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Recovery in Acid Aluminium Induced Toxicity in Gills with Silicon Dioxide in *Channa punctatus* (Bloch)

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Abstract : Water pH affects the solubility of Al metal. Gills in fishes serve vital functions like gaseous exchange and acid- base balance which is damaged due to metal toxicity. Under acidic condition at pH < 5 aluminium ($Al_2(SO_4)_3$) at sublethal dose (140 mg/l) brings morphological alterations in the gills including lamellar fusion, dealignment of microridged epithelial cells and hyperplasia of epithelial cells. These abnormalities can be attributed to response to prevent the entry of metal in the gills. The toxicity of aluminium on the gills of fish *Channa punctatus* (Bloch) can be reduced with the application of Silicon dioxide. Damage to the gill epithelium was discernibly lessened by the ameliorating effect of Silicon dioxide, which reduced the alterations in the gills effectively and in turn it increased survival in the fish.

Key Words: *Acid, Aluminium, Fish gill, Silicon dioxide, Amelioration*

Introduction

Water pH is an important chemical property for the survival of aquatic organisms because it affects the ability of fish and other aquatic organisms to regulate basic life- sustaining processes, primarily the exchanges of respiratory gases and salts with the water in which they live (Evans *et al*., 2005). The pH value of aquatic ecosystem affects the solubility of aluminium metal. Aluminium is the third most prevalent element which forms about 8% of the earth's crust. It a silvery white, ductile and malleable metal and belongs to group IIIA of the periodic table. It is extremely reactive and is not found as the free metal. Bauxite is the most important raw material for the production of aluminium, which contains up to 55% alumina (aluminium oxide). Bentonite, and zeolite are natural aluminium minerals which are used in water purification, sugar refining, brewing and paper industries. In the soil, in clay fraction aluminium comes through chemical weathering of rocks.

Both natural processes and anthropogenic sources contribute to the release of aluminium in the environment. It enters environmental media naturally through the weathering of rocks and minerals. Anthropogenic sources are air emissions, waste water effluents, and solid waste primarily associated with industrial processes such as smelting and mining etc. Aluminium becomes more soluble and more toxic to freshwater biota as pH of ambient water decreases below 5 (Gensemer and Playle, 1999). The acute toxicity of Al has been studied in different experiments by many authors (Gensemer and Playle, 1999; Berthon, 2002; Naskar *et al*., 2004; 2006; 2009). Aluminium acts as a gill toxicant to fish and cause ionoregulatory imbalance as well as haematological, respiratory disturbances (Neville and Campbell, 1988). Aluminium becomes soluble at low pH value <5 and are easily absorbed by fish and other aquatic organisms. Metal toxicity causes adverse effects on organism's activity, growth, metabolism, reproduction which are considered as sublethal effects(Wright and Welbourn,2002). When the pH of water is below 5.5, fish die or become seriously ill.

Silicon helps in the elimination of acute aluminium toxicity in fish when silicic acid reacts with aluminium to form hydroxialuminosilicates (HAS), one of the predominant forms of Al in acidified environment, which are extremely insoluble and play an important role in controlling the release of Al from soil to the aquatic environment (Exley *et al*., 1997; Exley *et al*., 2002). Hence the SEM study was undertaken to assess the ameliorating effect of Silicon dioxide in acid aluminum induced toxicity in gills of *C. punctatus* at sublethal concentration.

Material and Methods

Healthy fish *Channa punctatus* (Bloch) of approximate Weight 0.2±0.03 Kg Length 20.8+0.4 cm were procured and acclimated for 15 days under normal laboratory conditions(water temperature 25± l°C , pH

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7.1, Dissolved oxygen 6.2 mg/L ,total hardness 168 mg/L). After acclimation to laboratory conditions, fishes were divided in three groups , 20 in each and placed in separate glass aquaria. Fishes were fed *ad libitum* with *Tubifex tubifex* and *Daphnia* and were starved prior to experiment. Group I was maintained as control. Group II was exposed to Acidified water at pH < 5 with stock solutions of 1 M H_2SO_4 for period of 7 days. Group III was exposed to Acidified water along with sublethal dose of 140 mg/l of Aluminium Sulphate $Al_2(SO_4)_3.16H_2O$ (Qualigens) (LC_{50} is 220mg/l, Chakraborty *et al.* 2012) for 7 days. Group III fishes were treated with 150 mg of Silicon (metal) powder (Loba Chemie) for another 7 days to ascertain the ameliorating effect of Silicon in aluminium toxicity. The pH of ambient water of treated fish was maintained less than 5.0 with stock solution of 1M H_2SO_4.

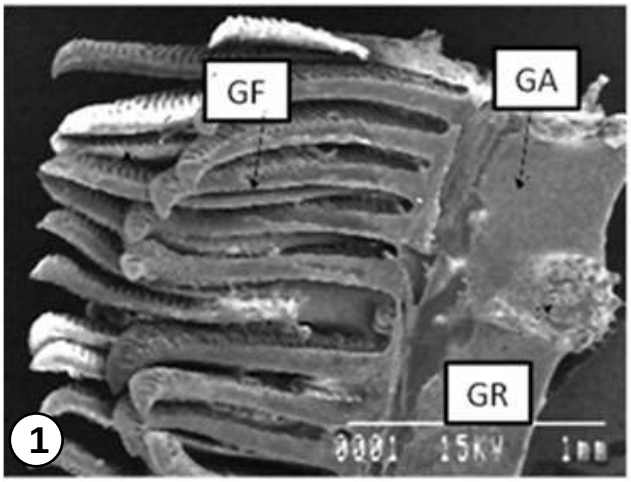
At the end of exposure period, gills from control and three experimentally grouped fishes (Acid treated, Al treated and silicon dioxide treated) were excised immediately and were washed repeatedly in normal saline for the removal of mucus. Gill tissues were fixed in 2.5% gluteraldehyde buffered with 0.1 Sodium cacodylate buffer (pH 7.4) for 24 hours at 4°C. Then they were washed for 2-3 times in washing buffer for 10-15 mins in each wash. Gill tissues were post fixed in 2% OsO_4 in buffer for 2 hours. Then gills were dehydrated in graded ethanol solution with final treatment in amyl acetate. The dehydrated tissues were critical point dried, mounted on stubs, gold coated and examined under the Scanning Electron microscope (Hitachi S-530).

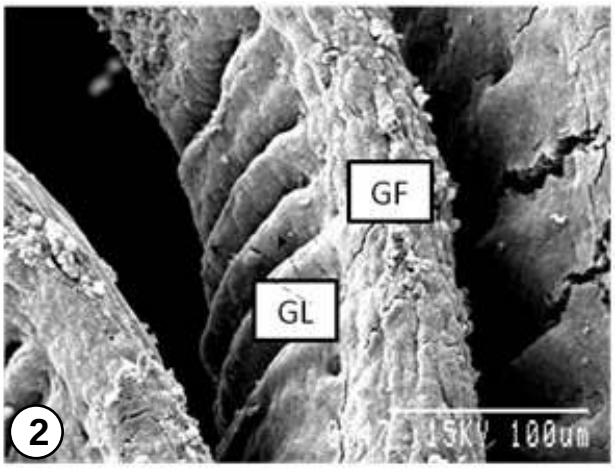
Results

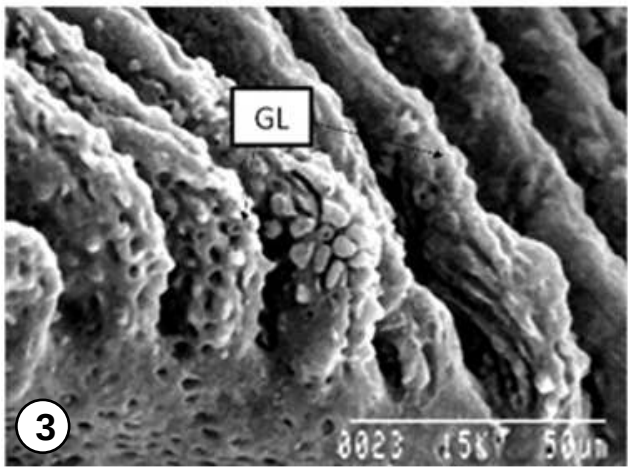
Fish mortality was absent in acid treated fish, while exposure to acid with aluminium caused death of fishes. Treatment with silicon as ameliorating agent, fish mortality reduced significantly. In SEM study of control gills, arrangement of gill filaments with free ends on the gill arch was observed. Secondary lamellae were arranged at equal space separated with each other. Microridged epithelial cells and mucous gland cells in secondary lamellae can be seen under higher magnification (Fig.1-6).

No discernable damage of gill epithelium was found under acidic condition. Normal alignment of microridges on epithelial cells was observed (Fig.7-8). Exposure to aluminium under acidic condition at pH<5 severe damage to gills along with excessive amount of mucus secretion was observed. Severe fusion of secondary lamellae which varies from partial to complete was seen. Swelling and clumping of secondary lamellae in the gills was due to gill epithelial hyperplasia (Fig.9-13). Microridges were dealigned in EC (Fig.14). Epithelial cells were fused with each other which altered the normal morphology (Fig 11).

Upon treatment with silicon as ameliorating agent, recovery in fish was observed. Structural damages to gill reduced with silicon. Fusion of lamellae was lacking in silicon treated gills. Normal gill architecture of epithelial cells and alignment of microridges were observed. Gills resumed the normal structure which increased the survival in fish (Fig 15-18)..



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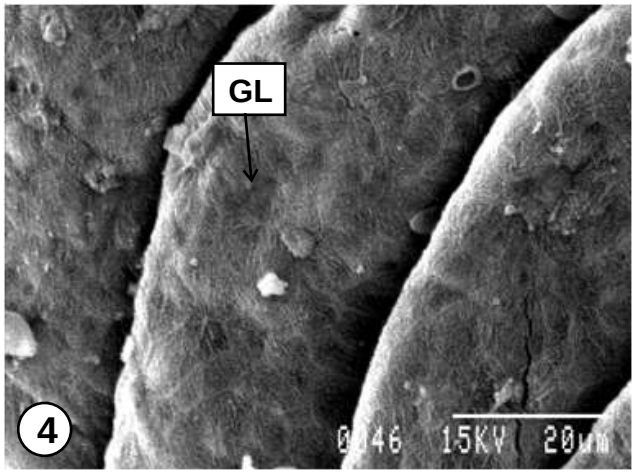
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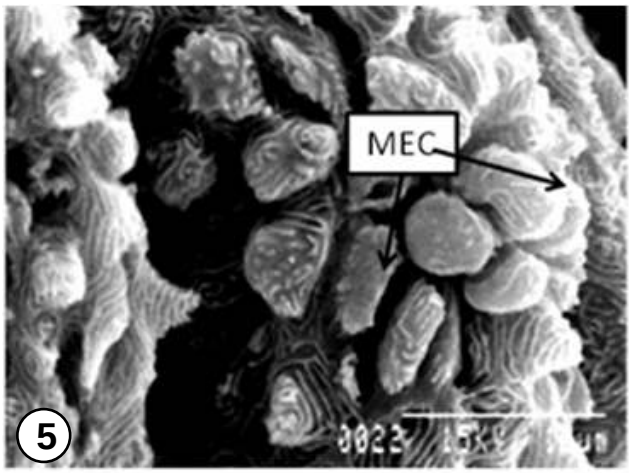
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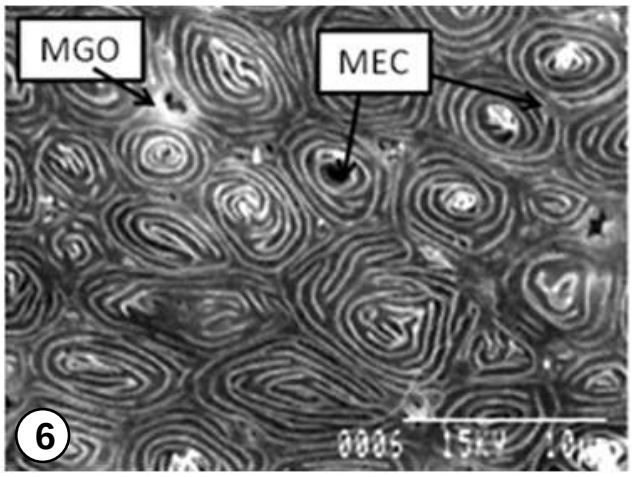
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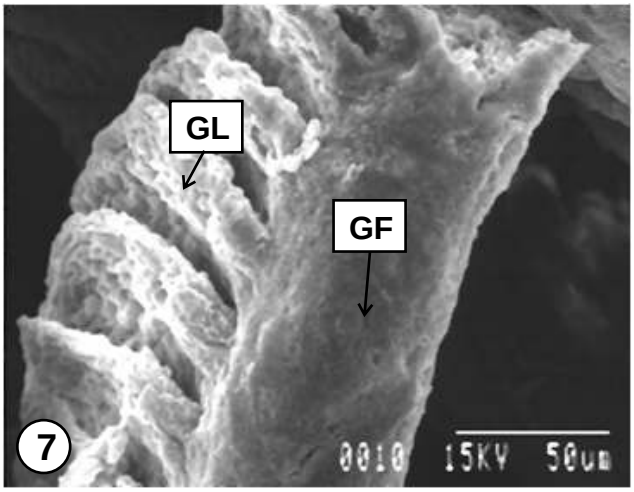
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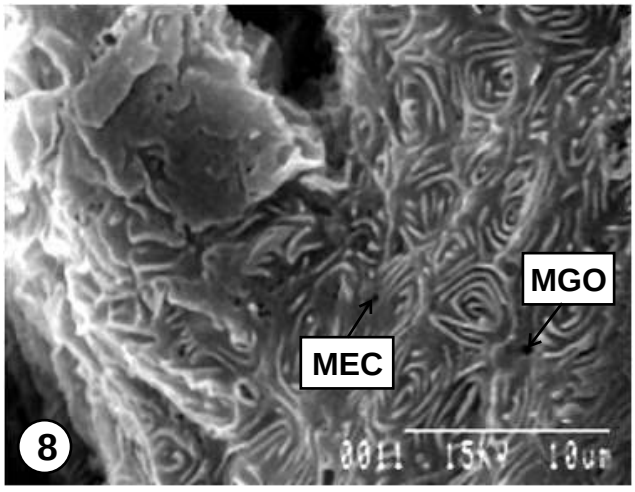
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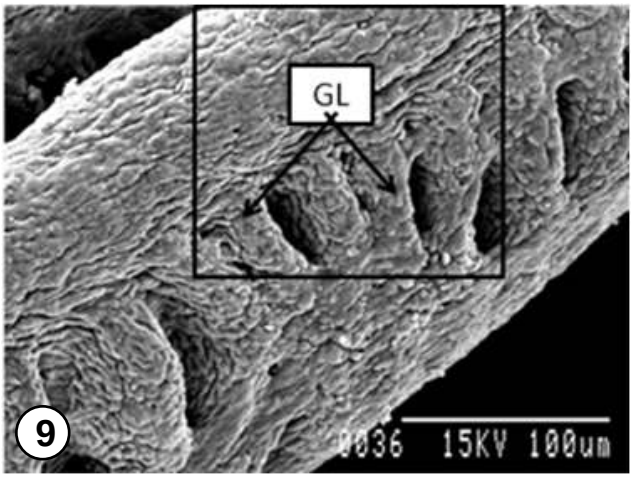
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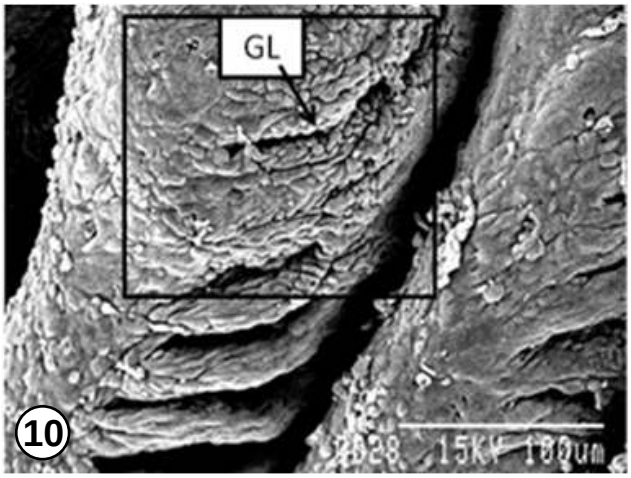
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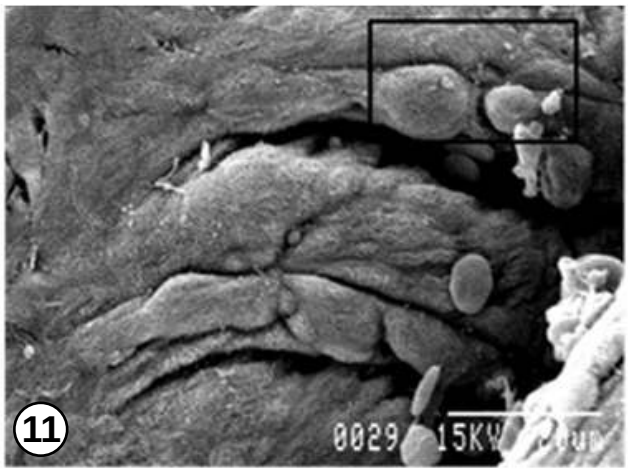
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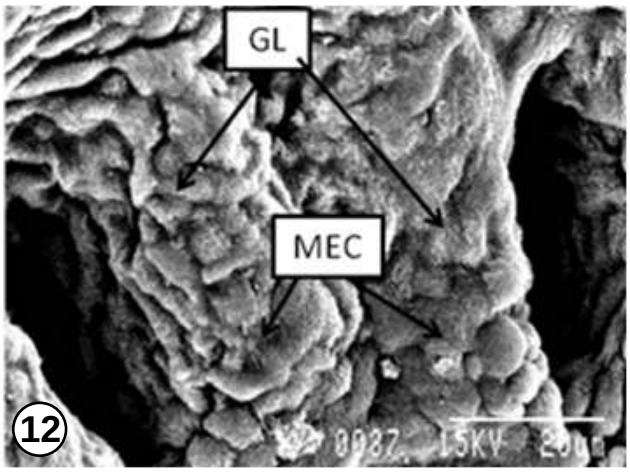
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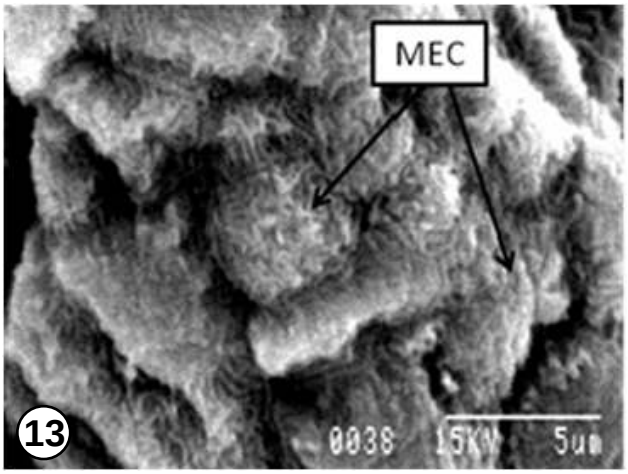
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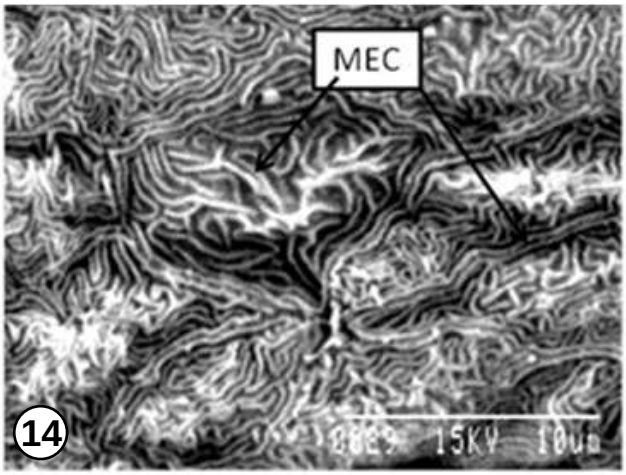
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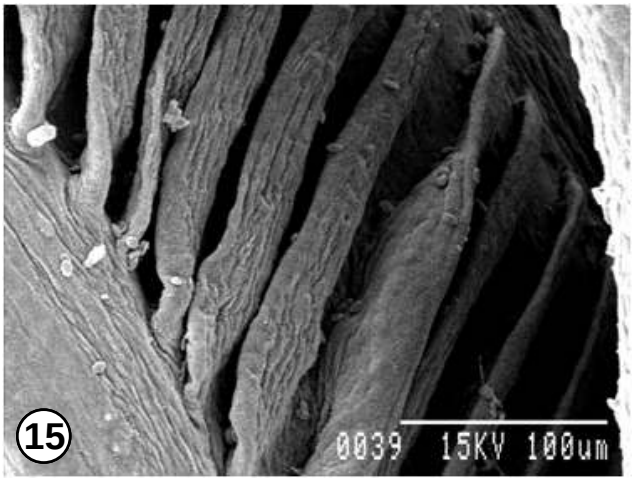

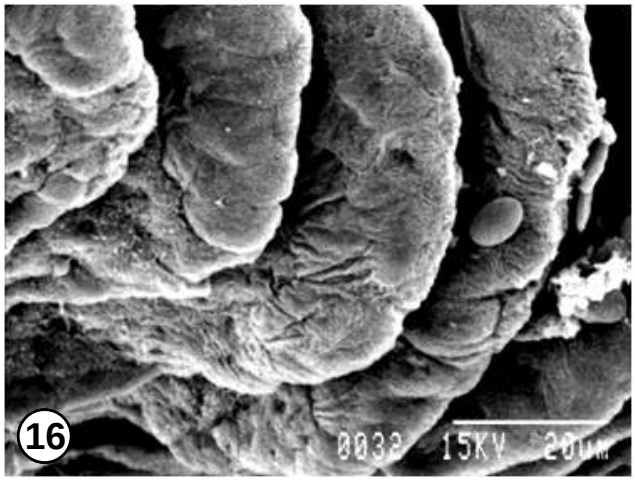

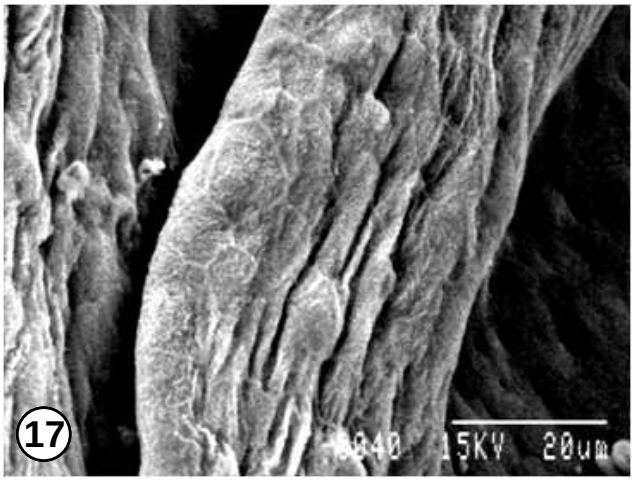

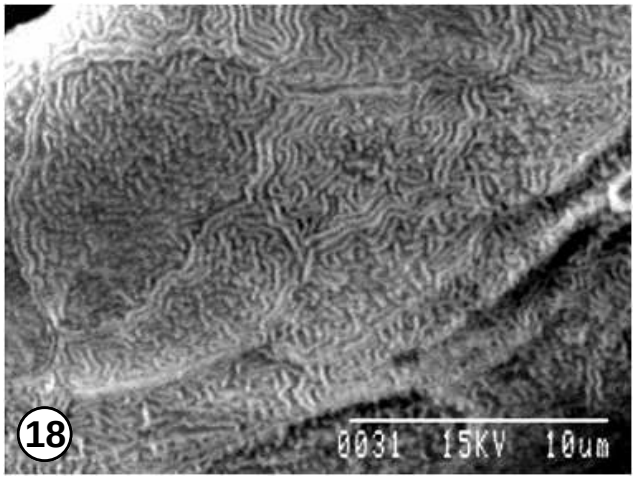

Explanation to Figures

Scanning electron micrographs of gill of control fish, *C. punctatus* (Figure 1-6) Fig. 1 reveals normal architecture of Gill Filaments (GF) on Gill Arch(GA), Gill Racker (GR) is also present.

Fig. 2 shows arrangement of the gill Filament(GF) and Gill Lamellae(GL) under high magnification. Compact arrangement of epithelial cells is present in Gill Lamellae (GL).

Figs. 3, 4 and 5 showing Gill lamellae under higher magnification reveals Microridges in Epithelial Cells (MEC).

Fig. 6 showing Alignment of microridges on Microridge Epithelial Cell (MEC) and Mucous Gland Openings (MGO) is visible under higher magnification.

Fig.7 Scanning electron micrographs of gill of Acid treated fish, *C. punctatus* reveales no discernable damage to gill lamellae (GL; Microridges alignment are not disturbed in microridge epithelial cells(MEC).

Fig. 8.SEM Photomicrographs of gill of fish *C. punctatus* exposed to sublethal dose 140 mg/l Aluminium Sulphate. Severe fusion of gill lamellae(GL) partial and complete.

Figs. 9 and 10. Hyperplasia of epithelial cells in gill lamellae/

Figs. 11 and 12. Fusion of microridge epithelial cells (MEC) under higher magnification(Fig 13); Dealignment of microridges in epithelial cells.

Fig. 14. SEM Photomicrographs of gill of fish *C. punctatus* exposed to ameliorating agent 150mg/l of Silicon. Fig. 15 reveals absence of fusion in gill lamellae (GL); space between the gill lamellae are equal like in control condition.

Fig. 16. Epithelial cells of gill lamellae are returning to the normal architecture.

Figs.17&18. Alignments of microridges are resuming the normal arrangement of microridge epithelial cells.

Discussion

Relationships between pH and survival of fish in the laboratory and in selected natural systems have been studied since early times (Baker *et al.,* 1990). Acidification of soil may cause leaching of Al metal which affect water quality in the drainage area (Ahtiamen, 1992). Al mobilizes in its soluble form from soil to aquatic ecosystems and affects aquatic organisms and become the most important factor responsible for fish kills in acidified lakes (Walker *et al*., 2001).

The solubility of aluminium is highly pH dependant, increasing at both low and high pH values reflecting the amphoteric nature of the element. This relationship, coupled with the substantial reservoir of aluminium in soils and sediments, means that dissolved aluminium concentrations can be substantially higher in acidic or poorly buffered environments when subjected to sustained or periodic exposure to strong acid inputs. Under such circumstances aluminium may be transported from soil to surface waters. Acidic deposition, afforestation, the cessation of liming and sulphide oxidation all contribute to acidification and the release of previously bound aluminium. For most freshwater fish, aluminium toxicity is maximum at pH values around 5.5 (Palmer *et al.*,1988; Guibaud and Gauthier, 2003; Naskar *et al.,* 2009,), with little toxicity being exhibited above pH 6.5.The mobilization of Al in soluble forms from soil to the aquatic ecosystem is an important consequence of acidification of lakes and streams (Stutter *et al.,* 2001; Palmer and Driscoll, 2002; Driscoll *et al.,* 2003).

Gills act as an interface between fish body and its environment. It is an important organ for gaseous exchange, acid-base balance, ion regulation and ammonia excretion (Das *et al*, 2006). The gills are a multi-functional organ playing vital role in osmoregulation of fish (Hwang and Lee, 2008). This organ represents the main target-organ of pollutants due to its extensive surface area in contact with the external environment and the very thin barrier between the environmental water and internal milieu of fish (Dang *et al.,* 2000; Cerqueira and Fernandes, 2002). The mechanism of aluminium toxicity to fish has been attributed to the inability of fish to maintain their osmoregulatory balance and respiratory problems associated with coagulation of mucus on the gills, the former effect being associated with lower pH levels. Fish produces mucus to combat the aluminium in their gills. This mucus builds up and clogs the gills so that oxygen and salt transport is inhibited. Fishes are then unable to regulate their body salts and it results in osmoregulatory disturbance. The fusion of the lamellae together with the accumulation of Al on the gills in the exposure at pH<5 caused low oxygen uptake and asphyxiation of the fish. Clogging of the gills with mucus will raise the diffusional resistance to O_2 and reduces the water flow through the secondary lamellae which leads to asphyxia. Aluminium also precipitates in the gills and interferes with the transport of Oxygen, so that fish die of suffocation.

When Aluminium is in acidified ambient water the effect is concentrated mainly in the gills and the physiological processes related to this organ (Waring and Brown, 1995; Cole *et al.,* 2001; Teien *et al.,* 2006). Toxic levels of aluminum can have negative impacts on the fish gill epithelium, which is an important structure for gas exchange, ion regulation, acid-base balance, and the excretion of nitrogenous wastes (Evans, 1987). Respiratory effects are thought to be caused by the polymerization or precipitation of aluminum in acidic water as the water enters gills that are higher in pH (Gensemer and Playle 1999). The inorganic mononuclear aluminium complexes are more toxic than the organically-complex forms to aquatic organisms. Toxicity appears to be caused only by aluminium hydroxyl complexes, especially Al $(OH)^{2+}$ (Helliwell *et al.,* 1983). Exposure to Al induces gill damage due to increased mucus production, which alters osmoregulation and respiratory processes (Exley *et al,* 1997) and thereby causes hypoxia, hypercapnia, metabolic acidosis and finally respiratory failures (Allin and Wilson, 2000; Royset *et al.* 2005). In the gills, hypoxia has been associated with an adaptive increase in lamellar surface area in fishes (Chapman *et al.* 2000; Sollid *et al.*2003; van der Meer *et al.*. 2005) as well as gill epithelial hypertrophy and hyperplasia, goblet cell proliferation with increased mucus secretion, hemorrhage, edema and telangiectasis (Scott and Rogers, 1980).

Present investigation reports a substantial decrease in the extent of gill damage following treatment by Silicon dioxide in Al-Acid treated fish. It has been reported earlier that ligands such as humic, fulvic, and tannic acids, fluoride, phosphate, and silicate decrease toxicity by complexing aluminium (Helliwell *et al.*,1983). Complexity with Silicon dioxide tends to lower the Al toxicity. Under equilibrium condition, Silicon dioxide forms orthosilicic acid with water and Hydroxyaluminosilicates (HAS) are formed by the reaction of silicic acid with aluminium (Exley & Birchall,1992,1993) whereas Birchall (1995) has suggested that soluble silica is essential to living organisms because it binds endogenous aluminium and prevents its toxicity and reduces aluminium bioavailability, toxicity or both (Carlisle, 1987; Quartley *et al.,* 1993; Birchall *et al.,* 1989; Edwardson *et al.*,1993; Bellés *et al.*,1998; Exley *et al.*;1997). Oligomeric silica has much higher affinity for

aluminium than monomeric silica (Jugdohsingh,2000). Biological importance of Silicon has become increasingly recognized and the major importance of silicon is in limiting the bioavailability of aluminium (Birchall,1991). Modest levels (100µmol/l) of silicic acid in water can protect against aluminium toxicity in fish (Birchall *et al.*, 1989; Exley,1991). Aluminium has a close chemical affinity with silicon, with which it reacts to form hydroxyaluminosilicates (Birchall, 1992). Silicon has also been reported to play a preventive role both in accumulation of aluminium and in aluminium related disease in dialysis patients (Parry *et al*.1998).

Hence it can be concluded that in acid-aluminium toxicity induced fusion of the lamellae, hypertrophy and hyperplasia of the epithelial cells significantly increased the diffusion distance across the gills. The precipitation of Al on the gills may also have increased this diffusion distance which in its turn has a negative effect on oxygen uptake of the fish and causing mortality. Silicon dioxide has an ameliorating effect in Al toxicity by preventing the lamellar fusion and hypertrophy in the gills. It also helps to resume the normal alignment of microridges in epithelial cells.

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