International Journal of Fauna and Biological Studies
Available online at www.faunajournal.com

I J F B S International Journal of Fauna And Biological Studies

ISSN 2347-2677
IJFBS 2015; 2 (4): 33-37
Received: 12-05-2015
Accepted: 11-06-2015

Spirita Sharmili V
Department of Zoology and Research Centre, Scott Christian College (Autonomous), Nagercoil

Avila Varshini R
Department of Zoology and Research Centre, Scott Christian College (Autonomous), Nagercoil

Kanagapan M
Department of Zoology and Research Centre, Scott Christian College (Autonomous), Nagercoil

Correspondence:
Spirita Sharmili V
Department of Zoology and Research Centre, Scott Christian College (Autonomous), Nagercoil

Alkylbenzene sulphonate, a detergent, induced toxicity on the gill of Zebrafish *Danio rerio* (Hamilton)

Spirita Sharmili V, Avila Varshini R, Kanagapan M

Abstract
Gills are the prime organs for gaseous exchange and perform several other physiological functions including osmoregulation and excretion. In the present study, the gill histological damage of the Zebra fish *Danio rerio*, exposed 1/4th (6.82 mg/l) and 1/10th (2.73 mg/l) 96h LC_{50} concentrations of Alkylbenzene Sulfonate (ABS) for long term intervals (30,45 and 60 days) is assessed. The histological changes to gills were edema, fusion of lamellae, lamellar aneurism and regeneration of the primary and secondary lamella occurred.

Keywords: Zebrafish *Danio rerio,* Alkylbenzene Sulfonate (ABS), histopathology, gill, lamellar aneurism.

1. Introduction
Pollutants adversely affect different organs, organ systems and tissues. Liver, kidney, muscles, brain, stomach, intestine and gills are the common target organs in fish. Histopathological changes in these organs vulnerable to pollutants are biomarkers indicating the impact of xenobiotics on the physiological constitution of the exposed organisms [1, 2].
Anionic surfactants are reported to be acutely toxic to aquatic organism [3, 4]. It has been observed in fish that the external organs were affected due to toxic chemicals. They cause loss of equilibrium, increasing opercular movement and finally lead to death. This may be attributed with significant damage to internal organs [5]. Several authors have reported that anionic surfactants (LAS) cause destruction in gill epithelium, impair chemoreceptor organs and damage epidermis and pharyngeal wall [6, 7].
The close contact of gills to the water favors the influence of dissolved chemical compounds, leading to morphological alterations [8]. Pollutant effects on gill structure of fish taken from polluted environments or exposed to laboratory test provide an indication of water contamination [9].
Tilapia caught from streams that received untreated domestic wastewater present gills with hyperplasia and detachment of the epithelium from the filament and lamella, complete and incomplete fusion of lamella, hypertrophy and hyperplasia of chloride cells, hemorrhage with epithelial rupture and aneurysm [10]. The adult tilapia exposed to wastewater from sewage treatment plant had edema with detachment of lamellar and filament epithelium and lamellar fusion, cell proliferation with thickening of gill filament [11].
The effect of several heavy metals seriously damages the gills of teleostean fish [12]. The toxic impact of the trace element zinc chloride (ZnCl2) on the gills and accessory respiratory organs of *Heteropneustes fossilis* [13]. The zebra fish belongs to the family Cyprinidae, the most species rich vertebrate family. The name Danio derives from the Bengali name "dhani" meaning "of the rice field". *D. rerio* was first described by Francis Hamilton, a surgeon with the British East India Company, stationed principally in West Bengal at the beginning of the 19th century. In the present investigation an attempt has been made to observe the possible histological changes in the vital organ, gills of the fish, *D. rerio* exposed to sub lethal concentration of ABS.

2. Materials and methods
The present study was carried out during the study period of 2013-2014 in the laboratory, Department of Zoology and Research Centre, Scott Christian College (Autonomous), Nagercoil, Tamil Nadu.

2.1 Collection and acclimatization of the test fish

Healthy *D. rerio* were obtained from the fish farm at Azhagiamandapam, Kanyakumari District. They were acclimatized and maintained under laboratory condition for 15 days, feeding with commercial fish–food prepared from "Dried Spirulina, Daphnia and Mysis". The fish was acclimatized in large FRP tanks containing tap water. Care was taken to avoid any sudden changes in temperature, salinity and pH. The fish was acclimatized for about 15 days before the commencement of the experiments.

2.2 Experimental set up

The histopathological studies were carried out for Alkyl benzene sulfonate. About 300 mg of the ABS powder was weighed and thoroughly mixed with tap water and made into 150 ml.

The exposure solution was renewed completely each day in order to ensure constant concentrations. Physical and chemical factors of water, such as temperature, pH and dissolved oxygen were maintained at appropriate values. Based on the 96 h LC50 value of ABS for Zebra fish, 27.31 mg/l, two sublethal concentrations ($1/4^{th}$ and $1/10^{th}$ of the 96 h LC_{50}) were chosen. So, the fishes were exposed to 6.82 and 2.73 mg/l concentrations of commercial ABS. Exposure to the different concentrations was conducted in triplicates and three control assays were run simultaneously. The control tanks were kept under the same conditions without addition of surfactant. Sampling of fishes was done after 30, 45 and 60 days from the beginning of experiment. Daily Samples of water were taken and measured for ensuring that the concentration of toxicant maintained as near as possible to the nominal value.

2.3 Histological analysis

After anesthetizing of fishes, the gills were removed and fixed by Bouin's solution. Then tissues were processed in a routine paraffin embedding procedure. Sections of 5 mm thick were taken which were later stained by haematoxylin and eosin method. Stained sections of liver were examined by light microscope.

3. Result

The gills from control specimens showed normal conditions. It is fresh and red in colour and the gill lamella were arranged normally (Fig -1). In the gills of *D. rerio* exposed to $1/10^{th}$ LC_{50} of Alkylbenzene sulfonate for 30 days, peeling and removal of gill epithelium, haemorrhage, loss of normal structure, loss of cartilage and heavy damage to all parts were observed. Afferent and efferent blood vessels and branchial cartilage of gill rachis revealed absence of RBCs in one of the vessels and partial erosion of connective tissue sheath of core cartilage. Inter branchial passage of gill shaved scattered blood clots and loose RBCs as debris in a stream. Altered histology and delamination of branchial epithelium from the gill rakers (Fig -2) was vividly noticed.

Fishes exposed to $1/4^{th}$ 96h LC_{50} concentration of Alkylbenzene sulfonate for 30 days lead to aneurysm in major blood vessels, haemorrhage in almost all blood vessels and capillaries, damage to cartilage, blebs in secondary branchial filaments. The consequent debris of damaged and discarded tissues were found scattered around (Fig - 3).

Enlarged mucous cells on the side of gills facing pharynx under high magnification were observed in fishes exposed for 45 days to $1/10^{th}$ 96h LC_{50} of Alkylbenzene sulfonate. These cells showed manifold increased at several gill rakers. This condition indicated a counter action against the irritation caused to the tissues by the chemical for a prolonged period of 45 days even in low concentration (Fig - 4).

Gill suffered hemorrhage in almost all blood vessels and capillaries, cartilage was damaged, blebs occurred in the secondary branchial filaments and debris of damaged and discarded tissues were scattered around. Origin, middle and tip of a branchial filament were totally lost owing to the effect of $1/4^{th}$ concentration of Alkylbenzene sulfonate for 45 days exposure (Fig - 5).

In D. *rerio* exposed for 60 days to $1/4^{th}$ and $1/10^{th}$ 96h LC_{50} of Alkylbenzene sulfonate, the primary and secondary branchial filaments suffered from oedema, fusion of secondary branchial filaments suffered and detachment of distal parts of secondary branchial filaments, leading to stumpy blebs (Fig – 6, 7).

4. Discussion

Toxicants in the environment mainly enter into fish by means of their respiratory system [14]. When exposed to toxic substances, the gills presented lesions, such as epithelial lifting, necrosis, hypertrophy, hyperplasia, lamellar fusion, rupture of gill tissue, hyper secretion and proliferation of mucosal cells [15, 8]. The presence of oedema accompanied by the detachment of lamellar epithelium is the first sign of severe gill lesion [16].

This lesion occurs after more severe handling and may be related to parasitic lesion, metabolic waste or chemical contaminants and when many lamellae are affected, the respiratory function may decrease mainly at high temperature, when the oxygen levels are low and the metabolic demand is high [17].

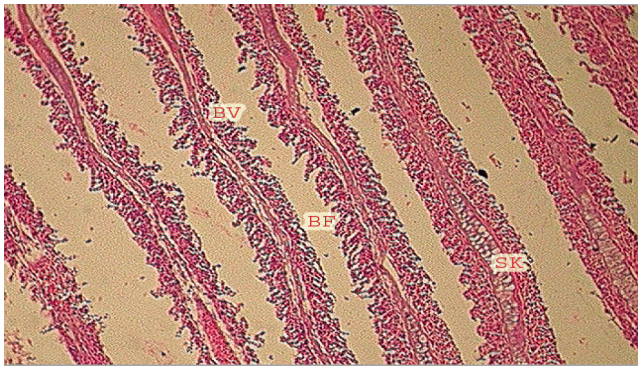

Fig 1: Section of the gill of control Zebrafish D. *rerio*
BV – Blood Vessel, SK – Skeleton, BF – Binary Filament

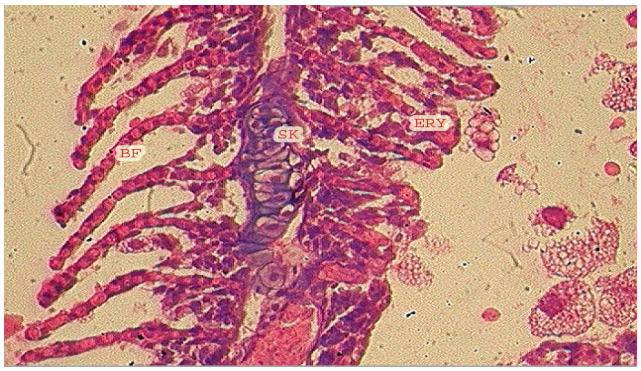

Fig 2: Gill of D. *rerio* exposed to $1/10^{th}$ 96hof ABS for 30 days
BF – Damaged binary Filament, SK – Skeleton, ERY – Erythrocytes

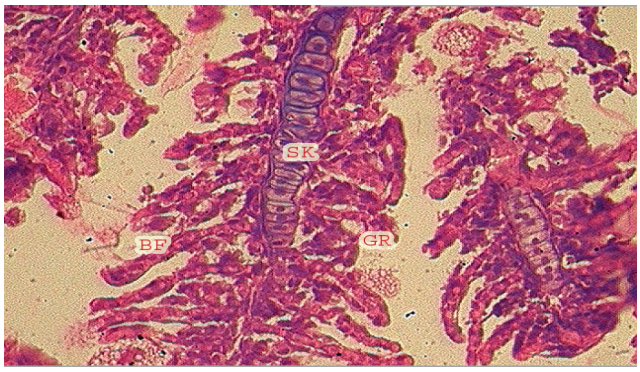

Fig –3 Gill of D. *rerio* exposed to 1/4th 96hLC50of ABS for 30 days
BF – Damaged binary Filament, SK – Skeleton
GR –Fusion of gillrackers

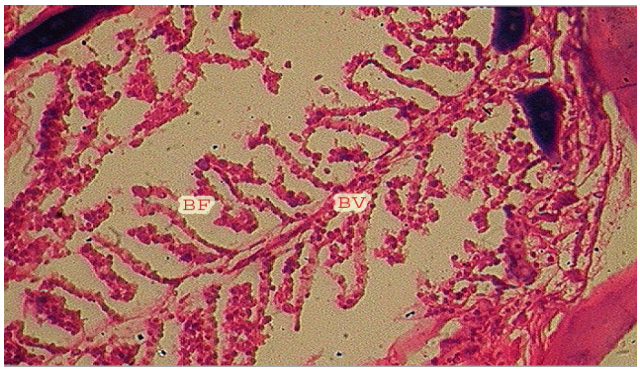

Fig 4: Gill of D. *rerio* exposed to 1/10th 96h LC_{50} of ABS for 45 days
BF – Damaged binary Filament, BV– Congestion of blood vessels

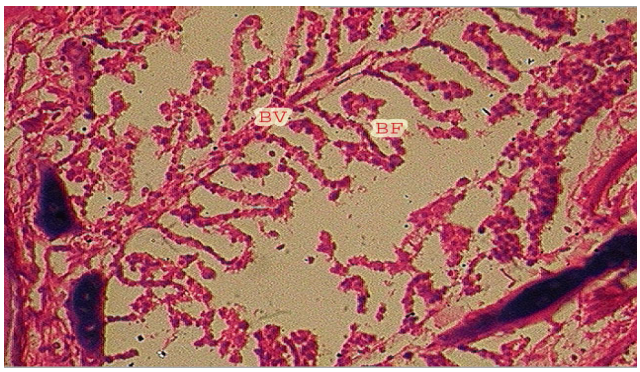

Fig 5: Gill of D. *rerio* exposed to 1/4th 96h LC_{50} of ABS for 45 days
BF – Damaged binary Filament, BV– Congestion of blood vessels

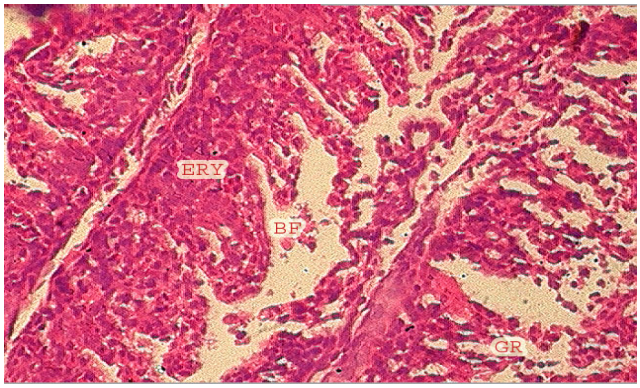

Fig 6: Gill of D. *rerio* exposed to 1/10th 96h LC_{50} of ABS for 60 days
BF – Fusion of binary Filament, ERY- Damaged erythrocytes

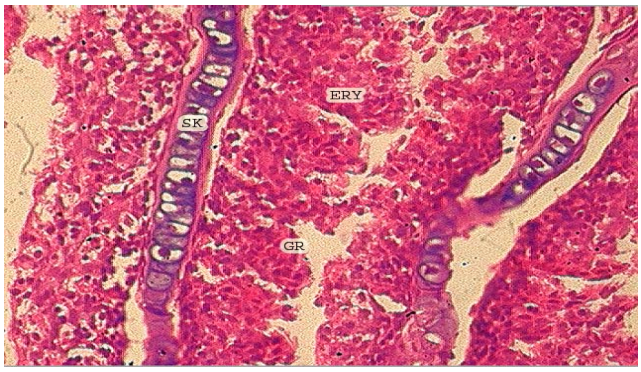

Fig 7: Gill of D. *rerio* exposed to 1/4th 96h LC_{50} of ABS for 60 days
SK – Skeleton, ERY – Damaged erythrocytes
GR – Fusion of gill rackers

Lifting of the lamellar epithelium is one of the first changes in fish gills under acute exposure to toxic substances, such as oils, detergents, ammonia, phenols, acids and metals like mercury [18, 19]. Oedema and leucocyte infiltration were also very common gill changes in all studied species and can be interpreted as defense responses to toxic agents, as described by Heath [19].

Histopathological changes of gill such as hyperplasia and hyper-trophy, epithelial lifting, aneurysm and increase in mucus secretion have been reported after the exposure of fish to a variety of noxious agents in the water such as herbicides, phenols and heavy metal [20].

According to [21], the mucus layer creates a microenvironment that may act as an ion trap, concentrating trace elements in the water. The histomorphological response of the gills of fish exposed to ambient insecticides (including metal salts) is often manifested by a prominent increase in the density of its mucus cells [22, 23]. The large amount of mucous secretion acts as a defense mechanism against several toxic substances [24, 25]. The regular elimination of mucous layer from the gill surface into aquatic media helps to remove the bound pathogens, toxicants and foreign matters [26] which remain stick to the gills. Peuranen [27] noticed that the gill microenvironment differs considerably from that of the surrounding body and water causing deposition of metals on the gill surface [28].

Death in fish is the end product of the various effects caused in the various tissues and organs. When these tissues collectively stop functioning due to the toxin, the fish dies. For instance, the degeneration of gills causes a dysfunction of its gas exchange ability causing an anoxic internal environment [29].

As the gills play a role in the gaseous exchange and osmoregulation, the tissue transformations directly affect the morphophysiological mechanisms [30, 31]. Because the gills are in direct contact with water, toxic substances can easily interfere in the morphophysiology of these organs, as observed in the use of organic pesticides [32, 15], detergents [33], acids [24], and salts [34]. In the gills of *D. rerio* exposed to 1/10th LC_{50} of Alkylbenzene sulfonate for 30 days, peeling and removal of gill epithelium, haemorrhage, loss of normal structure, loss of cartilage and heavy damage to all parts were observed.

The gills are considered to be one of the most delicate structures because of their external location and intimate contact with water which exposes them to various aquatic pollutants [35]. The morphological anomalies of the gills due to lead exposure are similar to the gill structural damages reported for a variety of toxicants like detergents [36] pesticides and heavy metals [37]. Roncero *et al*., (1990) [38] showed that

experimental lead nitrate poisoning caused various microscopic and ultrastructural abnormalities in the gills of *Tinca tinca* and pointed out that the histopathological alterations in the gills of fish coupled with reduction in the surface area of the respiratory barrier and inhibition of mitochondrial enzymes leads to inevitable death of the fish.

5. Conclusion

From the results of the present study, it appears that the ABS detergent has acute toxic and severe histopathological effects on the gill of Zebrafish *D. rerio*. So, *D. rerio* can be used as a model organism to detect the toxicity of different chemicals contaminated in the aquatic environment.

6. References

1. Hinton OE, Bauman PC, Gardner GR, Hawkins WE, Hendricks JD, Murlhelano RA *et al.* Histopathological biomarkers. In: Rand, G.M and Petrocelli, S.R (eds.): Fundamentals of aquatic toxicology methods and applications. Hemisphere publishing corporation Washington, New York, 1985, 155-209.
2. Stentiford GD, Longshaw M Lyons BP, Jones G, Green M, Feist SW. Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. Marine Environmental Res 2003; 55:137-159.
3. Abel PD. Surfactants and the environment. Journal of Surfactant and Detergent 2009; 1:109-117.
4. Tovell PWA, Newsome CS, Howes D. Adsorption metabolism and excretion by goldfish of the anionic detergent sodium lauryl sulphate. Toxicol 2007; 4:17-29.
5. Soegianto MA, Fawole OO, Adewoye SO. Pathologic lesions in the gills structure of *Clarias gariepinus* on exposure to sublethal concentrations of soap and detergent. J. Cell and Animal Bio 2009; 3(5):078-082.
6. Pozo C, Rodels B, Calvo D, Toledo MVM. Linear Alkylbenzene sulfonates (LAS) and soil microbial activity. Food. Agric. Environ 2003; 1(2):348-350.
7. Fontaínhas-Fernandes A, Luzio A, Garcia-Santos S, Carrola J, Monteiro S. Gill histopathological alterations in Nile tilapia, *Orechromis niloticus* exposed to treated sewage water. Brazilian Archives of Biology and Technology 2008; 52(5):1057-1063.
8. Pawert M, Müller E, Triebskorn R. Ultrastructural changes in fish gills as biomarker to assess small stream pollution. Tissue and Cell 1998; 30(6):617- 626.
9. Schwaiger J, Wanke R, Adam S, Pawert M, Honnen W, Triebskorn R. The use of histopatological indicators to evaluate contaminant-related stress in fish. Journal of Aquatic Ecossystem, Stress and Recovery 1997; 6:75-86.
10. Lupi C. Avaliação da poluiçãoambientalatravés dasalteraçõesmorfológicasnasbrânquias de *Oreochromis* niloticus (tilapia) noscórregos Retiro, Consulta eBebedouro, município de Bebedouro-SP. Revista Hispeci e Lema 2006; 9(3):30-36.
11. Fontaínhas-Fernandes A, Luzio A, Garcia-Santos S, Carrola J, Monteiro S. Gill histopathological alterations in Nile tilapia, *Orechromis niloticus* exposed to treated sewage water. Brazilian Archives of Biology and Technology 2008; 52(5):1057-1063.
12. Dutta HM, Munshi JSD, Roy PK, Singh NK, Adhikari S, Killius J. Ultra structural changes in the respiratory lamellae of the catfish *Heteropneustes fossilis* after sublethal exposure of malathion. Environ. Pollut. 1996; 3:329-341.
13. Hemalatha S, Banerjee TK. Histopathological analysis of sublethal toxicity of zinc chloride to the respiratory organs of the air breathing catfish *Heteropneustes fossilis* (Bloch). Biol. Res 1997a; 30:11-21.
14. Tovell PWA, Howes D, Newsome CS. Absorption, metabolism and excretion by gold fish of the anionic detergent, Sodium lauryl sulphate. Toxicol 1975; 6:17-29.
15. Mallat J. Fish gill structural changes induced by toxicants and other irritants: a statistical review. Ottawa. Canadian Journal of Fisheries and Aquatic Sciences 1985; 42(4):630-648.
16. Thophon SM, Kruatrachue M, Upatham ES, Pokethitiyook P, Sahaphong S, Jaritkhuan S. Histopathological alterations of white sea bass, *Lates calcarifer* in acute and subchronic cadmium exposure. Environmental Pollution 2003; 121(3):307-320.
17. Roberts RJ. *Patologia de los peces*. Madrid: Mundi-Prensa, 1981.
18. Muller R, Lloyd R. Sublethal and chronic effects of pollutants on freshwater fish. Oxford: Oxford Blackwell Scientific. Arellano, J. M., V. Storch, and C. Sarasquete, (1999). Histological changes and copper accumulation in liver and gills of the Senegales sole, *Solea senegalensis*. Ecotoxicol. Environ. Saf 1994; 44:62-72.
19. Heath AG. Water Pollution and Fish Physiology. BocaRaton, FL: CRC Press/Lewis Publisher, 1995.
20. Nowak B. Histological changes in gills induced by residues of endosulfan. Aquat. Toxicol 1992; 23:13-84.
21. Part P, Lock RAC. Diffusion of calcium, cadmium and mercury in mucous solution from rainbow trout. Comp. Biochem. Physiol. 1983; 76:259-263.
22. Baker JTP. Histological and electron microscopical observations on copper poisoning in the winter flounder (*Pseudopleuronectes americanus*) J. Fish. Res. Board. Can 1969; 26:2785-2793.
23. Dutta HM. A composite approach for evaluation of the effectof pesticides on fish. In Fish morphology: Horizon of new research (Munshi, J. S. D., H. M. Dutta Eds.) Science Publisher Inc., USA, 1997, 249-277.
24. Mcdonald DG. The effects of H+ upon the gills of fresh water fish. Canadian Journal of Zoology 1983; 61(4):691-703.
25. Mazon AF, Cerqueira CCC, Monteriro EAS, Fernandes MN. Acute copper exposure in freshwater fish: Morphological and physiological effects. *In: Biology of Tropical Fishes*, (Val, A. L.,Almieidaval, V. M. F., Eds.) INPA, Manaus, 1999, 263-275.
26. Powell MD, Speare DJ, Burka JF. Fixation of mucus on rainbow trout (*Onchorhynchus mykiss*, Walbaum) gills for light and electron microscopy. J. Fish Biol 1992; 41:813-824.
27. Peuranen S, Vuorinen PJ, Vuorinen M, Hollender A. The effect of iron, humic acids and low pH on the gills and physiology of brown trout (*Salmo trutta*). Ann. Zool. Fennici 1994; 31:389-396.
28. Playle R, wood CM. Water chemistry changes in the gill microenvironment of rainbow trout: experimental observations and theory. J. comp. Physiol. B 1989; 159:527-537.
29. Ajani F, Olukunle OA, Agbede SA. Hormonal and Haematological Responses of *Clarias gariepinus* (Burchell, 1822) to Nitrate Toxicity. J. Fisheries Int 2007; 2(1):48-53.

30. Hibiya T. An atlas of fish histology: normal andpathological features. New York: Kodansha Tokio, 1982.
31. Meyers TR, Hendricks JD. Histopathology. In: Rand, G. M. E.; Petrocelli, S. R. (Ed.). Fundamentals of aquatic toxicology: methods and applications. Washington, D.C.: Hemisphere Publishing Corporation, 1985, 283-331.
32. Rao KSP, Rao KVR. Lipid derivatives in the tissues of the freshwater teleost, *Saurotherodon mossanbicus* (alias *Tilapia mossanbica*) (Peters) - Effect of methylparathion. Proceedings of the National Academy of Sciences 1981; 47(1):53-57.
33. Bolis L, Rankin J. Interactions between vascular actions of detergent and catecholamines in perfused gills of European eel, *Anguilla anguilla* L. and brown trout, Salmo trutta L. Journal of Fish Biology 1980; 16(1):61-73.
34. Fanta E, Luvizotto MF, Meyer AP. Gill structure of antartic fishes *Notothenia* (Gobinotothen) *gibberifrons* and *Trematomus newnesi* (Notothenidae) stressed by salinity changes and some behavioral consequences. Antarctic Record 1995; 39(1):25-39.
35. Roberts RJ. Fish pathology, Baillore Trindall, London, 318, 1978.
36. Schaperclaus W. Fish diseases vol. 2 Oxonian Press Ltd., New Delhi, 1991.
37. Paulose PV. Histological changes in relation to accumulation and elimination of inorganic and methyl mercury in gills of *Labeo rohita* (Hamilton). Indian J. Exp. Biol 1989; 27:146-150.
38. Roncero V. Experimental lead poisoning: Microscopic and ultrastructural study of the gills of Tench (*Tinca tinca*, L). Env. Heth Perspectives 1990; 89:137-144.