International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **3(11),** 18-22, November **(2014)** *Int. Res. J. Biological Sci.*

Toxic effects of Cadmium and Popper on Gillsurface Ultra structure of *Anabas testudineus* Bloch: A scanning Electron Microscopic Study

Chakpram Rita and Gupta Abhik
Department of Ecology and Environmental Science, Assam University, Silchar, Assam, INDIA

Available online at: www.isca.in, www.isca.me
Received 29th May 2014, revised 5th September 2014, accepted 8th November 2014

Abstract

The effects of a 96 hexposure to 40.898 mg L^{-1} Cd and 0.980 mg L^{-1}Cu, which represented the 96 h LC_{50} of these two metals, on gill surface ultra structure of Anabas testudineus was studied using scanning electron microscopy. Fish gills comprise primary and secondary lamellae, which have three different cell types: epithelial pavement cells, chloride cells and mucous cells. The changes in gill surface ultra structure were characterized by fusion of adjacent secondary lamellae, oedema, disruption of gill epithelium, and excretion of large amount of mucous on lamellar surface. These alterations demonstrate the morphological as well as physiological responses of the fish when exposed to Cd and Cu. The study shows scanning electron microscopy to be useful tool for identifying morphological biomarkers on the gills of Anabas testudineus.

Keywords: *Acute toxicity,LC_{50}, scanning electron microscopy, anabas testudineus.*

Introduction

The occurrence and concentrations of various contaminants have increased in the environment along with increasing anthropogenic activities in the industrial, agricultural and other sectors[1]. Water is a valuable resource and plays a vital role in supporting all forms of life[2]. Water pollution is, therefore, of universal concern. Among the various contaminants, environmental concentrations of heavy metals have been increasing in aquatic environments in recent years. Cadmium and copper are highly toxic to aquatic animals[3,4]. Cadmium is a xenobiotic with no known metabolic role and is genotoxic, mutagenic, carcinogenic and teratogenic[5-7]. On the contrary, several metabolic processes including neurotransmission, iron absorption in intestine, erythropoiesis through hemoglobin synthesis, and others are mediated by copper, which is an essential trace element, and a constituent of many enzymes[8].

The gills of fishes are characterized by their large surface area, which come in contact with the aquatic environment. Because of this, they become the targets of various toxicants including heavy metals. Gills are lined by a thin epithelium that separates the internal and external media. Thus they are the organs which are the first to come in contact with pollutants present in water, which in turn impair the structure and function of gill epithelium. Furthermore, gills also have a tendency to bioaccumulate heavy metals at levels higher than that in muscles[9]. Important physiological functions of fish, e.g., gas and ion exchanges, osmoregulation, removal of nitrogenous wastes and acid-base equilibrium are affected due to disruptions in the normal surface ultra structure of gills[10,11]. Because of these reasons, gills are considered as suitable tissue for detecting the damages caused by environmental toxicants on fish[12].

Fishes an important source of protein supplement to our body[13]. *Anabastestudineus* (Bloch) is acommon freshwater fish belonging to the family Anabantidae of the order Perciformes. It is a popular food fish of India and other south and Southeast Asian countries, and is one of the vulnerable fish species affected by environmental pollution, unsustainable capture and improper culture methods[14]. The presence of accessory respiratory organ helps this fish to survive in unfavourable environmental conditions such as outside of water for a short duration and to tolerate oxygen deficient conditions in water. *Anabas testudineus* is used as a test fish for our study because it is a food fish of common occurrence, and can be easily transported and maintained under laboratory conditions.

Teleostean gill arch bears primary lamellae which give rise to rows of secondary lamellae. The lamellar epithelium comprises pavement cells, chloride cells and mucous secreting cells. The thin pavement cells are found in large numbers on the lamellae and mainly perform the role of ionic and acid- base regulation along with excretion of nitrogenous waste materials. Chloride cells are involved in ion uptake and exchange such as those involving sodium and calcium ions and others[15,16]. They are oval to round in shape and rich in mitochondria with tubules and vesicles of different size[12]. Increase in size of chloride cells has been reported in fish after heavy metal exposure in order to compensate the loss of ions as well as to enhance the removal of toxicants. Mucous cells are completely protected by neighboring pavement cells and contain granules of varying electron density[17]. It has been suggested that histological alterations provide more precise evaluation of both the health of the fish and the effects of pollutants than any single biochemical parameter[18]. Such changes are more sensitive and occur earlier

in comparison to reproductive and developmental changes. This paper, therefore, investigates the effect of Cd and Cu at their acutely toxic concentrations on the gill surface ultra structure of *Anabas testudineus.*

Methodology

Healthy *Anabas testudineus* were collected from water bodies in Cachar district, Assam, India. Fish specimens were subjected to prophylactic treatment by bathing them in 0.05% (w/v) potassium permanganate ($KMnO_4$) for two minutes to cure any fungal or bacterial infection and to ward off further infections. In the laboratory, fishes were maintained in aquaria and acclimatized for 10 days before toxic exposure. They were fed adequately with commercial food pellets ('Tokyu') during this period, and water in the aquaria was changed every 24 h. However, the fish were not fed during the last 24 h of acclimatization and throughout the exposure period of 96 h for each metal. Only disease-free, healthy fish were used for the experiments. Analytical-grade cadmium chloride ($CaCl_2$. H_2O) (98%) and copper sulfate ($CuSO_4.5H_2O$) manufactured by Himedia Lab. Ltd., Mumbai, India, were used as the test compounds.

Scanning electron microscopic study: The acute toxicity tests from a previous study reported a 96 h LC_{50} of 40.898 mg L^{-1} and 0.980 mg L^{-1} for Cd and Cu, respectively, for *Anabas testudineus*[19]. Fishes were exposed to the two above-mentioned concentrations of cadmium and copper separately for 96 h, gills were removed from the treated fish, washed in distilled water and fixed in 3%glutaraldehyde for 4h at 4^0C. They were then washed in 0.1 M Sodium Cacodylate buffer for 15 minutes followed by dehydration in 30 - 100% acetone at 4^0C and drying with tetra methyl silane[20]. A control set of fish was maintained in unchlorinated tap water without added Cd or Cu. Dried specimens were mounted onto aluminium stubs and coated with gold in a fine coat sputter coater (JFC-1100), and examined using scanning electron microscope (JEOL-JSM 6360).

Results and Discussion

Control fish showed gills with normal arrangement of cell components and primary and secondary lamellar organization patterns (figure-1). In control fish, the four gill arches had normal structure and they supported many gill filaments or primary lamellae. A row of secondary lamellae was present on the lower and upper side of each primary lamella.

Exposure of fish to 40.898 mg L^{-1} Cd resulted in major ultra structural changes which included cell hypertrophy and other alterations on lamellar surface. Scanning electron micrographs showed swelling and fusion of lamellae especially at their tips along with mucous deposits on gills. Epithelial lifting and breakdown of surface epithelium were the major effects due to cadmium intoxication (figure-2).

Fish treated with 0.980 mg L^{-1}Cu showed gill with copious amount of mucous deposited on its surface. Thinning of secondary lamellae was accompanied by smothering and obliteration in some places. Swollen and fused tips of lamellae were more pronounced and there was disruption of surface epithelium in copper exposed gill (figure- 3).

Oedema and rupture of lamellar epithelium are among the first symptoms which indicate that the fish is suffering from some pathological effects[21]. Proliferation of mitochondrial rich cells and stem cells may result in partial or complete fusion of secondary lamellae[22]. Such adaptive mechanisms of fish comprise defensive responses to reduce the surface area of gill in contact with the toxicant, with the extent of gill damage reflecting the toxic potential and mode of action of the xenobiotic[23,24]. Parashar and Banerjee[25] observed that some gill lesions in *Heteroneustesfossilis* represented direct deleterious effects of the toxicant, while some others were defence responses of the exposed fish.

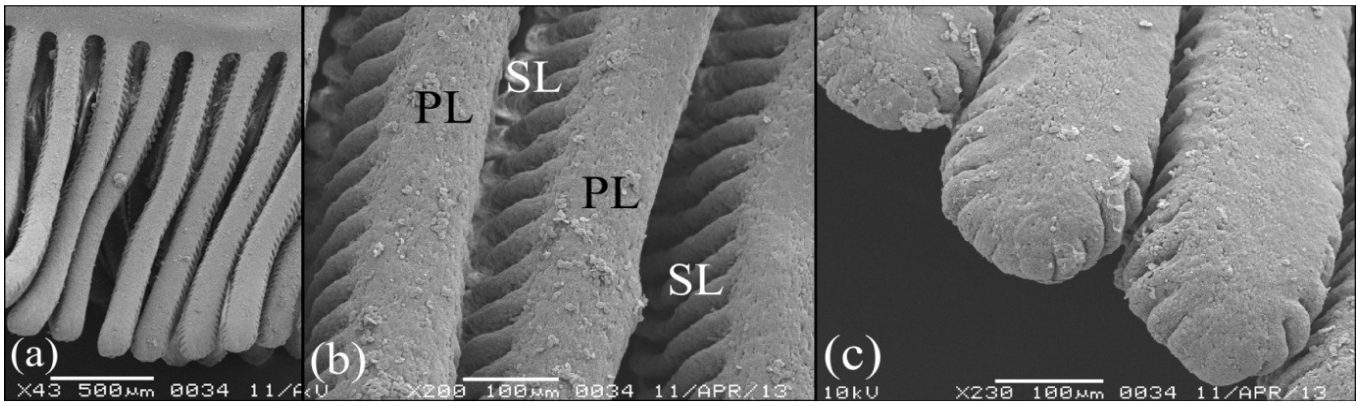

Figure-1 (a- c)
Scanning electron micrographs of gill of control*Anabas testudineus*showing normal gill filament (primary lamellae, PL, and secondary lamellae, SL)

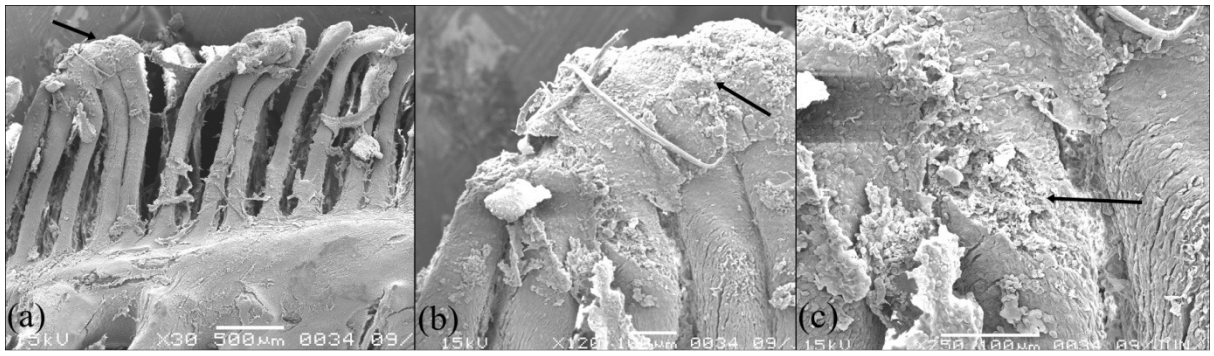

Figure-2(a-c).
Scanning electron micrograph of gill of *A.testudineus* exposed to 96 h LC_{50} Cd (40.898 mg L^{-1}). Swollen and fused tips of lamellae are prominent. Arrows point to epithelial lifting and erosion of gill epithelium

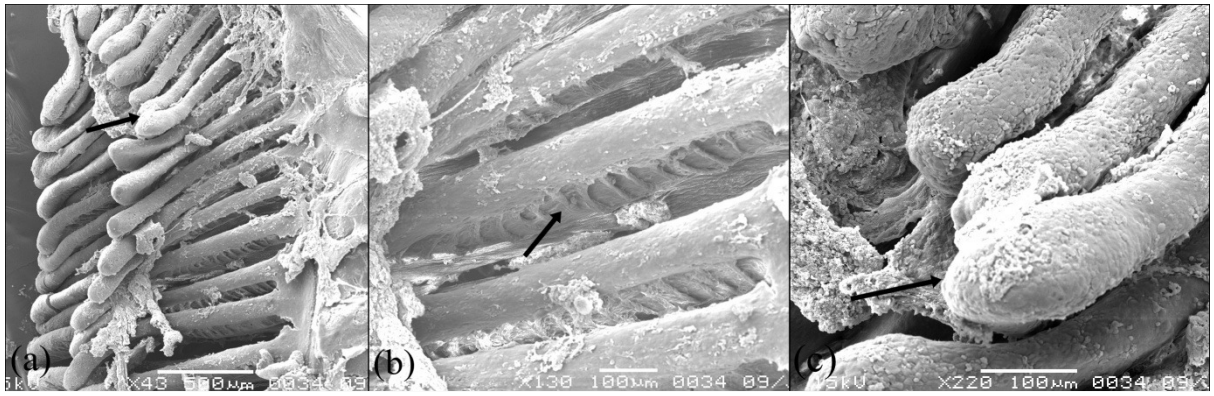

Figure-3(a - c)
Scanning electron micrograph of gill of *A.testudineus* exposed to 96 h LC_{50} Cu (0.980 mg L^{-1}). Copious amount of mucous is deposited on gills; arrow points to thin secondary lamellae with wider interfilamental space; secondary lamellae swollen and fused especially at the tips

The structural damages in the gill of *A. testudineus* observed in the present study after exposure to cadmium and copper were similar and comparable to those described in fishes exposed to other heavy metals[26-28]. *Catlacatla* after treatment with 15.5 mg L^{-1} lead nitrate exhibited cell hypertrophy, hyperplasia, and lamellar fusion accompanied by various changes in lamellar ultrastructure[29]. Epithelium of the filament and lamellae, mucous secreting cells and chloride cells were mainly affected in the gills of *Oreochromisniloticus* after copper exposure[28]. Profound histological changes had also been reported in gills of *Astyanaxaff. Bimaculatus* after acute exposure to zinc which include dhyperplasia, lamellar fusion, aneurysm, destruction of lamellar epithelium, rupture of membrane and obliteration of secondary lamella[30].

A 96 h exposure of cadmium (300 µg L^{-1}) to *Cyprinuscarpio*, *Australoherosfacetum* and *Astyanaxfasciatus* produced structural changes, oedema and fusion of adjacent secondary lamellae on the gills[31].The profuse mucous secretion on the gill surface epithelium of the lamellae of *A. testudineus* observed in the present study indicated a high mucous secreting character of the gills[32-34]. This mucous secreting nature served as a protective barrier[32,35] and at the same time played a role in ionoregulation[36]. The secretion of large amount of mucous was the first response observed in the external tissues of fish to toxic substances present in water[37]. Such responses represented defence mechanisms that aidedthe fish in getting rid of pathogens, toxic compounds and foreign materials[25].

Conclusion

The gills emergedas sensitive indicators of the toxic effects of Cd and Cu because of their direct contact with water, their large surface area and high permeability, and the characteristic responses of their surface epithelial cells to the effects of the toxicants. This study showed that cadmium and copper have adverse effects on gillsurface ultrastructure of *Anabas testudineus* and further studies are needed to relate these histological changes to those occurring at the physiological and biochemical levels.

Acknowledgements

Rita Chakpram gratefully acknowledges the University Grants Commission, New Delhi, India, for award of a UGC- AUS fellowship. The authors are thankful to the Head, Sophisticated

Analytical Instrument Facility (SAIF-NEHU) at North Eastern Hill University, Shillong, India, for allowing use of SEM.

References

1. Manoj K. and Padhy P.K. Oxidative stress and heavy metals: an appraisal with reference to environmental biology, *Int. Res. J. Biological Sci.*, **2(10)**, 91-101 **(2013)**

2. Rajiv P., Hasna A.S., Kamaraj M., Rajeshwari S. and Sankar A., Physico-chemical and microbial analysis of different river waters in Western Tamil Nadu, India. *Int. Res. J. Environment Sci.,* **1(1)**, 2-6 **(2012)**

3. Jezierska B. and Witeska M., Metal toxicity to fish. University of Podlasie, Siedlce **(2001)**

4. Mendez-Armenta M. and Rios C., Cadmium neurotoxicity, Mini review, *Environ. Toxicol. Pharmacol.,* **23,** 350–358 **(2007)**

5. Walkes M.P., Cadmium carcinogenesis in review, *J. Inorg. Bichem*., **7**, 241–244 **(2000)**

6. Gabbianelli R., Lupidi G., Villarini M. and Falcioni G., DNA damage induced by copper in erythrocytes of gilthead sea bream *Sparusaurata* and mollusc, *Scapharcainaequivalvis. Arch. Environ. Contam. Toxicol*., **45,** 350–356 **(2003)**

7. Cavas T., Garanko N.N. and Arkhipchuk V.V., Induction of micronuclei and binuclei in blood, gill and liver cells of fishes subchronically exposed to cadmium chloride andcopper sulphate, *Food Chem Toxicol.,* **43**, 569–574 **(2005)**

8. Fedeli D., Carloni M. and Falcioni G., Oxidative damage in trout erythrocyte in response to ''in vitro'' copper exposure, *Marine Environ. Res*., **69,** 172–177 **(2010)**

9. Nwajei G.E., Obi–Iyeke G.E. and Okwagi P., Distribution of selected trace metal in fish parts from the River Niger, *Res. J. Recent Sci.,* **1(1)**, 81-84 **(2012)**

10. Maina J.N., *The Gas Exchangers. Structure, Function and Evolution of the Respiratory Processes*, Springer, New York, 498 **(1998)**

11. Wendelaar Bonga S.E. and Lock R.A.C., The osmoregulatory system, in Di Giulio, R.T. and Hinton, D.E. (Eds) : *The Toxicology of Fishes*, CRC Press-Taylor and Francis Group, Boca Raton, FL, 401–415 **(2008)**

12. Vigliano F.A., Aleman N., Quiroga M.I. and Nieto J.M., Ultrastructural characterization of gills in juveniles Argentinian Silverside, *Odontesthesbonariensis* (Valenciennes 1835) (Teleostei : Atheriniformes), *Anat. Histol. And Embryol.*, **35**, 76–83 **(2006)**

13. Ganguly S., Human Health benefits from fish consumption and environmental toxicity issues in fish flesh, *Int. Res. J. Biological Sci.,* **2(9),** 84-85 **(2013)**

14. Kartika B., Diana A., Marsoedi and Hakim H., Effects of protein levels on the growth of climbing perch, *Anabas testudineus* Galam type, in Peat water, *Int. Res. J. Biological Sci.,* **2(4)**, 55-58 **(2013)**

15. Kirschner L.B., The mechanism of sodium chloride uptake in hyper regulating aquatic animals, *J. Exp. Biol.*, **207**, 1439–1452 **(2004)**

16. Evans D.H., Piermarini P.M. and Choe K.P., The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiol. Rev.*, **85**, 97–177 **(2005)**

17. Fernandes M.N. and Perna-Martins S.A., Epithelial gill cells in the armoredcatfish, *Hypostomus CF. Plecostomus* (Loricariidae), *Revista Brasileira de Biología*, **61**, 69–78 **(2001)**

18. Poleksic V., Lenhardt M., Jaric I., Djordjevic D., Gacic Z., Cvijanovic G. and Raskovic B., Liver, gills, and skin histopathology and heavy metal content of the Danube starlet (*Acipenserruthenus Linnaeus*, 1758), *Environ. Toxicol. Chem*., **29(3)**, 515–521 **(2010)**

19. Chakpram R. and Gupta A., Effects of cadmium and copper on survival and growth of *Anabas testudineus*, Bloch, *Res. J. Chem. Environ. Sci*. **2(2) (2014)**(In Press)

20. Gupta S. and Gupta A., Scanning electron microscopic study of the cuticular structures on the head of *Gerris Sp*. (Hemiptera: Gerridae) and *Cloeon Sp*. (Ephemeroptera : Baetidae), *Entomen*., **29(1)**, 25-30 **(2004)**

21. Thophon S., Kruatrachue M., Upatham E.S., Pokethitiyook P., Sahaphong S. and Jaritkhuan S., Histopathological alterations of white seabass *Latescalcarifer*, in acute and subchronic cadmium exposure. *Environ. Pollut.*, **121**, 307-320 **(2003)**

22. Dang Z., Lock R.A.C. and Flik G., Wendelaar Bonga S.E., Metallothionein response in gills of *Oreochromismossambicus* exposed to copper in fresh water, *Am. J. Physiol–Regul. Integr, Comp. Physiol.*, **277**, 320–331 **(1999)**

23. Cengiz E.I. and Unlu E., Histopathological changes in the gills of mosquito fish *Gambusiaaffinis* exposed to endosulfan, *Bull. Environ. Contam.Toxicol.*, **68**, 290–296 **(2002)**

24. Poleksic V. and Jermic S., Effects of acute cyanide poisoning on the fish gills, *Ind.Pol*., **4**, 802–808 **(2003)**

25. Parashar R.S. and Banerjee T.K.., Toxic impact of lethal concentration of leadnitrate on the gills of air-breathing catfish *Heteropneustesfossilis* (Bloch), *Vet.Arch*., **72**, 167–183 **(2002)**

26. Stouthart A.J.H.X., Spanings F.A.T., Lock R.A.C. and Wendelaar Bonga S.E., Effects of water pH on chromium toxicity to early life stages of the common carp (*Cyprinuscarpio*), *Aquat. Toxicol.*, **32**, 31–42 **(1995)**

27. Mazon A.F., Cerqueira C.C.C. and Fernandes M.N., Gill cellular changes induced by copper exposure in the South American tropical freshwater fish *Prochilodusscrofa*, *Environ. Res.*, **88A**, 52–63 **(2002)**

28. Monteiro S.M., Rocha E., Mancera J.M., Fontaínhas-Fernandes, A. and Sousa M. A stereological study of copper toxicity in gills of *Oreochromisniloticus*, *Ecotox. Environ. Saf*., **72**, 213–223 **(2009)**

29. Palaniappan P.L.RM., Selvi Sabhanayakam, Krishnakumar N. and Vadivelu M., Morphological changes due to lead exposure and the influence of DMSA on the gill tissues of the freshwater fish, *Catlacatla*, *Food and chemical Toxicol.,* **46**, 2440-2444 **(2008)**

30. Daiane C.M.S., Sergio L.P.M., Juraci A.O. and Jorge A.D.S., Histological alterations in gills of *Astyanaxaff. bimaculatus* caused by acute exposition to zinc, *Exp. Toxicol. pathol*., **64(7-8)**, 861-866 **(2012)**

31. Ferrari L., Eissa B.L., Ossana N.A. and Salibián A., Effects of sublethal waterborne cadmiumon gills in three teleosteanspecies : Scanning electron microscope study, *Int. J. Environ. Health.,* **3(4),** 410-426 **(2009)**

32. Laurent P., Hobe H. and Dunel-Erb S., The role of environmental sodium chloriderelative to calcium in gill morphology of fresh water salmonid fish, *Cell Tissue Res*., **240**, 675–692 **(1985)**

33. Pisam M. and Rambourg A., Mitochondria-rich cells in the gill epithelium ofteleost fishes : An ultra structural approach, *Int. Rev. Cytol*., **130**, 191–232 **(1991)**

34. Saboia-Moraes S.M.T., Hernandez-Blazquez F.J., Mota D.L. and Bittencourt A.M., Mucous cell types in the branchial epithelium of the euryhaline fish *Poeciliavivipara*, *J. Fish Biol*., **49**, 545–548 **(1996)**

35. Mc Cahon C.P., Pascoe D. and Kavnagh M., Histochemical observations on the salmonids *Salmosalar L.* and *Samotruta L.* and the ephemeropterans *Baetisrhodani* (Pict.) and *Ecdyonurusvenosus* (Fabr.) following a simulated episode of acidity in an upland stream, *Hydrobiologia.,***153**, 3–12 **(1987)**

36. Handy R.D., Eddy F.B. and Romain G., In vitro evidence for the ionoregulatory role of rainbow trout mucus in acid, acid/aluminium and zinc toxicity, *J. Fish Biol*., **35**, 737–747 **(1989)**

37. Kossakowski M.K. and Ostaszewska T., Histopathological changes in the Juvenilecarp *Cyprinuscarpio*, *Arch. Pol. Fish*., **11**, 57–67 **(2003)**