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Histological Effects of Copper-treated Water Media on the Kidney of *Oreochromis niloticus* fingerlings

ERMELINDA GOC-ONG TOBIAS
VIRGINIA S. CARINO

Abstract

Sublethal concentrations of copper as copper sulphate ($CuSO_4.5H_2O$) were used to treat the water media where **Oreochromis niloticus** *fingerlings were reared. Samples of fingerlings were taken after 15, 30 and 45 days of exposure for light microscope studies. In both concentrations used, dense bodies, interstitial damage, vacuolations, casts in the lumina, tubular necrosis and other degenerative changes were observed in varying degrees and frequency of occurrences.*

Key Words : *Oreochromis niloticus*, kidney, copper sulphate, light microscopy

Introduction

Anthropogenic activities, in many cases, with best motive in mind, led to their toxicity not only to humans but also to other non-target biological resources in the ecosystem. These activities may include the use of agrichemicals in the farm with the hope of increasing its productivity in order to alleviate their economic conditions. One of these may be application of copper sulphate in aquaculture to prevent fungal attacks to fishes, shrimps and other cultured aquatic organisms.

Copper, an essential heavy metal, is also widely distributed in the environment through industrial discharges, mining operations, weathering and leaching (Schreck and Lorz, 1978). Though copper is a normal component of the living organisms where it is being recognized as an important growth factor for protein synthesis (Cousins, 1985), it can also be toxic especially at high concentrations. Copper occurs in low concentration in the blood bound to ceruloplasmin, a plasma protein. In addition, it is present in the structure of several enzymes like superoxide dismutase, ascorbic acid oxidase, tyrosinases, cytochrome oxidase and in important hemocyanin molecule (Torres et al., 1987).

This study was funded by a grant from the National Research Council of the Philippines (NRCP). [1]Department of Biological Sciences, College of Science and Mathematics, MSU-Iligan Institute of Technology, Iligan City [2]Institute of Biology, University of the Philippines, Diliman, Quezon City.

Exposure to heavy metals like copper at levels beyond the organism's capacity to tolerate constitutes a stress. In a stress response alarm reactions is first observed followed by a stage of resistance then finally death (Sathyanathan et al., 1988). In trying to resist to whatever in excess is present in the body, the organism undergoes some activities to compensate, one of which is excretion. In excretion, unnecessary materials are eliminated from the blood or tissue fluids. Furthermore, potentially toxic materials may become combined with other substances in the blood or tissues and rendered harmless and excreted.

Organisms are known to accumulate metals in their tissues approximately in proportion to the degree of environmental contamination and thus can be used as bioindicators of metal pollution (Holcombe et al., 1979; Lobel 1978a, 1978b, 1978c; Ishii et al., 1985; Bradley and Morris, 1986; Wahbeh, 1985).

Channa punctatus (Bl.) exposed (Saxena and Parashari, 1981). Xenobiotic transformation activities was observed in the kidney of rainbow trout after intraperitoneal administration of cadmium. This response in the kidney was accompanied by histological changes in the proximal convoluted tubules associated with plasma hypocalcemia observed in cadmium-poisoned fish (Foerlin et al., 1986). In addition, Baker (1969) exposing the winter flounder (*Pleuronectes americanus)* to low, medium and high levels of copper as copper sulphate observed that at medium and high levels, the kidneys showed considerable changes. The hemopoietic tissues were necrotic and very much reduced in volume. The tubule cells seemed to disintegrate and their lumina contained much dense materials. Likewise, renal lesion and liver damage in *Fundulus heteroclitus* exposed to 1.0 and 5.0 mg/l copper were observed (Gardner and LaRoche, 1973).

The objective of the present study is to shed more information on the toxic effects of copper in the aquatic ecosystem using freshwater fish as a test animal. Furthermore, this study aimed to determine if using sublethal concentrations (lower than used by previous authors) of copper as copper sulphate to treat the water media for rearing *Oreochromis niloticus* fingerlings, despite their normal physical appearance, can induce histopathological effects on one organ of elimination, the kidney.

Materials and Methods

Experimental Animal. *Oreochromis niloticus*, locally named as "tilapia" and is sturdy and prolific, which is of great importance as a source of protein in the Filipino diet, was used. Fingerlings of 20-25 mm in length were obtained from the College of Fisheries, University of the Philippines Diliman, reared and acclimated in the Natural Sciene Research Institute wet laboratory conditions in a well-aerated water media contained in 25-gal. glass aquaria for at least a week, afterwhich 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 salt of copper sulphate ($CuSO_4.5H_20$) (Merck) was obtained from the Institute of Biology, College of Science, University of the Philippines Diliman. The preliminary bioassay performed on early fingerling stage (20-25 mm body length) was estimated using arithmetic graphic method to be at 2.857 mg/liter from which sublethal concentrations used in this experiment was derived. Two different concentrations, 1.0 mg/liter (1.0 ppm Cu) and 0.5 mg/liter (0.5 ppm Cu) copper as copper sulphate were used to treat the water media which were changed once a week for the entire duration. One hundred fingerlings per 40-liter water media. A replicate experiment for each set-up was done.

Sampling period and sampling size. In all set-ups, 10 fingerlings each were taken at random on the 15th, 30th and 45th day of exposure to copper as copper sulphate.

Tissue preparation for light microscopy. The tail kidney, being the principal organ for excretion, was dissected and sliced into desired sizes and fixed in 2.5 % glutaraldehyde for one hour. After which, the tissues were washed with 5% sucrose phosphate buffer (pH 7.2) for three changes at an interval of 15 minutes. Postfixation with 1% osmium tetroxide followed by washing with 8 % sucrose phosphate buffer was done in preparation for dehydration. A series of increasing acetone concentrations was used to dehydrate the tissues. Proportions of propylene dioxide and araldite mixtures were used for infiltration. Pure araldite resin was used to embed the tissues in Beem capsules and were allowed to polymerize for 4 to 5 days at 600 degrees Celsius inside the oven. Sectioning was done using LKB ultratome. Semithin sections were mounted on the glass slides and stained with toluidine blue for light microscope analysis.

Light microscope analysis. All kidney samples taken in all sampling periods were sectioned for histopathological analysis. General conditions of the entire organ and conditions of the renal tubules and glomeruli were noted. Photomicrographs were taken.

Results and Discussions

Table 1 shows the experimental set-ups with the corresponding concentrations of copper as copper sulphate used.

Table 1. *Summary of the treatment groups and the concentrations of copper as copper sulphate used.*

TREATMENT GROUPS	CONCENTRATIONS USED
Control	0
Group 1	1.0 mg/l
Group 2	0.5 mg/l

The results of the toxic effects of copper exposure using average cumulative mortality rate (ACMR) as index had been published somewhere (Tobias et al., 1997).

Light microscopy: The normal histology of the tail kidney of *Oreochromis niloticus* fingerlings was studied and documented. It is made up of nephrons embedded in the interstitial tissues. The nephron consists of renal corpuscle, proximal segment, distal segment and collecting tubule (Tobias and Carino, 1997). The renal corpuscle consists of well-developed thin-walled glomerular capillaries surrounded by Bowman's capsule (Figure 1). The proximal tubules have pyramidal cells with centrally-located nuclei and distinct brush borders. The distal segment has cuboidal cells with not so distinct brush borders. The collecting tubules have simple columnar cells with few brush borders (Figure 2).

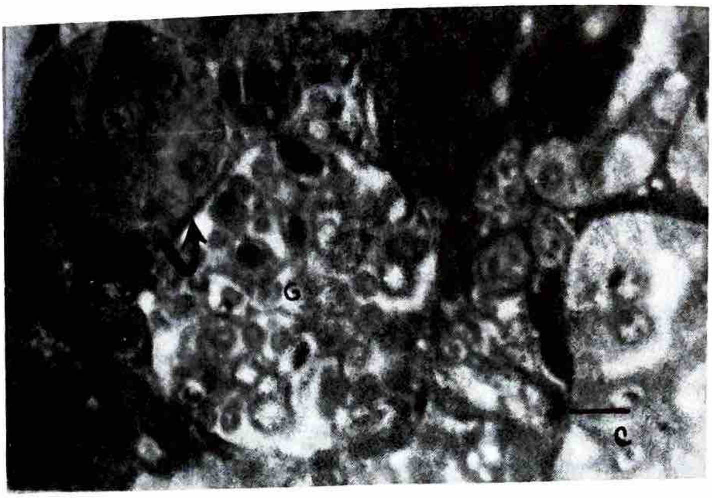

Figure 1. Photomicrograph of kidney from the control group of *Oreochromis niloticus* fingerlings showing the normal glomerulus (G) in thin Bowman's capsule (arrow) surrounding the capillary tuft. Bar = 2 um.

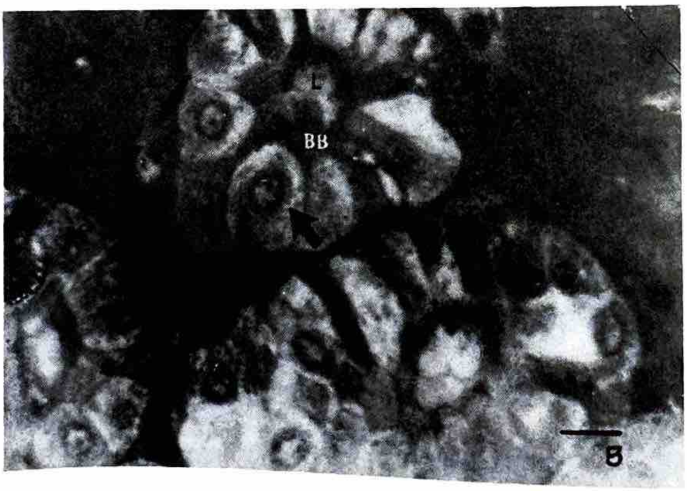

Figure 2. Photomicrograph of kidney from the control group of *Oreochromis niloticus* fingerlings showing the pyramidal cells (arrow) with very distinct brush borders (BB) projecting into the lumina (L). Bar = 2 um.

Histopathological effects of copper concentrations on the tail kidney of *O. niloticus* fingerlings. Table 2 summarizes the percentage incidence of the histopathological effects of copper as copper sulphate on the tail kidney of *Oreochromis niloticus* fingerlings sampled on the 15th, 30th and 45th-day of exposure and are presented in the succeeding figures.

Table 2. Summary of the percentage incidence of the different histopathological effects of two sublethal concentrations of copper (1.0 ppm and 0.5 ppm) as copper sulphate on the tail kidney of *Oreochromis niloticus* fingerlings sampled on the 15th, 30th and 45th –day of exposure.

HISTOPATHOLOGICAL EFFECTS	SAMPLING PERIODS								
	15th day			30th day			45th day		
	Ctrl	Grp 1	Grp 2	Ctrl	Grp 1	Grp 2	Ctrl	Grp 1	Grp 2
1. General									
Hyperemia	0	0	25	0	0	0	0	0	50
Interstitial damage	0	50	0	0	25	75	0	0	0
2. Tubules									
Presence of dense bodies	0	50	50	0	25	0	0	0	0
Tubular necrosis	0	75	75	0	50	50	0	75	75
Dilated tubules	0	0	0	0	75	75	0	75	0
3. Renal Corpuscles									
Dilated Bowman's capsule	0	0	0	0	50	75	0	0	0
Compacted glomerulus	0	0	0	0	50	50	0	0	25

Hyperemia which was characterized by dilated blood vessels with aggregation of blood cells was observed only among the 25% of the samples taken from the lower sublethal concentration (Grp 2) on the 15th day and 50% on the 45th day. None was observed among the samples taken on the 30th day. Such condition can be attributed to the organism's effort to protect and defend itself from its toxic effects hence biologic amines are released to the bloodstream in order to increase circulation (Gill and Pant, 1981) and be able to get rid of the xenobiotics, be it a heavy metal like zinc (Tobias and Carino, 1997) or the present study.

Damage to interstitial tissues (Figure 3) was observed in 50% of the Grp 1 samples on the 15th day; only 25% on the 30th day and none on the 45th day. Likewise, such effect was also observed in 75% of the Grp 2 samples after 30 days of exposure. This was also observed by Wolmaram and others (1986) on snails after exposure to copper. The swollen connective tissues of the epidermal ridge pulled away some parts from each other resulting in the formation of large spaces. Moreover, damage to connective tissues of the liver of *Heteropneustes fossilis* (Bloch) was also observed after 30 days of exposure to sublethal cadmium (Ghosh and Chakrabarti, 1993). This can be ascribed to the toxic effects of the metal ions on the organizations of the cytoskeletal proteins (Amdur et al., 1991). It had been recognized that exposure to heavy metals causes some alterations of calcium homeostasis which results to dissociation of cytoskeletal proteins and a corresponding injury.

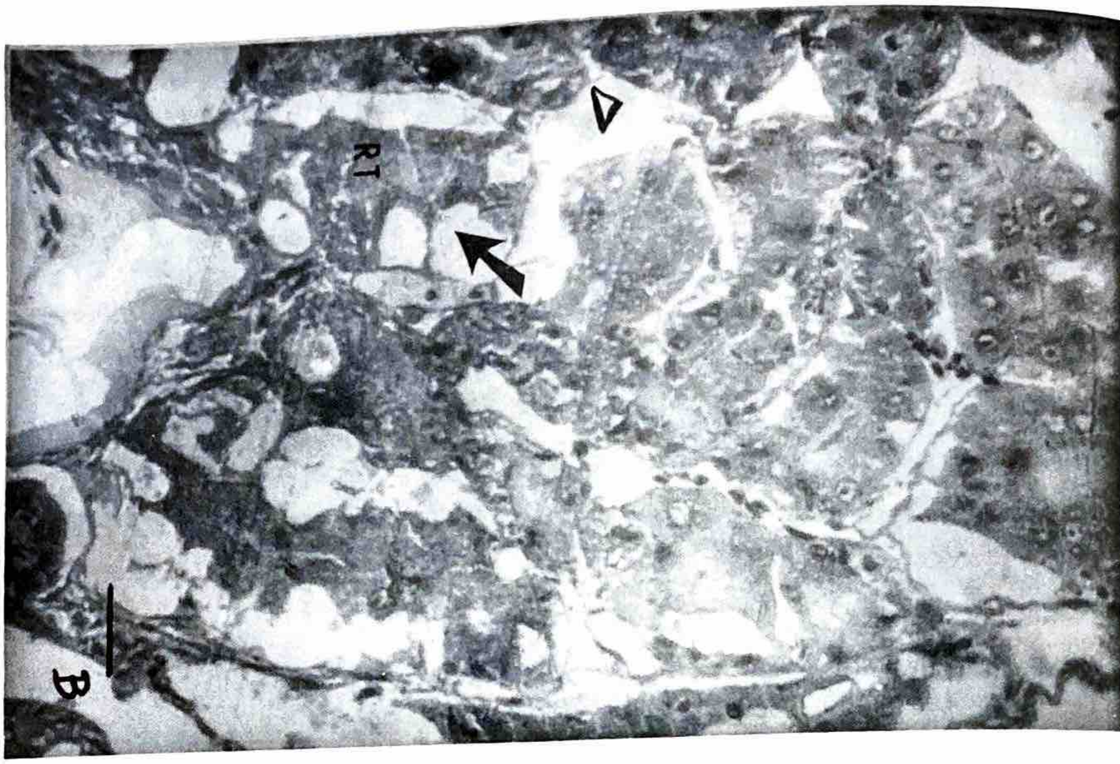

Figure 3. Photomicrograph of kidney tissues of *Oreochromis niloticus* fingerlings exposed to copper as copper sulphate showing interstitial damage (arrowhead) that resulted to widened space between tubules. Also shown are tubular epithelia exhibiting cytolysis (arrow) which is indicative of necrotic condition. Bar = 5 um.

The presence of dense bodies in the nuclear region of the tubular epithelia (Figure 4) was observed in 50% of the samples in both treatment groups on the 15th-day sampling period. This persisted in 25% of the Group 1 samples on the 30th day. None was observed on the 45th day sampling period for both groups. These observations which were prevalent in the early sampling period, are similar to the studies done by a number of researchers. Pirie et al. (1984) observed black or brown granules in amoebocytes of oysters collected from highly metalliferous environment. These granules correspond to these dense bodies and were confirmed to contain either copper or zinc deposits or both through histochemical tests. Such that these dense bodies are accumulations of heavy metals that are sequestered in membrane-enclosed structures and may act as detoxifying system (Mason et al., 1984). Similar structures named as inclusion bodies were observed in the liver of trout exposed to dietary copper. It is suggested that the sequestration of all heavy metals in membrane-bound cytoplasmic inclusions is a generalized response of an organism to detoxify the effects of these elements in addition to its role in the uptake and serving as an intracellular storage site where metals can be deposited in a relatively inert form..

Figure 4. Photomicrograph of kidney tissues of *Oreochromis niloticus* fingerlings exposed to copper as copper sulphate showing dense bodies (slender arrow), onset of necrosis (big arrow) and presence of casts (CAST) in the lumina of the collecting duct. Bar = 2 ùm.

Likewise, tubular necrosis at varying degrees (Figures 3, 4, and 5) was prevalent in both treatment groups in all sampling periods. This effect ranges from hydropic degeneration, cytolysis, karyolysis, karyorhexis, casts in tubular lumina to severe necrotic condition of the kidney. Seventy five percent of the samples showed this effect on the 15^{th} day, 50% on the 30^{th} -day and 75% on the 45^{th} day.

On the 30^{th}-day sampling period, dilated tubules (Figure 5) was first observed among 75% of the samples in both concentration groups. This became intense on the 45^{th}-day sampling period among Group 1 samples.

The presence of dilated and thinned tubules was observed only in the later sampling period. Similar with necrosis, this can also be an atrophic reaction of the tubules to the hypoxic conditions brought about by the damage of the interstitia (Kumar and Pant, 1981; Torres et al., 1987). Furthermore, with copper attacking various renal enzymes (Baker, 1969) and with damaged renal interstitia, the process of reabsorption is hindered, thus, resulting to atrophy.

Likewise, dilated Bowman's capsule (Figure 6) was observed in both groups. Almost always, dilated Bowman's capsule is associated with compacted glomeruli which was observed in 50% of the samples in both groups.

Figure 5. Photomicrograph of kidney tissues of *Oreochromis niloticus* fingerlings exposed to copper as copper sulphate showing degenerative changes indicating necrosis include much dilated and thinned renal tubules (RT) with casts in their lumina (arrows). Bar = 5 um.

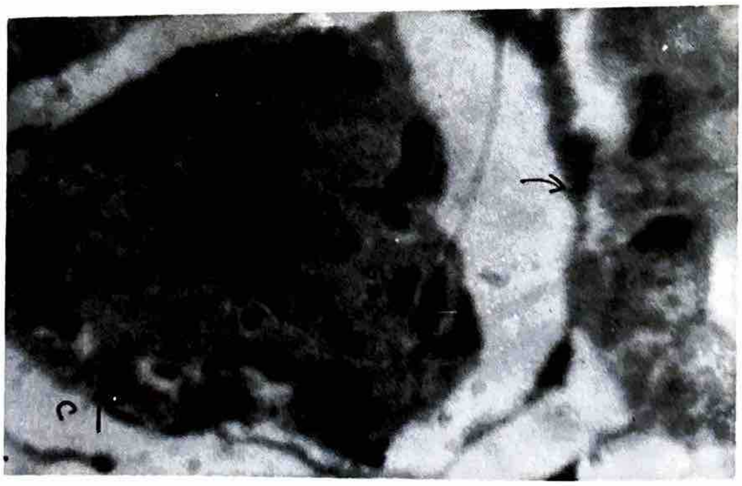

Figure 6. Photomicrograph of kidney tissues of *Oreochromis niloticus* fingerlings exposed to copper as coppers sulphate showing dilated Bowman's capsule (arrow) and compacted glomerulus (G). Bar = 2um.

Similar findings of shrunken glomeruli were observed among freshwater fish, *Puntius conchonius* Ham after a 12-week sublethal cadmium exposure (Gill et al., 1989) and in the catfish, *Heteropneustes fossilis* after a 30-day exposure to 0.2ppm mercuric chloride (Bano and Hassan, 1990). Moreover, similar change called marked contraction of the glomeruli *of Barbus conchonius* Ham was also observed 24 hrs after exposure to sublethal concentrations of monocrotophos, a systemic insecticide (Kumar and Pant, 1984). Most nephrotoxins can cause marked constriction of renal arteries. A tubuloglomerular feedback mechanism shuts down glomerular vessels following necrosis of the tubular epithelia (Amdur et al., 1991). Such vascular changes may affect the microcirculation and can be responsible for the development of the atrophic condition.

The results of the present study showed that copper sulphate which is being used as fungicide in aquaculture industry also exert its negative impact on the fish causing some histopathological effects on osmoregulatory organ which can not be detected in its external morphology for the fishes sampled appear to be physically normal. Similar conditions may also be true with other organisms cultured.

References Cited

Amdur, M. O., J. Doull and C. D. Klaasen. 1991. Eds. Casarett and Doull's Toxicology: The Basic Science of Poisons. 4th ed. Pergamon Press, NY. pp. 600.

Baker, J. T. P. 1969. Histological and electron microscopical observations on copper poisoning in the winter flounder (*Pleuronectes americanus*). J. Fish. Res. Bd. Canada. 26: 2785-2793.

Bano, Y. and M. Hassan. 1990. Histopathological lesions in the body organs of catfish (*Heteropneustes fossilis)* following mercury intoxication. J. Environ. Sci. Health B. B25: 67-85.

Bradley, R. W. and J. R. Morris. 1986. Heavy metals infish from a series of metal-contaminated lakes near Sudbury, Ontario. Water, Air and Soil Pollut. 27: 341 - 354.

Cousins, R. N. 1985. Absorption, transport and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiol. Rev. 65: 238 - 309.

Foerlin, L., C. Haux, I. Karlsson-Norrgren, P. Runn and A. Larsson. 1986. Biotransformation activities of enzymes and histopathology in rainbow trout, *Salmo gairdneri*. Aquat. Toxicol. 8: 51 - 64.

Gardner, G. R. and G. LaRoche. 1973. Copper-induced lesions in estuarine teleosts. J. Fish. Res. Bd. Canada. 30: 363 - 368.

Ghosh, A. R. and P. Chakrabarti. 1993. Histopathological and histochemical changes in liver, pancreasand kidney of the freshwater fish *Heteropneustes fossilis* (Bloch) exposed to cadmium. Environ. Ecol. 11: 185 - 188.

Gill, T. S., J. C. Pant and H. Tewari. 1989. Cadmium nephrotoxicity in a freshwater fish, *Puntius conchonius* Ham. Ecotoxicol. Environ. Saf. 18: 165 - 172.

Holcombe, G. W., D. . Benoit and E. N. Leonard. 1979. Long-term effects of zinc exposure on brook trout (*Salvelinus fontinalis*) Trans. Amer. Fish. Soc. 108 : 76 - 87.

Ishii, T., M. Ishikawa and T. Koyanagi. 1985. High accumulations of elements in the kidney of the marine bivalve *Cyclosunetta menstrualis*. Waste Mange. Res. Abst. 16: 257.

Kumar, S. and S. C. Pant. 1981. Histopathologic effects of acutely toxic levels of copper and zinc on gills, liver and kidney of *Puntius conchonius* (Ham). Indian J. Expt. Biol. 19: 191 - 194.

__________ **and** ________. **1984.** Organal damage caused by aldicarb to a freshwater teleost *Puntius conchonius* (Ham). Bull. Environ. Contam. Toxicol. 33: 50 - 55.

Lobel, P. B. 1978a. Intersite, intrasite and inherent variability of the whole soft tissu zinc concentrations of individual mussel *Mytilus edulis* : Importance of the kidney. Mar. Environ. Res. 21: 59 - 71.

_________. **1978b.** Inherent variability in the ratio of zinc to other elements in the kidney of the mussel *Mytilus edulis*. Comp. Biochem. Physiol. 87C: 47 - 50.

_________. **1978c.** Short-term and long-term uptake of zinc by the mussel, *Mytilus edulis*: A study of individual variability. Arch. Environ. Contam. Toxicol. 16: 723 - 732.

Mason, A. Z., K. Simkiss and K. P. Ryan. 1984. The ultrastructural localization of metals in specimens of *Littorina littoria* collected from clean and polluted sites. G. Mar. Biol. Ass. U. K. 64: 699 - 720.

Pirie, B. J. S., G. George, D. G. Lytton and J. D. Thomson. 1984. Metal-containing blood cells of oysters: Ultrastructure, histochemistry and X-ray microanalysis. J. Mar. Biol. Ass. U. K. 64: 115 - 123.

Sathyanathan, B. S., M. Nair, J. Chacko and P. N. K. Nambisan. 1988. Sublethal effects of copper and mercury on some biochemical constituents of the estuarine clam, *Villorita cyprinoides* var. *cochinensis* (Hanley). Bull. Environ. Contam. Toxicol. 40: 510 - 516.

Saxena, O. P. and A. Parashari. 1981. Cadmium-induced tumors in liver and kidney of *Channa punctatus* (Bl.). Int. J. Acad. Ichthyol. , Modiganar. 2: 55 - 59.

Schreck, C. B. and H. W. Lorz. 1978. Stress response of coho salmon (*Oncorhynchus* *kisutch*) elicited by cadmium and copper and potential use of cortisol as an indicator of stress. J. Fish. Res. Bd. Canada. 35: 1124 - 1129.

Tobias, E.G. and V. S. Carino. 1997. Light microscope studies on the effects of zinc-treated water media on the kidney of *Oreochromis niloticus* fingerlings. Mindanao Forum. 12(2): 1-10.

Torres, P., L. Tort and R. Flos. 1987. Acute toxicity of copper to Mediterranean dogfish. Comp. Biochem. Physiol. 86C: 169 - 171.

Wahbeh, M. I. 1985. Levels of zinc, iron, magnesium and cadmium in the tissues of fish from Aquaba, Jordan. Sci. Georgr. 12: 35 - 42.

Wolmaram, C. T.,. J. van Aardt and J. Coetzee. 1986. Histopathological effects of copper on selected epitheial tissues of snails. Bull. Environ. Contam. Toxicol. 36: 906 - 911.