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Physico-Chemical Characteristics and Impact of Aquatic Pollutants on the Vital Organs of a Freshwater Fish *Glossogobius giuris**

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Abstract: Environmental pollutants such as pesticides, industrial wastes and other organic wastes pose serious risks to many aquatic organisms. Accordingly a great deal of previous research has characterized physiological mechanisms of toxicity in animals exposed to contaminants. In contrast, effects of aquatic contaminants on fish pathology and physico-chemical characteristics of aquatic system are less frequently studied. To investigate present position of the dam water physico-chemical characteristics and histopathological changes in fish was undertaken. Analysis exhibited richness in nitrogen, phosphates, in addition to high value of total dissolved solids, total alkalinity, DO, BOD and organic matter. The histological damage caused to the fish *Glossogobius giuris* by various aquatic pollutants present in polluted water body. A microscopic study exhibits the Swelling Gill Lamellae (SGL) and fusion of secondary lamellae (FL) of gill filaments and oedematous separation of basement membrane from epithelial lining of the Secondary Lamellae (SL) was also noticed. Cellular hypertrophy followed by vacuolization in Thyroid Follicles (TF), irregular pluripotent haemoblasts (hae) in haematopoietic tissue and vacuolated erythrocytes (RBC's), neutrophils and monocytes in the peripheral blood. The results obtained suggests that the gills, thyroid, haematopoietic tissue and peripheral blood cells were structurally altered in polluted water body fishes. Such alterations could affect vital physiological functions such as respiration, osmotic and ionic regulation of the gills, storage and secretion in thyroid follicles, haemopoiesis of the kidney and oxygen carrying capacity, haemoglobin content in erythrocytes and loss of immune mechanism, phagocytosis in leucocytes, which in turn could ultimately affect the survival and growth of *G. giuris*. Thus all possible remedial measures should be adopted to prevent the occurrence of contamination in the aquatic environment.

Key words: Histopathology, pollutants, BOD, nitrogen, TDS, *G. giuris*

Introduction

The environmental quality of freshwater ecosystem has deteriorated markedly over the last two decades. Pollution of water bodies is assuming alarming proportions with increased population, industrialization, urbanization and intensive agriculture.

Pollution may take place due to the discharge of domestic and industrial wastewater, toxic chemicals used for agriculture and other purposes, solid waste due to garbage, and cleaning of vehicles, drainage from farms and land surface, dustfall, wastes due to recreational use and much more such

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activities as suggested by Singh and Mahaveer (1997) and Neera *et al.* (2003). Pollution caused by plethora of human activities primarily affects physico-chemical characteristics of water leading to destruction of community disrupting delicate food webs, deteriorating aquatic environment (Khan, 1996; Ara *et al.*, 2003).

Pollution of the aquatic environment generally causes changes in the physiological and structural aspects of the inhabitant organisms, particularly the fishes. Histopathological affects by pollutants vary with the body parts, nature of the pollutant, medium and duration of exposure (Venkataramana *et al.*, 2001). Water quality characteristics influence histopathological manifestations of toxic effects (Galat *et al.*, 1985; Bhavan and Geraldine, 2000). As the freshwater fishes in our country constitute an important part of animal protein in rural as well as in urban areas; their direct or indirect pollution and the consequent toxicity are of great importance to the environmental biologists. Fish is considered as an important tool in the aquatic toxicology and the toxic pollutants significantly alter certain physiological and biochemical processes when they enter into the body. *Glossogobius giuris* is a bottom dweller, edible fish, a good sensitive indicator, spawning is intense during Septembers and distributed widely in freshwater ponds, tanks and dam in and around Bangalore. Hence, the present investigation was conducted to monitor the water quality and impact of water pollutants in vital tissues such as gills, thyroid, haematopoietic tissue and blood cells of the freshwater gobiid fish, *G. giuris*.

Materials and Methods

Study Area

Avalapalli dam in between Bangalore and Hosur is located at 13°20' 58" to 13°21'39" S latitude and 77°06'22" to 77° 06'38" W longitude, about 23 miles North East of Bangalore. It supports agriculture and small-scale fishing and serves as a water source for the forest nursery nearby, the water is also used for drinking after recycling. The dam covers an area of 200 acres and has a catchment area of about 2.5 km². It receives sewage and municipal wastes and used as huge public sanitary dump. The water samples from surface (max. depth 25 cm) were collected with necessary precaution in plastic cans from different sites of the dam. The different physico chemical parameters of water were analyzed as per the methods recommended by APHA AWWA WPCF (1995), Trivedy and Goel (1984) and Mackereth (1963).

The freshwater gobiid fish *G. giuris* (body length 8-10 cm, body wt. 30-40 g) from other water bodies (Sankey tank: The tank has a well-maintained park and a corporation swimming pool at the Southern part and a nursery towards the North, adjacent to the tank. It also attracts large populations of migratory birds apart from small time fishing activities. The tank presently has no major source of pollution) as control fish and polluted water body (Avalapalli dam) fish were collected by using cast and gill net (mesh:10 mm). Both (control) and polluted water (experimental) live fishes brought to the laboratory and maintained in separate glass aquaria (60"x 30"x 20") with respective of their tank water. The tissues of gills of both control and experimental fishes dissected immediately and fixed in Bouin's fluid, thyroid in buffer neutral formalin dehydrated in ethanol and embedded in paraffin (58-60°C) and midlongitudinal sections of 5-6 μ thickness were cut and stained Ehrlich haematoxylin stain for histopathological studies. Imprints of head kidney were used for haemopoiesis. A thin slice of excised organ of both was collected and placed on a methanol coated clean microslides for preparing smears of head kidney by adapting the technique as described by Ashley and Smith (1963). The haematopoietic tissue smears are air dried and stained with Giemsa for cytomorphological studies. The blood smears were also made immediately by severing the caudal peduncle on a methanol coated clean microslides. The slides were air-dried and blood smears were stained with Giemsa for cytomorphological studies.

Results

Physico-chemical Parameters

The water quality monitored for a period of six months at two points towards the periphery and one at center of the dam. The pollution status of the dam was evaluated by analyzing the various physico-chemical parameters from three sampling sites were shown in the Table 1. The samples collected at the inlet (A), center (B) and outlet (C) (sampling at center was done on the availability of a boat and absence of water hyacinth) were analyzed. The colour of the water body was dark gray towards the inlet, greenish towards center and outlet. The temperature of the water during sampling time was in the range of 23.4-27°C. pH is a dynamic parameter in an aquatic system varying with changes in physical and chemical properties over a period. pH of the dam was found to be alkaline range from 7.67 to 8.52 at the sample points. The electrical conductivity noticed during the study period was varying from 2122 to 2590. The alkalinity and nitrates ranged from 479-569 mg L⁻¹ and 235-362 mg L⁻¹ and chloride values of the samples were 211.4, 179.0 and 188.0 mg L⁻¹ at sampling points A, B and C, respectively. The TS in the dam was 804 mg L⁻¹ in point A, 590 mg L⁻¹ in sampling point B and 580 mg L⁻¹ in point C. The suspended solids ranged from 150 to 184 mg L⁻¹ and TDS of the water body were found to be 620 mg L⁻¹ in sampling point A, 425 mg L⁻¹ in point B and 430.6 mg L⁻¹ in point C. The DO level ranged from 4.2 to 6.3 mg L⁻¹ and of BOD is 122.5 mg L⁻¹ in point A, 37 mg L⁻¹ in point B and 32 mg L⁻¹ in point C. The COD was found to be 386.0, 348 and 362.0 mg L⁻¹ at the sampling point A, B and C, respectively (Table 1).

Gills

Histology

The gills of freshwater gobiid fish, *G. giuris* consist of laterally compressed leaf like gill filaments, Primary Gill Lamellae (PL) arranged alternately on either side of the interbranchial septum. Each primary filament bears a row of Secondary Lamellae (SL) on both sides perpendicular to its long axis comprising of a central core of Cartilaginous Rod (CR). The secondary lamellae also termed, as Respiratory Lamellae (RL) are highly vascularised and covered with a thin layer of epithelial cells (E), blood vessels are extended into each of the secondary gill filaments (Fig. 1). The region between the two adjacent secondary gill lamellae is known as Inter Lamellar Region (ILR). Mucous cells are scattered over the arch, support epithelium, filament and lamellae. Chloride cells are found on the epithelium, lamella portion of the filament and medial margin of the lamellae. These cells are most frequent on the filament surface between the lamellae (inter lamellar filament epithelium) and around the afferent-trailing edge with respect to water flow.

Table 1: Physico-chemical characteristics of polluted dam water (Avalapalli dam)

Parameters	Sampling point A	Sampling point B	Sampling point C
pH	8.52	7.82	7.67
Temperature	27.00	23.40	24.90
Electrical conductivity	2590.00	2273.00	2122.00
Alkalinity	569.00	532.00	479.00
Chlorides	211.40	179.00	188.00
Nitrates	362.00	235.00	292.00
TS	804.00	590.00	580.00
TSS	184.00	165.00	150.00
TDS	620.00	425.00	430.60
DO	4.20	6.30	5.70
BOD	122.50	37.00	32.00
COD	386.00	348.00	362.00

*All values in mg L⁻¹, except pH, Electrical conductivity

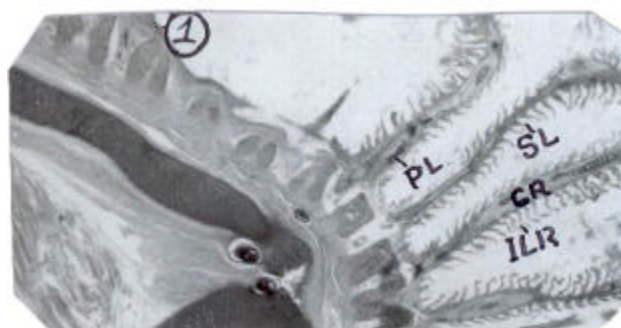


Fig. 1: Typical organization of the gills of *G. giuris* (control). Primary Lamellae (PL), Secondary Lamellae (SGL) and Cartilaginous Rod (CR), with uniform interlamellar space (ILS), x 100 (bar = 10)

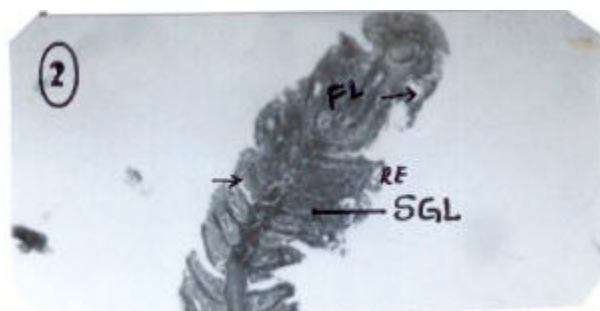


Fig. 2: Pollutants induced alterations in the histoarchitecture of the gills of *G. giuris*. Swelling of gill lamellae (SL) and fusion of gill lamellae (FL), x160 (bar = 10)

Histopathology

Gills, mainly concerned with respiration and osmoregulation showed certain degenerative changes like the Fusion of secondary Lamellae (FL) (Fig. 2), bulging of tips of gill filaments and atrophy of the Primary gill Lamellae (PL) (Fig. 4). In some cases a complete degeneration of gill filaments and respiratory epithelium was observed. Hyperplasia of interlamellar cells (IL) and necrosis of Secondary Lamellae (SL) was also noticed in few cases compared to control (Fig. 3). The secondary gill filament lost their original shape and curling of secondary gill filaments was observed. The pillar cell nucleus showed necrosis and development of vacuoles in the secondary gill epithelium (Fig. 4). There is a tendency of fusion of disorganized secondary gill filament compare to control (Fig. 2). In some cases disappearance of walls of pillar cells, necrotic changes in epithelium of secondary gill filaments resulting in the development of vacuoles in epithelium was also noticed (arrow).

Thyroid

Histology

Thyroid gland is composed of a large number of follicles each of which is in the form of hollow ball consisting of a single layer of epithelial cells enclosing fluid filled space (Fig. 5). These follicles vary in shape and size and are bound together by connective tissue (CT). The gland is highly vascular and is well supplied with blood. The epithelium (E) surrounding the follicle and the height of the cells

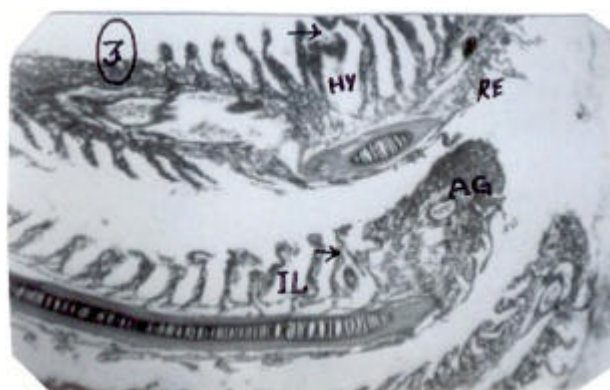


Fig. 3: Pollutants induced alterations in the histoarchitecture of the gills of *G. giuris*. Hyperplasia (HY) of secondary lamellae and Abnormal gill tips (AG), x160 (bar = 10).

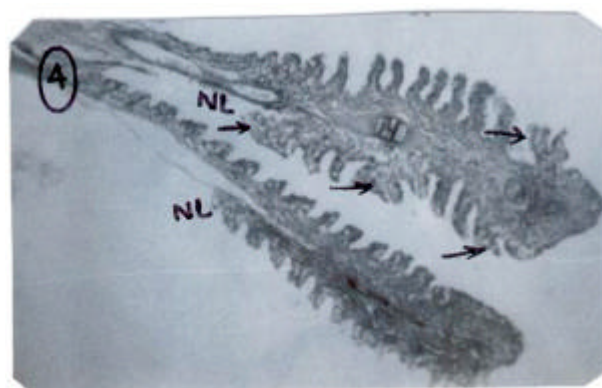


Fig. 4: Pollutants induced alterations in the histoarchitecture of the gills of *G. giuris*. Necrotic gill lamellae (NL) and oedematous changes at the base of the Primary Lamellae (PL), lamellar telangectases (arrow) x160 (bar = 10)

depends upon its secretory activity. It is composed of two kinds of cells, the chief cells and cuboidal cells contain droplets of secretory material and the lumen of each follicle is full of colloid (C) depending upon the secretory activity of the follicle.

Histopathology

The thyroid follicles of polluted water fish exhibited marked cytological changes. The epithelial cells were found to be cuboidal with dense colloid, thus many vacuoles recognized in the colloid adjacent to the epithelium (E) (Fig. 6 and 7). Most of the follicles were devoid of colloid. In some follicles, the follicular lumens (FL) are totally obliterated with total loss of colloid, few follicles with little amount of colloid were also noticed (Fig. 7). Cellular hypertrophy and follicular hyperplasia (HY) accompanied such change, rupture follicles, peripheral vacuolization leading to glandular atrophy compare to control.



Fig. 5: Typical organization of the thyroid gland of *G. giuris* (control). Thyroid Epithelium (TE). These epithelial cells enclose a lumen filled with homogeneous colloid (C), x 400 (bar = 10)

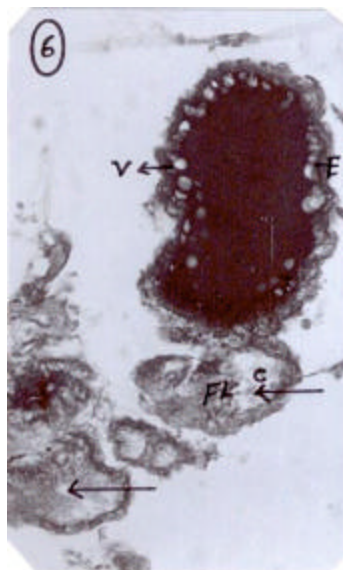


Fig. 6: Pollutants induced alterations in the histoarchitecture of the thyroid of *G. giuris*. Vacuolization (V) in the colloid adjacent to the epithelium (E) and concomitant loss of colloid (C) in few follicles (arrow), x 400 (bar = 10)

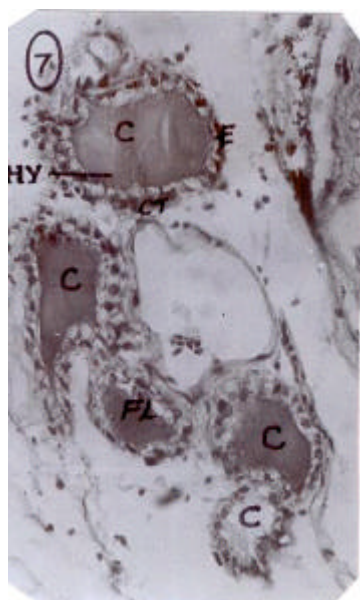


Fig. 7: Pollutants induced alterations in the histoarchitecture of the thyroid of *G. giuris*. Heterogeneous nature of colloid (C) and cellular hypertrophy and follicular hyperplasia (HY), x 400 (bar = 10)



Fig. 8: Photomicrograph of haematopoietic tissue of *G. giuris* (control). Spherical pluripotent haemoblast (hae), the thick darkly stained oval basophilic erythroblast (be), x 400 (bar = 10)

Haematopoietic Tissue

Cytomorphology

The normal haematopoietic tissue is characterized by the presence of stem cells called haemoblast (hae) and interstitial tissue (It) (Fig. 8). Haemoblast is the pluripotent nature, which gives

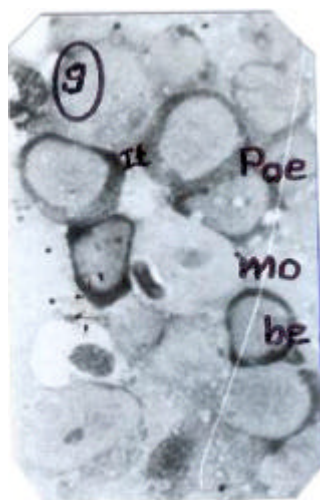


Fig. 9: Photomicrograph of haematopoietic tissue of *G. giuris* (control). Spherical or oval or amoeboid distinct membrane polychromatophilic erythroblast (poe), eccentric monoblast (mo), x 400 (bar = 10)



Fig. 10: Photomicrograph of haematopoietic tissue of *G. giuris* (control). Round or oval distinct darkly stained haemoblast (hae) and small and large lymphoblast (ly), x 1000 (bar = 10)

rise to four stages of unipotent precursor cells (Flow chart). These are in clumps and scattered in the imprints of head kidney. Haemoblast (hae) is spherical or oval stains moderate to dark blue with Giemsa. The nucleus is large with diffuse chromatin material. The developmental stages of erythroblast, lymphoblast (ly), granuloblast (gr) and monoblast (mo) are spherical, oval or round with distinct cell membrane (Fig. 9 and 10).

Cytopathology

The histopathological alterations due to the aquatic pollutants in the haematopoietic tissues are the changes in the cellular nature of haemoblast (hae) (Fig. 11), granuloblast (gr), erythroblast, lymphoblast (ly) cells, interstitial tissue and desquamation of the epithelium was observed when

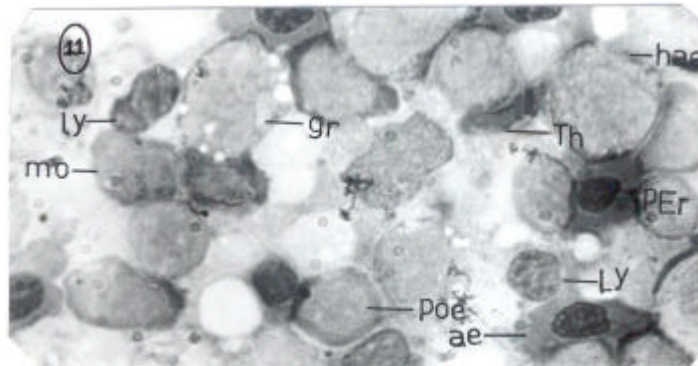
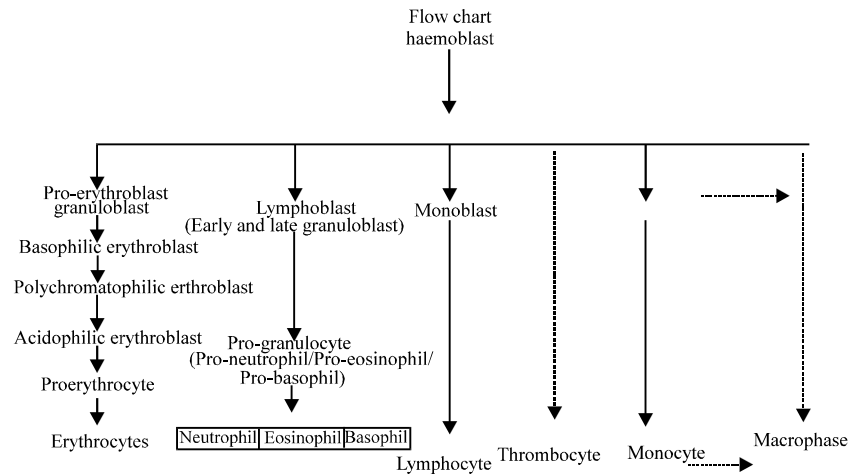


Fig. 11: Pollutants induced alterations in cytoarchitecture of the haematopoietic tissue of *G. giuris*. Hypertrophy in haemoblast (hae), vacuolar granuloblast (gr), serrated cell membrane in acidophilic erythroblast (ae) and clumped cytoplasm in lymphoblast (ly) and lymphocytes (Ly), x 1000 (bar = 10)

compare to control. Majority of the imprint shows severe vacuolization in granuloblast (gr), monoblast (mo) and necrosis was also noticed. The imprint was relatively high number of degranulated granulocytes (Fig. 11).

Blood Cells

Cytomorphology

The peripheral blood cells of *G. Giuris* are broadly classified into erythrocytes, thrombocytes and leucocytes. The mature erythrocytes (RBC's) are oval, oblong or elliptic with distinct rounded or oval nuclei containing chromatin material (Fig. 12). Thrombocytes varied from round to horseshoe and spiked form. They are differentiated by their scant, pale grey to colourless cytoplasm with Giemsa stain. The leucocyte population composed of lymphocytes (Ly), neutrophils (Ne), monocytes (Mo) and macrophages (Mp). The lymphocytes are amoeboid or spherical in shape with pseudopoid formation, nucleus contains coarsely clumped chromatin material which stained deeply with Giemsa. Neutrophils are spherical, oval and fairly large with distinct cell membrane and the nucleus appears to be spherical or dumb-bell in shape (Fig. 12). Manocytes and macrophages is the rare type of cells with larger in size and eccentric irregular nucleus.

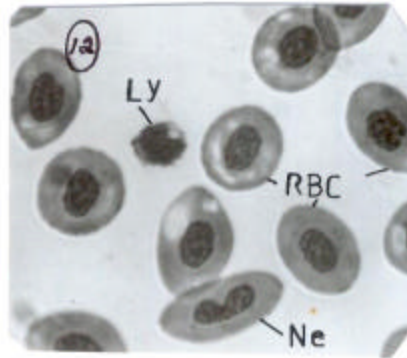


Fig. 12: Photomicrograph of blood smear of *G. giuris* (control). Showing homogeneous cytoplasm and distinct cell and nuclear membrane of RBC's, small granular lymphocyte (Ly) and dum-bell shaped nucleus of neutrophil (Ne), x 1000 (bar = 10)

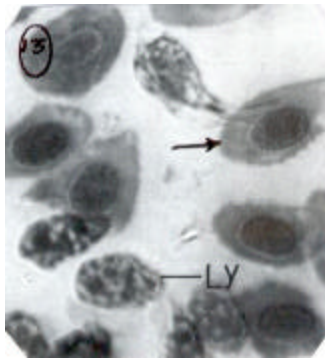


Fig. 13: Pollutants induced alterations in the cytoarchitecture of the blood smears of *G. giuris*. Clumping nature of cytoplasm in lymphocytes (Ly) Serrated cell membrane in RBC s, x 1000 (bar = 10)

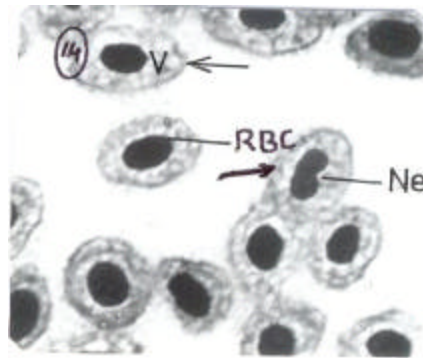


Fig. 14: Pollutants induced alterations in the cytoarchitecture of the blood smears of *G. giuris*. Vacuolated cytoplasm in RBC's and neutrophil (Ne) and condensed darkly stained nuclei, x 1000 (bar = 10)

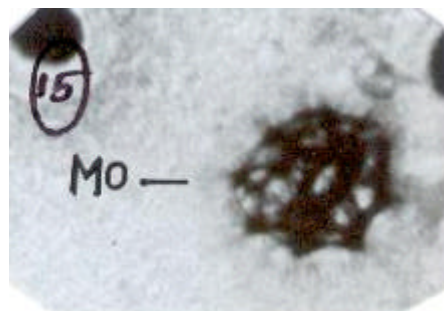


Fig. 15: Pollutants induced alterations in the cytoarchitecture of the blood smears of *G. giuris*. Hypertrophied, vacuolated and eccentric nucleus in monocyte (Mo), x 1000 (bar = 10)

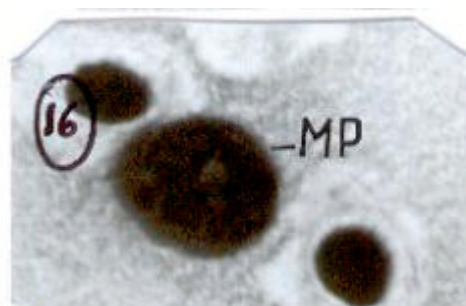


Fig. 16: Pollutants induced alterations in the cytoarchitecture of the blood smears of *G. giuris*. Darkly stained condensed nucleus in macrophage (Mp), x 1000 (bar = 10)

Cytopatology

Erythrocytes were extensively deregulated and cellular hypertrophy was evident (Fig. 13 and 14), some cells showed cytoplasmic vacuolization with dark, condensed nucleus. The shrinkage of erythrocyte (RBC's) cell membrane was also significant (arrow). The lymphocytes showed indistinct cell membrane and the cytoplasm was chromophobic (Fig. 13). Neutrophils are hypertrophied, in most of these cell granules were compact, deeply stained and confined to the periphery of the cell membrane as a result of accumulation of chromatin on one side of the nucleus. The cell membrane became shrunken or in serrated in nature (Fig. 14). Monocytes and macrophage exhibits sign of necrosis, indistinct cell membrane and these cells are large and pycnotic eccentric nuclei with diffused chromatin material (Fig. 15 and 16).

Discussion

The balance in an ecosystem is maintained when pH is between 5.5 and 8.5; extremes of pH hinder the survival of living organisms. During the present study pH of dam was found to be within the permissible limit of 5.5-9.0 according to BIS standard. Temperature is one of the important ecological factors, which controls the physiological behavior and distribution of organisms. Jain *et al.*, (1996) have observed diurnal variations in temperature. In the present study, diurnal variations were noted and water temperature was found to be lower than atmospheric temperature at all the three sites.

A number of bases, viz, carbonates, bicarbonates, hydroxides, phosphates, nitrates, silicates, borate etc., contribute to alkalinity (Garg, 1998; Chandrasekhar *et al.*, 2003). Present work is in agreement with the report of Pandey and Soni (1993) who had observed high values of free carbon dioxide,

alkalinity and pH along with low dissolved oxygen in highly polluted lake water. In the present study, lowest dissolved oxygen is recorded (4.2 mg L^{-1}) and high total alkalinity (569 mg L^{-1}). Pandey and Soni (1993) observed higher amounts of total alkalinity at highly polluted Naukuchiyatal Lake.

The presence of chlorides in the wastewater streams is due to industrial sector using organic and inorganic chloride compounds. The presence chloride concentration in water source is used as an indicator of organic pollution by domestic sewage (NEERI 1979). Nitrate is the oxidized form of nitrogen and in water it is most important source is biological oxidation of nitrogenous organic matter of both autochthonous and allochthonous origin which include domestic sewage, agricultural runoff and effluents from industries (Saxena, 1998). The disposal of both suspended solids leads to sedimentation (Saxena, 1998). The settleable solids include inorganic and undissolved solids, which settle to the bottom of the ponds causing siltation of dam (Verma, 1978). The BOD and COD variation is due to of more oxidizing organic compounds in the water (Kumar *et al.*, 2001). The present findings indicate that the water of Avalapalli dam is most polluted due to high alkalinity, free carbon dioxide, hardness, pH and low level of dissolved oxygen.

In fish, the gill is the most important organ for respiration and osmoregulation and it is the first organ to which the pollutant comes in contact. Hence, it is more vulnerable to damage than any other tissue. Present study in *G. giuris* the pathological condition include separation of epithelial layer over secondary gill filaments, hyperplasia of primary filaments and secondary lamellae, necrosis in interlamellar spaces and fusion of secondary gill lamellae, swelling of secondary gill filaments. Some of these changes were reported in the gills of *Punctius ticto* exposed to Khan river water with industrial sewage by Chouhan and Pandey (1987) and Romao *et al.* (2001) exposed to seawater dilution. Mucous cells in the secondary lamellae of the experimental (polluted) fish are more than the in control fish, indicating the response of the fish to the toxicant. The sub epithelial space between pillar cells and epithelial lining reduces the effective respiratory and osmoregulatory surface over the central lamellar blood space since flow through this region reduced (Rios and Fanta, 1998; Eiras-Stofella *et al.*, 2001). Degeneration of epithelial cells indicates the damage in the gill lamellae, which reduces the activity this might be one of the reasons for fall in oxygen consumption of the fish in polluted water body. These changes reduce the respiratory area thereby reducing the respiratory and osmoregulatory potential (Gardner and Yerish, 1970; Lindesjoo and Thulin, 1994). It also indicates a decrease in energy metabolism due to degeneration of respiratory epithelium and the damage of the gill tissue may finally result in tissue hypoxia.

In the light of present findings on the *G. giuris* cause damage on the structure of follicle, amount of colloid and function of the cell. Singh and Singh (1983) reported suppressed thyrotrophic secretion from the pituitary gland of *Heteropneustes fossilis* after cyathion and hexadrin exposure. These works suggested that the aquatic pollutants probably retard the hypophysial TSH out put was followed by hypo thyroidal activity. In the present study led to remarkable enlargement of the thyroid follicle, vacuolization, follicular epithelial cell height increases as manifestation of dysfunctions. The present work is also harmony with the findings of Lata (1995) and Shrivastava *et al.* (1996). The follicular lumen was obliterated with total loss of colloid, similar observation were made by Van Overbreeke and Mc Bride (1971) in Sockeye salmon *Oncorhynchus nerka*, Hyperactivity of thyroid follicles of few teleost were also reported by Baker Cohen (1961) and Latey and Rangeker (1983).

Alterations in cell morphology and fluctuation of cells in response to several internal and external factors (Tanka *et al.*, 2004). The degeneration of the cells suggests impairment in the developing cell lines and infiltration process leading to imbalance in osmotic regulation of the body fluids. The present study, the penultimate developmental stages of both erythrocytes and leucocytes in the haematopoietic tissue, is decreased significantly during the stress conditions Dheer *et al.* (1986). These pathological changes may be due to the preferential accumulation of pollutants that causes degeneration leading to lymphocyte infiltration as a measure of resistance to the toxicants and tissue susceptibility (Kumari and Kumar, 1997; Kelenyi and Larsen, 2005).

Erythrocytes are oxygen-carrying devices; the quantitative decrease in their levels might have led to the dearrangement of the oxidative metabolism with concomitant decrease in the tissues of respiratory potential. The decrease in erythrocytes leading to anaemia as a result of inhibition of erythropoiesis, haemosynthesis and increase in the rate of erythrocyte destruction in the haemopoietic organs. Similar reports have also been made by Goel and Kalpana (1985) and Sampath *et al.* (1998). Natarajan (1981) reported the reduction of haemoglobin (Hb) content, erythrocyte count and haematocrit values, resulting in hypochromic anaemia, due to deficiency of iron and decreased utilization for Hb synthesis. The increase of neutrophil may be due to tissue damage and lymphocytes could lead to loss of immune mechanisms of fish. The present study indicates that produce deformities in the cytomorphology of blood cells (Kurde and Singh, 1995; Mc Leay and Gordon, 1977).

Avalapalli dam is economically important. It remains dry during summer and refills during monsoon. However of late, due to continuous flow of municipal and industrial effluents from urbanized Bangalore and Hosur City, the dam is overflowing throughout the year, thus changing the characteristics of the dam from being a natural ecologically healthy dam to artificial reservoir of sewage and industrial wastes. If the present state of affairs continues, situation may arise that Avalapalli dam might become an ecologically inactive. From the present study it is also inferred that histopathological changes in fishes would serve useful purpose in evaluating the toxic effects of various pollutants present in large amounts in a heavily polluted Avalapalli Dam.

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