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Preliminary Laboratory Investigation of the Bioconcentration of Zinc and Iron in Selected Tissues of the Banded Tilapia, *Tilapia sparrmanii* (Cichlidae)

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Most metals are essential for the normal functioning of the physiological processes in fish (Khalaf *et al.* 1985). Abnormally high concentrations of metals can, however, cause death while sublethal concentrations may lead to cellular and histological changes. Exposure of fish to zinc can result in gill damage and various behavioral, physiological and biochemical changes (Holcombe *et al.* 1976; Heath 1987). Iron toxicity may be associated with the precipitation of the metal on the gills which may lead to suffocation (Muniz and Leiverstad 1980). Smith and Sykora (1976) conclude that the maximum range of 0.97 to 1.27 mg/L iron would result in successful hatching, survival and growth of coho salmon, *Oncorhynchus kisutch*. The main routes of accumulation of metals by fish is via the gills, skin and food. Studies have shown that zinc accumulates in the blood, skin, muscular, liver, bone, gill, gut and gonadal tissue of fish. If fish are exposed to iron, this metal will accumulate mainly in the gut, blood, gills, liver and milt (Holcombe *et al.* 1976; Hilmy *et al.* 1987; Grobler-van Heerden *et al.* 1991).

In South Africa high concentrations of metals are present in both treated and untreated industrial and mining effluent. Zinc and iron are important constituents of industrial and mining effluent that may reach the aquatic environment. The present study was initiated to investigate the bioconcentration of zinc and iron in selected tissues of the banded tilapia, *Tilapia sparrmanii*, which is distrubuted widely in Southern Africa.

MATERIALS AND METHODS

Adult *T. sparrmanii* were collected from ponds at the Lydenburg Provincial Hatchery, South Africa. The fish were maintained in the laboratory for a minimum of five weeks (acclimation period) in 1,000L aquaria supplied with well aerated, continuous-flowing well water. The acclimation period allowed superficial wounds to heal and voluntary feeding to commence. The temperature during this toxicological study was kept at 23.0 ± 0.5°C (average summer surface water temperature on the Witwatersrand). The physicochemical characteristics of the water were: pH, 7.0; conductivity (at 25°C) 164.0 us cm^{-1}; alkalinity ($CaCO_3$) 520 mg L^{-1}; hardness ($CaCO_3$), 61.0 mg L^{-1}. A photoperiod of 12hr darkness and 12hr light was maintained in the laboratory.

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After acclimation, fish for each experiment were selected randomly and exposed to sublethal concentrations (Table 1) of zinc chloride or iron chloride for 72 hr (acute) and for four wk (chronic) in continuous flow bioassays. For each test the fish were exposed to either zinc or iron in a continuous flow, recirculating system. The system consisted of a reservoir tank (100 L) which supplied well water (47 L h^{-1}) to each of eight aquaria (60 L). The overflow water was caught in a recipient tank (100 L) and pumped through a swimming pool filter back to the reservoir. Each aquarium was supplied with an airstone and the water temperature (23.0 ± 0.5°C) was regulated with a heater in the recipient tank. This experimental design, therefore, allowed the exposure of 32 fish (four individuals per tank) to one test concentration of zinc or iron (Table 1) at a time. During the acclimation and the chronic exposure periods, the tilapia were fed (at 10H00; 3% of live body weight) commercially available trout pellets every other day. The fish were not fed during the acute exposure tests.

After the exposure period, twenty fish from each test concentration and control were measured, weighed (Table 1) and tissue samples (axial muscle, liver, gonads, heart and brain) removed for analysis. After dissection one gram of tissue was weighed into a 100 mL Erlenmeyer flask to which 10 mL concentrated nitric acid and 5 mL concentrated perchloric acid were added. The acid digestion was performed on a hot plate (200-250°C) for at least four hours during which total digestion and clearing of the samples were achieved. Each of the digested samples was then filtered using a millipore 6 um paper filter and a vacuum filter system (Van Loon 1980). After filtration, the filter system was rinsed with double distilled water to remove all traces of dissolved metal from the filter paper and the filter system. Each sample was then made up to 50 mL with double distilled water and stored in clean storage bottles for the analysis of the metals. Due to the small sample weight, the heart, gonads and brain samples were pooled to achieve the required one gram wet tissue sample weight.

The total metal concentration (dissolved plus suspended) in the water was determined by adding 10 mL concentrated nitric acid, 5 mL concentrated perchloric acid and 20 mL of well mixed water into a 100 mL Erlenmeyer flask, and then evaporating the mixture to 2 to 5 mL on a hot plate until it cleared. Each sample was then made up to 20 mL with double distilled water and stored as described previously. All glassware used in the experiment was washed with soap, rinsed in double distilled water, washed with acid (1 M HCl) and rinsed again in double distilled water (Giesy and Wiener 1977).

Samples of tissue and water were analyzed with a Varian AA-835 atomic absorption spectrophotometer (detection limits: Zn, 0.002 ug mL^{-1}; Fe, 0.006 ug mL^{-1}). Analytical standards for these metals were prepared from stock solutions of Holpro chemicals. No tissue reference standards were, however, available. The metal concentrations in the water are expressed as ug L^{-1} while the metal concentrations in the tissues were recalculated and expressed as ug metal per g wet tissue weight. Bioconcentration factors were calculated for the metals and were defined by the ratio of ug metal per g tissue to ug metal per g water. Statistical analysis was performed on paired groups using the Student's t-test. The significance level was taken as $P < 0.05$.

Table 1. Test conditions for *Tilapia sparrmanii* exposed to zinc chloride or iron chloride for either 72 hours or four weeks. The volumes in brackets indicate fish mortality.

Test groups and initial concentrations of metal chloride ($Mg\ L^{-1}$)	Exposure Time	Fish weight (g) mean±SE	Fish length (mm) mean±SE	N	Concentration of metal at the end of test ($mg\ L^{-1}$)
Exposure to zinc chloride					
A (0.0)	control	83.8±6.6	172.4±4.3	20	0.23
B (9.8)	acute 72hr	87.5±7.3	168.8±4.2	20(1)	0.40
C (49.0)	acute 72hr	79.3±6.4	162.6±4.0	20	1.30
D (98.0)	acute 72hr	70.5±9.0	156.9±6.3	20(7)	1.40
E (147.0)	acute 72hr	64.0±4.2	153.7±2.8	20(12)	1.50
F (98.0)	chronic 4 weeks	69.4±5.0	165.0±4.3	20(12)	1.40
Exposure to iron chloride					
A (0.0)	control	83.8±6.6	172.4±4.3	20	0.50
B (8.8)	acute 72hr	72.3±2.7	159.1±1.9	20	1.80
C (44.0)	acute 72hr	88.1±6.8	167.7±4.1	20	9.60
D (88.0)	acute 72hr	61.8±3.1	152.6±2.3	20(2)	18.60
E (132.0)	acute 72hr	83.9±6.2	166.2±2.3	20(6)	29.20
F (88.0)	chronic 4 weeks	79.1±5.9	168.0±4.0	20(12)	18.60

RESULTS AND DISCUSSION

Detectable levels of zinc and iron were found in the muscle, liver, gonads, heart and brain tissue of *T. sparrmanii* from the various experiments. The bioconcentrations of these metals, however, depended on tissue type, exposure level, duration of exposure and the metal under investigation.

Levels of zinc in the tissue in descending order of accumulation was heart > liver > testis > brain > ovary > muscle for fish from acute exposure regimes and ovary > brain > heart > testis > liver > muscle for fish from the chronic exposure regime (Fig. 1). These results are in agreement with data from studies which recorded the lowest zinc concentrations in the muscle tissue of fish (Khalaf *et al.* 1985; Hilmy *et al.* 1987). After exposure of *T. sparrmanii* to iron, the lowest iron concentrations in the tissues were not detected in the axial muscle tissue. The bioconcentration order of iron was liver > ovary > heart > muscle > testis > brain after acute exposure and liver > muscle > heart > ovary > brain > testis after chronic exposure. These results indicate that the bioconcentration orders of zinc and iron were different and were influenced by the duration of exposure.

The concentration of zinc in the axial muscle increased with an increase in exposure dose (Fig. 1A). Fish from the control group (exposure group A) had significantly lower ($P<0.005$) zinc concentrations in muscle tissue when compared with fish exposed to zinc. There were, however, no significant differences ($P>0.05$) in muscle zinc levels between fish from exposure groups B and C, as well as between exposure groups D and E. Duration of exposure had no significant effect ($P>0.05$) on the bioconcentration of zinc in the muscle tissue. Fish from acute exposure group D had significantly lower ($P<0.005$) iron concentrations in their axial muscle when compared with fish subjected to chronic iron exposure (exposure group F). At a zinc exposure concentration of 1.5 mg L^{-1}, *T. sparrmanii* (exposure group E) still regulated muscle zinc concentration, the mean muscle zinc concentration being lower than that of fish exposed to 1.4 mg L^{-1} zinc (exposure group D). Furthermore chronic exposure did not result in significantly higher zinc concentrations in the muscle tissue. During acute exposure to iron, the bioconcentration in the axial muscle was generally low, with only the concentration in fish from exposure groups D and E being significantly higher ($P<0.005$) than that of the control group A (Fig. 1A). The significant increase in muscle iron concentration during chronic exposures, indicates that when fish are exposed for four weeks to 18.6 mg L^{-1} iron, the iron is less effectively regulated in the muscle.

The zinc concentrations in the liver of fish from exposure groups B,C,D and E were significantly higher ($P<0.05$) than in the control group A (Figure 1B). The largest difference of 395 ug g^{-1} wet weight was between the control group and the fish exposed to 1.4 mg L^{-1} zinc (exposure group D). The data confirm that zinc would accumulate in the liver tissue to a certain level whereafter the concentration will be regulated (Romanenko *et al.* 1985). Zinc homeostasis in the liver of fish was achieved by various regulatory mechanisms which lead to the excretion of zinc in the urine, feces, bile and via the gills (Romanenko *et al.* 1985; Everall *et al.* 1989). *T. sparrmanii* from the acute exposure regime (exposure group D) had significantly higher ($P<0.005$) zinc levels in their liver tissue than fish from the chronic exposure regime (exposure group E). These two groups of fish were exposed to the same zinc concentration and it therefore appears that

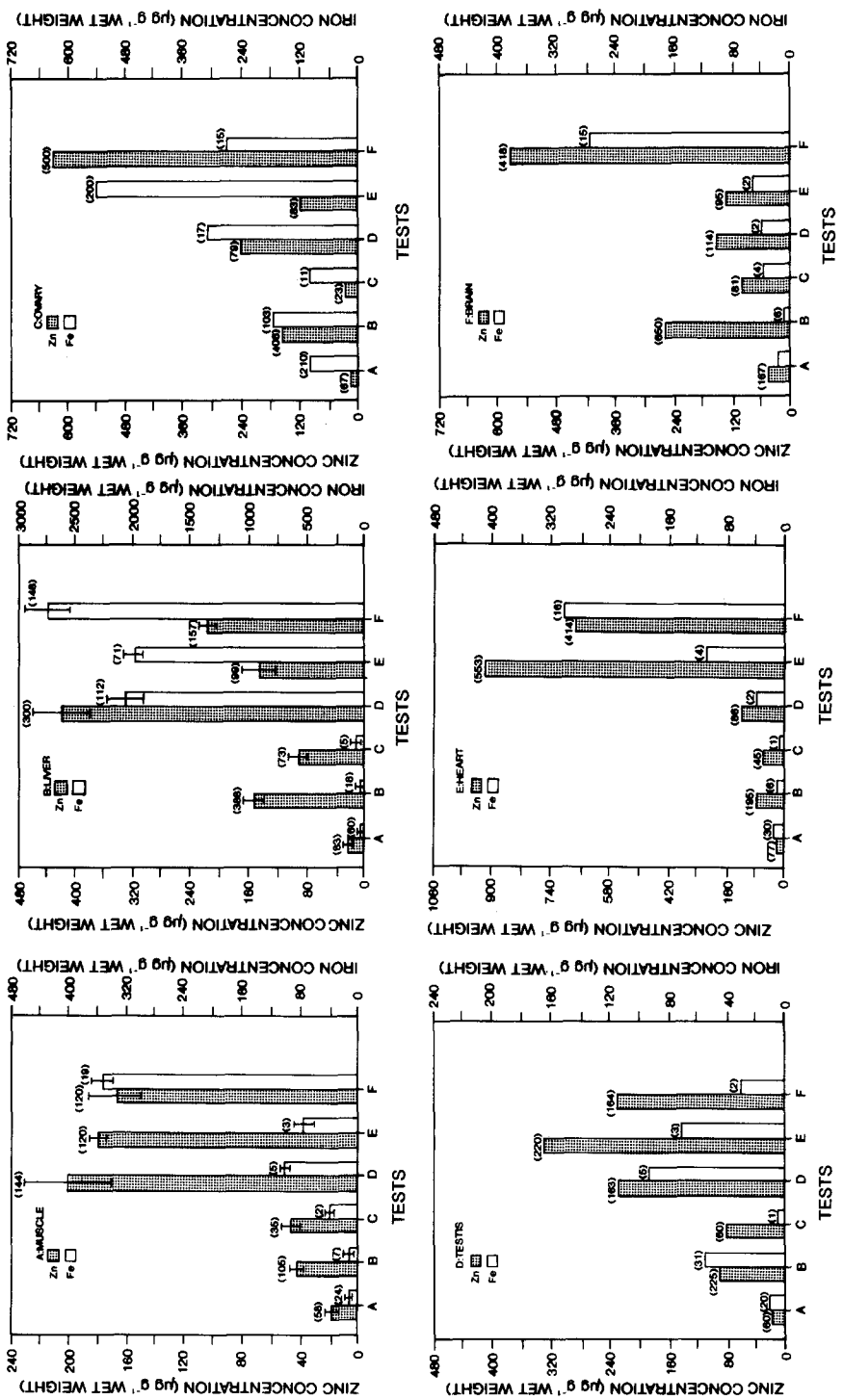

Figure 1. The bioconcentration of zinc and iron in muscle (A), liver (B), ovary (C), testis (D), heart (E), brain (F), tissues of *Tilapia sparrmanii* during acute and chronic exposure to either zinc or iron. The value in brackets indicates the bioconcentration factor. Tests as in Table 1.

the regulation of zinc improved with time. This may be a result of an increase in the levels of metallothionein, a metal binding protein which may increase the ability of *T. sparrmanii* to regulate zinc (Bradley *et al.* 1985). However, further investigations will be needed to support this contention.

The iron concentration in the liver of the control group (mean: 30 ug g^{-1} wet weight) was significantly lower ($P<0.05$) than the values recorded for fish from exposure groups D, E and F, but was not significantly different ($P>0.05$) from the lower concentrations recorded for fish from exposure groups B and C (Fig. 1B). The high bioconcentration in the liver tissue after acute (groups D & E) and chronic (group F) exposure can be attributed to the formation of iron-protein complexes such as hemoglobin, ferritin and transferritin in the presence of excess iron in the ferric ionic form (Fe^{+3}) in the body of the fish (Morgan *et al.* 1974). The large number of blood vessels in the liver may also contribute to high levels of iron (Saad *et al.* 1981) since the liver may remove iron complexes from the blood. The increase in the bioconcentrations of iron during chronic exposure suggests that at this exposure level the mechanisms of iron regulation are not well-established or that iron is not so well regulated in the liver tissue.

Zinc and iron concentrations in the ovaries were higher than in the testis of fish from the same exposure regime (Fig. 1C & 1B). The ovaries of *T. sparrmanii*, as in other teleosts, tend to have a higher bioconcentration capacity than the testis (Baumann and Gillespie 1986). During acute exposure to zinc, the zinc concentrations in the ovaries and testis of *T. sparrmanii* were the highest in fish from exposure groups D and E (Fig. 1C & 1D). Bioconcentration of zinc in the testis tissue generally increased with exposure concentration, but was more varied in the ovarian tissue. Bioconcentration of iron in ovarian tissue generally increased (excluding the exposure group C) with exposure concentration (Fig. 1C). The concentrations detected in testis tissue showed no clear trend, with the concentrations in fish from exposure group C and E being lower than that of fish from exposure groups B and D (Fig. 1D). The ovaries accumulated metals from the blood plasma and indirectly from the liver during oocyte and embryonic development (Fletcher and King, 1978a). This explains the high variation in zinc and iron concentrations in the ovaries of *T. sparrmanii* which were at different stages of development. After spawning, a decrease in testis zinc concentration was recorded for sockeye salmon, *Oncorhynchus nerka* (Fletcher and King 1978b). It is therefore evident that when comparing metal bioaccumulation in the gonads of fish, the stage of gonad development must be considered before meaningful conclusions can be drawn.

As the center of the blood circulation system, a large volume of blood is pumped through the heart. During this phase of blood circulation,pollutants such as metals can be accumulated from the blood. Bioconcentration of zinc in the heart tissue of fish from the control exposure group (exposure group A: 23 ug g^{-1} wet weight) and exposure groups B, C and D (range: 58 to 120 ug g^{-1} wet weight) were much lower than the concentration in the heart tissue (830 ug g^{-1} wet weight) of fish from exposure group E (Fig. 1E). Iron concentrations in the heart tissue of fish from the chronic exposure group E were the highest (Fig. 1E). Fish from the acute exposure group D had lower zinc and iron concentrations in the heart tissue when compared to the concentrations measured in fish from the chronic exposure group F. The influence of the exposure concentrations on

the heart tissue of *T. sparrmanii* was not investigated. Crandall and Goodnight (1963) concluded that zinc can cause the degradation of heart tissue, while Wong *et al.* (1977) observed a decrease in heart activity and heart blockage when *Cyprinus carpio* were exposed to zinc and copper.

The bioconcentration of zinc and iron in the brain tissue was generally low (Fig. 1F). The brain tissue of fish from the chronic exposure regime had higher zinc and iron concentrations than did the brain tissue of fish from the acute exposure regimes. During the exposure to high zinc concentrations (1.4 and 1.5 mg L^{-1}) *T. sparrmanii* showed some behavioral changes which included fin flickering, convulsions and muscle spasms. These behavioral changes indicate that the brain may be affected. Behavioral changes were also noted when other fish species were exposed to metals (Holcombe *et al.* 1976; Henry and Atchison 1979). The behavioral changes, mortalities and the high zinc and iron concentrations detected in the brain tissue of *T. sparrmanii* exposed to high concentrations of these metals may indicate that the brain-barrier system (Collier *et al.* 1980) is affected, resulting in ineffective regulation. The role and function of the brain-barrier system are, however, not well understood (Collier *et al.* 1980).

The bioconcentration factors for zinc and iron depended not only on the exposure regime but also on the tissue examined (Fig. 1). Bioconcentration factors for zinc ranged from 23 to 650 and were the highest in the brain (650) and heart (553) of fish from exposure groups B and E, respectively. Iron bioconcentration factors were generally less than 40, with higher values only being calculated for liver and ovarian tissue. The higher zinc bioconcentration factors indicated that zinc was more readily accumulated than iron.

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