Effects of Copper on Haematological Parameters in the African Freshwater cichlid, *Oreochromis Urolepis*

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Abstract: *The effect of copper on blood parameters in the African freshwater Cichlid Oreochromis urolepis were investigated. This was an experimental study conducted in the aquaria in the Department of Zoology and Wildlife Conservation, University of Dar Es Salaam. Exposure of Oreochromis urolepis to different concentration of copper for a period of up to 35 days, at average water temperature 25ºC, dissolved oxygen 5.4mg/l and pH 6.7, revealed a significant change in some heamatological parameters. For example, Red blood cell counts (RBC) in fish exposed at 100, 150 and 200µgCu/l increased significantly on day 7. Packed cell volume (PCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Volume (MCV) significantly changed with increasing copper concentration and exposure time. Plasma sodium ion concentration [Na^{+}] was significantly altered on day 2, in all copper exposed fish. Chloride ions significantly decreased in higher exposures and increased, on day 35. No significant changes were observed in plasma Ca^{+2} in all exposed fish. Potassium ions significantly increased only in fish exposed to higher copper concentration. It is recommended that copper at lower concentrations of up to 50µg/l can be used in aquaculture for a short period without causing any significant heamatological changes in the cultured fish.*

Keywords: Copper, haematological parameters, *Oreochromis urolepis*.

INTRODUCTION

Freshwater bodies have undergone immense physicochemical and biological changes due to rapid Urbanization, industrial development and modern agrochemical practices resulting into deterioration of water quality, and affected fish health. Therefore, the assessment of the effect of copper on haematological parameters in *Oreochromis urolepis* was necessary in the present experiment.

Copper concentration in the body may reach higher than normal and cause homeostatic ironic imbalance, reduced growth and reproduction (Lauren and McDonald, 1987). For this reason, monitoring copper accumulation in fish tissue and homeostatic imbalance serves an important function as an early indicator of sediment contamination or related water quality problems. This will also permit detection of concentrations of toxic levels of copper in fish that may be harmful to consumers, and take appropriate action for public health and the Tanzanian environment. Haematological parameters are useful to detect the intensity of

pollution in water or in the organisms. Fishery biologists have used haematological procedures to assess the state of health in fish stocks, in hatcheries and in fish biology research. Thus, haematological parameters have been applied and used to investigate health condition and parasite infestation in fish (Blaxhall, 1972).

In general, copper affects the blood characteristic in fish, as its exposure leads to an increase in haemoglobin with subsequent decrease in leucocytes and lymphocytes in brook trout, rainbow trout and carp (Dick and Dixon, 1985; Svobodova *et al.,* 1994). The Mozambique tilapia (*Oreochromis mossambicus)* showed haemodilution, lowered haemoglobin and increased haematocrit values after a short-term exposure to copper and mercury (Cyriac *et al.,* 1989). In addition copper may cause lowering of red blood cell count, white blood cells, packed cell volume and a gradual decline in oxygen carrying capacity (Khangarot and Tripathi, 1991). Sublethal doses of aluminum also cause physiological changes in the blood of Oreochromis hybrid such as swelling of red blood cells, haemodilution and impaired haemoglobin synthesis (Khangarot and Tripathi, 1991).

The study on the effect of copper on haematological indices of fish is important for the evaluation of physiological changes and may forecast the consequences of long-term exposure (Cyriac *et al.,* 1989). Rainbow trout exposed to 26.9ε gCu/l, manifested a transient increase of haemoglobin and haematocrit (Dethloff *et al.,* 1999). Similarly an increase in haemoglobin and haematocrit concentration was observed in *Oreochromis mossambicus* after 24 hours exposure to lower copper concentration. Vosyliene, (1999) observed an increase of erythrocytes in fish exposed to 0.5mgCu/l concentration. Also found that haemolysis and anaemia occurred in catfish (*Claries lazera*) after 96 hours exposure to 3.2mg/l of copper.

In most cases anaemia appears to be a common sensitive response to heavy metal intoxication in higher vertebrate as well as in several fish species. Nonetheless, it is reported that fish have the ability to adapt and recover from heavy metal stresses after prolonged exposure (Pelgrom *et al*., 1995).

The goal of the present study was to evaluate the effect of ambient copper on haematological parameters, gill structure and osmoï ionic regulation in *Oreochromis urolepis*. Therefore in this study both, the effects of copper on fish exposed to short term (2, 7 and 14 days) and long-term exposure (21 to 35 days) were determined.

Materials and Methods

Adult Male and female *Oreochromis urolepis* of mean body weight 18.75g and length 12.2cm were collected using a small seine-net and brought to the laboratory. Fish were acclimatized to laboratory conditions for two weeks in a big tank with dechlorinated freshwater. The tank was aerated and monitored to maintain constant temperature, pH and copper concentrations. Fish were fed with a locally prepared food pellets (a mixture of chick meal and dagaa powder).

Thereafter, Fish were randomly divided into five aquaria each with 27 individuals and fed daily with 8% food per body weight, divided into two portions.

Fish were then exposed to a series of increasing copper concentrations (50,100,150 and 200µgCu/l), while the fifth tank served as a control where no copper was added. The average water temperature was maintained at 25^{o}C, dissolved oxygen 5.4mg/l and pH 6.7. Fish were observed for any behaviour changes and details were recorded.

Replicate experiment was done and a total of 250 fish, 25 fish for every exposure were sampled, that is 5 fish per each exposure, after 2, 7, 14, 21, and 35 days. Blood samples were collected from each sampled fish.

Blood sample was then centrifuged and the plasma was stored at ï 20°C for further analysis. Total plasma calcium was determined spectrophotometrically using sigma diagnostic kits. Sodium and potassium was analyzed using flame atomic absorption spectrophotometer (AAS, Varian). The packed cell volume (PCV) was read using a haematocrit reader after centrifugation. Haemoglobin was determined by cynomethaemoglobin method using spectrophotometer (Dacie and Lewis, 1994). Total red blood cell (RBC) count was determined according to Baker *et al.* (1966). The other haematological indices for example, Mean Corpuscular Volume (MCV); Mean Corpuscular Haemoglobin (MCH); and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated according to Green (1976) and Dacie and Lewis (1994

Results and Discussion

Results were recorded from replicated experimental setup and the values of unexposed fish (controls) were considered as the normal readings and used for comparison with treated samples throughout the experiment.

In this study, most of the changes were due to toxic effect of copper which also resulted in hyperventilation and reduced exploratory behaviour in higher copper concentration of 150 and 200µgCu/l particularly on long term exposures of 21 to 35 days. Similar findings were reported by Stagg and Shuttelworth, (1982) in *Platichthys flesus L.* However, when fish were exposed beyond 35 days, they appeared to acclimatize to the new environment and about 90% in the 100µgCu/l and 50% in 150 and 200 µgCu/l concentrations started behaving as those in the control aquarium.

Some fish exposed in high copper concentrations had a tendency to swim with the mouth wide open and frequently coming to the surface. This was probably due to higher secretion of mucous in the gills as large amount of mucus interfere with the process of gas uptake in the gills.

Analysis of heamatological indices gives a picture of the health status in fish and other conditions such as anaemia. They also provide information on stress conditions due to environmental pollution, and diseases, so altered haematological parameters reflects the poor condition of fish more quickly than other parameters and they are also predetermined both by the concentration of heavy metals in water

and duration of exposure. Since blood responds quickly to changes in environmental conditions it is widely used as an environmental indicator of stress.

In the present study, low concentration of copper (e.g. 50 to 100µgCu/l) caused an increase in erythrocyte count, although not significant and packed cell volume (PCV) on day 21, while haemoglobin concentration [Hb] showed a slight increase on days 7 to 21. Similar results of copper pollution were reported by McKim *et al.,* (1970) in the blood of *Rainbow trout* after a long exposure (three months) to 0.1mg/l copper. Blaxhall and Daisley, (1973) reported changes in haematological values of a healthy *Rainbow trout* exposed to lethal and sublethal copper concentrations which, indicated a compensatory response in the blood oxygen carrying capacity of the species.

The decrease in RBC in the present study on day 7, upon exposure to 100, 150 and 200µgCu/l, could be due to sudden response to changes in water quality, or a reflection of the beginning of stress reaction or could be an indication of haemodilution in fish which was caused by increased copper concentrations. Also the decrease in haemoglobin values in the fish after exposure to sublethal copper could be a result of impaired water balance (osmotic stress) (Tort *et al.*, 1987). This could moreover cause a decrease in oxygen supply to various tissues, thus resulting in a slow metabolic rate and low energy production.

The swelling of erythrocytes was inferred from the increase in the MCV values reported in the present study. This was also reported by Larsson *et al.,* (1984) who attributed it to the swelling of the red blood cells due to hypoxic conditions or impaired water balances (osmotic stress) or abnormal cell division during erythropoiesis. It was also reported that in order to optimize gas exchange fish do release catecholamine under stressful condition that helps to increase both blood flow and permeability of gill tissue. Svobodova *et al.,* (1994), suggested that heavy metal exposure induce change in blood parameters in fish by increasing red blood cells disintegration or in case of more sensitive species damage of the haemopoitic system. An increase of Hb concentration in fish exposed to 100, 150 and 200µgCu/l after 35 days could be explained as a process where the body produces an increased amount of Hb to replace the oxidized or denatured Hb formed as a result of metal exposure.

Significant decreases in MCHC at day 21 in 100, 150 and 200µgCu/l exposures are probably an indicator of red cells swelling and or decrease in haemoglobin synthesis. This was also reported by Bhagwant and Bhikajee (2000), in *Oreochromis hybrid* (Cichlidae) exposed to 100µg/l in sublethal doses of aluminium, and Mazon *et al.*, (2002), reported similar observations in *Prochilodus srofa* exposed to short term sublethal and lethal copper concentration. Since fish survival is closely associated with their environment (water), any changes to this medium will be reflected in their haematological indices (Koyama and Ozaki 1984; Van Vuren 1986; Srivastva and Narain 1985; Bhagwant and Bhikajee, 2000).

In the present study where PCV showed low values, which could be an indication of worsening of fish condition, that led to development of anaemia. MCV decreased after exposure for 14 days but increased on subsequent exposure towards the end of 21 days, followed by a sudden drop on day 35 of the experiment, while the MCHC showed significant increase until the end of the experiment.

Changes in haematological indices of fish caused by heavy metals and their mixtures are pre-determined both by the concentration of heavy metals in water and time of exposure. The haematological indices can be a secondary response of an organism to irritants. A short-term exposure to low concentration of heavy metal mostly induces an increase of these values such as red blood cell counts as shown on day 2 at 50µgCu/l in the present experiment.

The difference and variation in haematological indices among the fish species may be due to individual deviation in terms of age, nutritional uptake and genetic dissimilarity. Plasma Ca^{2+} and Cl^{-} contents were found to be sensitive to water-borne copper and increased in the copper exposed fish (Lauren and McDonald 1987). Similar results were observed in this study on day 35 in fish exposed to 100 and 150µgCu/l. The values of potassium ions increased significantly ($p<0.001$) especially in exposure to higher copper concentrations of 100, 150 and 200µgCu/l for 7 days (8.96mml/l. Stagg and Shuttelworth, (1982) revealed higher concentration of plasma potassium in freshwater adapted *Platichthys L.* (115.2mml/l). A rise in plasma potassium levels indicates that there was haemolysis or tissue damage as a result of the toxic effect of copper which could cause impairment of kidney excretion. A drop in plasma calcium (hypocalcaemia) was observed in the present study in fish exposed to high copper concentrations of 150 and 200µgCu/l on days 21 and 35. This could be attributed to either the increased passive efflux of ion across the gills due to a more or less non selective rise of permeability of the gills especially the gill lamellae to water and ions, which leads to haemodilution by enhancing osmotic uptake of water.

Conclusion and Recommendations

Monitoring of the health status of fish using standardized non-lethal and inexpensive methods will be needed in the currently growing aquaculture industry in Africa and Tanzania in particular. The authors suggest that, there is a need to conduct more experiments in areas where copper is extensively used, so that precaution can be taken to minimize harmful effects. Also the knowledge will help to raise aquaculture industry, production of good quality fish and consequently foster the economic growth in the fish farming communities.

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