RESEARCH ARTICLE

Histological changes in gills and liver of fishes in river Sutlej as an effect of Buddha Nullah pollution at Ludhiana.

Aditi Jaidka and Hundal SS

Department of Zoology, Punjab Agricultural University, Ludhiana- 141004 (Punjab), India
Corresponding author E mail: jaidka.aditi@yahoo.in

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ABSTRACT

Buddha nullah serves as a reservoir of industrial and sewage effluents of Ludhiana, Punjab containing high levels of toxic chemical discharged directly into river Sutlej. This leads to deterioration in the water quality and is a serious threat to fish fauna of the river. The study aims to investigate the level of changes in the histological architecture of gills and liver of fish from 3 sampling sites - 2km downstream in river Sutlej after the confluence with Buddha Nullah, 2 km upstream in river Sutlej after the confluence with Buddha Nullah and 5 km upstream in river Sutlej after the confluence with Buddha Nullah caused by Buddha Nullah pollution. Significantly higher histological abnormalities were observed in fish species collected from downstream area than upstream. Complete secondary lamellar fusion, breakage and degeneration of secondary epithelium, detachment of secondary epithelium, necrosis of lamellae, vasodilation of secondary lamellae, haemorrhage and vasodilation in primary lamellae were found in the gills of fish inhabiting polluted waters. In contrast, fish captured from 5 km upstream site (non polluted site) showed milder alterations limited to ruptured secondary epithelium only. Histology of liver showed vacuolization of hepatocytes, degenerative hepatocytes, necrosis and disruption of normal architecture in the fish from polluted sites whereas the sections of the tissues from fish collected from non polluted site of the river Sutlej exhibit a normal structure of hepatocytes with a rare occurance of mild necrosis The study clearly exhibits the deleterious effect fish vital organs even after a small extent of exposure to contaminated natural waters.

Keywords: Histopathology, *Cyprinus carpio*, Gills, Liver, Water pollution

INTRODUCTION

The Sutlej river, longest of the five rivers, flows through the region of Punjab and then crosses into Pakistan. Ludhiana, the most developed industrial city of the Punjab State (India) today, was founded on the ridge of Buddha Nullah which earlier was a clear brook and catered to the needs and requirements of Ludhiana city. The rapid pace of industrialization and increasing population pressure has brought in a host of environmental problems and Buddha Nullah which enters and (30°95'N 75º.55'E) after passing through densely populated Ludhiana city, drains into Sutlej river (29º.38' N, 71°.03' E) at Walipur Kalan (near Humbran), in the north western corner of Ludhiana city. Budhha Nullah used to be a fresh water channel with about 56 types of fish species prior to 1964, but because of high level toxicity in the water no fish was reported even by Saxena and Jior (1992). Once an asset to the city, the Nullah is polluted after it enters Ludhiana city and is now a source of public nuisance posing a serious threat to the aquatic biodiversity of river Sutlej (Kaur 1997), in addition to the stress on fresh water resources in Punjab (Thakur *et al.* 2014). Presently, the discharge of sewage and industrial waste in the form of untreated composite mixture into Buddha Nullah, has contaminated it to the extent that no fish species probably exist even at the confluence of Buddha Nullah and river Sutlej (Anonymous 2015).

Fishes are main organisms in the aquatic food chain and are particularly vulnerable and heavily exposed to pollutants because they cannot escape from the detrimental effect of pollution (Yarsan and Yipel 2013, Mahboob *et al.,* 2014, Ahmed *et al.,* 2016) and are therefore considered to be the most significant bio fauna indicators in aquatic systems for the estimation of metal pollution level (Authman 2008). It is imperative that histopathological investigations be conducted to assess the overall health of the entire population in a polluted ecosystem as compared to rather unpolluted locations (Arslan *et al.* 2010). The gills account for over 50% of the surface area of a fish, and are one of the first major target organs of waterborne toxicants (Bols *et al.* 2001). Hepatic alterations are considered as non specific biomarkers as many different toxicants can produce liver changes. The organ most associated with the detoxification and biotransformation process is the liver and due to its function, position and blood supply, it is also one of the organs most affected by contaminants in water (Sultana *et al.* 2016). Thus the present study was undertaken to interpret the effect of pollution on histological structure and function of gills and liver of fish from selected spots in river Sutlej.

MATERIAL AND METHODS

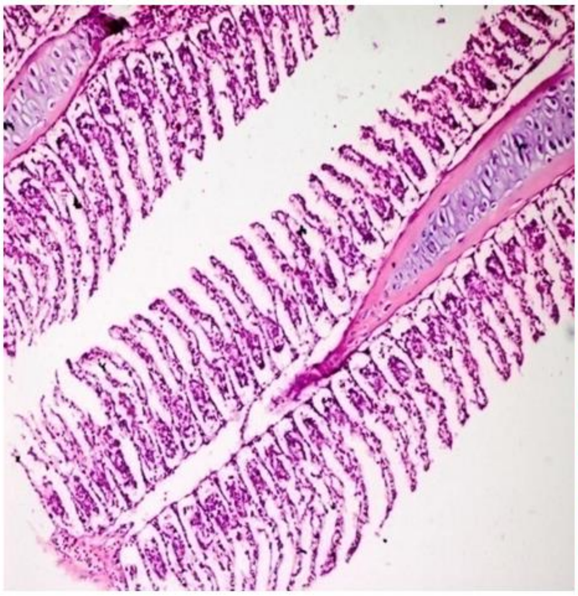
Sampling sites and fish Collection: 3 sites were selected in river Sutlej for fish collection - 2 km downstream (approx) after the confluence with Buddha Nullah in river, 2 km (approx) upstream after confluence with Buddha Nullah and 5 km (approx) upstream in river after its confluence with Buddha Nullah. Live specimen of *Cyprinus carpio* measuring about 20-25 cm and weighing 50-60 gm were captured for experimental study from selected spots in river Sutlej.

Histopathological Procedure:
The fish specimens were brought to labortary, dissected, liver and gills were removed and excised of surrounding fat and tissues then fixed in aqueous Bouin's fixative for 24 hours. Preserved tissues were dehydrated in graded series of ethanol and embedded in paraffin blocks. Sections of 5-7 µm thickness were taken using rotatory microtome and stained with Hematoxylin and Eosin (H&E) for histopathological examination by light microscope.

RESULTS AND DISCUSSION

Gills: The histological observations in the gills of the fish collected from 5 km upstream of Sutlej from the confluence with Buddha Nullah, showed an ordinary structure in which lamellae were lined by squamous epithelium. Rare observations revealed mild alterations in the form of ruptured secondary epithelium were also observed (Fig 1 and 2).

Alteration	Site		
	2Km downstream	2 Km Upstream	5Km upstream
Ruptured secondary lamellae	+++	++	+
Clubbed secondary lamellae	+++	++	-
Necrosis of lamellae	+++	++	-
Haemorrhage	++	+	-
Hyperplasia	+++	++	-
Blood from degeneration of secondary lamellae	++	-	-



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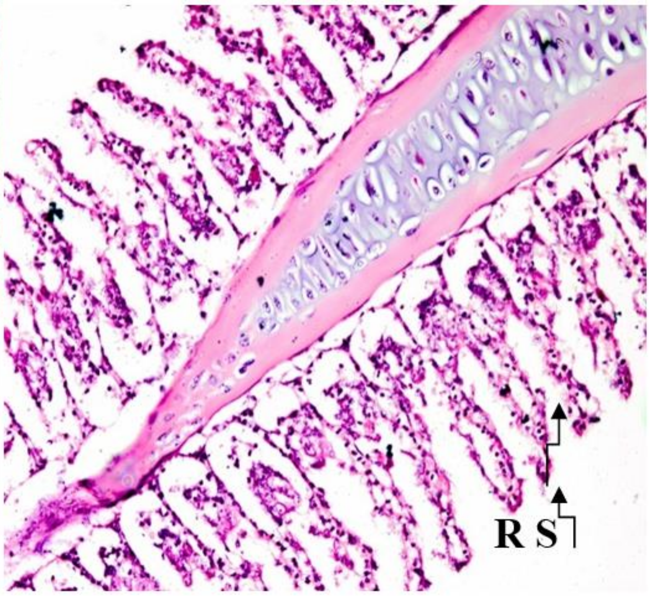

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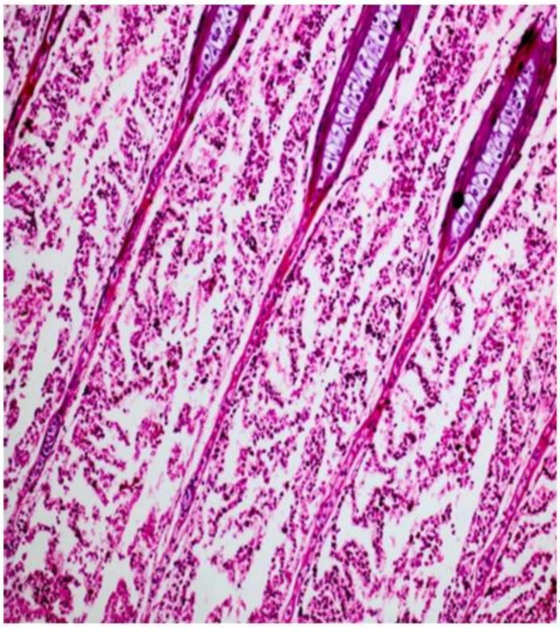

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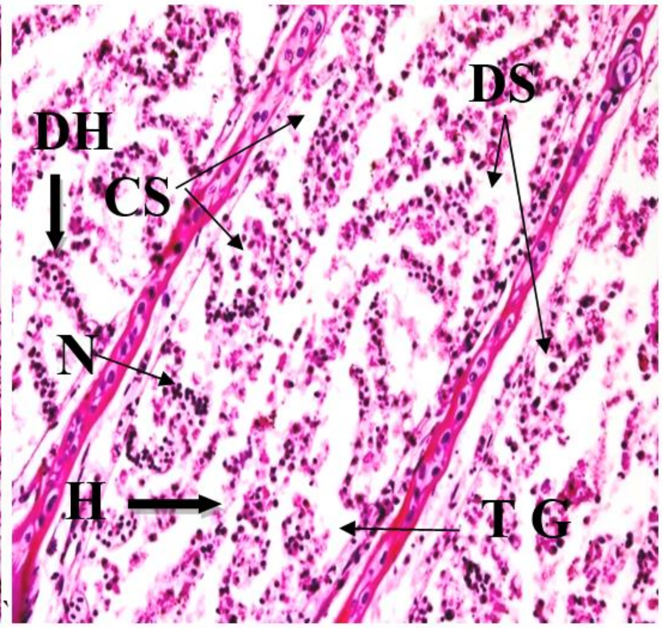

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Fig 1 and 2: T.S. of Gills of fish from 5 km upstream site showing slightly ruptured secondary epithelium (R S). H.E x 20X and H.E x 40X respectively. **Fig 3 and 4:** T.S. of Gills of fish from 2 km upstream site showing lamellar haemorrhage (H), clubbing in secondary epithelium (C S), disorganisation of secondary lamellae (D S) and distal hyperplasia of secondary lamellae (D H). H.E x 20X and H.E x 40X respectively.

Cell proliferation with thickening of gill filament epithelium, lamellar fusion was the prime histological alteration observed in the gills of fish collected from 2 km upstream site in the river. Lamellar haemorrhage, clubbing in secondary epithelium, necrosis, disorganisation of secondary lamellae and distal hyperplasia of secondary lamellae have also been observed (Fig 3 and 4). Light microscopy of the sections of gills from samples collected from the downstream area in the river after the confluence, which has been reported to be heavily contaminated, illustrates severe histological alterations including complete secondary lamellar fusion, breakage and degeneration of secondary epithelium, detachment of

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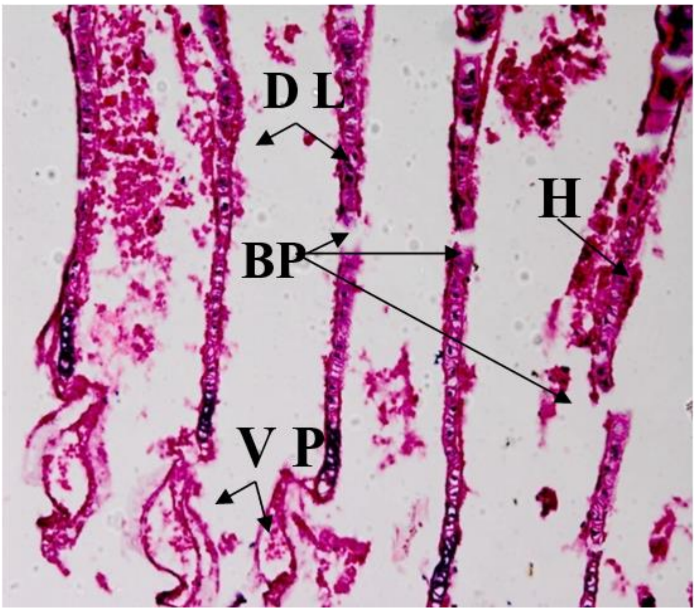

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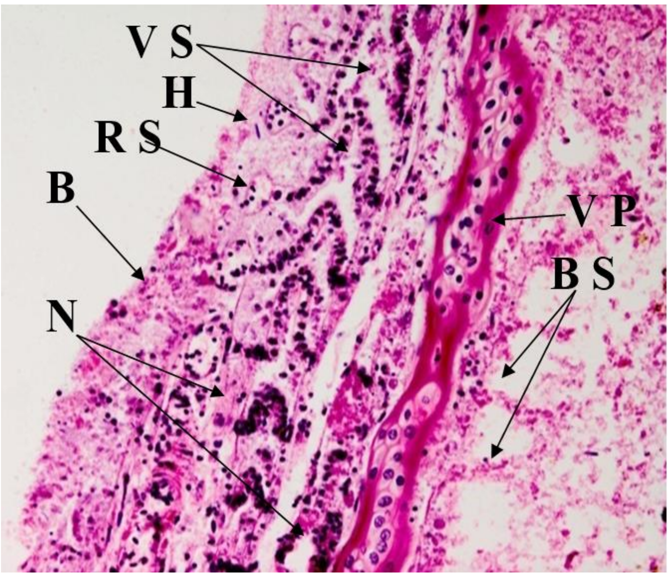

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Fig 5. T.S. of Gills of fish from 2 km downstream site H.E x 20X.

Fig 6. T.S. of Gills of fish from 2 km downstream site showing breakage in primary lamellae (B P), haemorrhage (H), secondary lamellar fusion (F S) and Degeneration of secondary lamellae (D L) and vasodilation in primary lamellae (V P) H.E x 40X.

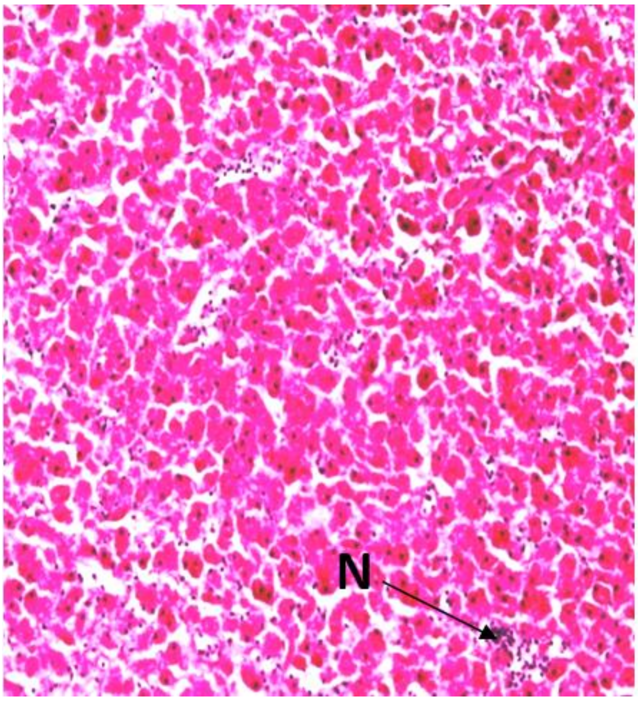
Fig 7. T.S. of Gills of fish from 2 km downstream site showing ruptured secondary epithelium (R S), vasodilation of secondary lamellae (V S), haemorrhage (H), Blood from lamellar haemorrhage (B), necrosis (N), blood from degeneration of secondary lamellae (B S)and vasodilation in primary lamellae (V P) H.E x 40X.

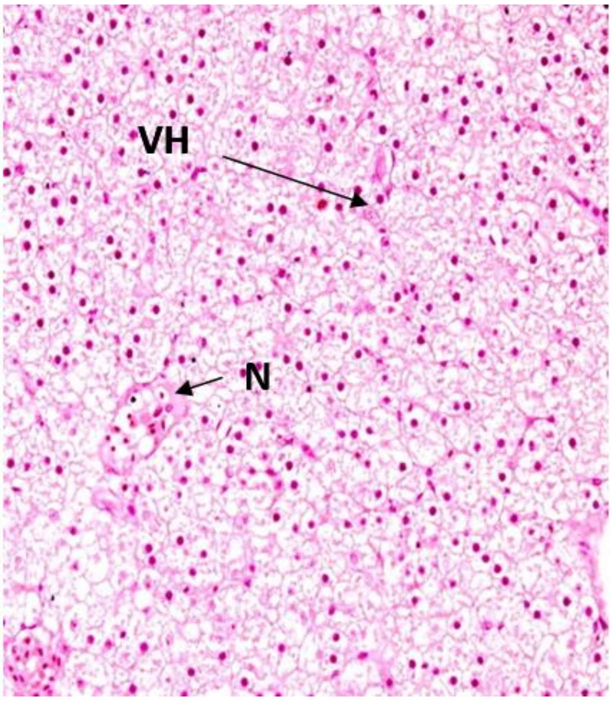
secondary epithelium, necrosis of lamellae, vasodilation of secondary lamellae, haemorrhage and vasodilation in primary lamellae (Fig 5,6 and 7).

The aberrations observed in gills sectioning are comparable with the observations of Sultana *et al.* (2016) in fish inhabiting polluted water of river Ravi in Pakistan. The fish collected from polluted site in river Ravi showed fusion of various secondary lamellae, degeneration and necrosis of lamellae as well as hyperplasia in secondary epithelial cells. The epithelial proliferation (hyperplasia) and the detachment of the gill epithelial cells observed along with the partial to complete lamellar fusion are non-specific responses of the gills to toxic irritants , which are the natural attempts by the exposed fish to increase the diffusion distance between their blood and the toxic external environment (Olurin *et al.* 2006). Lamellae dilatation at the site of toxic damage might be responsible for the observed lamellar edema (Abalka 2015). The observed cells proliferation and hypertrophy, leads to increased mucous secretion, are more often associated with heavy metal exposure than with organic pollutant exposures (Kaptaner *et al.* 2008).

Liver:

Light microscopy has revealed that the liver sections of the fish collected from non polluted site i.e. from 5 km upstream of the Buddha Nullah and river confluence, in the Sutlej exhibit a normal structure of hepatocytes





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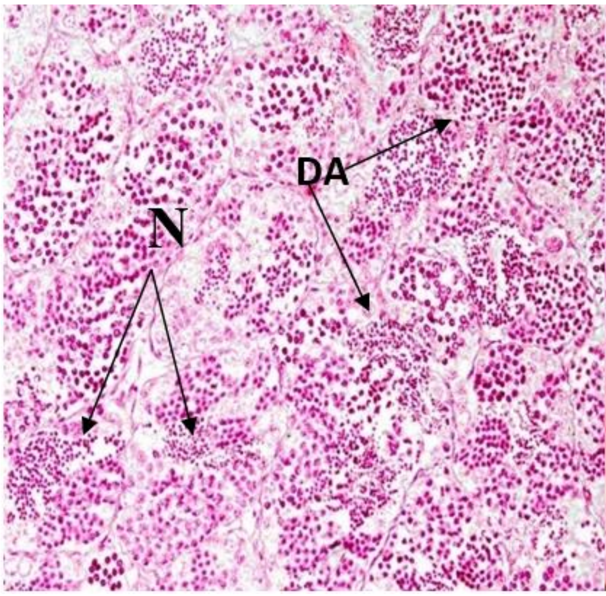

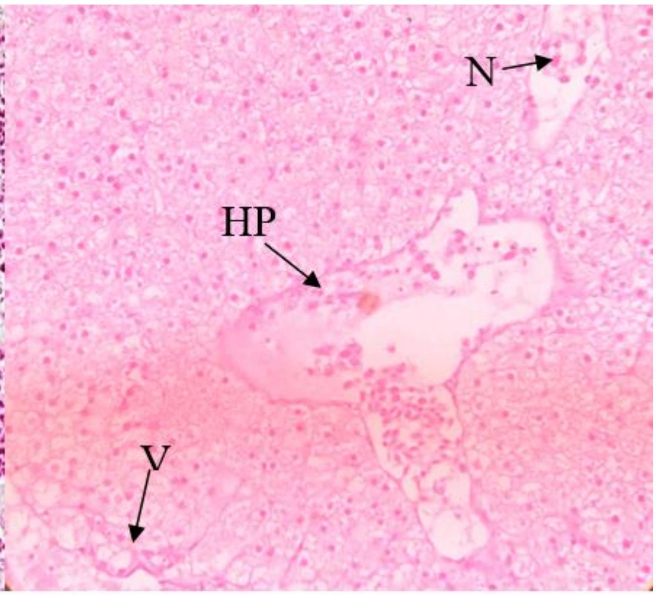

(10) **(11)**

Fig. 8: T.S. of Hepatopancreas of fish from 5 km upstream site showing mild necrosis (N) leading to disruption of normal architecture (DA) H.E 400 **Fig. 9**: T.S. of Hepatopancreas of fish from 2 km upstream site showing severe vacuolar degeneration (V H), and necrosis (N) H.E 400 **Fig. 10**: T.S. of Hepatopancreas of fish from 2 km downstream site showing degenerative hepatocytes, necrosis (N) and disruption of normal architecture (D A) H.E 400 **Fig. 11**. T.S. of Hepatopancreas of fish from 2 km downstream site showing massive degenerative hepatocytes (D P), haemolysis in pancreatic acini (HP) and necrosis (N) H.E 400.

with a mild necrosis. No infiltration of leucocytes was observed in the sinusoids (Fig 8). Histological findings in liver of fish collected from 2 km upstream showed severe vacuolar degeneration and necrosis (Fig 9), whereas from sampling site located downstream of the confluence showed number of pathological alterations, including vacuolization of hepatocytes, degenerative hepatocytes, necrosis, haemolysis in pancreatic acini and disruption of normal architecture thus caused histopathological alterations in hepatic tissue (Fig 10 and 11).

Abalka 2015 also reported hepatic vacuolations, haemorrhage, necrosis and cellular infiltration in fish inhabiting heavy metals loaded water of Tiga dam, Nigeria.

The observed hepatic vacuolations may be due to lipid or glycogen deposition suggestive of metabolic disorders as a consequence of the exposure to toxic agents (Pacheko *et al* .2002). Hepatic cellular infiltrations were suggestive of inflammatory responses in the affected liver. The observed haemorrhage might be due to the toxic damage to the liver of the fish inhabiting toxic loaded water, while the necrotic changes in the affected liver were a consequence of the toxic effects of the metals (Mohamed *et al.* 2008). Although liver lesions are not usually specific to pollutants, a casual relationship between me-tal concentrations and fish liver lesions has been established (Au 2008).

CONCLUSION

The results of the present study indicate that environmental contaminants systematically disturbs an aquatic ecosystem, which induce severe histological changes in fish tissues.

Conflicts of interest: The authors stated that no conflicts of interest.

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