



## Review Paper

# Assessment of Aquatic Pollution Using Histopathology in Fish as a Protocol

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## Abstract

*In chemical monitoring the presence of pollutants in tissues are evaluated by chemical analysis, but biomonitoring methods evaluate not only the presence, but also the response of the organisms to these pollutants by the assessment of various cellular, biochemical and histopathological biomarkers. Hence the use of these biomarkers integrates in chemical monitoring programmes to determine the toxic effects of pollutants. This paper presents an overview on the development and application of histopathology of fish as biomarkers. Because histopathological alterations in fish are mainly depend on the water quality. Aquatic pollution with different pollutants has a subject of great concern. Hence there is an urgent need for bio-monitoring tools in impact assessment to indicate the effects of pollution. Various biochemical studies of fish have been used to assess the impact of aquatic pollution. But histopathology provides direct and reliable evidence by identifying the cellular destructions. Therefore, the present study is aimed to study the histopathological alterations in various organs in various fish species in different environmental conditions. Moreover, it offers a means of detecting harmful effects of pollution in different tissues in different fish species. The present study reviews the most important histo-cytopathological alterations in various organs of fish, which have been used as monitoring tools in various pollution monitoring programs. This review provides useful information of effects of aquatic pollution on different organs in different species of fish. We believe that this study may provide a powerful and useful tool for the assessment of biological effects and to identify target organ toxicity.*

**Keywords:** Histopathology, biomarker, fish, contamination.

## Introduction

Aquatic pollution is one of the current global environmental issues. Due to rapid industrialization and unplanned urbanization many Rivers in India are experiencing complicated problems of pollution. As a result aquatic sources are highly at risk due to accumulation high concentrations of chemicals from surroundings. It causes reduction in the quality of water (pH, DO, BOD, COD, TDS, TSS etc). Thus, water bodies are frequently stores for a large variety of xenobiotics which cause the biochemical and histopathological alternations in fish<sup>1,2</sup>.

Chemical monitoring of water helps to measure the degree of contamination. But for the assessment and evaluation of pollution effects on various organisms including fish can be studied by using biomarker response. But most biomarkers are narrow in their expression whereas histopathology is broad in its evaluation<sup>3,4</sup>. Histopathological changes in animals tissues are reliable and direct indicators to environment stressors<sup>5</sup>. It is also the easiest method assessing both short and long term toxic effects<sup>6</sup>.

Histopathological evaluation is a sensitive tool in toxicant impact assessment to indicate the effect of toxicants on fish health and also allows for early warning signs of disease and injury in cells, tissues, or organs. Such structural changes in

fish as biomarkers in various tissues in different species have also been studied by many researchers<sup>7-12</sup>.

Therefore, Histopathology is the gold standard when defining toxicological effects, but it is invasive, time consuming and expensive. Furthermore, as histological testing is often impractical in human subjects, using biomarkers with a known histological distribution may fill the need of localizing toxic injury to distinct organs or tissues.

Therefore, histopathological evaluation remains an important part of the assessment of the adverse effects of xenobiotics on the whole organism. The present study reviews the most important histo-cytopathological alterations in various organs of fish, which have been used as biomarkers in various pollution monitoring programs.

## Histopathological Changes of Gill

The gills of a fish comprise a multifunctional organ and constitute over 50 percent of the total surface area of the animal that make it sensitive to chemicals in water. It is the site of gaseous exchange and osmoregulation. The fish gills play an important role in maintaining of whole animal ionic homeostasis<sup>13</sup>. Consequently many pollutants come in contact with gill epithelium and causes injury. However damage level

depends on the concentration and period of exposure of the toxicants.

The reviews of Wood<sup>14</sup> and Au<sup>15</sup> have provided extensive information on gill structural alterations in fish as a result of toxicants exposure. Lifting of the lamellar epithelial cells away from the basement membrane due to a penetration of fluid is the most common lesion, which could be give rise to reduce respiratory gas exchange by increasing diffusion distance and decreasing interlamellar distance. Fusion of neighboring lamellae and epithelial rupture are perhaps the direct results of pavement cell lifting and represent more severe gill damage<sup>8,15</sup>. Lamellar fusion, hyperplasia, necrosis of different lamellar and filament cells like chloride and pavement cells is another most commonly reported responses, but is more common for metals than for organics or other pollutants, possibly since metals directly interact with ion transport proteins and inhibit their activity<sup>8,9</sup>. Necrosis would be expected to increase diffusion of ions and water. In true necrosis, Transmission Electron Microscopy shows that organelles and cytoplasmic volume swell and become more electrons dense in necrotic cells. Ultimately cell membranes would be ruptured and the contents possibly would be lost by swamping to the external water. Leukocyte infiltration should be also considered an adaptive response<sup>16</sup>. Hypertrophy of the pavement cells is possibly an event associated with necrosis cell swelling. This lesion is also more commonly associated with metals. However, cell hypertrophy sometimes indicates the origin of pavement cells which occurs when they shrink back to expose increased chloride cell-surface area in return to acid-base and ionic interruptions<sup>17</sup>. Proliferation of mucous cell, associated with excess mucus secretion, seems to occur more frequently in result of exposure to metals than to organic pollutants. Proliferation of pavement cells, mucous cells and chloride cells seem to be protective which limit the accesses of chemicals with the branchial surface, on other hand they may also block respiratory gas exchange and then lead to animal smothering<sup>18</sup>. Uplifting of epithelium, necrosis, and increased density of the cells on secondary lamella hyperplasia and hypertrophy of the epithelial cells are common defense mechanisms. It result in the increase of the gap between the external environment and the blood and thus serve as a barrier to the entry of the contaminants. These alterations, more commonly associated with chronic exposures than acutely lethal exposures, are greatly increase the blood-to-water diffusion distance decrease interlamellar distance and lead to a total reduction in the diffusive conductance of the gills to respiratory gases<sup>19</sup>. Lamellar aneurysms and blood congestion with dilation of marginal channels together with leukocyte infiltration could be considered part of an inflammatory response and occur when fishes suffer a more severe type of stress<sup>20</sup>.

A variety of pollutants and xenobiotics cause various gill lesions in response to a wide range of contaminants<sup>15</sup>. The most of gill alterations reported in the literature, even though concentration dependent (i.e. more severe in acute lethal exposures than in

chronic sub lethal exposures), are actually non-specific and are not correlated with the kind of toxicant, exposure level (acute or chronic), exposure medium (freshwater or seawater), or fish species. Uplifting of epithelial lining and fusion of gill lamella were observed in rainbow trout (*Oncorhynchus mykiss*) exposed to petroleum residues<sup>21</sup>. The same changes have also been reported in the gills of the fishes exposed to organic toxicants<sup>20</sup> and metals and industrial effluent<sup>7,8</sup>.

Definitely, the respiratory epithelium changes cooperates the host respiratory ability. Moderate changes don't lead to mortality directly, but can harmfully affect the functioning of the fish. On the other hand, severe or extensive damage may directly cause death. Overall, gill histopathology can be used as direct and promising biomarker for the assessment of water quality and level of environmental pollution.

### Histopathological Changes of Liver

Liver is the site of metabolism. It plays a key role in biochemical transformations of pollutants under detoxification process. Due toxicant exposure and accumulation lesions and other histopathological alternations are common in liver. Contaminants also affect function and its pronounced metabolic capacity<sup>22</sup>. Numerous workers used liver histopathology as reliable biomarkers of various contaminants<sup>7,23,24</sup>. Therefore such studies have been incorporated in national marine biological effects monitoring programs in many countries including Europe and USA<sup>25</sup>. Myers *et al.*<sup>26</sup> generally classified fish hepatic alterations into several groups and ranked them according to their relative importance as indicators of toxicant exposure. Inflammatory changes consists a third group of liver alterations, which is considered as minimal significant indicator of pollutant exposure, although this group can offer more information on the general health status and condition of the fish. Thus it was observed that histopathological lesions of liver are not specific to pollutants. For example, exposure to organic pollutants and pesticides increases the presence of liver lesions such as foci of cellular alteration (FCA), megalocytic heptoses (MH), hepatocellular nuclear polymorphism (NP), hydropic vacuolation, non-neoplastic proliferative lesions and non-specific necrotic lesions significantly increased<sup>27</sup>. Fanta *et al.*<sup>28</sup> reported abnormalities such as irregular shaped hepatocytes, cytoplasmic vacuolation and nucleus in a lateral position in the siluriform *Corydoras paleatus* exposed to organophosphate pesticides. Reddy *et al.*<sup>8</sup> and Reddy and Baghel<sup>23</sup> observed signs of degeneration (cytoplasmic and nuclear degeneration and nuclear vacuolation) and the focal necrosis in the liver parenchyma of fishes exposed to the industrial effluent. These alterations have been reported as more severe changes, which are more commonly associated with the exposure of the fishes to contamination by various metals.

Lesions including hepatocellular cytoplasmic vacuolization, leucocytes infiltrations, blood congestion necrosis and fatty infiltrations were found in the liver of catfish *Clarias gariepinus*

treated with fenvalerate<sup>29</sup>. The same changes were reported by Teh *et al.*<sup>30</sup> in the liver of 7-day-old larvae of the fish *Sarcamento splittail*. However, using of liver histopathology as a biomarker of contamination exposure may not be a highly cost-effective method for pollution screening because it needs much time and effort to prepare liver samples and expected pathologists are also required to distinguish hepatological alterations<sup>15</sup>.

## Conclusion

On the basis of the information presented in different studies, there is no doubt that the application of histopathological changes as a biomarker of organism exposure to contaminated sites, offers important information that can contribute to environmental monitoring programs designed for surveillance, hazard assessment or regulatory compliance.

One of the most important benefits of the use of histopathological biomarkers in environmental screening is possibility of examining specific target organs, including gills, kidney and liver. However, the fish are responding to the direct effects of the pollutants as well as to the secondary effects caused by stress. This information verifies that histopathological changes are valuable biomarkers for field evaluation, especially in tropical regions that are naturally affected by variety of environmental variations. It should be highlighted that histopathology is able to assess the initial effects and reactions to acute exposure to chemical stressors.

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