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Studies on the histopathological changes in selected tissues of fish *Labeo rohita* exposed to phenol

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Abstract

Histopathological changes in vital tissues like gills, liver and kidney in the fish *Labeo rohita* exposed for 8 days to sublethal (5.2 mgl^{-1}) and lethal concentration (25.09 mgl^{-1}) of phenol were studied. The observed histopathological changes in the gills were epithelial hyperplasia with lamellar fusion, epithelial hypertrophy, edema, general necrosis, increased mucous production and degeneration of primary and secondary gill lamellae at sublethal (5.2 mg l^{-1}) and degenerated primary and secondary gill lamellae, lamellar fusion and lamellar disorganization at lethal (25.09 mg l^{-1}) concentration. In the liver, the changes include as: formation of number of vacuoles, enlargement of nuclei of some cells, enlarged sinusoids with numerous blood cells and atrophic areas at sublethal (5.2 mg l^{-1}) concentration and nuclear and cytoplasmic degeneration and melanomacrophages aggregates at lethal (25.09 mg l^{-1}) concentration. In case of kidney, the changes were: degeneration of proximal and distal convoluted tubule, vacuolation of renal interstitial tissue and deformation of the nuclear membrane of some cells at sublethal (5.2 mg l^{-1}) and occlusion of tubular lumen, cloudy swelling degeneration and hyaline droplets degeneration at lethal (25.09 mg l^{-1}) concentration.

Key words

Phenol, *Labeo rohita*, Histopathology

Introduction

Pollution of the aquatic environment is a serious and growing problem. Increasing number and amount of industrial, agricultural and commercial chemicals discharged into the aquatic environment have led to various deleterious effects on the aquatic organisms (Van der Oost *et al*., 2003). Phenol and its compounds are ubiquitous water pollutants which come to the natural water resources from the effluents of chemical industries such as coal refineries, phenol manufacturing, pharmaceuticals and industries of resin, paint, dyeing, textile, leather, petrochemical and pulp mill (Avilez *et al*., 2008). They are commonly used in agriculture as non-specific pesticides, herbicides, bactericides and fungicides (Avilez *et al*., 2008). From industrial and agricultural operations, these compounds find their way into the natural water resources and affect the aquatic organisms (Tilak *et al*., 2007). They also give an unacceptable taint to water and fish, especially chlorophenols which are formed from the chlorination of phenols (Loh *et al*., 2000).

Bioconcentration and bioaccumulation of phenol or its metabolites in the tissues of fishes, birds, wild animals and human beings have been reported to cause morphological alterations in vital tissues of the organisms even at very low levels (Tilak *et al*., 2006; Abdel-Hameid, 2007). It is generally reported that the histopathological biomarkers are useful indicators of the general health of the fish and are considered as a mirror that reflects exposure to a variety of anthropogenic pollutants (Mohamed, 2009). They also allow examining specific target organs, including gills, kidney and liver that are responsible for vital functions, such as respiration, excretion and accumulation and biotransformation of xenobiotics in the fish (Gernhofer *et al*., 2001). Tilak *et al*. (2006) in *Catla catla* and Abdel-Hameid,

(2007) in *Oreochromis aureus* reported pathological changes in fish exposed to phenol. Though sufficient literature exists on fishes there is paucity of information on the carp, *Labeo rohita*. Hence, in the present study an attempt has been made to study the possible histopathological changes in selected vital tissues like gill, liver and kidney of, *Labeo rohita*.

Materials and Methods

Phenol was purchased from Qualigens Fine Chemicals, Mumbai. Though phenol was fairly soluble in water, to aid in the uniform dispersion of the toxicant, especially at higher concentrations used, acetone was used as a carrier solvent. Stock solutions of phenol were prepared in acetone and working standards were prepared in distilled water (APHA, 2005). The fish, *Labeo rohita* (size 4-6 cm in length, 4-5 g in weight) were brought from the fish farms at Nandivelugu. They were acclimated in the laboratory for 96 hr. The water used for acclimation and conduction of experiments was clear ground water. The hydrographical condition of the water was as follows: temperature 28 $\pm$ 2°C, pH 7.8, total hardness 232 mgl^{-1}, chloride 30 mgl^{-1}, nitrate 0.06 mgl^{-1}, iron 0.05 mgl^{-1}, phosphate 0.112 mgl^{-1}, dissolved oxygen 9-10 mgl^{-1}, phenols nil. Standard protocols of APHA (2005) were followed for the collection of materials, storage, handling, feeding, biomass loading and for the analysis of water.

The fish were exposed for 8 days to sublethal ($^1/_5^{th}$ of 96 hr LC_{50} of 5.2 mgl^{-1}) and lethal concentration (96 hr LC_{50} of 25.09 mgl^{-1}) of phenol by static renewal bioassay. In lethal exposures, the mortality was 80% within 8 days. Surviving fish (100% in sublethal and 20% as lethal concentration) were randomly selected after the exposure period for histopathological examination. Tissues like gill, liver and kidney were isolated from normal and experimental fish. Physiological saline solution (0.85% NaCl) was used to rinse and clean the tissues. They were fixed in aqueous Bouin's solution for 48 hr processed through graded series of alcohols, cleared in xylene and embedded in paraffin wax. Gills alone were processed by double embedding technique. Sections of 6µ thickness were cut, stained with Ehrlich hematoxylin/Eosin (dissolved in 70% alcohol) and mounted in canada balsam (Humason, 1972). Sections were observed in digital microscope (Intel Play QX3) at 200x magnification.

Results and Discussion

Phenol caused marked pathological changes in the gills of fish exposed to sublethal concentration (5.2 mgl^{-1}) as compared to control (Fig. 1A). The changes include: epithelial hyperplasia with lamellar fusion, epithelial hypertrophy, telangiectasia, edema, general necrosis, increased mucous production and degeneration of primary and secondary gill lamellae (Fig. 1B). Damages of the gills at lethal concentration (25.09 mgl^{-1}) were much more extensive with severe degenerated primary and secondary gill lamellae, lamellar fusion and lamellar disorganization (Fig. 1C) compared to sublethal concentration (Fig 1B).

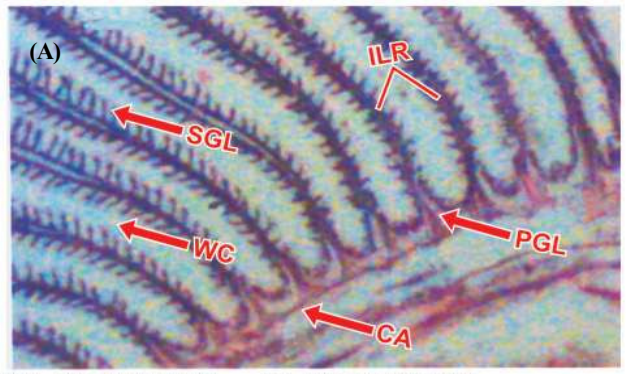
The following histological changes observed in the liver exposed to sublethal (5.2 mgl^{-1}) concentration were formation of number of vacuoles, enlargement of nuclei of some cells, nuclear hypertrophy, enlarged sinusoids with numerous blood cells and atrophic areas (Fig. 2B) as compared to control cells of liver (Fig. 2A). Under lethal (25.09 mgl^{-1}) exposure the changes observed were more intense as compared to sublethal concentration (Fig. 2B) with the proper structure of the liver obscured, vacuolated and atrophied. Nuclear and cytoplasmic degeneration and melanomacrophages aggregates were also found (Fig. 2C).

Under sublethal (5.2 mgl^{-1}) exposure, the proximal convoluted tubules and secondary convoluted tubules were moderately degenerated and cellular contours were not clearly distinguished. Renal corpuscle with glomerular expansion and absence of bowmans space, nuclear hypertrophy and formation of vacuoles were observed (Fig.3 B**)** on comparison to control histology (Fig. 3A). Under lethal (25.09 mgl^{-1}) exposure occlusion of tubular lumen, cloudy swelling degeneration and hyaline droplets degeneration were observed (Fig. 3C) which are not evident at sublethal concentration.

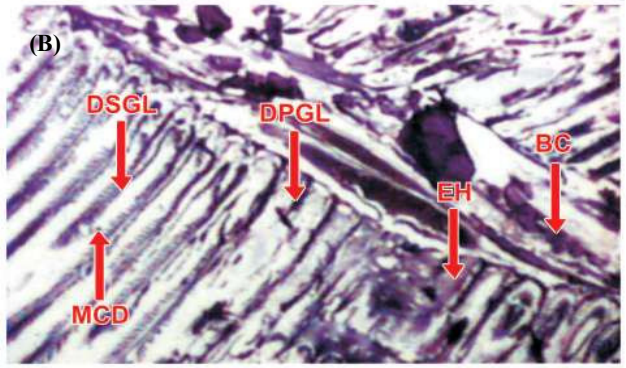
The organ most associated with the detoxification and biotransformation process is the liver and due to its function, position and blood supply it is also one of the organs most affected by contaminants in water (Camargo and Martinez, 2007). The liver also exhibited considerable damages like enlargement of sinusoids, vacuolation and necrosis of hepatic cells. These damages probably diminish the detoxification function of the liver (Kolanczyk and Schmieder, 2002; Solem *et al*., 2003).

Tilak *et al*. (2006) reported acute toxicity of phenol in Catla Catla. The fish were exposed to 5 ppm of phenol for 12 days. In mild damage the epithelial layer of gill was swollen whereas in severe cases this layer was stripped away. In case of liver, sinusoids became enlarged and contained numerous blood cells, vacuolated and atrophic areas were visible. In kidney extravasations and necrosis of the nephron were reported. In severe cases the kidney lost their affinity towards Hematoxylin-Eosin staining to a considerable degree with some parts of the structure obliterated.

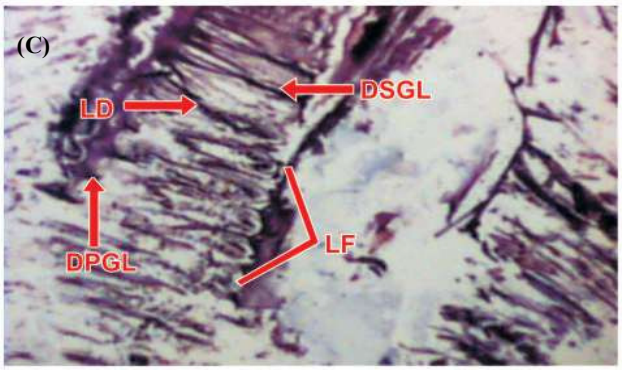
Abdel-Hameid (2007) described histopathological changes in the liver of blue tilapia, *Oreochromis aureus* exposed to 5.8, 11.6 and 23.2 mgl^{-1} of phenol for 7 days. The



PGL - Primary Gill Lamella
SGL - Secondary Gill Lamella
CA - Central Axis
ILR - Inter Lamellar Region
WC - Water Channel

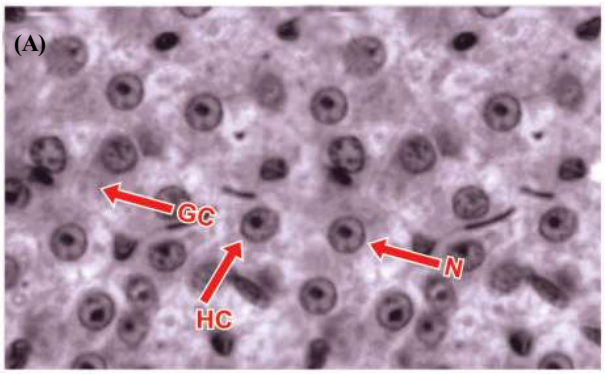


DPGL - Degenerated Primary Gill Lamella
DSGL - Degenerated Secondary Gill Lamella
MCD - Marginal Channel Dilation
EH - Epithelial Hyperplasia
BC - Blood Congestion

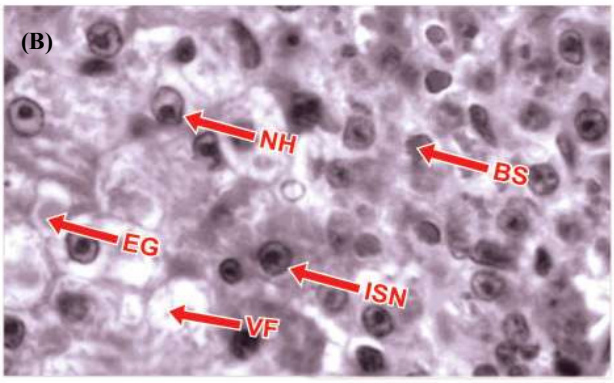


DPGL - Degenerated Primary Gill Lamella
DSGL - Degenerated Secondary Gill Lamella
LD - Lamellar Disorganization
LF - Lamellar Fusion

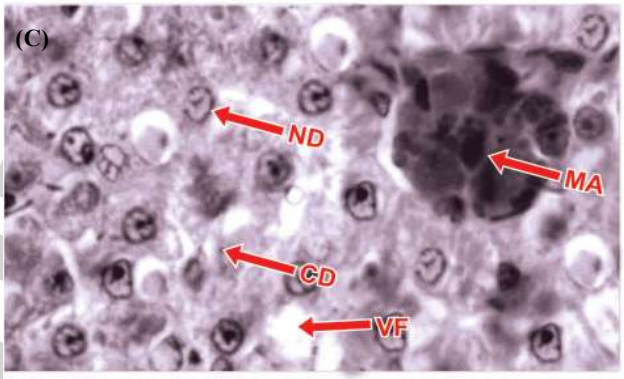
Fig. 1 : Microscopic observation of gill of fish *Labeo rohita* (A) control (B) sublethal (5.2 mgl^{-1} phenol) (C) lethal (25.09 mg l^{-1} phenol)



N - Nucleus
GC - Granular Cytoplasm
HC - Hepatic cell



NH - Nuclear Hypertrophy
BS - Bile Stagnation
VF - Vacuole formation
EG - Eosinophilic Granules
ISN - Irregular Shaped Nucleus



ND - Nuclear Degeneration
CD - Cytoplasmic Degeneration
MA - Melanomacrophages Aggregate
VF - Vacuole formation

Fig. 2 : Microscopic observation of liver of fish *Labeo rohita* (A) control (B) sublethal (5.2 mgl^{-1} phenol) (C) lethal (25.09 mg l^{-1} phenol)

changes included inflammation, central necrosis and cell degeneration and were found to be concentration dependent where the fish exposed to high concentration recorded high damages.

The pathological changes observed in the present study were similar to those reported by Tilak *et al.,* (2006) and Abdel-Hameid (2007). During the experiment, the phenol exposed fish produced excessive quantities of mucous, demonstrating unequivocally the aggressive action of phenol on surface tissues. Increased mucus production in the gills and skin were earlier reported by Tilak *et al.,* (2006). Increased density and dimensions of the mucous glands of phenol exposed fish is related to greater mucus secretion which in turn cleans the branchial epithelium and minimizes the toxicity of the chemical.

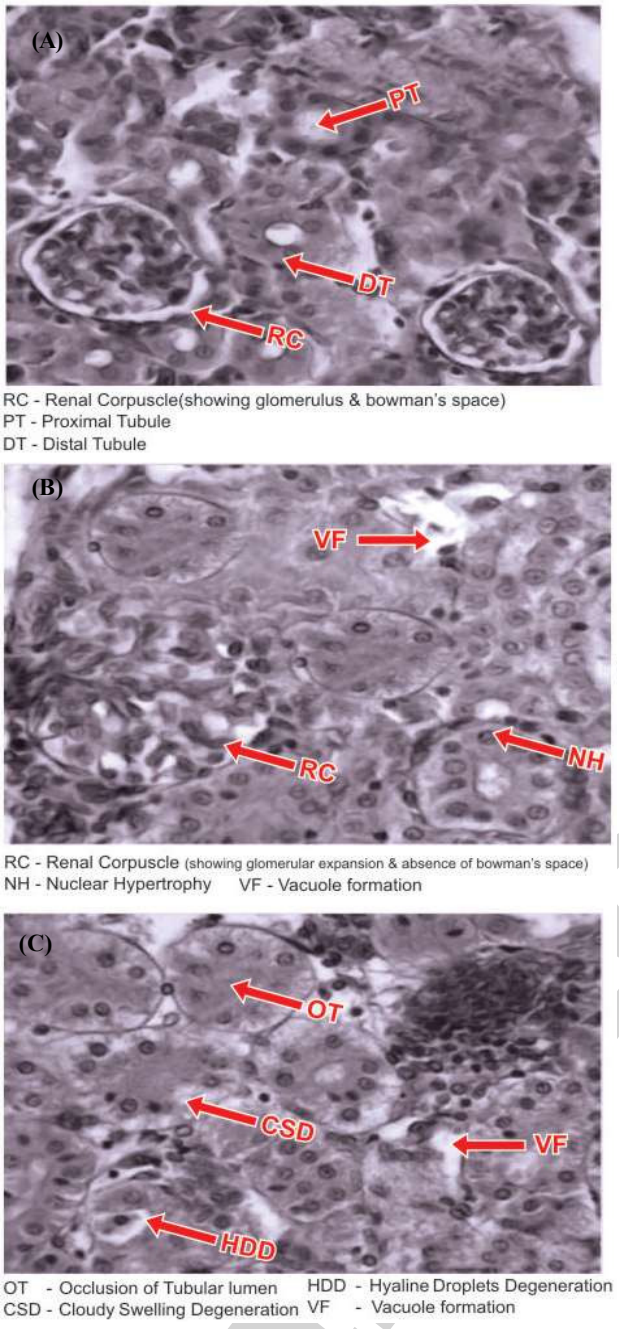


Fig. 3 : Microscopic observation of kideny of fish *Labeo rohita* (A) control (B) sublethal (5.2 mgl^{-1} phenol) (C) lethal (25.09 mg l^{-1} phenol)

As phenol is rapidly metabolised by the tissues of the fish (Solem *et al*., 2003), an exposure period of 8 days was found in the present study to produce histological changes. The gills which participate in many important functions in fish, such as respiration, osmoregulation and excretion remain in close contact with the external environment and particularly sensitive to changes in the quality of water are considered the primary target of the contaminants (Poleksic and Mitrovic-Tutundzic, 1994; Mazon *et al*., 2002; Fernandes and Mazon, 2003). Fish swimming at the water surface and gulping of air was possibly due to respiratory disturbances. The intensity of gill damage was severe as indicated by lamellar fusion and basal hyperplasia. Alterations like epithelial lifting, hyperplasia and hypertrophy of the epithelial cells, besides partial fusion of some secondary lamellae are examples of defense mechanisms, 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 (Fernandes and Mazon, 2003; Akaishi *et al*., 2004; Butchiram *et al*., 2009). Such alterations were non-specific and may be introduced by different types of contaminants. As a consequence of the increased distance between water and blood due to epithelial lifting, the oxygen uptake is impaired. In addition, hyperplasia may also reduce secretary and excretory functions of the gills (Tilak *et al*., 2006). The epithelial membrane of the gill filaments and secondary lamellae were swollen and in severe cases the epithelial wall was destroyed. Probably, destruction of the gill structure caused asphyxia which was the most probable cause of death. The most pronounced reactions were seen in lethal groups, in some cases with a degree of tissue damage that due to the resulting drastic reduction in respiratory surface, would have caused death within a very short time.

The kidney is a vital organ of body and proper kidney function is to maintain the homeostasis. It is not only involved in removal of wastes from blood but is also responsible for selective reabsorption which helps in maintaining volume and pH of blood and body fluids and erythropoiesis (Iqbal *et al*., 2004). The kidney is one of the first organs to be affected by contaminants in the water. The common alterations were necrosis of nephron and extravasations. These damages possibly caused malfunction of this organ, therefore the removal of the poison could not take place actively. Thus, the lethal concentration of phenol has produced significant histopathological changes in comparison to sublethal concentration in this species of fish.

References

Abdel-Hameid, N.A.H.: Physiological and histopathological alterations induced by phenol exposure in *Oreochromis aureus* juveniles. *Turk. J. Fish. Aquat. Sci*., **7,** 131-138 (2007).

Akaishi, F.M., H.C. Silva de Assis, S.C.G. Jakobi, D.R. Eiras-Stofella, S.D. St-Jean, S.C. Courtenay, E.F. Lima, A.L.R. Wagemer, A.L. Scofield and C.A. Oliveria Ribeiro: Morphological and neurotoxicological findings in tropical freshwater fish (*Astynax* sp) after waterborne and acute exposure to water soluble fraction (WSF) of crude oil. *Arch. Environ. Contam. Toxicol*., **46,** 244-253 (2004).

APHA: Standard methods for the examination of water and wastewater. 21th Edn. APHA, AWWA, WEF, Washington DC, USA (2005).

Avilez, I.M., T.S. Hori, L.C. de Almeida, A. Hackbarth, C. Netojda, V.L. Bastos and G. Moraes: Effects of phenol in antioxidant

metabolism in matrinxa, *Brycon amajonicus* (Teleostei: Characidae). *Comp. Biochem. Physiol. C Toxicol. Pharmacol*., **148,** 136-142 (2008).

Butchiram, M.S., K.S. Tilak and P.W. Raju: Studies on histopathological changes in the gill, liver and kidney of *Channa punctatus* (Bloch) exposed to alachlor. *J. Environ. Biol*., **30,** 303-306 (2009).

Camargo, M.M. and C.B. Martinez: Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. *Neotrop. Ichthyol*., **5,** 327-336 (2007).

Fernandes, M.N. and A.F. Mazon: Environmental pollution and fish gill morphology. In: Fish adaptations (Eds.: A.L. Val and B.G. Kapoor). Enfield Science Publishers, p. 203-231 (2003).

Gernhofer, M., M. Pawet, M. Schramm, E. Muller and R. Triebskorn: Ultrastructural biomarkers as tools to characterize the health status of fish in contaminated streams. *J. Aqua. Ecosys. Stress Recovery*, **8,** 241-260 (2001).

Humason, G.L.: Animal tissue technique (Eds.: W.H. Freeman and Co.). 3rd Edn., San Fransisco (1972).

Iqbal, F., I.Z. Qureshi and M. Ali: Histopathological changes in the kidney of common carp, *Cyprinus carpio* following nitrate exposure. *J. Res. Sci*., **15,** 411-418 (2004).

Kolanczyk, R. and P. Schmieder: Rate and capacity of hepatic microsomal ring hydroxylation of phenol to hydroquinone and catechol in rainbow trout (*Oncorhynchus mykiss*). *Toxicol*., **176,** 77-90 (2002).

Loh, K.C., T.S. Chung and A. Wei-Fern: Immobilized-cell membrane bioreactor for high-strength phenol wastewater. *J. Environ. Eng*., **126,** 75-79 (2000).

Mazon, A.F., G.H.D. Pinheiro and M.N. Fernandes: Hematological and physiological changes induced by short-term exposure to copper in the freshwater fish, *Prochilodus scrofa*. *Brazilian J. Biol*., **62,** 621-631 (2002).

Mohamed, F.A.S.: Histopathological studies on *Tilapia zilli* and *Solea vulgaris* from lake Qarum, Egypt. *World J. Fish Marine Sci*., **1,** 29-39 (2009).

Poleksic, V. and V. Mitrovic-Tutundzic: Fish gills as a monitor of sublethal and chronic effects of pollution. In: Sublethal and chronic effects of pollutants on freshwater fish (Eds.: R. Muller and R. Lloyd). Oxford, Fishing News Books pp. 339-352 (1994).

Solem, L.E., R.C. Kolanczyk and J.M. McKim: An *in-vivo* microdialysis method for the qualitative analysis of hepatic phase I metabolites of phenol in rainbow trout (*Oncorhynchus mykiss*). *Aquat. Toxicol*., **62,** 337-347 (2003).

Tilak, K.S., K. Veeraiah, M.S. Butchiram and P.B. Thathaji: Acute toxicity of phenol to the freshwater fish *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*. *J. Ecotoxicol. Environ. Monit*., **16,** 311-317 (2006).

Tilak, K.S., K. Veeraiah, M.S. Butchiram and P.B. Thathaji: Histopathological changes in the gill and liver of *Catla catla* exposed to phenol. *J. Ecotoxicol. Environ. Monit*., **16,** 425-430 (2006).

Tilak, K.S., K. Veeraiah, P.B. Thathaji and M.S. Butchiram: Toxicity studies of butachlor to the freshwater fish *Channa punctatus* (Bloch). *J. Environ. Biol*., **28,** 485-487 (2007).

Van der Oost, R., J. Beyer and N.P.E. Vermeulen: Fish bioaccumulation and biomarkers in environmental risk assessment: A review. *Environ. Toxicol. Pharmacol*., **13,** 57-149 (2003).