Indian J.Sci.Res. 20(1): 85-90, 2018

ISSN: 0976-2876 (Print)
ISSN: 2250-0138(Online)

EFFECT OF WATER POLLUTION: A HISTOLOGICAL EXAMINATION IN *Rasbora dandia* (HAMILTON, 1822)

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ABSTRACT

The histological alterations induced by the polluted waters of Thanikkudam River in gill and liver tissues of freshwater fish, *Rasbora dandia* have been studied. The Thanikkudam River, Thrissur, Kerala is polluted by the dumping of domestic wastes, automobile waste and also by runoff from the agricultural field nearby. In the present study, fishes collected from the river and incubated in water collected from two different sites showed histological alterations in the gill and liver tissues. It has been revealed that the gill and liver tissues of the fish incubated in water collected from Site 2 was much more affected than the fish incubated in water collected from Site 1 which maybe as a result of Site 2 being more polluted than Site 1 from the results obtained after water analysis.

KEYWORDS: Thanikkudam River, *Rasbora dandia*, Histopathology, Gill, Liver

Water quality, defined as the suitability of water to sustain different uses or processes, is influenced by a variety of natural as well as human activities. These factors all interact and may vary seasonally depending on weather conditions, run-off volumes and water levels. Water quality deterioration has a serious economic impact because the value of the resource is grossly affected needing significant restoring and management input (Meybeck et al., 1996). Measuring physical and chemical water quality along with health of biota can be used to assess the health of the ecosystem (bio-monitoring) (Herricks and Cairns, 1982). Macroscopic signs of toxicity are almost always preceded by histological, physiological and biochemical alterations in an organism. The biota therefore act as indicators of the overall ecological condition of the aquatic ecosystem, by acting as ongoing monitors of the water in which they live, thereby allowing long-term analysis of both regular and intermittent discharges, changeable concentrations of pollutants, single and multiple pollutants along with synergistic or antagonistic effects (Dallas and Day, 2004).

The toxicity of water pollutants, and the potential danger related to anthropogenic substances in water, can be determined by assessing pathological changes in fish organs. Fishes are more sensitive to numerous pollutants and are a convenient test subject for indication of ecosystem health (Simon, 2000).

Kerala is blessed with abundant water resources, with backwaters and rivers that exist throughout the State. The growing population density, migration of people from other states and unplanned urbanization is giving new dimensions to the problems of water pollution in terms of both quality and quantity. So the present study was carried out with the aim to assess the physical and chemical water quality parameters of water collected from two sites of Thanikkudam River, Thrissur, Kerala and to compare the degree of impact on the fish species incubated in water collected from two different sites through histopathology.

MATERIALS AND METHODS

Study Area

The main study area was Thanikkudam River, 29 kms long and originating from the slopes of Vazhani ranges and Chenkalli. The river has become mostly perennial over the last few decades owing to the change in climate and land use. However, it has important effect on the lifestyle, water use and weather of the place. Two sites were chosen for the investigation of water pollution in Thanikkudam River and were marked as Site 1, (Fig 1A) Peringavu ($10^0$54'N, $76^0$22'E) and Site 2, (Fig 1B) Puzhakkal ($10^0$54'N, $76^0$18'E).

Figure 1A Site1: Peringavu

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Figure 1B Site2: Puzhakkal

Water Analysis

Surface water samples (10 L) were taken from a depth of 0.5 m below surfaces in May, 2015(summer). 1 L of water samples were also collected from each sites in clean plastic bottles and physico-chemical parameters, presence of heavy metals were analyzed with the help of Envirocare Lab, Thrissur.

Test Animal

For this study, *Rasbora dandia* (Hamilton, 1822) was chosen as they were most common and abundantly found in the rivers.

Experimental Design

21 fishes with body weight of the fishes varying between 1-2g was obtained from the river and maintained in the lab with temperature of 37 ^{0}C. The fishes were fed with artificial pellets and acclimatized for a week. It was then divided into 3 groups, each containing 7 fishes. The first group was maintained as control in which tap water was taken. The second group of fishes was exposed to the polluted waters collected from Site 1 and the third group to the polluted waters collected from Site 2. The fishes were fed twice daily with artificial pellets. The exposure period was 15 days.

Histological Analysis

Fishes were taken from each group on the first, fifth, tenth and fifteenth day of exposure period. The gill arches and the liver excised were quickly fixed in Bouin's solution for 24 h (Tao et al., 1999). The specimens were dehydrated (Humason 1967), cleared in xylene, embedded in paraffin wax before being sectioned at 5 µm using a rotary microtome. The specimens were stained with hematoxylin and eosin. Finally, the prepared sections were examined using Olympus CX21i biological microscope and was photographed using Canon Powershot A570 IS Digital Camera.

RESULTS

Water Quality Analysis

The physico-chemical parameters of water samples collected from two different sites of Thanikkudam River are summarized in Table 1.

Morphological and Behavioural Changes

The fish, *Rasbora dandia* showed erratic swimming and struggled hard for breathing. The fishes of Group 2 & 3 developed a thick mucus covering over the whole body surface.

Histological Effects On Gill

Control (Plate 1, Fig 1)

In the control fish, the primary gill lamellae (PL) were flat leaf like structures with a central rod like supporting axis and a row of secondary gill lamellae on each side of it. The secondary lamellae (SL) were equally spaced along the columnar structures with intact cellular layer, attached at their bases with the primary lamellae and free at their distal ends. Between the secondary lamellae, the primary lamellae lined by a thick stratified epithelium and this region contained numerous mucous and chloride cells.

Test Site 1- Peringavu

On the 5th day of exposure there was proliferation in the epithelium of gill filaments and secondary lamellae, resulting in fusion of secondary lamellae. (Plate 1, Fig 2). On the 10th day of exposure oedematous changes, characterized by epithelial detachment were observed in the gill filaments and secondary lamellae (Plate 2, Fig 1). On the 15th day of exposure swelling and curling of and degeneration of secondary gill lamellae was observed (Plate 2, Fig 3).

Test Site 2- Puzhakkal

On the 5th day of exposure there was proliferation of gill epithelium and fusion of secondary lamellae. (Plate 1, Fig 3). On the 10th day of exposure intercellular oedema, detachment of epithelial cells, erosion and degeneration of secondary lamellae in the gills was observed. (Plate 2, Fig 2). On the 15th day of exposure necrosis of the epithelial layer resulted in the erosion of the respiratory epithelium. Hyperplasic cells were seen (Plate 2, Fig 4).

Histological Effects On Liver

Control (Plate 3, Fig 1)

The control liver comprised of hepatocytes (parenchymal cells) arranged in typical tubular architecture, sinusoids and blood vessels filled with numerous blood cells. The hepatocytes were morphologically polygonal and had conspicuous nuclei with densely stained nucleoli.

Test Site 1- Peringavu

On the 5th day of exposure the hepatocytes had lost their polygonal shape and hence the gaps between the cells increased. (Plate 3, Fig 2). On the 10th day of exposure slight degeneration and aggregation of the hepatocytes was noted. (Plate 4, Fig 1). On the 15th day of exposure some of the cells showed lysis. (Plate 4, Fig 3).

Test Site 2- Puzhakkal

On the 5th day of exposure, loss of the shape and vacuolization among the hepatocytes were observed. (Plate 3, Fig 3). On the 10th day, the cell membrane underwent lysis and was exposed. Aggregation of hepatocytes was observed. (Plate 4, Fig 2). On the 15th day of exposure, aggregation and necrosis of the hepatocytes was observed. Some of the hepatocytes had ruptured leading to denucleated cells. (Plate 4, Fig 4).

Table 1: Physicochemical parameters of water sample collected from Site 1 (Peringavu) and Site 2 (Puzhakkal).

PARAMETERS TESTED	UNIT	RESULTS		DESIRABLE LIMIT
		Site 1 Peringavu	Site 2 Puzhakkal	
PHYSICAL EXAMINATION				
Colour	Hazens	19.3	25.1	5
Turbidity	NTU	21.7	43.1	5
Electrical Conductivity	µs/cm	83	102	
Total Dissolved Solids	mg/l	50	61	500
CHEMICAL EXAMINATION				
pH		6.1	6.3	6.5-8.5
Iron	mg/l	0.20	0.29	0.3
Alkalinity	mg/l	10	10	200
Acidity	mg/l	11.5	4.9	
Total Hardness	mg/l	25.2	31.5	300
Silica	mg/l	5	8	
Sulphate	mg/l	0.86	1.41	200
Chloride	mg/l	5	10	250
Nitrate	mg/l	0.05	0.11	45
Biological Oxygen Demand	mg/l	0.07	0.15	30
BACTERIOLOGICAL ANALYSIS				
E.coli	Per 100ml	21	28	Nil

PLATE 1

FIG 1- Plate showing the photomicrograph of section of gill of control fish
(PL - Primary Lamellae, SL - Secondary Lamellae)

FIG 2 - Plate showing the photomicrograph of section of gill of fish exposed for 5 days to water collected from site 1 (40X)

FSL

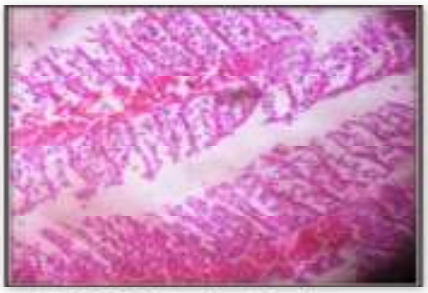

FIG 3 - Plate showing the photomicrograph of section of gill of fish exposed for 5 days to water collected from site 2 (40X)

(FSL - Fusion of Secondary Lamellae)

PLATE 2

FIG 1 - Plate showing the microphotograph of section of gill of fish exposed for 10 days to water collected from site 1 (40X)

FIG - 2 Plate showing the microphotograph of section of gill of fish exposed for 10 days to water collected from site 2 (40X)

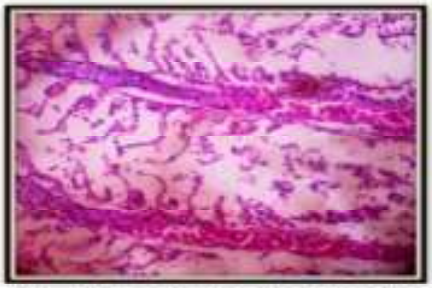

FIG - 3 Plate showing the microphotograph of section of gill of fish exposed for 15 days to water collected from site 1 (40X)

FIG - 4 Plate showing the microphotograph of section of gill of fish exposed for 15 days to water collected from site 2 (40X)

(E - Edema, EpD - Epithelial detachment, DeSL - Degeneration of Secondary lamellae, SSL - Swelling of Secondary lamellae, CSL - Curling of Secondary lamellae, NEp - Necrosis of epithelium, HC - Hyperplasic cells)

PLATE 3

FIG 1 - Plate showing the photomicrograph of section of liver of control fish (40X)

(H - Hepatocytes)

FIG 2 - Plate showing the photomicrograph of section of liver of fish exposed for 5 days to water collected from site 1 (40X)

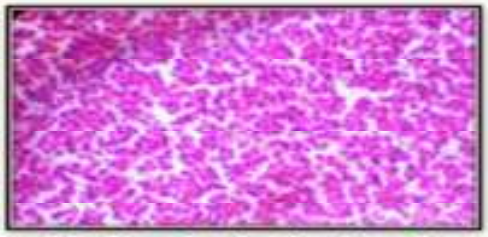

FIG 3 - Plate showing the photomicrograph of section of liver of fish exposed for 5 days to water collected from site 2 (40X)

(LPS - Loss of polygonal shape, VH - Vacuolization of hepatocytes)

PLATE 4

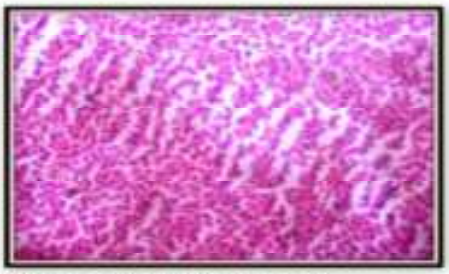

FIG 1 - Plate showing the photomicrograph of section of liver of fish exposed for 10 days to water collected from site 1 (40X)

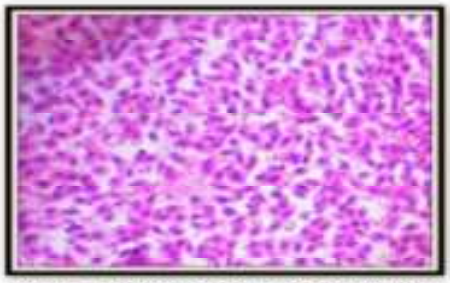

FIG 2 - Plate showing the photomicrograph of section of liver of fish exposed for 10 days to water collected from site 2 (40X)

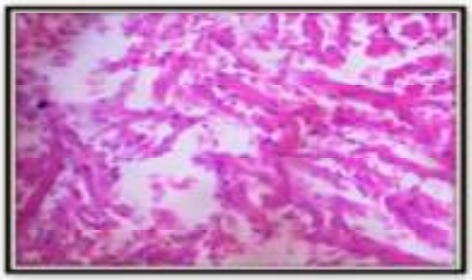

FIG 3 - Plate showing the photomicrograph of section of liver exposed for 15 days to water collected from site 1 (40X)

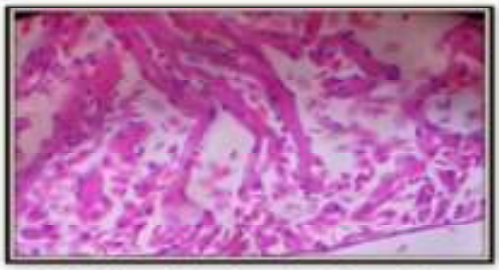

FIG 4 - Plate showing the photomicrograph of section of liver exposed for 15 days to water collected from site 2 (40X)

(CL - Cell lysis, AH - Aggregation of hepatocytes, NH - Necrosis of hepatocytes, DH - Denucleated Hepatocytes)

DISCUSSION

The physico-chemical analysis of water of Thanikkudam River revealed that it was not suitable for drinking, bathing and washing purposes, that the river water was slightly acidic, turbid with the presence of *E.coli*. Electrical conductivity value and turbidity (Table 1) was observed to be low in Site 1. Total Dissolved Solids of Site 1 (50Mg/l) and Site 2 (61mg/l) might be attributed to Site 2 receiving more automobile waste than Site 1 disposed from an automobile workshop nearby. Slightly low pH in Site 1 (6.1) and Site 2 (6.3) might be due to differences in dumping of domestic wastes. The high nitrate value in Site 2 (0.11mg/l) maybe due to runoff from fertilized paddy fields or due to automobile wastes. Comparatively higher BOD in Site 2 (0.15mg/l) than in Site 1 (0.07mg/l) might indicate high concentration of aerobic bacteria and lack of healthy submerged plants. The result of water quality analysis shows that Site 2 is more polluted than Site 1. Environmental condition of the river is not good due to the continuous dumping of waste materials especially automobile and domestic wastes. Fishes are known to bioaccumulate, bioconcentrate and biomagnify toxicants in their tissue and are widely used as bioindicators for pollution in marine and freshwater environment (Casini et al,. 1995). Fish gill is very sensitive to changes of the environment and injury to gill epithelium is observed in fish exposed to a variety of contaminants; the severity of damage depending on the concentration and the period of exposure to toxicant. The control group of fish retained a normal morphology of the structure through the duration of the experiment. According to the results obtained from the experiment, gills of fishes reared in water collected from Site 2 showed more histological changes than those of Site 1and may be attributed to exposure to urban sewage. The polluted waters of Thanikkudam River induced obvious histological changes in the liver of *R. dandia*. During the experiment, the liver of fish in the control group did not show any morphological changes in the structure. By the 15th day aggregation and necrosis of hepatocytes with some rupturing leading to denucleated cells was seen. The changes were more in the liver of fishes incubated in water collected from Site 2.

The histological changes in fish are noteworthy in understaning the changes in the structural organization of an organism due to environmental pollution. The changes observed are to its extreme during summer season i.e. at the time of complete dryness. The present results are in agreement with those observed in other fish species under the influence of different pollutants.

ACKNOWLEDGEMENT

Authorities of 27th Swaeshi Science Congress.

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