

An electron microscopic investigation on the effect of aquatic pollutants in an edible fish of Ashtamudi lake, the Ramsar site, Kerala.

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Abstract

In the last few years aquatic pollution and its effects on the health of aquatic ecosystems is a great problem that has been studied intensively. Ashtamudi lake, the second largest backwater in Kerala is prone to several kinds of toxic aquatic pollutants. Domestic garbage, sewage, discharge of pollutants from coconut husk retting area, toxic heavy metals from industries, oil hydrocarbons from fishing trawlers, fertilizers and pesticides through surface run off are the major sources of pollution. The presence of pollutants in Ashtamudi lake is a serious threat to the aquatic organisms inhabiting the lake. When compared to the control samples with that of polluted ones, many histological alterations can be noticed in the targetted organs of *Etroplus suratensis*. These predominant changes were due to the combined effect of aquatic pollutants. The result of the present study revealed the fact that the normal histological structure of the vital organs of *Etroplus suratensis* such as gill, liver, kidney and muscle got completely altered due to the toxicity.

Key words – *Etroplus suratensis*, pollutants, SEM, TEM, gill, liver, kidney, muscle.

Introduction

Ashtamudi Lake in Kollam district, Kerala has been classified as a coastal estuarine lake of brackish water. The Lake is harboring a wide variety of fishes such as *Aplocheilus* sp, *Terapon* sp, *Heteropneustes* sp, *Etroplus* sp, *Channa* sp, *Mugil* sp, *Mystis* sp, *Penaeus* sp etc. There has been a huge interest in the aquaculture of these species due to their high demand or price, high flesh content and rapid growth rates in captivity. For the last few decades Ashtamudi Lake is polluted extensively. Domestic garbage, sewage, discharge of pollutants from coconut husk retting area, toxic heavy metals from industries, oil hydrocarbons from fishing trawlers, fertilizers and pesticides through surface run off are the major sources of pollution. The human settlements and public effluent sources are the chief factors for the degradation of lakes, particularly the urban lakes. Infrastructure development, housing pressure and encroachments have resulted in converting all urban lakes into hyper eutrophic state (Reddy, 2009). The toxicity of aquatic pollutants in the water and sediment samples of Ashtamudi lake has been reported by Razeena *et al.*, (2015); Babu *et al.*,(2010); Suma *et al.*,(2012);

Geetha (1997) and many others. The presence of pollutants in Ashtamudi Lake is known to disturb the delicate balance of the aquatic ecosystem.

Fishes are notorious for their ability to concentrate various toxic pollutants in their body since they are in direct contact with the aquatic environment. Pollutants can be transferred through the upper classes of the food chain once accumulated by an aquatic organism and paves way for biomagnifications. It is very important to determine the accumulation levels of pollutants in fishes that making high proportion of protein sources in the food chain for human health. A commercially important fin fishes - *Etroplus suratensis* of Ashtamudi lake was selected for the present study. The effect of aquatic pollutants on the selected organs such as gill, fin, muscle, liver and kidney of *Etroplus suratensis* were examined under SEM and TEM.

Etroplus suratensis, popularly known as Karimeen or pearl spot is very expensive and is considered as a delicacy to humans. Fried *Etroplus* is most popular, but smoked, baked, and canned fishes are also eaten. This species has a unique breeding habit of nesting on submerged substratum on the lake bottom. Interest in the aquaculture of this species has been high due to the great demand and rapid growth rates in captivity. The main aim of the present study was to determine the toxic effects of aquatic pollutants on the targetted organs of this edible fish with special reference to electron microscopic investigation.

Materials and methods

Healthy and active *Etroplus suratensis* were collected from the fish farm, of Kollam district. Fishes were acclimatized under laboratory conditions for 30 days in dechlorinated water and the tank was provided with aerators and filters. The fishes were fed with fresh hydrilla and other formulated feed during the study period so that fish tissues does not show any changes due to starvation. The average length and weight of the fishes under investigations varied from 11.96 cm (\pm 15.5) and 16.75 gm (\pm 17.65) respectively. The gill, liver, kidney and muscle samples of these fishes were taken as control.

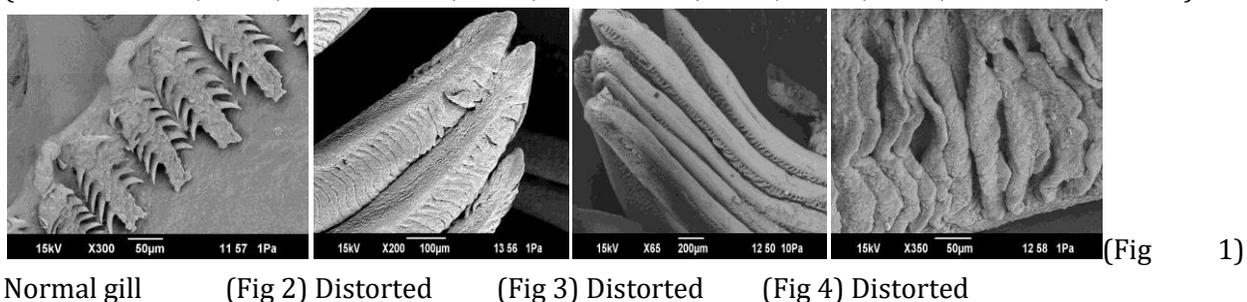
For comparing the pollution status of fish, the live fishes were collected from a polluted region (Kureepuzha) of Ashtamudi lake by using cast net with the help of traditional fishermen. This region of the lake is heavily contaminated with domestic garbage, industrial wastes, oil hydrocarbons, fertilizers and pesticides. The live fishes were transported to the laboratory carefully in plastic containers with the help of battery operated aerator. The length and weight of the fishes varied from 12.00 cm (\pm 15.0) and 17.00 gm (\pm 18.00) respectively were selected among the fishes .

Gill, liver, kidney and muscle samples of fishes (both control and polluted) were carefully taken and cut into small pieces of about 2 mm diameter. They were then examined carefully under light microscope. Samples were then selected among them for electron microscopic investigation. 2.5% gluteraldehyde was used as the fixative. Sample preparation for electron microscopy followed Caberoy and Qunitio (2000). Gross morphological study of the gills of fishes were done using SEM(

model JSM-6390 LV). Histological studies of the liver, kidney and muscle samples of fishes were done using TEM (model CM-10, Philips) .

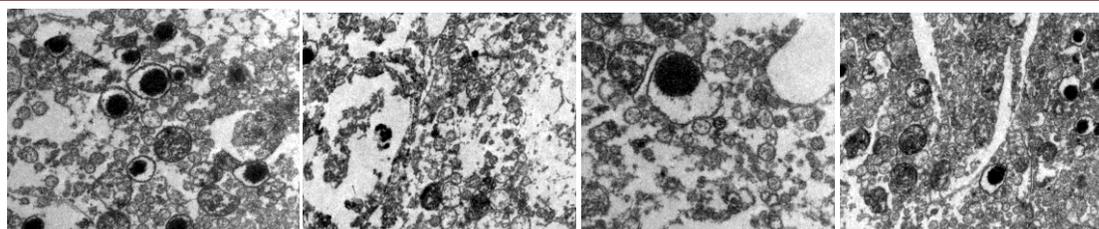
Results and discussion

Gill - The gross structure of control samples were studied using SEM, showed normal structures of gills like gill arch, gill rakers and gill filaments. Arising from the gill arch are a number of gill filaments which are arranged in a single row and are equidistant from each other giving it a leaf like structure (Fig 1). The gill samples collected from polluted region showed gill epithelium disorientation, hypertrophy and hyperplasia of epithelial cells, fusion of gill lamellae (Fig 2) . The clubbing of the ends of the secondary lamellae, fusion of adjacent secondary gill lamellae and necrosis in the primary lamellae were noticed (Fig 3). Epithelial lifting, necrosis and curling of secondary lamellae were also very well observed in many samples. (Fig 4). These patterns of changes have also been reported by many workers due to the accumulation of aquatic pollutants in the gills of fishes. (Yousafzai *et al.*, 2010; Parvathiet *al.*, 2011; Senthil *et al.*, 2011; Baker, 1969; Daoust *et al.*, 1984).



Normal gill (Fig 2) Distorted (Fig 3) Distorted (Fig 4) Distorted

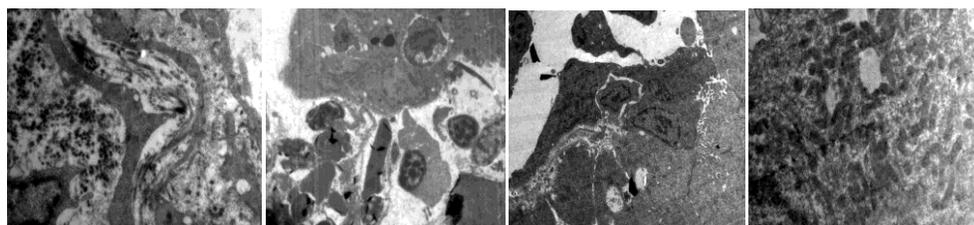
Liver - The liver samples of control fishes has a typical parenchymatous appearance which has a palisade arrangement around the central vein. The main hepatic structure includes hepatic parenchyma and blood vessels. The hepatic parenchyma are polygonal in shape and with centrally placed rounded nucleus and homogeneous cytoplasm (Fig 5). A close observation of electron microscopic studies of samples from polluted region showed distorted structures. Hepatocytes were under different patterns of degeneration with swollen cells, necrosis, shrinkage of nuclei and intracellular vacuolation were apparent (Fig 6). According to Hughes *et al.*, (1979) necrosis is the direct toxic effect of the pollutant. Disintegration of cell boundaries, loose arrangement of hepatic cells and histolysis were observed (Fig 7). Similar kinds of observations were made by earlier workers (Figueiredo-Fernandes *et al.*, 2007, Das and Mukherjee,2000) due to toxicity of pollutants in fish liver tissues. The sinusoids also undergo degeneration in many liver samples. The liver of fish tissues also showed marked changes like pyknotic nuclei and degeneration of blood vessels (Fig 8). Such histological effects in the liver of *Mollienesia* sp. were reported by Sultan and Khan (1981), in *Oreochromis niloticus* by António *et al.*, (2007) and in *Labio rohita* and *Channa striatus* by Loganathan *et al.*, (2006) due to the exposure of aquatic pollutants.



(Fig 5)

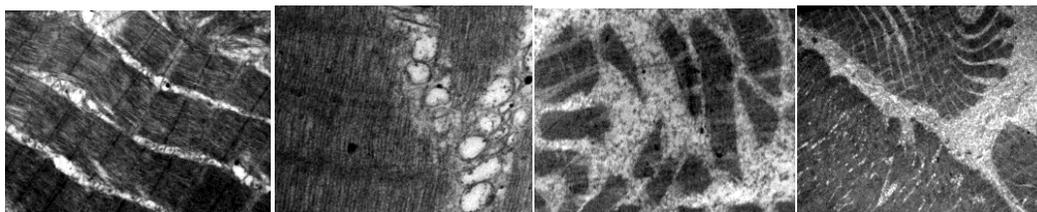
Normal liver (Fig 6) Distorted(Fig 7) Distorted(Fig8) Distorted

Kidney – The samples taken as control showed structures of normal kidney which composed of many nephrons and intestinal lymphoidal tissue. Nephron consists of malpighian capsule, proximal, distal and collecting tubule. The haemopoietic tissue, located in between the renal tubules, is composed of polygonal slightly basophilic cells with spherical nuclei. Few scattered blood corpuscles are seen in this haemopoietic tissue (Fig 9) . A number of histological alterations can be observed in many haemopoietic tissues of *Etroplus suratensis* collected from polluted region. The most obvious histologic response noticed was detachment of epithelial cells from the underlying tubular basement membrane and degenerative changes of the epithelial cells (Fig 10). Epithelial cells of kidney tubules with degenerative changes and vacuoles can be clearly visible on transmission electron micrographs. Large spaces were seen around the glomeruli which were atrophied (Fig 11). The haemopoietic tissue in some regions of kidney tissues showed an abnormal appearance, whereas aggregated clumps of cells as well as blood corpuscles were also seen (Fig 12). Similar results were also reported in various fishes such as *Anabas testudineus* (Supap, 2009), *Channa punctatus* (Mishra and Mohanty, 2008) and *Cyprinus carpio* (Parvathi et al., 2011) due to the chronic effect of toxic pollutants.



(Fig 9)Normal kidney (Fig 10)Distorted (Fig 11)Distorted (Fig 12)Distorted

Muscle - The control muscle samples of *E.suratensis* showed multiple nuclei which lay at the periphery of the muscle fibers. Groups of fibers were surrounded by a large pale area with loose connective tissues, the perimysium. The whole muscle or muscle bundle was surrounded by a denser connective tissue, the epimysium (Fig 13).Transmission electron microscopic studies on the muscle samples from polluted region showed vacuolar degeneration in muscle bundles (Fig 14) and splitting of muscle fibres (Fig 15). Focal area of necrosis and muscular oedema were other prominent changes observed in the study (Fig 16). Similar results were noticed in a variety of fish muscle tissues due to the impact of aquatic pollutants (Andreji *et al.*, 2006; Soegianto and Hamami, 2007; Haggag *et al.*, 1999; Elghobashy *et al.*, 2001).



(Fig 13) Normal muscle (Fig 14) Distorted (Fig 15) Distorted (Fig 16) Distorted

Conclusion

When compared to the control samples with that of polluted ones, many histological alterations can be noticed in the targetted organs of *Etroplus suratensis*. These predominant changes were due to the combined effect of aquatic pollutants. The result of the present study revealed the fact that the normal histological structure of the vital organs of *Etroplus suratensis* such as gill, liver, kidney and muscle got completely altered due to the toxicity. The continuous drainage of toxic pollutants into the aquatic environment would increase its level at alarming state, threatening seriously, the aquatic organisms including fish population. Aquatic organisms especially fishes have a tendency to accumulate pollutants in high values from their direct environment in which they inhabited. Toxic pollutants may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms. The result indicates that the pollutants in the aquatic system definitely affect the aquatic life of the fresh water fish.

The predominant factors responsible for deterioration of the Ashtamudi backwaters are pollution and encroachment. If the discharge of aquatic pollutants into the water bodies goes on at an elevated level it will seriously affects the survival of aquatic organisms. Commercially important edible fishes such as *Etroplus suratensis* is a popular food item and is often a delicacy to humans. Due to the consumption of these edible fish pollutants can easily enters into the human system through the process of biomagnification. Hence healthy and scientific measurements should be taken in order to protect the aquatic water body and to improve the health status of this economic fish.

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