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Water quality demonstrates detrimental effects on *Mystus* species indicating pollutant toxicity

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ABSTRACT

Water quality of some water bodies in Haldia. *Mystus gulio* was treated for 20 days in laboratory conditions from water collected from the selected sources. Post assessment they were assessed through histopathological studies as well as on some physical and biochemical parameters. The study provided noticeable histopathological lesions depending on the pollution level of the water source on tissue samples of muscle, gill and parts of skin which were stained with haematoxylin -eosin. Treatment wise variations were also observed in biochemical and physiological parameters. Cellular degeneration, splitting of muscle fibre, edema were common symptoms for muscle study and absence of secondary gill lamellae, epithelial rupture, epithelial lifting, cell hypertrophy were found for gill study. The study indicated that the water sources contained different types of pollutants which had different toxic effects on the aquatic pollution load. The study concluded not only the presence of pollutants but also the response of the organisms being in direct contact with surrounding environment where the fish species acted as a biomarker.

***Key words :** Environmental, Histopathology, Treatment, Water pollution, Biochemical analysis, Fish*

Introduction

With the boom of industrialization, increase in agricultural wastes that are generated from these activities be it of agricultural, industrial, commercial origin they have always found entry into aquatic environments. These result in to various deleterious effects on the aquatic organisms which induce plural changes. Not only that untreated sewage effluents consists of complex mixtures of toxicants that increase significantly, impact on aquatic ecosystems, decreasing the species diversity have also been observed in different regions of the world (Schmitt *et al*.,1999; Amisah and Cowx, 2000). High concentrations of chemicals cause reduction in the quality of water ($_pH$, BOD, COD, TDS, etc.). Thus, water bodies frequently stores a large variety of xenobiotic compounds which cause the biochemical and histopathological alterations in fish (Barnet *et al.,* 1999; Reddy, 2012) and other aquatic flora and fauna. According to Liebell *et al.* (2013) and Ayas *et al*. (2007) histopathological events are considered fast and efficient mode of detection of acute and chronic adverse effects in fish, and may express the health condition of exposed individuals (Oliveria Ribeiro *et al*., 2005; Miranda *et al*., 2008). The skin and gill being in direct contact with the surrounding environment and the muscle located just under the skin represent important target organs to study effect of dissolved pollutants in water. Morphological changes can represent adaptive strategies for conservation of some physiological functions or endpoints to evaluate aquatic and chronic exposer to chemicals present in water and sediments (Liebell *et al*., 2013; Tkatcheva *et al*., 2004). The organic and inorganic contaminants degrade the water quality and make it polluted which effect directly on fish health and fish quality. Maintenance of the aquatic system not only keeps the environment balanced but promotes fishery to become a profitable business to fulfill the need of the local present life. When aquatic environments are maintained properly it gives quality fish (Maity and Banerjee, 2016). Trace metals can be accumulated by fish directly from water body which effect physiological changes created by particular pollutant. So, deterioration may be one of the evidence to ensure the presence of pollutants of that particular water body. This study was considered with the establishment of relation between fish health and water quality on the base of histological and biochemical examination. Histopathological, biochemical and physical parameters investigation are considered necessary tool for aquatic environment monitoring and the status of fish health where it can be considered as useful bio indicator of environment pollution. The degree of distortion of the tissues serve as the warning signs of damage to fish health which indirectly affect human health.

Materials and Methods

Study area and sampling

The present study considered four different types of water from different water bodies located in Haldia area West Bengal including one control (Table 1, Fig.1). In the experiment *Mystus gulio* species of catfish of the family Bagridae, were used which were purchased from a commercial supplier, average length of fishes being seven to nine centimeter. At the beginning of the experiment, seven fishes were transferred to each glass aquarium with wherein a 50% diluted station water namely as A, B, C and D respectively. Control aquarium contained tap water and named as T.

Constant aeration was supplied to every treatment and all the fish were fed with commercially available fish feed pellets. The rearing of the fish was continued for 20 days. After 20 days the length and weight were measured separately for every treatment and mean value was taken.

Physico -chemical parameters

Biological oxygen demand was determined by using 5 days incubation method, chemical oxygen demand and total solids were determined following the standard methods by Andrew *et al*. (2005).

Physical and biochemical parameters

The length was measured by centimeter scale and weight was measured with weight balance separately for every treatment and mean value was taken. The body fluids of fish samples from each treatment were extracted in phosphate buffer. After centrifugation supernatant was used for biochemical analysis. Total protein was estimated by Bradford method (Bradford, 1976) and total DNA was estimated by diphenylamine test (IITG Lab Protocols).

Histopathological procedures

Gills and muscle with stratum compactum from fish of each treatment were preserved in fixative solution (formalin) for 12 hours, dehydrated in a graded series of ethanol baths. After cleaningthe tissue was embedded in paraffin which after solidification went through tissue sectioning in microtome. The slide sections (5µm) were stained in haematoxylin and eosin and were observed under light microscope (Paul and Chanda, 2017).

Table 1. Water sources for treatment of fish

Legend Used in Study	Type of Water Body	Latitude, Longitude
A	A domestic pond	22.050916, 88.068316
B	An Aquaculture (fish) pond and also used for domestic purposes	22.049066, 88.069395
C	An Aquaculture (fish) pond	22.037996, 88.057880
D	A canal where many industrial effluents are discharged	22.060393, 88.130769
T	Control or Tap water	————

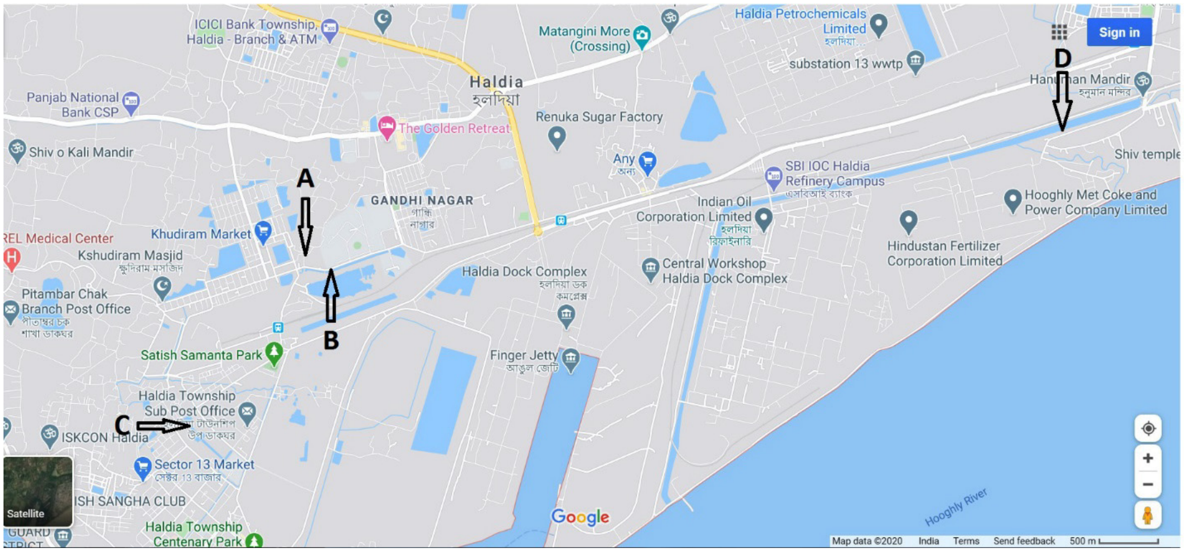

Fig. 1. Location of water bodies on map; (A) water body source for treatment A; (B) water body source for treatment B; (C) water body source for treatment C; (D) water body source for treatment D.

Results

Haldia is an industrial port city in Purba Medinipur district of West Bengal, located approximately 125 kilometres southwest of Kolkata near the mouth of the Hoogly River. The domestic ponds are used in Haldia area as water reservoir, where bathing, dishwashing, clothes washing, fish production, animals bathing, disposal of domestic waste are common anthropological activities. As the ponds are surrounded by paddy fields so water body constantly receive the drain-off the fertilizers. The source of water for treatment D is a canal which flows besides the premises of many industries like Exide, Indian Oil Corporation Limited, Hoogly Met Coke and Power Company, Bharat Petroleum Corporation Limited etc. So, there is a chance of toxic pollutants presence in this canal water which may come from industrial waste directly or indirectly.

Physico -chemical parameters of water

Biochemical oxygen demand (BOD), chemical oxygen demand and total solids values were different as different water bodies were taken to investigate. Treatment D revealed higher values and control showed lower values of physico- chemical parameters may be due to the presence of different pollutants. Findings are presented in Table 2.

Table 2. Physicochemical parameters of treated water

Treatments	BOD (mg/L)	COD (mg/L)	Total dissolved solids (mg/L)
A	1.5	38	350
B	1.2	34.4	150
C	2.0	37.2	420
D	10.4	91.2	980
T	0.58	29.6	105

Physical and biochemical parameters finding

In the investigation, it was observed that the fishes in treatment C showed higher increase in length, treatment A showed in weight and tap water treated specimen showed lower increase in weight and length than the other treatments. Observed results of physical and biochemical parameters are presented in Table 3.

Histopathological findings

Histopathology deals with the study of pathological changes in body tissue when comes in contact of toxic substances. The results showed severe lesions in microscopic structure of muscle with hypodermis and in gill structure of *Mystus*. The pathological findings in muscle bundles and in hypodermis included the cellular degeneration, splitting of muscle fiber and increase of muscle spaces, degenerative changes, necrotic change, edema between muscle fibers were common symptoms in every treatment (Fig. 2). In control edema between muscle bundles

Table 3. Physical and biological parameters analysis

Treatments	Physical parameters		Biochemical parameters	
	Weight (gm)	Length (cm)	Protein(mg/L)	Total DNA (mg/L)
A	6.5	0.42	520	74.25
B	2.4	0.36	390	242.00
C	5.7	0.52	410	151.25
D	2.0	0.25	270	134.75
T	1.3	0.23	360	129.25

were higher than any other treatment. There splitting of muscle fibers were more in treatment C than treatment A. In treatment D necrotic cells were high in numbers. In respect of skin histopathology the hypodermis layer of treatment A was very thin there in treatment B the same layer was found very thick than any other treatment. There was an edematous change in hypodermis in treatment D and in treatment C were also found.

The microscopic structure of fish gill showed a number of injuries in every treatment. The lifting of lamellar epithelium, cell hypertrophy, epithelial rupture, necrotic changes, absence of proper gill structure, cell hypertrophy were observed in fish gill of different treatment (Fig. 3). When other treatment was compared with treatment T, in treatment A and B severe damage was found. Proper Gill structure as well as the secondary lamellae was absent in treatment A and B in most cases. Cell hypertrophy, cell hyperplasia was found in treatment A and epithelial rupture, proliferation or thicker lamellar space was seen in treatment B. In treatment

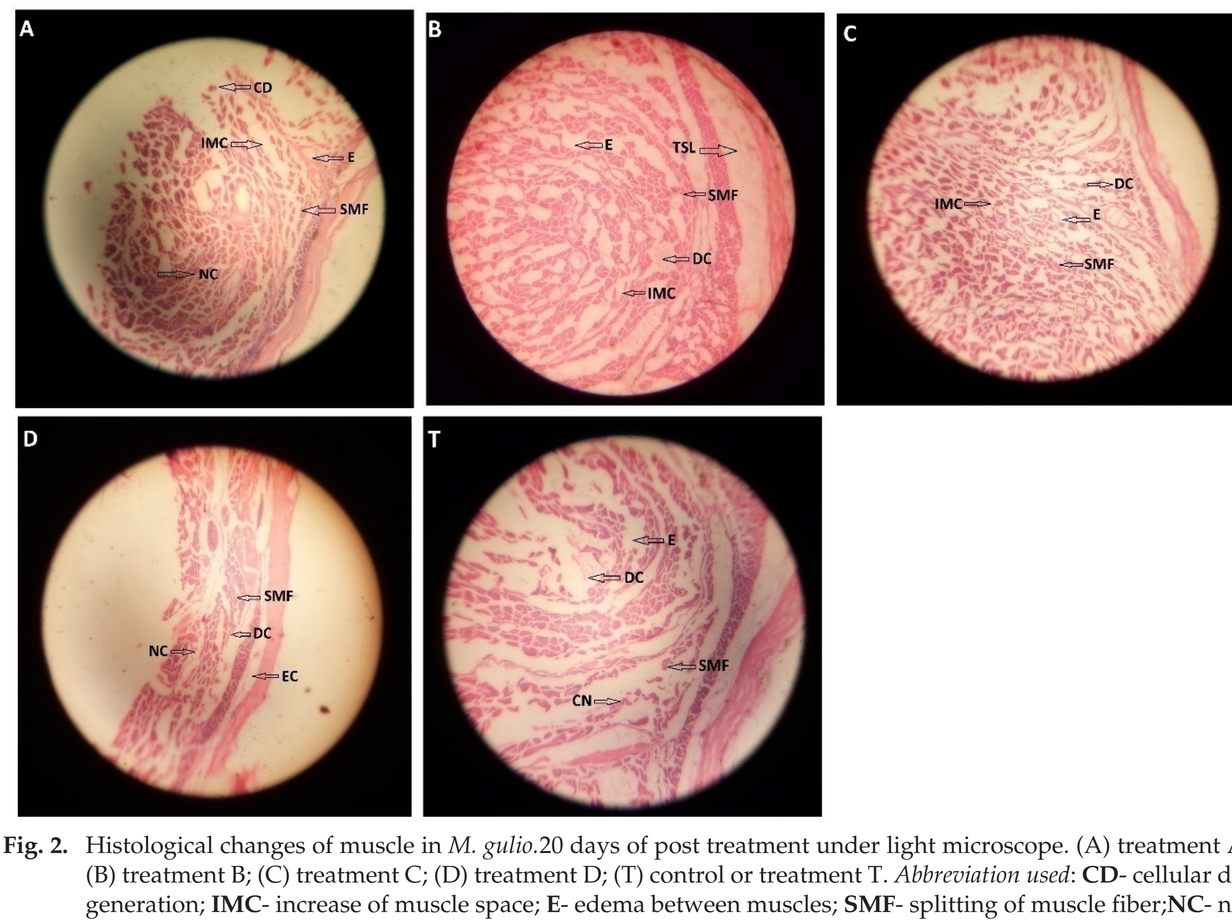

Fig. 2. Histological changes of muscle in *M. gulio.*20 days of post treatment under light microscope. (A) treatment A; (B) treatment B; (C) treatment C; (D) treatment D; (T) control or treatment T. *Abbreviation used*: **CD**- cellular degeneration; **IMC**- increase of muscle space; **E**- edema between muscles; **SMF**- splitting of muscle fiber;**NC**- necrotic changes; **DC**- degenerative changes; **TSL**- thick subcutaneous layer; **EC-** edematous change; **CN**- change in cell due to necrosis (Haematoxylin and Eosin, Bar 10 µm).

C overlapped cells on lamellar epithelium, epithelial rupture, lifting of lamellar epithelium and also the necrotic changes in lamellar epithelium were observed. Partial fusions of lamellar epithelium, lifting of lamellar epithelium, severe degenerative and necrotic changes in gill filament were noticed in treatment D. Over all the atrophy of secondary lamellae were seen in every treatment. The primary lamellae was also absent in few observations of treatment D.

Discussion

Physico- chemical quality of any medium have a marked impact on feed intake of the fish as they can affect the fish physiological condition, capable of creating all short of stress and neuro- endocrinological imbalance (Wynne *et al*., 2005). Martinez-Palacios *et al*. (1996); Koskela *et al*. (1997); Eriegha and Ekokotu, (2017) carried their study on the effect of varied feeding level and water temperature, where it was found a positive relationship between feed consumption and water temperature. There was a dramatic increase in feed intake with increasing temperature. Concentrations of total dissolved solids may increase the water temperature, which increase the feed intake, on other side it may lead to fish death due to the high TDS and it dehydrates the skin of animals which leads to the tissue damage. Among the five treatments as treatment B was less polluted so DNA content was high. Hussain *et al*., (2017) found significant differences were observed for DNA damage through comet assay in fish from polluted sites compared to control sites. BOD and COD frequently used as key determinants of chemical pollution assessment along with toxic contaminants (Kim *et al*., 2019). BOD has an inverse relationship with dissolved oxygen. So increased BOD is harmful for aquatic life. COD is used as a measurement of pollutants in water, wastewater hazardous wastes. At high concentration of pollutants (metals, pesticides, surfactants etc.) fish suffer from decrease of antibody production, in this way the disease resistance of fish may be decreased. Damaged caused to fish by too much oxygen dissolved in water as well as if they are exposed to a lower atmospheric pressure water. As because the BOD COD rate was

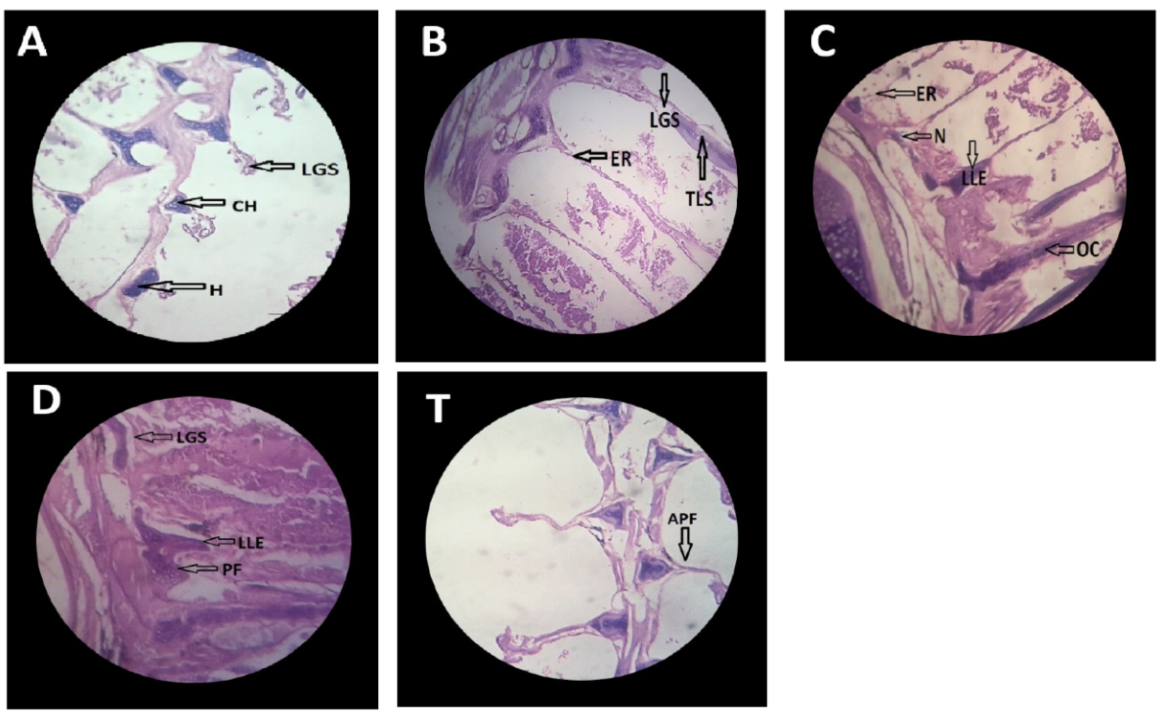

Fig. 3. Histological changes of gill in *M. gulio.* 20 days of post treatment under light microscope. (A) treatment A; (B) treatment B; (C) treatment C; (D) treatment D; (T) control or treatment T. *Abbreviation used*: LGS- loss of gill structure; CH- cell hypertrophy; H- hyperplasia; ER- epithelial rupture; TLS- thicker lamellar space(proliferation); LLE- lifting of lamellar epithelium; OC- overlapped cells on lamellar epithelium; N- necrosis; PF- partial fusion; APF- absence of partial fusion of primary lamellae (Haematoxylin and Eosin, Bar 10 μm).

high in treatment D, the specimen suffered or damaged the most.

The weight and length findings showed treatment A and C favorable for the fishery business. Maybe the fertilizers, pesticides added the growth and survival advantages but histopathological findings in these two treatments showed fairly unpleasant results which are detrimental for fish health. As treatment A water sample was collected from a domestic pond but there is a doubt that fishes treated were not exposed to any chemical fertilizers and pesticide because the lesions on organs indicated use of chemical products into water which could be domestic wash off compounds like detergents etc. Treatment A specimen showed highest protein content and treatment D showed lowest protein content, there highest total DNA count found in treatment B. The different values of protein content from different treatment may be due to the intake amount of fish feed as the supplied quantity and quality were same to all. There are many factors that affect the feed intake in cultured fish. Among them culture environment, stressors are important.

The present study revealed that with the increase in pollutant level of the water the histopathology of the fish's revealed drastic deleterious effects. The histopathology of muscle bundles were loose and showed marked spacing and severe lesions which may be due to uptake of pollutants. Similar observations were reported by Al- Halani *et al*. (2018) for *Clarias gariepinus* from Nile Delta. Effects of pollutants coming from sediment and its surrounding environment on fish muscle were also observed by Thophon *et al*. (2003); Kaoud and El- Dahshan (2010), they found that fish exposed to pollutants may undergo histological alterations in the form of degeneration of muscles bundles with certain focal areas of necrosis. They observed that fish exposed to pollutants show degeneration of muscles bundles with atrophy and splitting of muscle fibers. Das and Mukherjee, (2000) observed severe intramuscular edema in the Indian major carp *Labeo rohita* exposed to 1/10 and 1/5 sub lethal doses of hexachlorocyclohexane during 45 days of trial period. Ayoola, (2011) observed mild lesions, necrosis, and cellular degeneration in the muscle of Nile tilapia exposed to lethal concentrations (96 h LC_{50}) aqueous and ethanolic extracts of *Ipomoea* aquatic leaf. From Lakshmaiah's (2016) study it can be said that the alterations in fish muscle in all treatments may be due to significant concentration of pesticide accumulation in the muscle or maybe it was a part of defense mechanism. Moreover the pollutants present in water were the main cause of destruction in the organ structure. It is possible that the pathological alterations in the tissue could be a direct result of sewage, agriculture, industrial waste, heavy metals, fertilizers; salts somehow enter into water body (Tayel *et al.*, 2014). Abbas and Ali, (2007) observed destruction and vacuolation of the muscle cells in *Oreochromis spp.* exposed to chromium. Maharanjan *et al*. (2016) found progressive damage in the structure of muscle with increasing concentrations of copper. Similar observation has been made by Nagarajan and Suresh (2005) in the muscle tissue of the fish *Cirrhinus mrigala* with increasing concentrations of sago effluent.

Alterations in the muscles of several species of the fishes exposed to heavy metals have been described by Oliveria *et al*., (2002); Gupta and Shrivastava (2006); Kaoud and El Dahshan (2010). As seen from the investigations made on muscle histopathology, it can be termed that treatment D and treatment A were poor quality water than other water sources and hence the lesions were much more in these two treatments. As skin remains in direct contact with the water environment it is subjected to many type of stresses. It may be due to this stress exerted that the observed structural support edema found in hypodermis layer of treatment A and D and in treatment B subcutaneous layer formed were thick. Tabarraei *et al*. (2019) found inflammation in dermis, hypodermis and subcutaneous muscles after a two week exposure to essential oil of *Carum carvi* seed in rainbow trout. There was a severe edematous change characterized by epithelial detachment. The acute toxicity reduces the solubility of oxygen in the water which enhanced the fish death due to hypoxia.

The gills are most sensitive organs to hypoxia and are considered as good indicator for water quality. It is important for many functions like respiration, osmoregulation, excretion etc. It is very much sensitive to changes in the quality of water (Mazon *et al*., 2002). The treatments with different types of water resulted in several forms of histopathological changes in the gills. Epithelial lifting, hyperplasia, cell hypertrophy, epithelial rupture, loss of secondary lamellae, loss of proper gill structure are examples of defense mechanism. Since, these result in the increase of the distance between the external environment and the blood and thus serve as barrier to the entrance of contaminants (Akaishi *et al*., 2004; Butchiram *et al*., 2009) it becomes a line of defense for the fish when exposed to such polluted aquatic environments. Such alterations were non-specific and maybe introduced by different types of contaminants. Hyperplasia also reduces secretary and excretory functions of the gills (Tilak *et al*., 2006). The degree of tissue damage resulting in drastic reduction in respiratory surface, would have caused death within a very short time. Wood (2001) and Au (2004) had provided extensive information on gill structural alterations in fish as a result of toxicant exposure. Lamellar fusion, hyperplasia, necrosis were more common for metals than for organics or other pollutants, possibly since metals directly interact with ion- transport proteins and inhibit their activity (Reddy *et al*., 2010; Butchiram *et al*., 2013). From that it can be assumed that treatment A, C and D water body may contain any metal pollutants. According to Tkatcheva *et al*., (2004) some morphological changes in gills and liver may represent adaptive strategies to maintaining physiological functions, but the histopathological lesions indicated that fishes were affected by the exposure to pollutants presented in water. The presence of surfactants, phenols, metals and other urban pollutants certainly were the reason to the damages as well as described by Tkatcheva *et al*., (2004). The reduction of oxygen maybe also the source of physiological disturbances that can sometimes reflect morphological damages (Alberto *et al*., 2003; Fernandes and Rantin, 1994). Treatment A, C and D showed higher impact level on gill histology than the control and treatment B. Moreover in some previous studies many researchers stated that the gill lesions are common in fish which constantly are exposed to the water environment, external environment is susceptible to the toxins environmental pollution and pathogens (Abalaka *et al*., 2010). Several studies have shown that fish tissue alternations are reliable and efficient tools to detect and monitor environment that are influenced by anthropogenic activities (Gernhofer *et al*., 2001).

Conclusion

Fish organs are useful indictor of environmental pollution. The present study showed that according to the histopathological and biochemical analysis the skin and gill lesions in *Mystus gulio* species are biomarkers for assessing the water quality which were supported by the severity of lesions found differently for different quality of water. Also the result concluded that different water bodies were impacted by different pollutants and further monitoring and assessment program of the main water bodies are strongly suggested. The scope of study did not attempt to assess the source of effluent into the water bodies which we have considered in this study. But from the study it can be also concluded that fish was responding to the direct effects of the pollutants as well as to the effects caused by stress and the results were able to assess the initial effects to acute exposure to chemical stress. Moreover, the study acted as a direct and reliable evidence to find out the aquatic pollution and environmental deterioration.

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