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Effects of Long-Term Exposure to a Mixture of Cadmium, Zinc, and Inorganic Mercury on Two Strains of Tilapia *Oreochromis niloticus* (L.)

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In an effort to improve fish production because of increasing demand for fish, many countries worldwide have turned to both marine and freshwater aquaculture. However, pollution of aquatic habitats is an inevitable problem aquaculturists face. Various pollutants affect survival, growth and reproduction of organisms, particularly those of economic importance. Contaminants in aquatic environments include mixtures of heavy metals and for this reason, a more realistic approach to studying metal pollution effects is to conduct toxicity tests with two or more chemicals (Verriopoulos *et al.* 1987). The potential toxic effects of mixtures of heavy metals has recently become a subject of growing interest (Spehar *et al.* 1978; Finlayson and Verrue 1982).

Tilapia are an economically important group of fish. They have a short generation period of 3-6 months (Wohlfarth and Hulata 1983) and exhibit successive breeding (Jalabert and Zohar 1982). In addition, their fast growth, herbivorous or omnivorous feeding habits, high food conversion efficiency, ease of spawning, ease of handling, resistance to disease and good consumer acceptance (Chervinski 1982) make this group of fish highly popular in aquaculture in Asia, Africa and other developing countries.

Tilapia have been the subject of research on pollution effects over the last decade (Abel and Papoutsoglou 1986; Carino and Puzon 1986; and Cuvin-Aralar 1991). The purpose of this study was to determine growth, accumulation and depuration responses of 2 strains of the Nile tilapia, *Oreochromis niloticus,* chronically exposed to a mixture of heavy metals including cadmium, zinc and mercury. The rationale for choosing these metals was as follows: (1) Zn, Cd and Hg are group IIb metals with widely varying physico-chemical properties, (2) Hg can exist in 0, +1 and +2 valence states whereas Zn and Cd do not exhibit multiple valency, (3) Zn resembles transition metals rather than Cd and Hg in its ability to form complexes with inorganic ions, (4) Zn can form complexes with both oxygen donors and S donor bases and is involved in the biosysnthesis of nucleic acids and, hence, in tissue healing processes, and (5) Cd^{2+} and Zn^{2+} do not form stable methylated derivatives in the environment whereas Hg^{2+} undergoes biological and abiological methylation to form methylmercury and dimethyl mercury, which are highly lipid soluble (Ramamoorthy and Blumhagen 1984).

MATERIALS AND METHODS

One strain of *Oreochromis niloticus* (Nile tilapia), namely the NIFI or Chitralada strain, was obtained from the National Inland Fisheries Insititute (NIFI) in Bangkok, Thailand, and the CLSU strain from the Freshwater Aquaculture Center of the Central Luzon

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State University (CLSU) in Nueva Ecija, a province in Northern Philippines (Basiao and Doyle 1990). These two strains were bred separately in open-air concrete tanks. The resulting fry were transferred to glass aquaria and maintained in the laboratory until they grew to the desired fingerling size (16 to 22 mm standard length; 0.1-0.25 g). By then, the fingerlings were approximately a month old from the swim-up stage.

Fifty fingerlings were placed in each of twelve aquaria containing 40 L of laboratory tapwater. There were 3 replicate aquaria for the treated and control group of each strain. Treatment consisted of exposure of the fingerlings to a mixture of 0.01 mg L^{-1} Hg, 0.1 mg L^{-1} Cd and 1.0 mg L^{-1} Zn. The metal stock solutions were prepared from reagent grade mercuric chloride ($HgCl_2$), cadmium chloride ($CdCl_2$), and zinc sulfate ($ZnSO_4$). Laboratory tapwater (source was a deep well) was used in all tanks. The tapwater used for the exposure experiment had the following characteristics: pH 7.32 • 0.07; hardness 86.55 • 9.83 mg CaCO3 L^{-1}; alkalinity 244.83 • 12.69 mg CaCO3 L^{-1}; conductivity 0.425 • 0.13 ms cm^{-1}; temperature 30.0 • 0.6 °C. Hardness and alkalinity measurements were done using Hach Test Kit Model FF2 (Hach Company, Colorado, USA) while conductivity, pH and temperature were measured using the Horiba Water Checker Model U-7 (Horiba Ltd., Kyoto, Japan).

Half of the water in both treatment and control aquaria were replaced with fresh tapwater on Monday, Wednesday and Friday and additions of the three metals were made to maintain the desired nominal concentrations.

During the entire exposure period of 60 days, the fish were fed twice daily with live brine shrimp, *Artemia salina*, nauplii hatched from cysts (Sanders Brine Shrimp Co. Inc., Utah. USA). After 60 days, survivors in aquaria were transferred to corresponding hapa (fine mesh) net cages attached to bamboo poles in Laguna Lake, a freshwater lake surrounding our research station. The fish were kept in cages without metal additions or supplemental feeds for another 60 days. The experimental run lasted a total of 120 days.

Survival was recorded during the 120-day period. Standard length and weight measurements of each surviving fish were taken on the 30th, 60th (aquarium or exposure phase), 90th and 120th (lake or depuration phase) day. Comparisons of length and weight were made using mean measurements at each sampling period. Three fish from each replicate aquarium were also randomly sampled at each sampling period, pooled and frozen for later heavy metal analyses. The heavy metal concentrations in fish expressed in succeeding sections are the means of 3 replicates.

In the analysis of length and weight, ANCOVA (analysis of covariance) was used. Density was used as a covariate of strain and treatment to correct for the effect of population density (i.e., mortalities in treatment aquaria) on growth rate. T- test was used to compare accumulation and depuration data for the different metals between strains. All heavy metal analyses were done at the University of the Philippines' Natural Science Research Institute (UP-NSRI) using a Thermo Jarrel Ash Video 11E atomic absorption spectrophotometer. The procedures given by Bouchard (1973) for Hg analysis and by the AOAC (1975) for for Zn and Cd analyses were followed. The IAEA (International Atomic Energy Agency) fish reference samples were used for all the metals with recoveries of 98% for Hg, and 95-120% for Zn and Cd. Results of heavy metal analyses in fish samples are expressed on a dry wt basis.

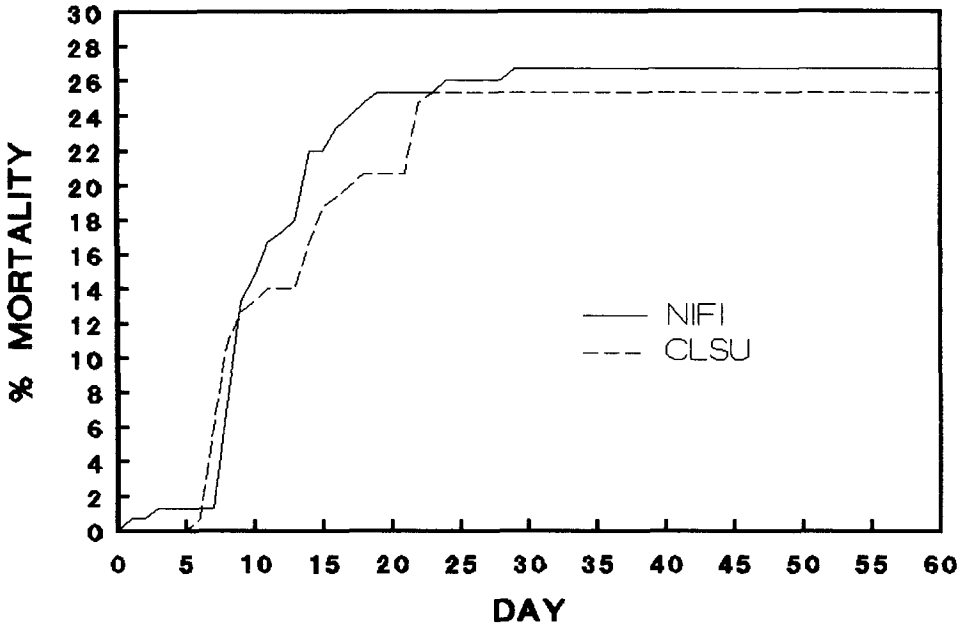

Figure 1. Mortality of two strains of *Oreochromis niloticus* during a 60-day exposure to the heavy metal mixture: 0.01 mg L^{-1} Hg, 0.1 mg L^{-1} Cd and 1.0 mg L^{-1} Zn. Control mortality $\leq$ 2.0%.

RESULTS AND DISCUSSION

Abel and Papoutsoglou (1986) reported negligible mortality of *Tilapia aurea* exposed to 0.1 mg L^{-1} Cd for 3 weeks. Zinc concentrations up to 30 mg L^{-1} induced gill damage to Nile tilapia fry but was sublethal after 21 days of exposure (Carino and Puzon 1986; Carino et al. 1987). Mercury concentrations of 0.02 mg L^{-1} and lower were also shown to be sublethal to *O. niloticus* fingerlings over a 96-hr period (Cuvin-Aralar 1991). Mortality of the two strains of tilapia tested to the mixture of Hg, Cd and Zn is shown in Figure 1. No post-exposure mortality was observed. No significant difference was observed between the survival of the NIFI and the CLSU strains. The control groups of both strains had 98% or better survival and were not included in Figure 1. High mortalities in the treated groups were observed between the 7th until the 25th day of exposure. Mortalities stabilized at approximately 25% in both strains by the 25th day of exposure and no deaths were observed after this period. It appeared that the fish of both strains had acclimatized to the toxicant levels after almost a month of continuous exposure. Comparing the results of exposure to a mixture of the 3 metals with the above-mentioned data on single exposure studies, it appears that Zn, Cd and Hg have a synergistic effect on tilapia. Whereas negligible or no mortality was observed in tilapia exposed to comparable concentrations of the 3 metals (Abel and Papoutsoglou 1986; Carino and Puzon 1986; and Cuvin-Aralar 1991), the combination of these metals resulted in mortalities significantly higher than control.

No significant differences ($P < 0.05$) in growth was observed between control and treated groups of the two strains and between strains (Figures 2a and 2b). In all

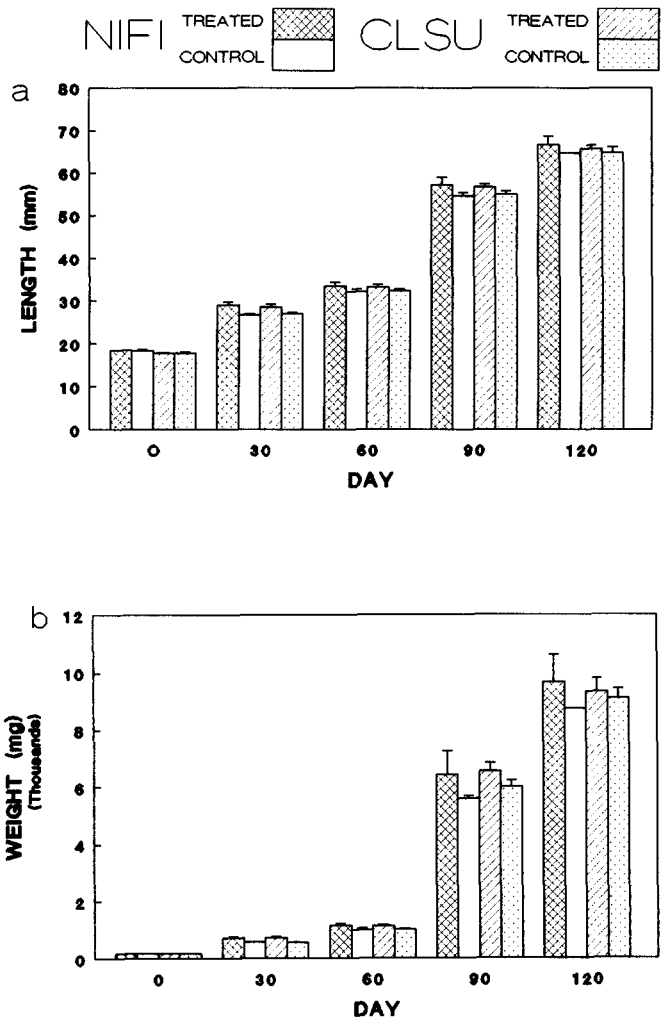

Figure 2. Growth of two strains of *Oreochromis niloticus* exposed to mixtures of Hg, Cd and Zn for 60 days with a subsequent 60-day depuration period in lake water. Bars indicate standard deviation.

instances, growth was slower during the exposure period (first 60 days) than during the lake grow-out period. This was due to the confined conditions in the aquaria where food was limited (feeding was only twice a day), unlike in the lake environment where natural food (i.e., plankton) is constantly available. Nevertheless, the results show that the low concentrations of the metals used had no effect on the growth of the fish. In fact, the treated groups of both NIFI and CLSU had better growth rates than control groups. This difference was most likely due to the decrease in stocking density of treated groups due to mortalities. Data analyses suggested no significant differences between the treated and control groups in both strains when stocking density was accounted for.

Heavy metal uptake and depuration of the two strains of *O. niloticus* are shown in Figure 3. Each data point represents the mean of 3 replicates. Cadmium uptake reached a mean peak of 78.5 • 7.6 $\mu g\ g^{-1}$ (range:71.2-86.4 $\mu g\ g^{-1}$) in NIFI and 82.4 • 10.8 $\mu g\ g^{-1}$ (range: 75.3-94.9 $\mu g\ g^{-1}$) in CLSU after 60 days exposure. This was twice the accumulated amount of 41.0 • 5.1 $\mu g\ g^{-1}$ (range:37.0-48.2 $\mu g\ g^{-1}$) and 40.3 • 3.9 $\mu g\ g^{-1}$(range:36.8-45.7 $\mu g\ g^{-1}$) for NIFI and CLSU, respectively, after 30 days of exposure. Accumulation of Hg peaked at 13.9 • 2.4 $\mu g\ g^{-1}$ (range: 11.5-16.4 $\mu g\ g^{-1}$) in NIFI and 14.2 • 3.1 $\mu g\ g^{-1}$ (range:10.7-16.4 $\mu g\ g^{-1}$) in CLSU after 60 days. Zinc levels reached a mean of 1447.0 • 75.9 $\mu g\ g^{-1}$ (range: 1389 - 1533 $\mu g\ g^{-1}$) in NIFI and 1591.3 • 120.9 $\mu g\ g^{-1}$ (range: 1463-1703 $\mu g\ g^{-1}$) in CLSU after 60 days. For both Hg and Zn, approximately two-thirds of the accumulated levels on the 60th day were taken up during the first 30 days of exposure. NIFI had accumulated 10.9 • 1.6 $\mu g\ g^{-1}$ (range: 8.7 - 11.5 $\mu g\ g^{-1}$) and CLSU 10.4 • 1.4 $\mu g\ g^{-1}$ (range:8.5 - 11.9 $\mu g\ g^{-1}$) of Hg after 30 days exposure. On the otherhand Zn in NIFI reached 1009.0 • 85.4 $\mu g\ g^{-1}$ (range:922 •1125 $\mu g\ g^{-1}$) and in CLSU 930.0 • 118.1 $\mu g\ g^{-1}$ (range: 820-1094 $\mu g\ g^{-1}$) after 30 days. Studies with flagfish, *Jordanella floridae*, exposed to Cd and Zn both individually and in mixture showed that metal residues reached an equilibrium by the 30th day of exposure, i.e., metal concentrations were similar after 30, 70 and 100 days (Spehar *et al.* 1978). In rainbow trout, *Salmo gairdneri*, Hg concentration reached its peak after only 24 hr and continuous exposure for 12 days did not increase the total Hg burden (Ramamoorthy and Blumhagen 1984).

Exposure of rainbow trout to a combination of Zn, Cd and Hg of equal concentration (10 mg L^{-1} each) resulted in almost comparable amounts of Cd and Hg taken up by the fish after 48 hr. The uptake of Zn reached as much as 40 times the concentration of Cd and Hg (Ramamoorthy and Blumhagen 1984). This differs with our observation with tilapia. Based on the nominal levels of heavy metals used for exposure (1.0 mg L^{-1} Zn, 0.1 mg L^{-1} Cd and 0.01 mg L^{-1} Hg), mercury and zinc were accumulated approximately 1,400 and 1,500 times more than their ambient concentrations in the water, respectively. On the other hand, Cd was accumulated approximately 800 times. In studies with minnows, *Phoxinus phoxinus*, peak accumulation factors for Hg was only approximately 8 times the ambient water concentration (Cuvin and Furness 1988); this was obtained within 20 days of exposure and after this period Hg accumulation leveled off. In the same study, the presence of the metal Se increased Hg accumulation. Similarly, in rainbow trout, exposure to a mixture of Hg, Cd and Zn increased the accumulation of Hg to almost twice the level than when the fish were exposed for the same period to Hg alone, suggesting a possible synergism between the 3 metals (Ramamoorthy and Blumhagen 1984). However, studies by Spehar *et al.* (1978) using flagfish exposed to mixtures of Cd and Zn showed that the uptake of one metal was not influenced by the presence of the other.

In this study with *O. niloticus*, depuration of the 3 metals was quite fast. Thirty days after exposure 89.39% of the Cd, 82.20% of the Zn and 86.02% of the Hg had been eliminated by the NIFI strain, while 92.92% of the Cd, 82.09% of the Zn and 91.03% of the Hg had been eliminated by the CLSU strain. The cumulative loss after 60 days was 99.10% for

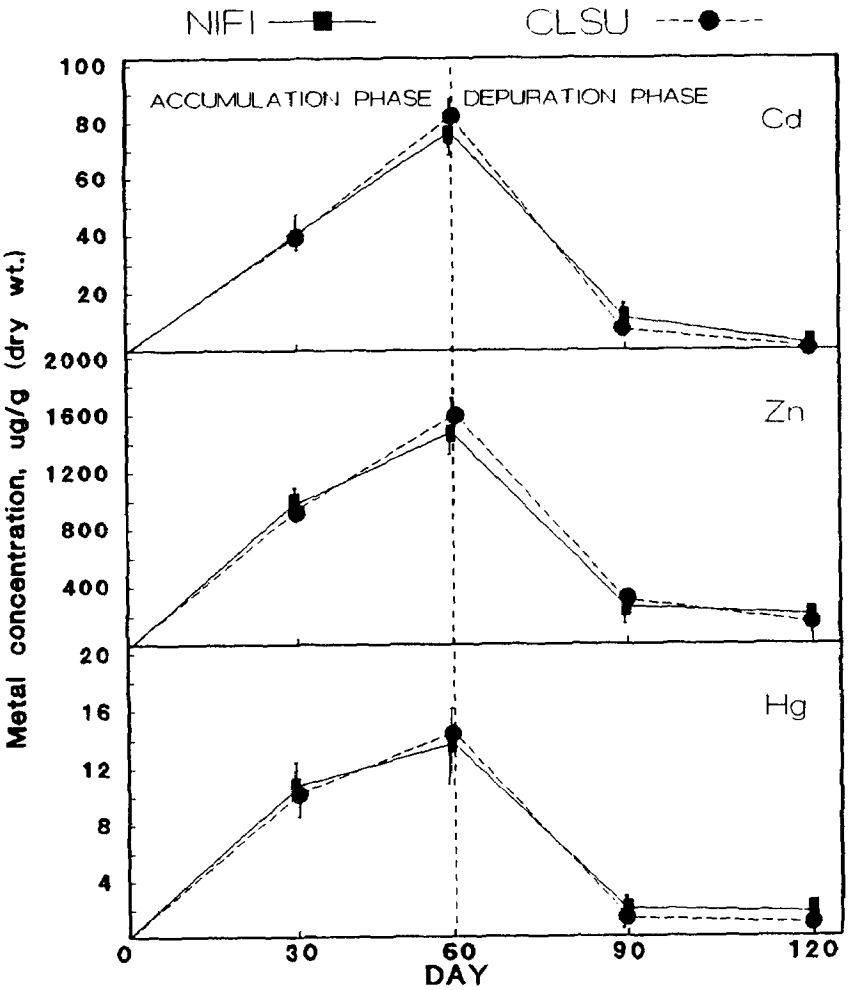

Figure 3. Uptake and depuration of Cd, Zn and Hg in two strains of *Oreochromis niloticus*. Each data point represents the mean of 3 replicates. Bars indicate standard deviation.

Cd, 87.93% for Zn and 88.78% for Hg in NIFI and 98.76% for Cd, 89.99% for Zn and 94.90% for Hg in CLSU. Approximately 90 to 97% of the metals lost had been eliminated during the first 30 days of depuration. It is possible that the bulk of depuration occured much earlier in the depuration period but since no sampling was done earlier than 30 days after exposure, this could not be ascertained. Data on mercury elimination in minnows showed a much slower rate of elimination with only approximately 50% of the Hg lost in 48 days (Cuvin and Furness 1988). The same data also showed that the presence of another metal, selenium, did not affect the elimination of Hg. In Hg elimination studies in plaice, *Pleuronectes platessa*, the biological half time of inorganic mercury was reported to be 61.6 days (Pentreath 1976). This is much longer than what we observed in the two *O. niloticus* strains in this study.

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