Toxic Effect of Aluminium in Oreochromis mossambicus (Peters)

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Aquatic systems are very sensitive to heavy metal pollutants and the gradual increase in the levels of such metals in aquatic environment, mainly due to anthropogenic sources, became a problem of primary concern. This is due to their persistence as they are not usually eliminated either by biodegradation or by chemical means, in contrast to most organic pollutants. Moreover, the decay of organic materials in aquatic systems together with detritus formed by natural weathering processes, provides a rich source of nutrients in both the bottom sediments and overlying water body. Microorganisms, microflora and algae are capable of incorporating and accumulating metal species into their living cells from various supply sources. Consequently, small fish become enriched with the accumulated substances. Eventually man, consuming the fish, inevitably suffers from the results of an enrichment taken place at each trophic level, where less is extracted than ingested (Forstner and Wittmann, 1983). In the present study, effect of Aluminium toxicity on carbohydrate, protein and lipid of *Oreochromis mossambicus* was seen. Our experiments demonstrate that increasing concentrations of aluminium decreases the carbohydrate, protein and lipid content of muscles and liver in the tilapia fish.

Key words: Heavy metal, Aluminium, Oreochromis mossambicus, Aquatic, Pollutant.

Water is the most abundant of all compounds on earth, and is essential for the continued existence of life on this planet. It covers approximately 71% of the planet and is present in all living matter, at times comprising as much as 90% of the body's tissues. Of all available water on earth, only 0.25% can be classified as surface

water (Davis and Day, 1998). Over the years there has been an increased interest in the effects of human habitation and industrialisation on water resources. With the earth's population increasing steadily every year, more pressures are being placed on this precious resource.

The aquatic environments are constantly being exposed to pollution from various sectors. These include industrial processes, mining operations, and agricultural and domestic run-off (Förstner and Wittmann, 1979). In order for aquatic ecosystems to be monitored, correct management policies need to be developed.

One of the main sources of pollution is from heavy metals, which regularly find their way into the aquatic ecosystem. Geological weathering, industrial processing of metals and

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ores, the use of metal compounds, leaching of metals from garbage and solid waste dumps, and animal and human excretions that may contain heavy metals have all contributed to the rise of metals in these fragile ecosystems (Förstner and Wittman, 1979). Being elements, metals cannot be broken down or destroyed by degradation, but instead accumulate within the environment in different forms.

Fish are the ideal models for addressing questions regarding pollution in the aquatic environment, as they are naturally exposed to a complex selection of environmental perturbations. When fish are subjected to stressors such as water pollution, they have immediate neuro-endocrine changes (regarded as the primary response) that produce secondary biochemical and physiological changes. These changes ultimately lead to changes at the individual, community or population level (Pickering and Pottinger, 1995).

Fish has also been extensively used in the study of physiological behavior of heavy metals in body organs (Suzuki et al., 1973; Goldberg, 1976; Oronsaye, 1989). The accumulation of toxic metals to hazardous levels in aquatic biota has become a problem of increasing concern (Dean et al., 1972; GESAMP, 1982; Manahann, 1994; Idodo-Umeh, 2002). Excessive pollution of surface waters could lead to health hazards in man, either through drinking of water and/or consumption of fish (Mathis and Cummings, 1973). The increasing importance of fish as a source of protein and the interest in understanding the accumulation of heavy metals at the trophic levels of the food chain, extend the focus towards finfish (Greig et al., 1978; Deb and Santra, 1997; Obasohan and Oronsaye, 2004). Pollution enters fish through five main routes: via food or non-food particles, gills, oral consumption of water and the skin. On absorption, the pollutant is carried in blood stream to either a storage point or to the liver for transformation and/or storage. Pollutants transformed in the liver may be stored there or excreted in bile or transported to other excretory organs such as gills or kidneys for elimination or stored in fat, which is an extra hepatic tissue (Heath, 1999; Nussey et al., 2000). The use of fish as bio-indicators of metal pollution of aquatic environments and suitability for human use from toxicological view point has been documented (Uthe and Bligh, 1971; Deb and Santra, 1997).

The aim of this study is to determine the toxicological effect of aluminium to one particular species of fish, *Oreochromis mossambicus* (Peters) which is very representative in the global productions from aquaculture and fisheries.

MATERIAL AND METHODS

The experimental fishes were procured from a local fish farm and stock was maintained in plastic tubs (90 lit). Irrespective of sex, young adult fishes weighing 25-30 grams were selected and used throughout the study. Animals are maintained in natural light-dark cycles. They were acclimated to the laboratory conditions for a period of three weeks. During acclimation, the fish were fed daily with a fish meal prepared in the laboratory, which is rich in protein, carbohydrate, minerals, and vitamins. The excreta and excess feed were siphoned out to avoid contamination and stress. Fish of uniform weight (20-30g) were selected for the study.

Aluminium sulphate is commercially one of the most important aluminium compound. It is used in the sewage treatment as a flocculating agent and in the purification of drinking water. Aluminium sulphate stock solution is prepared.

The acute toxicity of aluminium was estimated adopting static renewal bioassay procedure as described by American Public Health Association (APHA, 1975). Different concentrations of aluminium were selected and in each concentration ten fish were introduced to find per cent mortality. The per cent mortality in all concentrations was recorded at 24, 48, 72 and 96 hours of exposures. From the 96hour LC_{50} value, two sublethal concentrations were selected i.e. for long term exposures.

Healthy fish were chosen from the stock and exposed to two sublethal concentrations for 30 days (exposure period). A control group was kept in toxicant free water.

Estimation of biochemical constituents

The carbohydrate, protein, and lipid of muscle and liver of *Oreochromis mossambicus* were estimated by the following procedures. Carabohydrate content of the tissue was estimated by anthrone method (Seifter *et al.*, 1950). Protein

content of the tissue was estimated by the method of Lowry *et al.*, (1951). Lipids content of the tissue was estimated by Bligh and Dyer (1955) method. The mean (n = 5) \pm standard error, values of biochemical constituents were calculated and presented in tables. Student 't' test was performed to test the significant of difference between any two mean values. The significance was judged at 0.05% level of probability.

RESULTS AND DISCUSSION

The 96hr LC₅₀ value of *O. mossambicus* was observed as 8ppm of Aluminium. Based on this, two sublethal concentrations of Aluminium (2ppm and 4ppm) were chosen for exposure of *O. mossambicus* for 10, 20 and 30 days. The data presented in table 1 show the variations in carbohydrate content due to the exposure of *O. mossambicus* to Aluminium. The total carbohydrate (Table 1), protein (Table 2) and lipid (Table 3) in tissues decreased with increasing concentrations and durations of exposure.

It shows a gradual decreasing effect in the muscle carbohydrate content in both concentrations at 10, 20 and 30th day. In liver, the decrease in carbohydrate was gradual in both concentrations after 10 and 20th day. The decrease was suddenly increased at 30th day. Actually, in liver, the decrease was higher than in muscle. The effect of Aluminium in both the muscle and liver are almost similar. The observed depletion of carbohydrate in the present study explains the increased demand of these molecules to provide energy for the cellular biochemical process under toxic manifestations. Similar results were observed in Thalmile crenata. Anabas testudienues and Anabas scandens, when exposed to copper, lead, nitrate and mercury chloride, respectively (Villalan et al., 1988; Mary Candravathy et al., 1991). Under hypoxic conditions; fish derive the energy by anaerobic breakdown of glucose which is available to the cells with the increased glycogenolysis (Prasath and Arivoli, 2008).

Table 1. Carbohydrate content (mg/100mg w/w) of *O. mossambicus* muscle and liver exposed to two sublethal concentrations of Aluminium for 10, 20 and 30 days

Duration (days)	Muscle Concentration (ppm)			Liver Concentration (ppm)		
	0	2	4	0	2	4
0	6.2 ±0.01	6.2 ±0.01	6.2 ± 0.01	62.02±0.17	62.02±0.17	62.02±0.17
10	6.0 ± 0.005	5.91±0.004*	5.46±0.002*	62.41±0.06	59.9±0.17*	56.16±0.29*
20	5.57 ± 0.05	$5.0 \pm 0.02*$	$4.51 \pm 0.03*$	55.33±0.27	54.75±0.20	53.25±0.31
30	5.24 ± 0.005	$4.18\pm0.01*$	$3.53\pm0.02\texttt{*}$	$50.33 {\pm} 0.06$	$39.91{\pm}0.17*$	26.66±0.33*

* Statistically significant at 5% level

Table 2. Protein content (mg/100mg w/w) of *O.mossambicus* muscle and liver exposed to two sublethal concentrations of *Aluminium* for 10, 20 and 30 days

Duration (days)	Muscle Concentration (ppm)			Liver Concentration (ppm)		
	0	2	4	0	2	4
0	27.8 ± 0.1	27.8 ± 0.1	27.8 ±0.1	24.76±0.23	24.76±0.23	24.76±0.23
10	$28.4{\pm}0.09$	25.4±0.13*	24.1±0.05*	24.0 ± 0.05	22.4±0.08*	21.1±0.08*
20	27.8 ± 0.05	$21.3\pm0.09\text{*}$	20.3±0.11*	23.3 ± 0.05	19.3±0.21*	16.5±0.13*
30	27.5 ± 0.05	$17.8\pm0.1\texttt{*}$	16.8±0.05*	22.7 ± 0.05	$14.0\pm0.25*$	10.6±0.19*

* Statistically significant at 5% level

In both concentrations the protein content in muscle (Table 2) decreased gradually till 30^{th} day. In liver, the protein content decreases gradually in 10^{th} and 20^{th} day, but at 30^{th} day the protein content decreases suddenly in both concentrations. Depletion of protein content has been observed in the muscle, intestine and brain of the fish *Catla catla* as a result of mercury chloride toxicity (Prasath and Arivoli, 2008). The decrease in the protein content as observed in the present study in most of the fish tissues may be due to metabolic utilization of the ketoacids to gluconeogenesis pathway for the synthesis of glucose, or due to directing the free aminoacids for the synthesis of proteins, or for the maintenance of osmo and ionic regulation (Schmidt Nielson, 1975). When an animal is under toxic stress, diversification of energy occurs to accomplish the impending energy demands and hence the protein level is depleted (Neff, 1985).

It shows that the lipid content of muscle (Table 3) decreases rapidly at 10^{th} day in both concentrations, then the decrease was gradual after 10^{th} day. In liver, the lipid content decreased suddenly at 10^{th} day in both concentrations, and then the decrease was increased after 10^{th} day.

Table 3. Lipid content (mg/100mg w/w) of *O.mossambicus* muscle and liver exposed to two sublethal concentrations of Aluminium for 10, 20 and 30 days

Duration (days)	Muscle Concentration (ppm)			Liver Concentration (ppm)		
	0	2	4	0	2	4
0	0.018±0.0005	0.018±0.0005	0.018±0.0005	0.014±0.0005	0.014±0.0005	0.014±0.0005
10	$0.017{\pm}0.0005$	$0.014{\pm}0.0005$	$0.012{\pm}0.0005$	$0.014{\pm}0.0$	$0.010{\pm}0.0005$	$0.006 {\pm} 0.0005 *$
20	0.016 ± 0.0005	0.010±0.0005*	$0.008 \pm 0.0005*$	$0.0135 {\pm} 0.0005$	$0.008 \pm 0.0005*$	$0.004{\pm}0.0005*$
30	$0.014{\pm}0.0$	$0.006 \pm 0.0005*$	$0.005 \pm 0.0005*$	$0.013{\pm}0.0005$	$0.006 \pm 0.0005*$	$0.002 \pm 0.0005*$

* Significant at 5% level

Acute toxicity studies of Cadmium on the edible carp, *Catla catla* revealed significant changes in the biochemical constituents of the fish like glucose, glycogen, total proteins, lipids and free aminoacids (Sobha *et al.*, 2007).

CONCLUSION

Heavy metals may affect organisms directly by accumulating in their body or indirectly by transferring to the next trophic level of the food chain. One of the most serious results of their persistence is biological amplification through the food chain An increase in metal remnants in food chain present in an ecosystem reaches to thousands folds in birds and human fed on aquatic products. Fish is one of the main food sources, and as a part of aquatic life face to the toxic effects of metals. They cause serious impairment in metabolic, physiological and structural systems when present in high concentrations in the milieu.

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From this investigation, it is concluded that Aluminium is highly toxic to the teleost, *Oreochromis mossambicus* and its toxicity can be evaluated by physiological, biochemical and haematological parameters. The environmental contamination with this metal can represent a great threat for the fish populations and also a serious problem for the aquaculture.

REFERENCES

- 1. Barlas, N., A pilot study of heavy metal concentration in various environments and fishes in the upper Sakaryia river basin, *Turkey. Envion. Toxicol.*, 1999; **14**: 367-373.
- Bligh E.G. and Dyer W.J., A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 1955; 37 : 911-917.
- Davis, B. and Day, J., Pollution. In: Vanishing Waters. UCT Press, Cape Town, 1998; 166-200.
- 4. Dean JG, Bosqui FL, Lanouette VH, Removing heavy metals from *Waste Water*. *Environ. Sci.*

Technol. 1972; 6: 518-522.

- 5. Deb SC, Santra SC, Bioaccumulation of Heavy Metals in Fishes. An In-vivo Experimental Study of Sewage-fed Ecosystem. The Environmentalist 1997; **17**: 27-32.
- Förstner, U. and Wittman, T.W., Metal pollution in the Aquatic Environment. Springer-Verlag, Berlin, 1979; 30-43.
- GESAMP (Imo/FAO/UNESCO/WMO/WHO/ IAEA/UN/UNEP), Joint Group of Experts on Scientific Aspects of Marine Pollution. *Rep. Stud. GESAMP.* 1982; 15: 108.
- 8. Goldberg ED, The Health of the Oceans, Paris, UNESCO, 1976; 172.
- Greig RA, Wenzloff DR, Mckenzit CL, Merill AS, Zdanowicez VS, Trace metals in the sea Scallops *Pacepedent magelanicus* from Eastern United States. *Bull. Environ. Contam. Toxicol.* 1978; 19: 326-334.
- Hadson, P.V., The effect of metal metabolism uptake, disposition and toxicity in fish. *Aquatic Toxicol.*, 1988; 11: 3-18.
- Heath AG, Water Pollution and Fish Physiology. Lewis Publishers, Boca Raton, Florida, USA, 1991; 359
- Holcombe, G.W., Benoit, D.A. Leonard, E.N., McKim, J.M., Longterm effects of lead exposure on three generations of brook trout, *Salvalinus fontinalis. J. Fish. Res. Board. Can.*, 1976; **33**: 1731-1741.
- Idodo-Umeh G, Pollution assessments of Olomoro Water bodies using Physical, Chemical and Biological indices: PhD. Thesis, University of Benin, Benin City, Nigeria, 2002; 485.
- KargÝn, F., Effects of EDTA on accumulation of cadmium in *Tilapia zilli*. *Turkish J. Zool.*, 1996; **20**: 419-421.
- 15. Lowry D.H., Rosenbergh N.J., Far A.L. and Randall R.L., Protein measurement with Folin phenol reagent. *J. Biol. Chem.* 1951; **195** : 265-273.
- Manahann SE, Environmental Chemistry, 6th Edition, Lewis Publishers Ann. Arbor, London, Tokyo, 1994; 812.
- Mary Candravathy, V. M., Reddy, S. L. N., Lead nitrate exposure changes in carbohydrate metabolism of fresh water fish. *J. Env., Biol.*, 11991; 7: 75.79.
- Mathis BJ, Cummings TF, Selected metals in Sediments, Water and Biota of the Illinois River. J. Water Poll. Cont. Trop. 1973; 45: 1573-1583.
- 19. Neff, J. M., Use of biochemical measurement to detect polluatant medicated damage to fish.

ASTM spec. Tech. publ., 1985; 854: 154-183

- Nussay, G, VanVuren, J.H.J., DuPreez, H.H.: Bioaccumulation of aluminium, copper, iron, and zinc in the tissues of Moggel from Witbank Dam, upper Olifants river catchment (Mpumalanga). South African J. Wldlf Res., 1999; 29: 130-145.
- Nussey G, Van Vuren JHJ, Du Preez HH , Bioaccumulation of Chromium, Manganese, Nickel and Lead in the tissues of the Moggel, *Labeo umbratus* (Cyprinidae), from Witbank Dam, Mpumalanga. *Water SA* 2000; 26(2): 269-284.
- Obasohan EE, Oronsaye JAO, Bioaccumulation of Heavy metals by some Cichlids from Ogba River, Benin City, Nigeria. *Niger. Ann.Nat. Sci.* 2004; 5(2): 11-27.
- Onlu, E., G.mg.m, B.: Concentration of copper and zinc in fish and sediments from the Tigris river in Turkey. *Chemosphere*, 1993; 26: 2055-2061.
- Oronsaye JAO, Histological changes in the kidneys and gills of *Gasterosteus aculeatus*(L) exposed to cadmium. *Ecotoxicol. Environ. Saf.* 1989; 17: 279-290.
- Pickering, A.D. and Pottinger, T.G., Biochemical effects of stress. In: *Biochemistry* and Molecular Biology of Fishes, 5. Environmental and Ecological Biochemistry. (Eds.) P.W. Hochachka and T.P. Mommsen. Elsevier, Amsterdam, 1995; 349-347.
- Prasath P.M.D, and S. Arivoli, Biochemical study of freshwater fish *catla catla* with reference to mercury chloride *Iran. J. Environ. Health. Sci. Eng.*, 2008; 5(2): 109-116
- Schmidt Nielson B, Osmoregulation: Effect of Salinity and heavy metal. *Fed. Proc.* 1975; 33: 2137-2146.
- Seifter S., Wovie S.K. and Mutwyler E., The estimation of glycogen with the anthrone reagent. *Arch. Biochem. Biophys.* 1950; 20: 190-200.
- 29. Sobha, K., Poornima, A1., Harini, P1., Veeraiah, K2, A study on biochemical changes in the fresh water fish, catla catla (hamilton) exposed to the heavy metal toxicant cadmium chloride kathmandu university journal of science, engineering and technology Vol.i, no.iv, august, 2007.
- Suzuki T, Miyama T, Toyama C, The Chemical Form and Bodily Distribution of Mercury in Marine Fish. *Bull. Environ. Contam. Toxicol.* 1973; 10: 247-355.
- 31. Uthe JH, Bligh EG, Preliminary Survey of Heavy Metals Contamination of Canadian

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freshwater Fish. J. Fish Res. Board Can. 1971; 28: 786-788.

 Villalan, P., Narayanan, K. R. Ajmal Khan, S. and Natarajan, R., Proximate composition of muscle, hepatopancrease and gill in the copper exposed estuarine crab *Thalamita crenata* (Latreille). Proceeding of the second national symposium on eco-toxicology, Annamalai University, India. 1988.