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Bioaccumulation and effects of Copper exposure of *Aplocheilus lineatus*

R. Santhi and M. Seetha Lakshmi

Department of Zoology, Pioneer Kumaraswamy College, Nagercoil
* Department of Zoology, Sree Devi Kumari Women's College, Kuzhithurai.

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Abstract
The aim of the study to find out the effect of the copper moiety in copper oxychloride on a freshwater fish, *Aplocheilus lineatus* were exposure for 15, 30, 45, 60, 75 and 90 days. The experimental medium containing 1/4th and 1/10th of copper oxychloride were used for the present study. The accumulation of copper in liver, gill, muscle and intestine was carried out using Atomic Absorption Spectrophotometer. The results of chronic exposure of *Aplocheilus lineatus* to copper oxychloride at 1/4th and 1/10th 96h LC_{50} toxicant levels, the variation in bioaccumulation of copper in four different tissues tested, the maximum accumulation was observed to the liver and gill.

Keywords: *Aplocheilus lineatus,* copper, bioaccumulation

Introduction

Copper is an essential element for fish, but it can be toxic at higher levels (Handy, 1996). Copper-based compounds have been intentionally introduced into the environmental components as molluscicides, fungicides, algicides and herbicides. In addition to copper sulphate, copper oxychloride and copper hydroxide are commonly used as broad-spectrum fungicide for plant disease control in the agrifields. Copper oxychlordie may be sources of copper contamination in the agricultural environment and may also affect nontarget organisms such as snails and earthworms (Snyman *et al*., 2002). Fish mostly have a tendency to bioaccumulate metals and human consumers of fish can be at a great risk (Akan, *et al*., 2012). Sometimes certain heavy metals become lethal through the contamination of human food chain (Hesham *et al*., 2008). Bioaccumulation of heavy metals reflects the amount ingested by the organisms, the way in which the metals distributed among the different tissues and the extent to which the metals are retained in each tissue. Tissue –specific accumulation of metals has been proposed as a key indication of chronic exposure in aquatic toxicology (Luoma and Rainbow, 2005). Metal accumulation in the toxic sites depends on water chemistry, body mass, and sex (Di Toro *et al*., 2000: Serafim and Bebianno, 2001). There are many studies on the response of fish to metal contamination and fish are recognized as one of the most sensitive indicators of changes in the quantity of heavy metals in water bodies (Maheswaran *et al*., 2008). The present study aims to find out accumulation of copper present in the tissues of gill, liver, intestine and muscle of *Aplocheilus lineatus*, exposure of 15, 30, 45, 60, 75 and 90 days.

Materials and Methods

Experimental animal

Healthy fish *Aplocheilus lineatus* (6cm long, weighing about 3-4g) were selected for the experiments. The fish were obtained from the local fish vendor and reared under laboratory condition for about 10 days. These selected fish were fed regularly with minced sheep liver, boiled egg and live feed such as earthworm and mosquito larva. Water was renewed on alternate days (Sprague, 1973). The fish under chronic exposures were allowed into fungicide free water and fed with boiled egg and minced sheep liver for about one hour and transferred back to water containing the fungicide.

Experimental setup

After chronic exposures to copper oxychloride at 1/4th and 1/10th of 96h LC_{50} toxicant levels, *Aplocheilus lineatus* were removed for bioaccumulation studies on the 15, 30, 45, 60, 75 and 90th day of exposure. Liver, muscle, intestine and gill tissues were removed and pooled. The tissue samples were dried in hot air oven at 120^{o}C for about 24h. After drying, they were powered using an electrical blender. The powdered samples were wet washed with concentrated nitric acid (Doshi *et al*., 1969). For digestion, a known quantity of dried sample was taken in 250ml conical flask and digested with concentrated nitric acid until a white residue was formed. Finally, 1:1 mixture of concentrated nitric acid and hydrogen peroxide were added and the sample was kept in hot plate for evaporation. After complete evaporation the residue was dissolved in 1N hydrochloric acid and filtered using Whatman No.542 filter paper. The sample was then made upto 10 ml in volumetric flask. The copper concentration was analyzed using Atomic Absorption Spectrophotometer (Hitachi Zeeman Polarized, Z-5000 series).

Results and Discussion

The four different tissue types gills, liver, muscle and intestine were analysed for the copper accumulation of fish *Aplocheilus lineatus* and tabulated (Table1,2,3,4&5). The inorganic copper found in copper oxychloride gets

bioaccumulated in the different tissues of *Aplocheilus lineatus*. After chronic exposure of *Aplocheilus lineatus* to copper oxychloride at 1/4th and 1/10th 96h LC_{50} toxicant levels, the variation in bioaccumulation of copper in four different tissues tested, the maximum accumulation was observed to the liver and gill. After expose for 90 days, liver accumulated 2.004 ± 0.210 ppm, in 1/4th 96h LC_{50} and 1.768 ± 0.169 ppm in 1/10th 96h LC_{50} whereas the gill accumulated 1.431 ± 0,150 and 1.22 ± 0.101 ppm in 1/4th and 1/10th 96h LC_{50} respectively. These findings coincide with the results of Dallinger (1995) who reported that the highest copper and iron accumulation were found in the liver of *F. heteroclitus* and *S. senegalensis*. Liver accumulated maximum quantity of copper (Campenhout *et al*., 2008). The heavy metal form the medium gains entry into the tissues through water used for gaseous exchange (Pierce *et al*., 2008). Fishes accumulate higher concentration of heavy metals than their basal environmental values due to the process of bioamplification (Cooper, 1983) and are known to concentrate the metal in their body tissues in varying proportions depending upon the species and environmental conditions.

Table-1: Tissue accumulation of copper in *A. lineatus* exposed to 1/4th 96 LC_{50} of copper oxychloride

Sl. No	Tissue	Control	15days	30days	45days	60days	75days	90days
1	Liver	1.036 ± 0.107	1.384 ± 0.116 (33.59)	1.507 ± 0.141 (34.63)	1.710 ± 0.121 (65.06)	1.988 ± 0.181 (91.89)	2.001 ± 0.20 (93.15)	2.004 ± 0.210 (93.44)
2	Muscle	0.438 ±0.036	0.526 ± 0.041 (20.09)	0.653 ± 0.058 (49.09)	0.706 ± 0.061 (61.19)	0.791 ± 0.069 (80.59)	0.797 ± 0.077 (83.22)	0.804 ± 0.079 (83.56)
3	Intestine	0.539 ± 0.047	0.597 ± 0.049 (10.76)	0.704 ± 0.065 (30.61)	0.921 ± 0.082 (70.87)	1.150 ± 0.112 (113.36)	1.158 ± 0.112 (114.84)	1.170 ± 0.108 (117.07)
4	Gill	0.589 ± 0.048	0.746 ± 0.068 (26.65)	0.895 ± 0.079 (51.95)	0.913 ± 0.085 (55.01)	1.422 ± 0.139 (141.14)	1.427 ± 0.138 (142.28)	1.431 ± 0.150 (142.95)

Table-2: Tissue accumulation of copper in *A. lineatus* exposed to 1/10th 96 LC_{50} of copper oxychloride

Sl. No	Tissue	Control	15 days	30 days	45 days	60 days	75 days	90days
1	Liver	1.036 ± 0.107	1.229 ± 0.119 (18.63)	1.393 ± 0.129 (34.46)	1.514 ± 0.149 (46.14)	1.756 ± 0.166 (52.94)	1.762 ± 0.169 (70.08)	1.768 ± 0.169 (70.66)
2	Muscle	0.438 ±0.036	0.492 ± 0.039 (12.33)	0.536 ± 0.049 (22.37)	0.627 ± 0.057 (43.15)	0.676 ± 0.057 (54.34)	0.681 ± 0.061 (50.55)	0.687 ± 0.058 (56.85)
3	Intestine	0.539 ± 0.047	0.566 ± 0.048 (5.01) *	0.659 ± 0.061 (22.26)	0.710 ± 0.069 (59.37)	1.012 ± 0.09 (87.76)	1.019 ± 0.103 (102.23)	1.028 ± 0.098 (90.72)
4	Gill	0.589 ± 0.048	0.604 ± 0.059 (2.55) *	0.753 ± 0.073 (27.84)	0.793 ± 0.069 (34.63)	1.109 ± 0.111 (88.29)	1.114 ± 0.109 (89.13)	1.122 ± 0.101 (90.49)

Table-3: Tissue accumulation of copper in *A. lineatus* exposed to 1/4th 96 LC_{50} of copper oxychloride

Sl. No	Tissue	Control	15-days	30-days	45-days	60-days	75-days	90-days
1	Liver	1.036 ± 0.107	1.384 ± 0.116 (33.59)	1.507 ± 0.141 (34.63)	1.710 ± 0.121 (65.06)	1.988 ± 0.181 (91.89)	2.001 ± 0.20 (93.15)	2.004 ± 0.210 (93.44)
2	Muscle	0.438 ±0.036	0.526 ± 0.041 (20.09)	0.653 ± 0.058 (49.09)	0.706 ± 0.061 (61.19)	0.791 ± 0.069 (80.59)	0.797 ± 0.077 (83.22)	0.804 ± 0.079 (83.56)
3	Intestine	0.539 ± 0.047	0.597 ± 0.049 (10.76)	0.704 ± 0.065 (30.61)	0.921 ± 0.082 (70.87)	1.150 ± 0.112 (113.36)	1.158 ± 0.112 (114.84)	1.170 ± 0.108 (117.07)
4	Gill	0.589 ± 0.048	0.746 ± 0.068 (26.65)	0.895 ± 0.079 (51.95)	0.913 ± 0.085 (55.01)	1.422 ± 0.139 (141.14)	1.427 ± 0.138 (142.28)	1.431 ± 0.150 (142.95)

Table-4: Tissue accumulation of copper in *A. lineatus* exposed to 1/10th 96 LC_{50} of copper oxychloride

Sl. No	Tissue	Control	15-days	30-days	45-days	60-days	75-days	90-days
1	Liver	1.036 ± 0.107	1.229 ± 0.119 (18.63)	1.393 ± 0.129 (34.46)	1.514 ± 0.149 (46.14)	1.756 ± 0.166 (52.94)	1.762 ± 0.169 (70.08)	1.768 ± 0.169 (70.66)
2	Muscle	0.438 ±0.036	0.492 ± 0.039 (12.33)	0.536 ± 0.049 (22.37)	0.627 ± 0.057 (43.15)	0.676 ± 0.057 (54.34)	0.681 ± 0.061 (50.55)	0.687 ± 0.058 (56.85)
3	Intestine	0.539 ± 0.047	0.566 ± 0.048 (5.01) *	0.659 ± 0.061 (22.26)	0.710 ± 0.069 (59.37)	1.012 ± 0.09 (87.76)	1.019 ± 0.103 (102.23)	1.028 ± 0.098 (90.72)
4	Gill	0.589 ± 0.048	0.604 ± 0.059 (2.55) *	0.753 ± 0.073 (27.84)	0.793 ± 0.069 (34.63)	1.109 ± 0.111 (88.29)	1.114 ± 0.109 (89.13)	1.122 ± 0.101 (90.49)

The liver plays an important role in metal sequestration because heavy meals may be bound to matallothioneins or other ligands. Athikewavan *et al*., (2006) observed that when silver carp was exposed to zinc, maximum accumulation of zinc was recorded in the liver. One of the main reasons attributed to the increased presence of heavy metals in liver is its capacity to accumulate heavy metals like cadmium, zinc and copper brought by the blood from other parts effects of heavy metals by binding them (Atli *et al*., 2006). According to Klavercamp *et al*. (1884) the liver and gill along with kidney are the main sites of metallothioein production and metal retention. Followed by liver, the maximum amount of copper accumulated in the gill tissue. After 90 days of exposure, 1.431 ± 0.50 ppm copper accumulated in 1/4th 96h LC_{50} exposure, whereas in the same exposure 1.70 ± 0.10 ppm copper accumulated in the intestine and 0.804 ± 0.079 ppm in the muscle. These findings coincide with the results of Jeyakumar and Paul (2006). When the proximity of various tissues to the toxicants is analysed, gill is in direct contact with the toxic medium, whereas the intestine and muscle are exposed through media effect.

In the case of fish and crustaceans as well as molluscs, gill is one of the target organs to suffer instantaneously from ambient toxicants (Saha *et al*., 2006). Most of the heavy metals resist the action of enzymes and get bioaccumulated inside the gill tissue. After 75 days of exposure to the fungicide the intestine accumulated more amount of copper (1.15 ± 0.172 ppm) than muscle (0.797 ± 0.077 ppm). Even though intestine and muscle accumulate copper, higher accumulation is recorded in the intestine. The present results are in agreement with the result of Annume and Iyaniwura (1993) who reported higher accumulation and faster heavy metal uptake in the gills of *Oreochromis niloticus* and *Clarius gariepinus* followed by the gastro intestinal tracts. Of all the tissues investigated in the present study, the muscle accumulated the lowest level of copper even after 90 days of exposure. This finding confirmed the existing reports (Yang *et al*., 2007). The inorganic copper found in copper oxychloride gets bioaccumulated in the different tissues of *A. lineatus* the variation in bioaccumulation of copper in four different tissues of *A. lineatus* is in the order of liver> gill> intestine> muscle. The maximum accumulation of copper was recorded in liver (2.004 ± 0.210 ppm) in 1/4th 96 h LC_{50} and (1.768 ± 0.169 ppm) in 1/10th 96 h LC_{50}. Followed by liver the maximum amount of copper accumulation was recorded in gill tissue. Of all the tissue investigated the lowest accumulation was recorded in the muscle tissue (56.85 percent) of *A. lineatus* exposed to 1/10th 96h LC_{50} of copper oxychloride. In freshwater systems, the heavy metal ions remain biologically active affecting the physiology of fish and other aquatic organisms and get accumulated in their tissues.

References

Handy, R.D. 1996. Dietary exposure to toxic metals in fish. In Taylor E.W., eds, Toxicology of Aquatic Pollution: Physiology, Cellular and Molecular Approaches, *Cambridge University press, Cambridge, U.K*. pp: 29-60.

Snyman, R.G., Reinecke, A.J. and Reinecke, S.A., 2002. Field application of a lysosonal assay as biomarker of copper oxychloride exposure, in the snail *Helix aspesa. Bull. Environ. Contam. Toxicol*., 69: 117-122.

Akan, J.C., Mohmoud, S., Yikala, B.S. and Ogugbuaja, V.O. 2012. Bioaccumulation of some heavy metals in fish samples from River Benue in Vinikilang, Adamawa State, Nigeria. *American Journal of Analytical Chemistry*, 3: 727-736.

Hesham, M., Korashy, Ayman, O.S. and Kadi, E. 2008. Modulation of TCDD-mediated of cytochrome P4501A1 by mercury, lead and copper in human $HepG_2$ cell Line. *Toxicology in vitro,* 22(1): 154 – 158.

Luoma, S.N. and Rainbow, P.S. 2005. Why is metal bioaccumulation. So variable? Biodynamics as unifying concept. *Environ, Sci. Technol*., 39: 1921-1931.

Di., Toro, D.M., Allen, H.E. Bergmann, H.L. Meyer, J.S. Santore, R.C. and Paquin, P. 2000. The biotic ligand model: A Computational approach for assessing the ecological effects of copper and other metals in aquatic systems. *International Copper Association, Ltd.*, New Yark. Pp:106.

Serafim, M.A. and Bebianno, M.J., 2001. Variation of metallothinein and metal concentrations in the digestive gland of the clam *Rubitapes decussates*: sex and seasonal effects. *Environmental Toxicology and Chemistry*, 20: 544-552.

Maheswaran, R., Devapaul, A., Muralidharan, S., Velmurugan, B. and Ignacimuthu, S. 2008. Haematological studies of freshwater fish, *Clarias batrachus* (L.) exposed to mercuric chloride. *International Journal of Integrative Biology*, 2(1): 49 - 54.

Campenhout, K.V. Infante, H.G., Geomans, G., Belpaire, C., Adams, F., Blust, R. and Bervoets, L. 2008. A field survey of metal binding to metallothionein and other cytosolic ligands in liver of cells using an on-line istoper dilution method in combination with Size Exclusion (SE) High Pressure Liquid Chromatography (HPLC) coupled to Inductively Coupled Plasma Time Of Flight Mass Spectrometry (ICP- TOFMS). *Science of Total Environment,* 394(2-3): 379- 389.

Pierce, G.J., Stowasser, G., Hastie, L.C. and Bustamante, 2008. Geographic, seasonal and ontogenetic variation in cadmium and mercury concentrations in squid (Cephalopoda: Teuthoidea) from UK waters. *Ecotoxicology and Environmental safety*, 70 (3): 422- 432.

Cooper, J.J. 1983. Total mercury in fishes and selected biota in Lohotan reservoir. Navada. *Bull. Environ. Contom. Toxicol.*, 31:9-17.

Athikewavan, S., Vincent, S. and Velmurugan, B. 2006. Accumulation of zinc in the different tissues of the silver carp, Hypopthalmichthys molitrix. *Poll. Res*., 25 (1): 47-49.

Atli. G., Alptekin, O., Tukel, S. and Canli, M. 2006. Response of catalase activity to Ag^{+}, Cd^{+2}, Cr^{6+}, Cu^{2+} and Zn^{2+} in fish tissues of freshwater fish *Oreochromis niloticus*, Comparative biochemistry and physiology part C. *Toxicology and Pharmacology*, 143(2): 218-224.

Klavercamp, J.E., McDonald, W.A., Duncan, D.A. and Wagenann. 1984. Metallothionein and acclimation to heavy metals in fish, a review. In: *Contaminant effects on fisheries* (Cairns, V.W., Hodson, P.V. and Nriaga, J.O. Eds.) Wiley, New York, 99-113.

Jeyakumar, P. and Paul, V.I. 2006. Patterns of cadmium accumulation in selected tissues of the cat fish, *Clarias batrachus* L. exposed to sublethal concentration of cadmium chloride. *Veterinarski Arhiv*., 76(2):167-177.

Saha,M., Sarkar, S.K. and Bhattacharya, B. 2006. Interspecific variation in heavy metal body concentrations in biota of Sunderban mangroove wetland, northeast India. *Marine Pollution and Ecotoxicology*, 32(2): 203- 207.

Annume, P.A. and Iyaniwara, T.T. 1993. Accumulations of two trace metals in tissues of freshwater fishes, *Orecohromis niloticus* and *Clarias gariepinus*. *J Aqua. Food Prod. Technol*., 2(3): 5-18.

Yang, R., Yao, T., Xu, B., Jiang, G. and Xin, X. 2007. Accumulation features of organochlorine pesticides and heavy metals in fish from high mountain lakes and Lhasa River in the Tibetian Plateau. *Environment International,*33 (2): 151- 156.

Doshi, G.R., Sreekumaran, G., Mulay, C. and Patel, B. 1969. Ashing procedures for biomaterials. *Current Science*, 9: 206-207.

Sprague, J.B. 1973. The ABC's of pollutant bioassay using fish: Biological methods for the assessment of water quality. ASTM, STP 528, American Society for Testing and Materials.6-30.

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