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Relative Study on Haematology, Glycogen Content and Histological Changes in Organs of *Anabas testudineus* from Parvathiputhanar (Polluted) and Karamana River (Fresh Water)

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ABSTRACT

This study was carried out to evaluate the haematology and histology of the three main organs such as liver, kidney and intestine from the polluted water (Parvathyputhanar river) and fresh water (Karmana river). The release of heavy metals into the aquatic environment causes water pollution problems the blood cells although, liver, kidney and intestine like organs changes demonstrated by microtomy technique of histopathology. Haematological observation between fresh water and polluted water living *A. testudineus*: Haematocrit (PCV) was found decreased to 16%. A similar reduction was also observed in the haemoglobin content (4.8 g/100 mL). For instance, the haematological variation indicates in the Parvathiputhanar fish is due to this canal possessed slightly on the alkaline (7.1-8.5). Differential blood cells count also affected and glycogen levels were elevated in the polluted water living fish compared with fresh water *A. testudineus*. When compared these two different water bodies such as Parvathyputhanar and Karamana (polluted and unpolluted) collected fish *A. testudiensis* shows necrosis of tubular epithelium, cloudy swelling of epithelial cells of renal tubules, narrowing of the tubular lumen and contraction of the glomerulus and expansion of space inside the Bowman's capsule were observed in the kidney tissues than Karamanayar river. Moreover, hepatic lesions in fish living with Parvathyputhanar are characterized by hypertrophy of hepatocytes, cloudy degeneration, congestion, karyolysis, dilatation of sinusoids and focal necrosis. From this study focusing histopathological findings suggest the acidic and other heavy metal impurities caused severe damages as well as alterations occurred into the internal organs of fish and consequently change the physiological status. While, the polluted Parvathyputhanar River utilized by a peoples these findings highlight the need for adequate water treatment.

Key words: Haematology, histopahology, Karamana river, parvathyputhanar, *Anabas testudineus*

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 (Venkataraman *et al.*, 2007). It includes not only the influences of other plants and animals present but also those of the physico-chemical and materiological factors (Velmurugan *et al.*, 2007). Water pollution affects and kill all forms of wild life. Humans are the biggest threat to fish. Previously, reported due to this most organisms present in fresh waters face not only nutritional hazards but also marked diurnal and seasonal oscillations as well as man-made changes in the environment extremes may toxic even the physiological activities to the limit (Ehiagbonare *et al*., 2009). The effects of increasing pollution stress upon fresh water environments have become a cause of concern to man due to feed back to our own health, economic welfare and degradation in recreational facilities (Oluah, 2008). It is therefore necessary to evolve environmental management strategies and action plans for integration of environmental consideration in development activities of the aquatic environment.

It increase turbidity and may cause discoloration of water with possible adverse effects on fisheries and recreational activities. Some type of particulate matter may damage gill surfaces of fishes and vertebrate (Mohamed, 2006). Also, the materials settling at the bottom may alter the composition of sediment affecting benthic organisms. If the solids are organic, aerobic conditions may develop in the sediment overlying water layer, affecting in the organisms. Furthermore, Oluah (2008) assessed the pollution effects involves not only the chemical and physical character of the aquatic ecosystems but the biological parameters. Haematology is a reliable indicator of the physiological condition of the fish. This has been the incentive to the scientific interest and development of fish heamatology as chemical tool in monitoring fish health programmers (Gill *et al*., 1991). These also review background effects of water pollution and toxicity studies. A wide array of factors, both within and without the organisms affects the peripheral haematological make up of fishes (Mahajan and Dheer, 1983). Previously, Koca *et al*. (2008) reported sex, season and maturity stages are known to affect haematological values of fishes. The effects of biocides on target organism particularly on fishes may be exhibits to many ways. Majority of biocides produce detrimental and sometimes fatal side effects on fish fauna. Many researchers have been studied the bioassay of water pollutants of *A. testudiensis*. Even though, the water pollution properties extensive use in agricultural fields especially fields results in pesticide pollution (Kamal *et al*., 2007). Hence, the bioassay of water pollution and its histopathological effect on liver was investigated on *A. testudiensis* (Bloch.) which were a fresh water table fish with very high environment resistance and common in paddy fields.

MATERIALS AND METHODS

Five live fishes of equal size belonging to the species *A. testudeneus* where collected from the Parvathiputhanar canal located in Trivandrum. The fishes of same species where also collected from the fresh water Karamana river located in Trivandrum. They were brought into the laboratory and kept in glass tank at room temperature. Karamana River is maintained in another tank named as control. The fishes kept tank P. P were maintained as experimental fishes.

Haematological studies: The fishes were sacrificed and the body was cut open. Blood samples were collected from both control and experimental fishes. The samples were oxalated for preventing clotting. The following parameters of blood were studied.

Haematocrit or packed cell volume (PCV): Determined by centrifugalize samples of blood taken in the Wintrob tube. Haemoglobin content was determined using haemometer. Total count of RBC, WBC and Thrombocytes was made using the haemocytometer. Blood smear preparations were made and stained with Leishmanns stain to study the details of RBC and Thrombocytes. Differential count of Leucocytes was also made.

Studies on changes in the level of glycogen: The tissues were isolated from the body of fishes Parvathiputhanar and Karamana river and kept in separate Petri dish. From the sample and control fishes the following organs such as liver kidney and intestine were selected to identify the preliminary study of glycogen content present these three organs. Tissue were cut into small pieces and fixed separately in absolute alcohol. Paraffin sections were prepared and histochemical localization of glycogen was done by PAS technique. Photomicrographs were also prepared for detailed observation. The work had been carried out in January 2008 to June 2009 during the fourth semester M.Sc course in the department of Zoology Mar Ivanios College TVM.

RESULTS

Haematological studies: The following parameters of blood of *A. testudineus* such as PCV, haemoglobin, total count of RBC, WBC and Thrombocytes differential count of Lymphocytes, Granulocytes and macrophages of both control and experimental fishes were compared. The observation and result were summarized in Table 1.

The Haematocrit (PCV) was found to be 22% haemoglobin content was 7.5 g mL^{-1} of blood Erythrocytes were nucleate and oval in shape. Length of RBC varied from 12-14 m and their width varied from 8.5-9.5 m erythrocytes numbered 2.3×10^{6} mm^{-3} of blood.

Leucocytes: Leucocytes were numerous than erythrocytes. Leucocytes count was 0.14×10^{6} mm^{-3} blood. Three kinds of leucocytes were identified. Lymphocytes $\times10^{6}$ lymphocytes were most abundant leucocytes there were two kind of lymphocytes-small and large. Nucleus was round shaped. Lymphocytes constitute 65% of the total WBC. Diameter of these cells ranged from 4.2 to 8.2 m. The nucleus was seen deep violet eighth Leishmann Stain. Then the granulocyte possessed prominent granules in the cytoplasm. They formed 29% of the total WBC. Among the granulocytes Neutrophills were identified by virtue of their multilobed nucleus. The diameter of granulocyte varies from 9-12 m.

Macrophages: Large in size arranging in diameter from 30-35 m.

Thrombocytes: Spindle shaped cells having a length of 12-15 m Tejendra the cytoplasm was granulat and deeply basophilic in center and pale homogeneous periphery. Cytoplasm purplish in colour. Thrombocytes numbered 0.071×10^{6} mm^{-3} blood.

Haematological observation between fresh water and polluted water living *A. testudeneus*: Haematocrit (PCV) was found decreased to 16%. A similar reduction was also observes in the haemoglobin content (4.8 g 100/mL).

Erythrocytes: Erythrocytes had undergone several changes in shape and size. Shape varied from oval to irregular (Fig. 1) Plasma membrane ruptures and was disintegrating in many RBCs. Nuclei

Table 1: Study of blood cells characterization of the pollutant and fresh water fish of *A. testudiensis*

Blood cells	Length/width/diameter control of RBC 12-14×8.5-9.5 m	Length/width/diameter experimental of WBC 7-12.25×3.5-8.75 m
Lymphocytes	4.5-8.2 m	4-8 m
Granulocyte	9-12 m	9-11 m
Macrophages	30-35 m	32-38 m
Thrombocytes	12-15 m	11.5-15 m

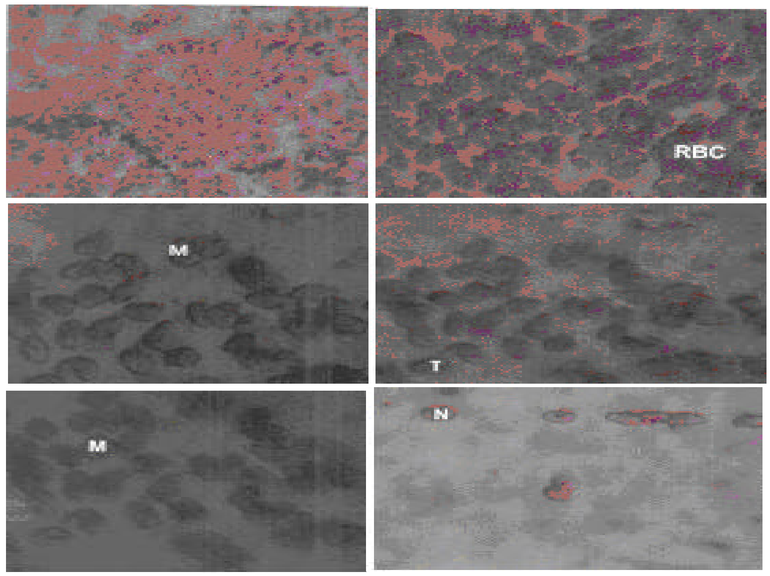

Fig. 1: Blood cells and its physiological on *A. testudineus* from Karamana river (fresh water)

was without change, nuclear size renamed more or less uniform length and width of the RBCs decreased to 1.7106 mm^{-3} blood.

Leucocytes WBC count decreased to 0.11×38106 mm^{-3} blood. When compared the Lymphocytes count there were a significant reduction in number observed than the control river fish *A. testudiensis* of lymphocytes could be Diameter remained unchanged. Large lymphocytes were more abundant. Differential count of lymphocytes was 55% showing a decrease of 10% over control fishes.

Granulocytes: There was a significant decrease in the number of granulocytes (8% WBC) and the difference between the control fish 21%. Though, size and shape of the cells remain unchanged.

Macrophages: There was a slight increase in the diameter in the diameter of macrophages (32-38 m) macrophages formed 37% of WBC showing an increase of 31% over control fishes.

Thrombocytes: A reduction in the number of thrombocytes was observed (0.055×10^6 mm^{-3} blood) (Fig. 1, 2).

Changes of glycogen content: The control slides were compares with experimental sections after localizing glycogen in liver, intestine and kidney. In the control slides, all regions of liver gave intense PAS positive reaction for glycogen. There was preponderance of glycogen in the outer region. The middle and inner region also colored deep pond. In the experimental sections, the reactions were slight to moderate. Many degenerating patches of parenchyma cells ranging from small to moderate size were also observed. The intestine showed intense PAS positive reactions including the serosa, muscularis, mucosa and villi on the control slides. Though, slight to abundant reactions were obtained in the experimental sections (Table 2). The serosa gives a slight PAS

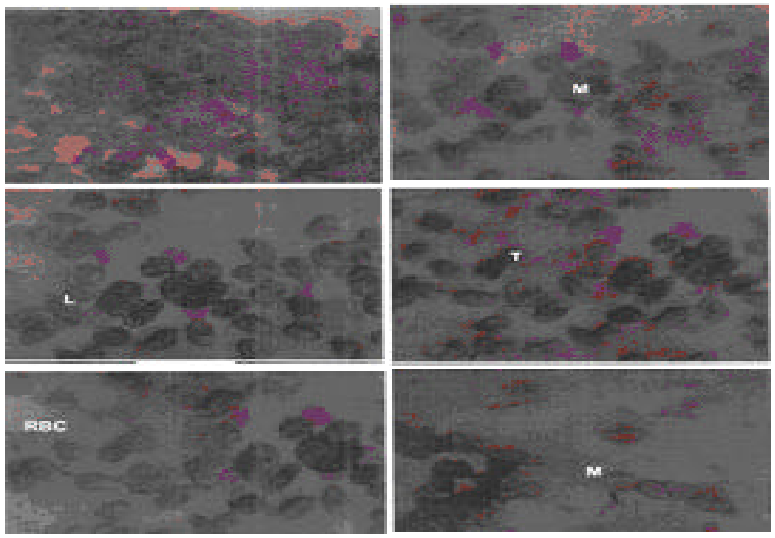

Fig. 2: Blood cells and its physiological on *A. testudineus* from Parvathyputhanar river (polluted water)

Table 2: Preliminary analysis of glycogen content from the 13 different regions of *A. testudiensis* (PAS)

Organ	Control	Experimental
Liver	++	+
Outer organ	+++	+
Middle region	+++	++
Inner region	+++	++
Intestine	++	+++
Serosa	+++	+
Muscularis	+++	++
Mucosa	+++	+++
Villi	+++	+++
Kidney	++	
Outer region	+++	+
Middle region	+++	++
Inner region	+++	+++

positive reaction. The glycogen concentration was moderate in the muscularis and abundant in the mucosa and villi. All regions of the kidney gave intense action for PAS in the control slides. In the experimental slides outer region showed slight PAS positive reaction. The middle region was moderate while the inner region showed abundant PAS positive reaction.

Histopathological studies: The pathological change in the blood cells of the fish from the polluted and non polluted river living fish has been observed. Haemorrhage, blood congestion and necrotic cells were generally observed in the liver tissue. Mononuclear cell focal infiltration was observed (Fig. 3b). The lesions in the kidneys of the fish exposed to acidic water in the unused lignite mine included degeneration of the epithelial cells of the renal tubules, degeneration of the

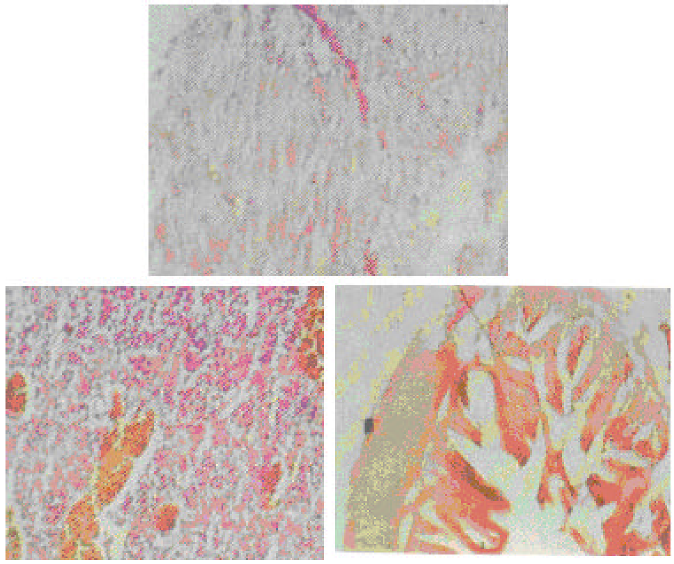

Fig. 3: Histological view of liver, kidney and intestine from the Parvathiputhanar (polluted) river *A. testudiensis*

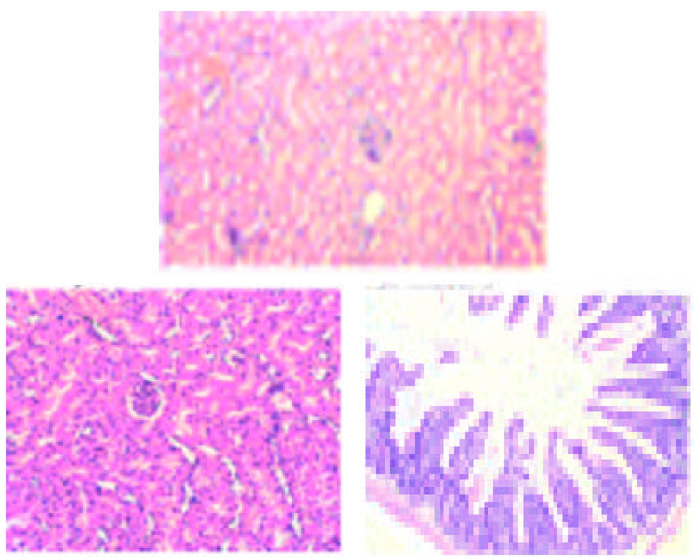

Fig. 4: Histological view of liver, kidney and intestine from the Karamana (fresh water) river *A. testudiensis*

glomeruli, hypertrophy of the epithelial cells of the renal tubules narrowing of the tubular lumen and glomerular contraction in the Bowman's capsule (Fig. 3). Such an observation doesn't present in the kidneys of the fresh water fish (Fig. 4).

DISCUSSION

One of the commonest pollutants of the fresh water ecosystem in all developing or undeveloped countries from sewage, raw or treated. In reasonable quantities, sewage can be harmless or even beneficial. The nitrate and phosphates in sewage fertile water, leading to increased growth of microscopic plant life, phytoplanktons in the water. This serve as the food for minute animals which is their turn end up as food for fish and other animals (Sarkar *et al.*, 2006). Particulate matter in the sewage water which depress according to its settling velocity can also influence this aquatic environment in many ways (Omitoyin, 2006). Assessment or interpretation of predicted inputs represent a vital activity in an environment impact study (Saloom and Duncan, 2005). Significant impacts on physical, chemical, biological environmental and socio-economic components of the environment are being caused human activities (Stone and Thormforde, 2003). The ecosystem under consideration in the Parvathiputhanar canal an artificial canal dug out for the purpose of inland water transport is now increasingly being used for the disposal of various waste materials. The present study is attempts undertaken to evaluate the variations in the biological characteristics include morphology, physiology, haematology and glycogen content of fishes of Parvathiputhanar a highly polluted river and fresh water river Karamana river.

The important of the hydrographic studies in aquatic environments is well realized in view of their value in assessing the biological potentialities of an ecosystem. The haematological Variations in the Parvathiputhanar fish is due to this canal slightly remained on the alkaline (7.1-8.5) because it one end having the influx of water from Veli lake and Poonthura water lake and also the garbage and sewages from the town and villages. The morphology and difference physiology of the fishes both in controlled and experimentalised shows variance due to environmental stresses. The experimental fish from Parvathiputhanar was highly adapted to the polluted condition. It can be clearly identified when the fishes are acclimated in the laboratory (Ademoroti, 1996). But the fishes were rejected it and very interested to feed earthworm. The regular feeding was stopped for two months. Meanwhile, the water has been changed every week. There was no infection identified and it survived. But the fishes from Karamana river were died after one day acclimated in the laboratory.

A poisoned fish become a more susceptible to bacterial and fungal infection. Anemia develops during infection (Biggs *et al.*, 2005). The reduction in the total RBC count (1.7810^7 mm^{-3} blood) in the poisoned fish also reflects the anemia accompanies in bacterial in bacterial and fungal infection. The RBCs had undergone several morphological changes after exposure of the fish to the pesticide. The poison getting in to the blood by absorption to integument may be causing changes in the permeability of RBCs. Still the physiological upset was bound to affect the manifestation of blood itself as perceivable in the circulatory blood. All species from polluted waters showed significantly lower erythrocyte numbers, haematocrit, haemoglobin and thrombocytes percentage and significantly higher Mean Cell Volume (MCV), leucocyte numbers and lymphocyte percentage, compared with the controls. The glycogen depletion observer in the liver, kidney and intestine hence the present study shows that glycogenolysis has occurred in these organs (Jamuna and Noorjahan, 2009). Freshwater fish *A. scandens* was exposed to sublethal concentration of lead nitrate (10 ppm) for a period of 15 days as a results revealed that the glycogen level reduced significantly in the liver and muscle during exposure this kind of similar observation has been made by Jamuna and Noorjahan (2009). For instance, the depletion of liver, kidney and intestine glycogen observed in the present study corroborates with the findings of the earlier investigators.

Previously, Tulasi *et al.* (1992) reported exposure of the freshwater fish *A. testudineus* to a sublethal (5 ppm) concentration of lead nitrate for a period of 30 days during the preparatory phase of its annual reproductive cycle reduced the total lipids, phospholipids and cholesterol levels in the liver and ovary tissues while the free fatty acid levels were increased and lipase activity was elevated. All the parameters in the blood were found to be increased.

Furthermore, Oluah (2008) studied the Haemoglobin, haematocrit and erythrocyte counts in fish exposed to wastewater were significantly lower than in a control group. There was a significant increase in the total leucocytes count in treated fish, which also suffered microcytic anaemia. The observed changes in the haematological parameters of the fish exposed to brewery wastewater may represent part of the physiological processes by which the wastewater exerts its deleterious impacts on the fish. The similar kind of results also explained by Roy and Bhattacharya (2006) reported irregularities in the renal tubule including apoptotic and necrotic cells were also common. Corresponding with the histopathological lesions, dose-dependent disturbances in liver and renal functions. Liver diffuse necroses, cordial disarrangement, individualization of hepatocytes, etc there significant changes induced by cypermethrin were hyperplasia, disintegration of hepatic mass, focal coagulative necrosis, etc.

From the two different water bodies collected fish *A. testudiensis* shows necrosis of tubular epithelium, cloudy swelling of epithelial cells of renal tubules, narrowing of the tubular lumen and contraction of the glomerulus and expansion of space inside the Bowman's capsule were observed in the kidney tissues of fish after exposure. Hepatic lesions in fish living with polluted water (Parvathyputhanar) be characterized by hypertrophy of hepatocytes, cloudy degeneration, congestion, karyolysis and karyohexis dilatation of sinusoids and focal necrosis been observed from polluted water living fish In order to that the intestinal lesions included infiltration of eosinophils into the lamina propria and atrophy of epithelial cells Fig. 3. The present study proves its toxic potential in terms of the damages in organ level observed from polluted canal. In natural condition pollutants will be less than the present study, but continuous usage of the pesticide might lead to the concentration that was used in the experimental condition (Venkataraman *et al.*, 2007).

Figure 2 indicates that the blood smear and its changes of pollutant free water living fish of *Anabas testudensis* (Karamana river). Qualitative study of Haematological parameters of the *A. testudineus* from various two water bodies of Parvathiputhanar (fresh water) and polluted water from Karamana river. Study of the intestine, liver and intestine in the control fish showed a typical structural organization of the internal cell organelles. Fish exposed to acid water had several histological alterations namely desquamation of lamellar epithelium, fusion of the lamellae and lamellar aneurisms (Ogbeibu and Edutie, 2006). The gill abnormalities observed in this present study were similar to previous studies of low environmental pH on fish gill morphology, which showed separation of the epithelial layers of secondary gill lamellae, deformation of secondary lamellae and degeneration of chloride cells accompanied by hyperplasia of undifferentiated cells in the primary lamellae (Gill *et al.*, 1991). Heavy metal compounds associated with acidification have also been associated with a reduction of both carbonic anhydrase and Na^{+}, K^{+} ATPase activities in salmonids, even at a relatively high pH (pH 5.0). The lamellar fusions are defense mechanisms that reduce the branchial superficial area in contact with the external surroundings, furthermore this mechanism also increase the diffusion barrier to the pollutant (Mishra, 1991). As in higher vertebrates, the kidneys of fish perform an important function relate to electrolyte and water balance and the maintenance of a stable internal environment (Das *et al.*, 1990).

CONCLUSION

An attempt to demonstrated the changes of the morphological, haematological parameters and the changes in the level of glycogen in the liver, kidney and intestine of *A. testudineus* from a highly polluted Parvathiputhanar river and fresh water river karamana where evaluated. The haematocrit and haemoglobin content drops considerable in experimental fishes. There is a significant reduction in the total count of RBC in experimental fishes believed to a resulting from anemia accompanying bacterial infection. There is a significant reduction in the total count of WBC except the macrophages. Granulocytes and lymphocytes reduce in number. Macrophages increase in number also thrombocytes reduced in number. The accumulation of pesticides in the Parvathiputhanar river flows to Thiruvallam and cause mass death of fishes. Since, the morphological variation and histological changes in liver, kidney and intestine of fishes (*A. testudineus*) from Parvathiputhanar and Karamana river shows remarkable variations depends upon the polluted materials in the water content. There is a depletion of glycogen in the liver, kidney and intestine of *A. testudineus* from the Parvathiputhanar river due to influx of pesticides.

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