A Comparative Study on the Average Cumulative Mortality Rate of *Oreochromis niloticus* Fingerlings Reared with Sublethal Concentrations of Zinc, Copper and Mercury, Singly or in Combinations

Ermelinda Goc-ong Tobias,[1] Daniel B. Tobias, Jr.[2] and Virginia S. Cariño[2]

Abstract

Preliminary bioassays of the three heavy metal salts such as zinc sulfate ($ZnSO_4.7H_2O$), copper sulfate ($CuSO_4.5H_2O$) and mercuric chloride ($HgCl_2$) were performed on Oreochromis niloticus *fingerlings and were estimated as 25 mg/l, 2.857 mg/l and 0. 833 mg/l, respectively. Sublethal concentrations of these metals were used to treat the water media singly or in combinations where the fingerlings were reared for 30 days. Daily mortality was recorded and average cumulative mortality rate during the two successive 15 days were computed. Results showed that copper in the concentration used was the most toxic and it acted synergistically with the zinc and mercury.*

Key Words : *Oreochromis niloticus* fingerlings, zinc, copper, mercury, interaction

Introduction

Contamination of aquatic environment by chemical pollutants has become a serious environmental problem. Some of the chemical pollutants are the trace elements such as mercury, cadmium, lead, cobalt, copper, fluoride, manganese, molybdenum, nickel, selenium, silicon, tin, vanadium and zinc. The first three metals are natural components of the environment which organisms can tolerate up to a certain optimum concentration. All the rest are present in healthy tissues of all organisms at relatively constant concentrations, hence, are essential in nature.

Heavy metals are known to be insidious toxic pollutants in the natural environment (Bender and Ibeanusi, 1987). Zinc is one commonly-occurring natural heavy metal often associated with many industrial and mining effluents (Narain and Nath, 1986) causing excessive amount in the aquatic medium . Beside being essential to life, it has been shown to be toxic to aquatic organisms (Hilmy et al., 1987). Copper, on the other hand, normally occurs

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[1] Department of Biological Sciences, College of Science and Mathematics, MSU-Iligan Institute of Technology, Iligan City

[2] Institute of Biology, College of Science, University of the Philippines, Diliman, Quezon City

in low concentrations in the blood bound to ceruloplasmin, a plasma protein (Cousins, 1985). Excess copper comes from electrical industry, alloys, chemical catalysts, paints, algicides and wood preservatives (Torres et al., 1987).

Another heavy metal of industrial and toxicological significance is mercury. Mercury is a non-essential metal and is a natural component of the environment. Organisms can tolerate its presence up to a certain optimum level only, beyond which adverse effects are readily observed. High levels of mercurials in the aquatic ecosystems associated with industrial and agricultural usage had received considerable attention primarily because of their notoriety and the consequences of toxicity in humans and other animals. Both organic and inorganic mercurials can be accumulated through diet, by transcutaneous absorption from physical contact or across respiratory surfaces (Olson et al., 1978). Mercuric chloride, an inorganic mercurial, is commonly used as moluscicide (Hanomante and Kulkami, 1979) to cúrb the population of freshwater vector snail which transmit trematode larvae that are of medical and economic importance. As such, mercuric chloride also acts as toxicant for extramolluscan coinhabitants, one of which may be fish population.

Exposure to heavy metals like zinc, copper or mercury at levels beyond the organisms' capacity to tolerate constitutes a stress. In a stress response, alarm reactions are first observed followed by a stage of resistance, then finally death (Sathyanathan et al., 1988).

Studies were done to determine the toxicity of these metals to fishes and aquatic invertebrates. In all these studies, the most sensitive stage in the life cycle was the embryo-larval or early juvenile period of development. Results showed differences in concentrations considered toxic to each one of them.

Water characteristics such as pH, hardness, temperature and presence of chelating agents (Pagenkopf et al., 1974) have been shown to alter the levels of most heavy metals needed or available to impair the continued survival of aquatic animals. The study of McLeese (1974) showed that decrease in temperature reduces the toxicity of copper to lobsters. Both zinc and copper have decreased toxicity with increased hydrogen ion concentrations (Miller and Mackay, 1980; Cusimano et al., 1986). The toxicity of both mercury and zinc increased with higher temperature (Rehwoldt et al., 1972; Smith and Heath, 1979).

Heavy metals are absorbed via the gut through the food, via the gills, skin and respiratory tract. These are transported in the blood to the internal tissues where a great bulk accumulates. These may then induce morphological and physiological defects with a consequent behavioral abnormalities which may lead to death.

Existing water quality criteria have been derived for single toxicants only, but it is rare to find natural waters in which single toxicants are present. Aquatic organisms are usually exposed to a wide variety of toxicants from direct effluent discharges or in nonpoint source of pollution from chemical runoffs. This study aims to simulate this kind of situation and be able to shed more information on the toxic effects of zinc, copper and mercury in the aquatic ecosystem using freshwater fish as a test animal using sublethal concentrations. With mortality as the index of toxicity, we aim further to determine some possible interactions of these heavy metals by using them to treat the water media either singly or in combination with each other.

Materials and Methods

Experimental Animal. *Oreochromis niloticus* (tilapia), an introduced fish species in the Philippine freshwater ecosystem, is of great economic significance as food. Fingerlings in early juvenile stage of 20-25 mm in length are sown in the ponds for stocking. It is therefore this stage of development and growth of tilapia that encounters, for the first time, different pollutants that may be present in the natural environment, hence the use of this stage in the present study. These were obtained from the College of Fisheries, University of the Philippines, Diliman, reared and acclimated in the NSRI wet laboratory conditions in a well-aerated water media contained in 25-gal. glass aquaria for at least a week and after which treatment commenced. For the whole duration of the experiment, the fingerlings were fed, *ad libitum*, with Tetra Fin commercial feeds.

Experimental Set-up. Reagent grade heavy metal salts such as ZnSO4.7H2O, CuSO4.5H2O and HgCl2 (Merck) were obtained from the Institute of Biology, College of Science, University of the Philippines, Diliman, Quezon City. Sublethal concentrations derived from the preliminary bioassay of each metal salt on this stage of tilapia were used in the chronic treatment experiments. Three different set-ups, specifically, single-metal, double metal and triple-metal exposures were done using 2.5 mg/l Zn, 0.5 mg/l Cu, and 0.15 mg/l Hg. In all set-ups, the water media had pH range of 6.7-7.2; temperature range of 19-230C and a total hardness of 49.35 mg CaCO3/L. In all of the concentrations used, whether singly or in combinations, 100 fingerlings per 40-liter water media were used. Water media were changed once a week. A replicate experiment for each set-up was done.

***Average Cumulative Mortality Rate* (ACMR).** Mortality was recorded daily until 30 days of exposure. Accumulated deaths were summed up for every 15 days of exposure and the rate computed and determined on an average daily mortality. Such is now the average cumulative mortality rate (ACMR). ACMR was determined after 15 and 30 days of exposure.

Statistical Analysis. Analysis of variance was utilized to compare the significance of average cumulative mortality rate (ACMR) of fingerlings in different concentrations of different treatment groups on the 15th and 30th day of exposure.

Results and Discussion

Toxicity Studies. Preliminary bioassay and 96-hr LC50 for all heavy metals used on *Oreochromis niloticus* fingerlings were estimated to be 25 mg/l, 2.856 mg/l and 0.833 mg/l for zinc sulfate, copper sulfate and mercuric chloride, respectively. These results conform to the trend of toxicity studies using other organisms where mercury is more highly toxic compared to copper, and copper being more toxic than zinc. The trend is Hg>>>Cu>>Zn. The different treatment groups and the corresponding concentrations of different heavy metals used are shown in Table 1.

Table 1. Summary of the different treatment groups of *Oreochromis niloticus* fingerlings and the concentrations of the different heavy metals.

TREATMENT GROUPS	HEAVY METAL USED	CONCENTRATION (mg/l)
Control		
Group 1	zinc sulfate	2.5
Group 2	copper sulfate	0.5
Group 3	mercuric chloride	0.15
Group 4	zinc sulfate & copper sulfate	2.5 and 0.5
Group 5	zinc sulfate and mercuric chloride	2.5 and 0.15
Group 6	copper sulfate and mercuric chloride	0.5 and 0.15
Group 7	zinc sulfate, copper sulfate and mercuric chloride	2.5, 0.5 and 0.15

Daily mortality was recorded until the 30th day of exposure to the different heavy metals and the average cumulative mortality rates were computed and determined after 15 and 30 days of exposure. These are shown in Table 2.

Table 2. Average Cumulative Mortality Rate (ACMR) of different treatment groups on the 15th- and 30th-day exposure.

TREATMENT GROUPS	ACMR	
	15th-day	30th-day
Control	0.0	0.0625
Group 1	0.0 X	0.03 X
Group 2	1.73 **	1.38 **
Group 3	0.0975 X	0.18 X
Group 4	2.0475 **	1.915 **
Group 5	1.095 **	1.3625 **
Group 6	3.68 **	1.43 **
Group 7	4.115 **	1.6625 **

Legends :

X - ACMR is not significantly different from the control group at P=0.05

** - ACMR is highly significant compared to the control group at P=0.05

Of the sublethal concentrations of heavy metals used singly in this study, copper (Group 2) resulted in highest ACMR in both sampling periods, and has highly significant difference compared with the control group, while both zinc (Group 1) and mercury (Group 3) resulted in ACMRs which are not significantly different from that of the control group. In all other combination treatment groups (4, 5, 6, 7), the ACMRs have highly significant difference for all sampling days compared with that of the control group suggesting a combined lethal action of zinc, copper and mercury at these sublethal concentrations used. Uptake of water-borne concentrations of these metals occurs mainly via the gills (Benedetti et al., 1989) where they exert lethal toxic effects (Everall et al., 1989b). The common trend of ACMR in the majority of the treatment groups was higher during the first 15 days of exposure than on the next 15 days. Uptake of these metals among the surviving fingerlings may be less or elimination may be efficient enough to result in a lower mortality. In addition, the liver may have been successful in detoxifying the blood and preventing lethal effects (Allen et al., 1988). Furthermore, the fingerlings that survived were believed to be the most resistant ones (Amdur *et al.,* 1991).

Comparison between the ACMR of double- and triple-metal treatment groups with those of the corresponding single-metal treatment groups of similar concentrations is presented in Table 3 .

Table 3. Comparison of Average Cumulative Mortality Rate (ACMR) of double-metal and triple-metal treatment groups with that of single-metal treatment groups on the 15th- and 30th-day sampling periods.

Treatment Groups	ACMR		
	Heavy metals used	15th-day	30th day
Set 1:			
Group 1	Zn	0.0 **	0.03 X
Group 2	Cu	1.73 X	1.38 X
Group 4	ZnCu	2. 0475	1.915
Set 2:			
Group 1	Zn	0.0 **	0.03 **
Group 3	Hg	0.0975 **	0. 18 **
Group 5	ZnHg	1.09	1.3625
Set 3:			
Group 2	Cu	1.73 X	1.38 X
Group 3	Hg	0.0975 **	0.18 **
Group 6	CuHg	3.68	1.43
Set 4:			
Group 1	Zn	0.0 **	0.03 **
Group 2	Cu	1.73 *	1.38 *
Group 3	Hg	0.0975 **	0.18 **
Group 7	ZnCuHg	4.115	1.6625

Legends:

X - means no significant difference in ACMR compared with double- and triple-metal treatment groups at P=0.05

* - means only a significant difference in ACMR compared with the double- and triple-metal treatment groups at P=0.05

** - means highly significant difference in ACMR compared with the double- and triple- metal treatment groups at P=0.05

In Set 1 of Table 3 where zinc and copper interaction was studied. it was observed that only group 1 had a highly significant difference in ACMR with group 4 but not group 2. The high ACMR in group 4 may be influenced mostly by the copper component in the medium as single-copper treatment group has high ACMR. This result which confirmed that copper is much more toxic than zinc is in accord with the studies of other workers on other organisms (Uma Devi, 1987; Torres et al., 1987). The higher toxicity of the copper group is probably related to the uptake rate into the lamellar epithelium or on the ability to damage cells with an osmoregulatory role. In addition, copper is chelated better by mucus compared to other metals and resulted in acceleration of internal hypoxia.

In Set 2 where zinc and mercury interaction was studied, highly significant differences in ACMR were observed between the combined treatment groups and the two single-metal treatment groups. The high mortality in the combined treatment group may be attributed to the synergistic interaction of zinc and mercury.

In Set 3 , the combined treatment group had a high ACMR compared with compared with the single-Hg treatment group but not with the single-Cu treatment group. Only less influence can be attributed to Hg , thus one can speculate a synergistic interaction between the two (Krishnakumar et al., 1990).

Synergistic interaction among the three heavy metals resulted in a high mortality rate as observed in Set 4. This is due to the stressful condition among the organisms exposed to combinations of toxicants (Ewing et al., 1982). In the 30th-day sampling period, only with both single Zn and Hg treatment groups is ACMR of group 7 significantly different and not with single-Cu treatment group. Thus, the high ACMR of group 7 may be attributed largely to the copper component due to its intense accumulation (Okazaki, 1976) that can damage the regulatory mechanisms and results to death. The results of this study showed the interactions of heavy metals used in the potentiation of mortality as indication or manifestation of their toxic effects though they may be in sublethal concentrations.

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