant increase in the cadmium concentrations of the scales on 2, 4, 14 and 35 days by 0.48 μg ($p<0.001$); 0.77 μg ($p<0.001$); 1.2 μg ($p<0.001$) and 1.79 μg ($p<0.001$) per gram dry weight was observed in fish exposed to waterborne cadmium (fig. 1). A linear relationship was observed between cadmium accumulation in the scales against the exposure period, with a correlation coefficient of $r^2 = 0.87$ and a regression line of $y = 0.752 + 4.436x$. After 2 days, the cadmium content of the opercular bone had significantly increased by 0.166 $\mu g.g.^{-1}$ dry weight ($p<0.001$) compared to the control fish. On further exposure the cadmium concentration of the opercular bone after 4, 14 and 35 days had returned to normal and was not significantly different from the controls. The cadmium concentration in the tailfin bone of the experimental fish was consistent and not different from the controls. In the gills, liver and kidneys the pattern of cadmium accumulation was not uniform during the exposure period (fig. 2). After 2 days, the highest cadmium concentration was observed in the kidneys followed by the gills and liver. The highest cadmium levels in the gills and liver were observed after 4 days. On further exposure for 14 and 35 days, a marked decline in the cadmium contents of the gills and kidneys was observed. However, the liver cadmium concentration was relatively high after 35 days of exposure (fig. 2). In the control fish very little cadmium (≤ 0.45 $\mu g.g.^{-1}$ dry wt) was detected in the gills, liver and kidneys.

Various studies have indicated that the metal content of the scales reflects the metal contamination of the water (Van Coillie and Rousseau 1974). Abdullah *et al.* (1976) found a strong correlation between Mn and Zn concentrations in salmonid scales and that of their freshwater environment. Similarly in brown trout a proportional relationship was observed between environmental and tissue zinc levels (O'Grady and Abdullah 1985). Likewise when mummichogs were exposed to different zinc concentrations, the average zinc content of the scales as well as the whole body was proportionally related to Zn concentrations in the water (Sauer and Watabe 1984). Other investigators have demonstrated that the addition of elements such as strontium and manganese to the food and/or water of growing salmons resulted in elevated concentrations of these metals in their scales and bones (Behrens-Yamada and Mulligan 1982, 1987).

Cadmium uptake, accumulation and elimination in the gills, liver and kidneys respectively have been reported for teleosts (Coombs 1979). A similar pattern was found in this study on tilapia. After 2 days, the highest cadmium accumulation was found in the kidneys and relatively low concentration in the liver and the gills. This suggests that some cadmium was probably bound to the mucus on the apical side of the gills and subsequently released to the water, while some cadmium that may have entered the gills was transported via the blood to the kidneys or the liver. Mount and Stephan (1967) observed substantial accumulation of cadmium in the gills, liver and

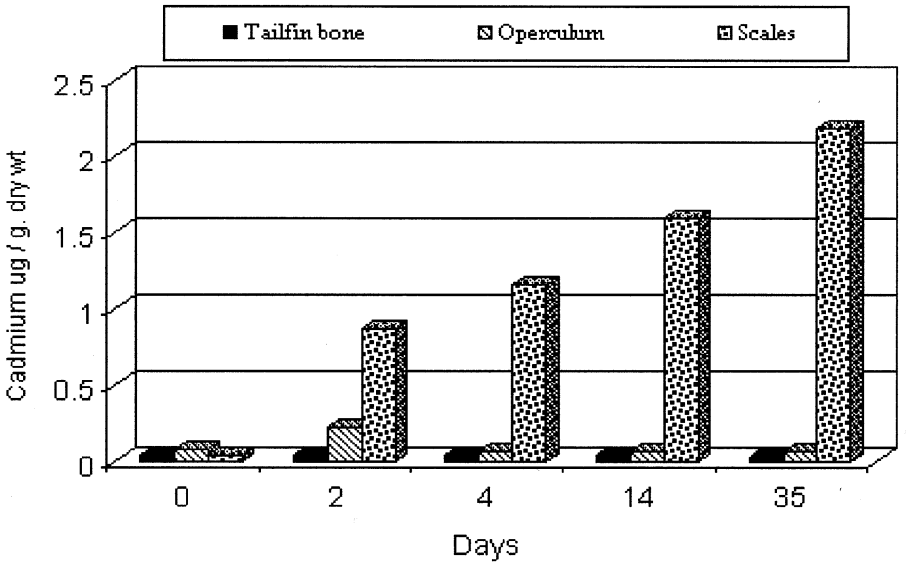

Figure 1. Cadmium levels (µg.g^{-1} dry weight) in tailfin bone, operculum and scales of *Oreochromis mossambicus* exposed to 10 µg.Cd.l^{-1} for 2, 4, 14 and 35 days.

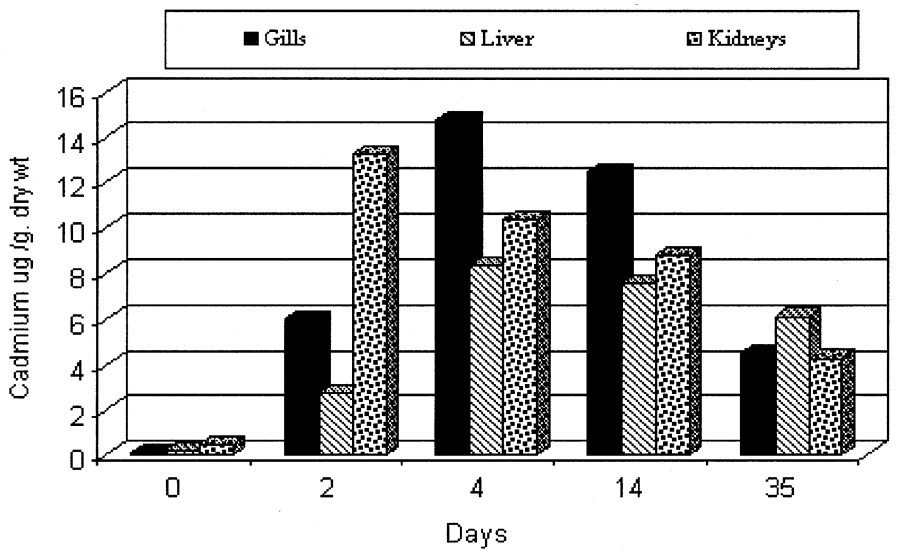

Figure 2. Cadmium levels (µg. g^{-1} dry weight) in the gills, liver and kidneys of *Oreochromis mossambicus* exposed to 10 µg.Cd.l^{-1} for 2, 4, 14 and 35 days.

kidneys of bluegills and bullheads exposed to lethal and sublethal concentrations of waterborne cadmium. Various studies on fish exposed to cadmium have shown that, although the highest metal concentration was found in the gills, liver and kidneys, the pattern of accumulation tended to vary between different species (Coombs 1979). In the present study, the cadmium content of the gills and kidneys decreased after prolonged exposure (35 days). However the liver cadmium levels remained elevated. Similarly in catfish given intragastric dose of cadmium (0.2 ppm), the gills contained high cadmium levels within one hour, followed by a decrease after 21 days, whereas cadmium levels of the liver and kidneys continued to increase during the exposure period (Rowe and Massaro 1974). Karlsson and Runn (1985) observed high activity of ^{109}Cd in the gills, liver and kidneys of zebrafish exposed to cadmium for 10 days which was followed by a reduction during the post-exposure period of 21, 42 and 84 days. Also high concentrations of cadmium were found in the gills, liver and kidneys of *Girella punctata* exposed to different concentrations of waterborne cadmium for 24 weeks. After the end of exposure, cadmium in the gills decreased while an increase in the liver was evident (Kuroshima 1987). In rainbow trout a progressive and dose-dependent accumulation of cadmium was found in the liver during the 30 weeks exposure period (Haux and Larsson 1984). The results obtained in this study on tilapia are compatible with the literature mentioned above. Although cadmium concentration in the gills, liver and kidneys of tilapia were relatively high after 35 days, previous studies with a similar cadmium exposure protocol have shown a complete physiological recovery in tilapia (Pratap *et al.* 1989, Pratap and Wendelaar Bonga 1993). This recovery process may be related to the induction of metal binding proteins like metallothioneins found in the gills, liver and kidneys of various species of teleost (Klaverkamp *et al.* 1984). Similar metallothionein like proteins have been shown in the gills, liver and kidneys of tilapia exposed to the same cadmium concentration as in this study (Fu *et al.* 1990). Therefore, the cadmium that was found in the gills, liver and kidneys after 35 days, was probably bound to metallothionein like proteins. In conclusion, this study shows that low waterborne cadmium concentration does not affect the mineral composition of the bone in tilapia. Since cadmium is a non-essential metal, its occurrence in the bony tissues such as scales indicates cadmium contamination of the environment. Moreover, during the 35 days exposure period, the scales of tilapia were found to gradually accumulate cadmium and therefore it may be a good practical approach to use the scales as an environmental indicator of cadmium pollution.

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