Original Article

Toxic effects of glyphosate-based herbicide, Excel Mera 71 on gill, liver, and kidney of *Heteropneustes fossilis* under laboratory and field conditions

Palas Samanta [a], Aloke Kumar Mukherjee [b], Sandipan Pal [c], Debraj Kole [a],
Apurba Ratan Ghosh [a],*

[a] Ecotoxicology Laboratory, Department of Environmental Science, The University of Burdwan, Golapbag, Burdwan 713104,
West Bengal, India
[b] P.G. Department of Conservation Biology, Durgapur Government College, Durgapur 713214, West Bengal, India
[c] Department of Environmental Science, Aghorekamini Prakashchandra Mahavidyalaya, Subhasnagar, Bengai, Hooghly 712611,
West Bengal, India

ARTICLE INFO

ABSTRACT

The effects of glyphosate-based herbicide Excel Mera 71 under field and laboratory conditions were investigated to evaluate the pathological symptoms through light and electron microscopic study in the gill, liver, and kidney of *Heteropneustes fossilis* (Bloch) for a period of 30 days. Histological alterations like hypertrophy and fusion in secondary lamellae, damage in chloride cells were more prominent in laboratory conditions under light microscopy. Topological changes such as complete loss of microridges, swelling, and irregular arrangement of microridges in the gills were prominent under scanning electron microscopic study under laboratory conditions. Transmission electron microscopy (TEM) study depicted vacuolation and degeneration in chloride cells, dilation in rough endoplasmic reticulum (RER), and mitochondria in gill epithelium. The liver showed enlarged and pyknotic hepatocytes, vacuolation, excess fat deposition, and necrosis under laboratory conditions, while enlarged acentric nuclei, increased sinusoidal space, and less vacuolation in cytoplasm were observed under field conditions. TEM displayed cytoplasmic vacuolation and a reduced number of endoplasmic reticulum and glycogen droplets in the laboratory, but this was less pronounced under field conditions. In the kidneys, loss of hematopoietic tissue, degenerative changes in glomeruli, proximal and distal convoluted tubule, and epithelial cell lining of the renal tubules were comparatively less prominent under field conditions. Under TEM, epithelial cell necrosis, endoplasmic reticulum fragmentation, and mitochondrial degeneration were more prominent under laboratory conditions. The present study evaluated the comparative toxicity under field and laboratory conditions under long-term exposure to glyphosate herbicide and identified pathological responses as indicators in monitoring the herbicidal contamination in aquatic ecosystems.

© 2016 Saudi Society of Microscopes. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Histopathological study has been widely used for toxicity testing of the effects of xenobiotic compounds at the suborganismal or organismal level, as well as

---

* Corresponding author.
E-mail address: apurbaghosh2010@gmail.com (A.R. Ghosh).

evaluation of overall health of the entire population in the ecosystem. The advantage of using histopathological symptoms in specific target organs like the gills, liver, and kidneys in environmental monitoring is that they are most effective to study the vital functions, such as respiration, accumulation and biotransformation, and excretion of xenobiotics in fish [1,2]. Histological changes appear as prime responses to sublethal stressors, while topological characterization of cell surface and subcellular organelles can be best analyzed under scanning electron microscopy (SEM) and transmission electron microscopy (TEM), respectively. Furthermore, the alterations in cells and tissues in vertebrates, especially fish, are recurrently used as biomarkers in many studies, but such changes also occur in all invertebrates inhabiting aquatic basins, and are normally easier to identify than functional ones [3], which ultimately serve as warning signs of damage to animal health [4,5]. Fish after exposure to these xenobiotic substances show several lesions in different tissue systems [6,7]. Gills [8,9], liver [4,10], and kidneys [11] are the most suitable organs for histological analysis in order to determine the effects of contamination. During xenobiotic exposure, the toxicants break down the adhesion between the epithelial branchial cells and the underlying pillar cells, accompanied by collapse of the structural integrity of the secondary lamellae and subsequent failure of the respiratory functions of the gills [12]. The liver is the central metabolic organ and plays a key role in biochemical transformations of the xenobiotic substances, which inevitably reflects on its integrity by creating lesions and other histopathological alterations in the liver parenchyma [13]. The kidneys perform an important function in maintenance of a stable internal environment and partially xenobiotic metabolism.

Aquatic bodies are contaminated by several pesticides, especially herbicides such as glyphosate, through irrigation water and surface run-off. Among the non-target aquatic organisms, fish represent the largest and most diverse group of vertebrates that are chronically exposed to these substances continuously. Therefore, the present study aimed to investigate the toxic effects of commercial formulations of the glyphosate herbicide, Excel Mera 71 (Excel Crop Care Limited, Mumbai, Maharashtra, India), at histopathological and ultrastructural levels through changes in the gills, liver, and kidneys of the fish *Heteropneustes fossilis* (Bloch) under laboratory and field conditions.

2. Materials and methods

2.1. Chemicals

Commercial formulation of the glyphosate herbicide (Excel Mera 71, Excel Crop Care Limited) was used in both the experiments. Excel Mera 71 is the trade name of glyphosate herbicide in the Indian Market. Delafield's hematoxylin stain, eosin yellow, xylene, Distyrene Plasticizer Xylene, amyl acetate, acetone, glutaraldehyde solution, sodium hydroxide, tricaine methonesulfonate, uranyl acetate (EM grade), ethanol, disodium hydrogen phosphate, dihydrogen sodium phosphate, lead citrate (EM grade), epoxy resin (EM grade), paraformaldehyde (EM grade), and araldite CY212 (EM grade) of analytical grade were purchased from Merck Specialities Private Limited (Mumbai, India). Osmium tetroxide was purchased from Spectrochem (Mumbai, India).

2.2. Fish

Freshwater teleostean fish *H. fossilis* (Bloch) of both the sexes with an average weight of 31.77 ± 3.440 g and total length of 16.58 ± 0.388 cm were procured from local markets and were acclimatized under congenial laboratory conditions for 15 days separately in aquaria of 250-L capacity. Fish were kept in continuously aerated water with a static system and experiments were conducted with a natural photoperiod (12-hour light/12-hour dark) and at an ambient water temperature. During acclimatization, the average values of water parameters were analyzed: temperature $26.49 \pm 0.127\,^{\circ}$C, pH 7.94 ± 0.04, electrical conductivity $392.22 \pm 0.62\,\mu$S/cm, total dissolved solids 279.33 ± 0.69 mg/L, dissolved oxygen 6.44 ± 0.05 mg/L, total alkalinity 204.0 ± 7.30 mg/L as $CaCO_3$, total hardness 180.44 ± 3.74 mg/L as $CaCO_3$, sodium 24.45 ± 0.56 mg/L, potassium 5.33 ± 1.02 mg/L, orthophosphate 0.03 ± 0.001 mg/L, ammoniacal nitrogen 1.66 ± 0.21 mg/L, and nitrate nitrogen 0.21 ± 0.030 mg/L. After acclimatization, fish were divided into two groups: one group was maintained in field ponds situated at Crop Research Farm premises of The University of Burdwan, West Bengal, India and the other group in laboratory aquarium. The fish were fed once a day with commercial fish pellets (32% crude protein, Tokyu® fish food, Thailand) during both acclimation and exposure periods. Therefore, the study was carried out under two different experimental conditions: field pond and laboratory, for a duration of 30 days.

2.3. Experimental design

2.3.1. Field experiment

Fish were maintained in two groups in two separate adjacent fields: three control groups containing 10 fish species in a cage in one field, and three glyphosate exposure groups containing 10 fish species in separate field and cages for 30 days. The desired dose of 750 g/acre, corresponding to the concentration recommended for use in rice culture, was dissolved in water and applied once. It was sprayed on Day 1 of the experiment on the surface of each glyphosate-treated cage. During experimentation, glyphosate-treated and control fish were subjected to the same environmental conditions. The cages were prepared for the culture of the experimental fish species as per Chattopadhyay et al. [14], with some modifications. All the cages were square in shape with an area of 2.5 m × 1.22 m and height of 1.83 m (submerged height was 0.83 m). The cages were framed by light strong bamboo. The four-sided wall, floor of the cage, and top of the cage cover was fabricated with nylon net and was embraced by two polyvinyl chloride nets: the inner and outer bearing mesh sizes of 1.0 mm × 1.0 mm and 3.0 mm × 3.0 mm, respectively. During the experimentation of 30 days, the field pond

water was analyzed: temperature $24.03 \pm 0.203\,°C$, pH 6.56 ± 0.087, electrical conductivity $347.00 \pm 1.15\,\mu S/cm$, total dissolved solids $247.67 \pm 1.45\,mg/L$, dissolved oxygen $7.0 \pm 0.157\,mg/L$, total alkalinity $221.33 \pm 3.53\,mg/L$ as $CaCO_3$, total hardness $140.0 \pm 2.31\,mg/L$ as $CaCO_3$, sodium $63.40 \pm 2.67\,mg/L$, potassium $15.96 \pm 2.10\,mg/L$, orthophosphate $0.24 \pm 0.026\,mg/L$, ammoniacal nitrogen $0.74 \pm 0.111\,mg/L$, and nitrate nitrogen $1.66 \pm 0.035\,mg/L$.

2.3.2. Laboratory experiment

Fish were segregated into two groups (control and glyphosate-treated) and maintained in six aquaria (3 control and 3 treated), containing 10 fish in each aquarium in the Ecotoxicology Laboratory, Department of Environmental Science, The University of Burdwan. The fish were exposed to sublethal doses of glyphosate, that is, $17.20\,mg/L$ in 40 L aquaria for a period of 30 days [15,16]. Doses were applied every alternate day, maintaining the same water quality. During experimentation, water parameters were measured: temperature $26.63 \pm 0.120\,°C$, pH 7.93 ± 0.075, electrical conductivity $426.0 \pm 5.93\,\mu S/cm$, total dissolved solids $302.89 \pm 4.69\,mg/L$, dissolved oxygen $5.06 \pm 0.43\,mg/L$, total alkalinity $209.80 \pm 10.50\,mg/L$ as $CaCO_3$, total hardness $163.11 \pm 3.04\,mg/L$ as $CaCO_3$, sodium $37.76 \pm 1.02\,mg/L$, potassium $7.26 \pm 1.12\,mg/L$, orthophosphate $0.04 \pm 0.002\,mg/L$, ammoniacal nitrogen $7.09 \pm 2.15\,mg/L$, and nitrate nitrogen $1.78 \pm 0.263\,mg/L$ on average. The quality of the water was assessed as per American Public Health Association [17].

2.4. Sampling

After completion of the 30 days experiment, on Day 31, the fish were collected from the control and treated aquarium and pond and were anesthetized with tricaine methanesulfonate (MS 222) and the gills, liver and kidneys were taken immediately after dissection and processed separately for histological and ultrastructural analysis.

2.5. Histopathological analysis

Fish gills, liver and kidneys from the control and treated groups were collected and fixed in aqueous Bouin's fluid solution, dehydrated through a graded series of ethanol, and finally embedded in paraffin. Paraffin sections were cut at $3–4\,\mu m$ using a Leica (Department of Environmental science, The University of Burdwan) RM2125 microtome. These sections were then stained with hematoxylin and eosin. Histopathological observations were made under a Leica DM2000 light microscope.

2.6. Ultrastructural analysis

For SEM, tissues were fixed in 2.5% glutaraldehyde in phosphate buffer (0.2 M, pH 7.4) for 24 hours at $4\,°C$ followed by post-fixation with 1% osmium tetroxide in phosphate buffer (0.2 M, pH 7.4) for 2 hours at $4\,°C$, dehydrated through graded acetone, subsequently by amyl acetate, and subjected to critical point drying with liquid carbon dioxide. The tissues were then mounted on

metal stubs and sputter-coated with gold to a thickness of $\sim 20\,nm$. The tissues were examined with a scanning electron microscope (Hitachi S-530) at the University Science Instrumentation Centre, The University of Burdwan.

For TEM, tissues were fixed in Karnofsky fixative (mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer) for 12 hours at $4\,°C$ and then post-fixed with 1% osmium tetroxide in phosphate buffer (0.2 M, pH 7.4) for 2 hours at $4\,°C$, dehydrated through graded acetone, infiltrated and embedded in epoxy resin, araldite CY212. Ultrathin sections (0.5–1 μm) were then cut by using a glass knife on an Ultracut E Reichart–Jung (at the Electron Microscope Facility, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India.) ultramicrotome at a thickness of $70\,nm$, collected on naked copper-meshed grids, and contrasted with uranyl acetate and lead citrate. The tissues were examined under a Technai G2 high resolution transmission electron microscope at the Electron Microscope Facility, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India.

2.7. Ethical statement

The experiment was carried out in accordance with the guidelines of the University of Burdwan and approved by the Ethical Committee of this University.

3. Results

3.1. Gills

Under light microscopy, the gills comprised lamellae (primary and secondary) composed of a cartilaginous skeletal structure, multilayered epithelium, and vascular system. Secondary lamellae were lined by squamous epithelium. Between the secondary epithelia, the primary lamella was lined by stratified epithelium. Secondary gill lamellae consisted of epithelial cells supported by pillar cells (Figure 1A). The most marked changes under the laboratory conditions after glyphosate exposure were hypertrophy and fusion of the secondary lamellae, and severe damage to chloride cells (Figure 1B), while partial fusion of some lamellae in *H. fossilis* was prominent under field conditions (Figure 1C).

Topographical study by SEM revealed that each gill filament in the control fish was composed of primary and secondary lamellae surrounded by stratified epithelial cells (Figure 1D). SEM showed complete loss of microridges in some stratified epithelial cells in the gills, swelling of microridges, and irregular arrangement of microridges under laboratory conditions after glyphosate exposure (Figure 1E) but under field conditions, there were no significant alterations in stratified epithelial cells and microridge structure (Figure 1F).

TEM observations of gill primary epithelium showed general appearance of chloride cells supported by tightly packed pavement cells under control conditions (Figure 1G). In gill epithelium of *H. fossilis* under laboratory conditions, TEM showed degenerative changes in chloride
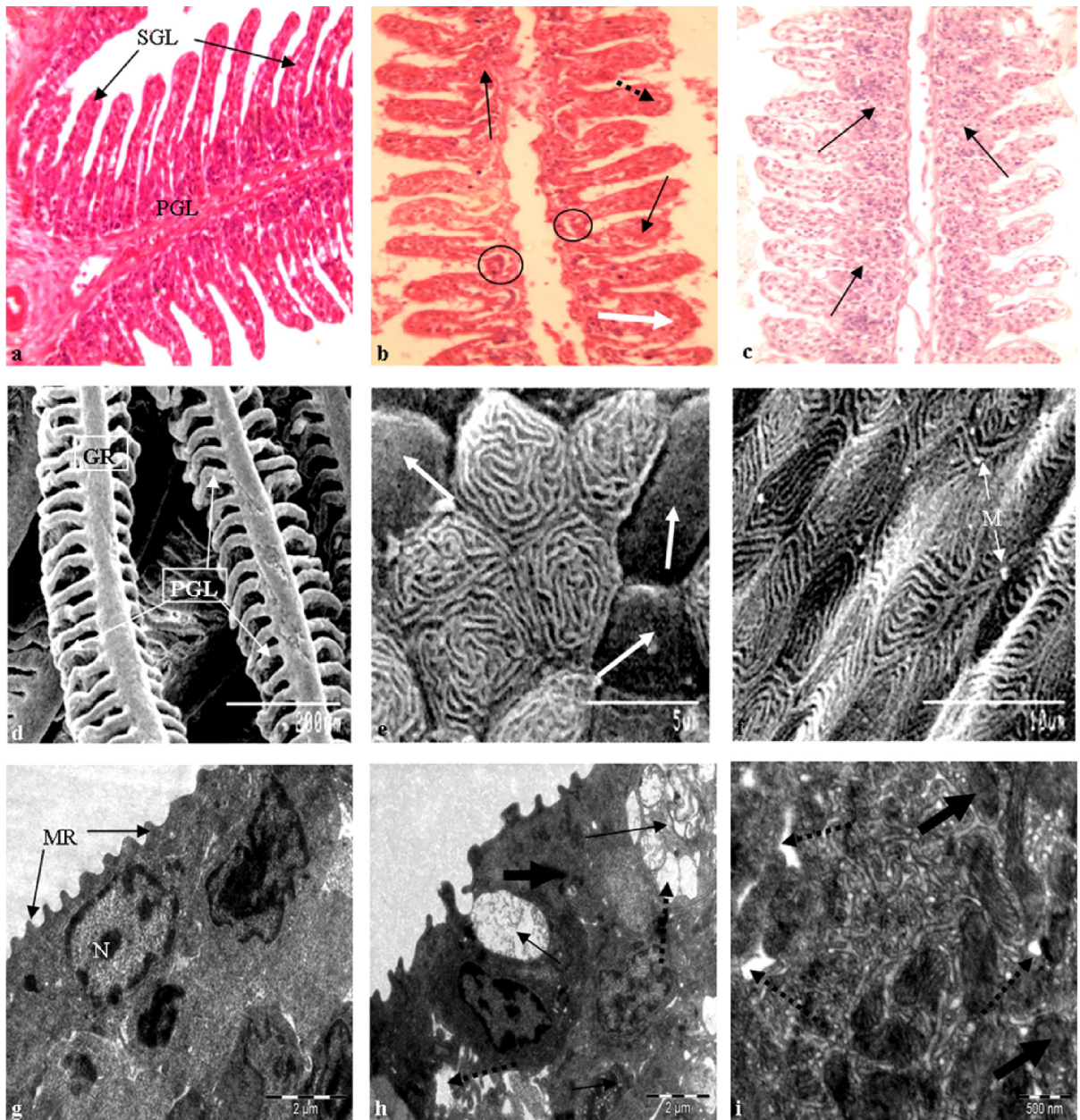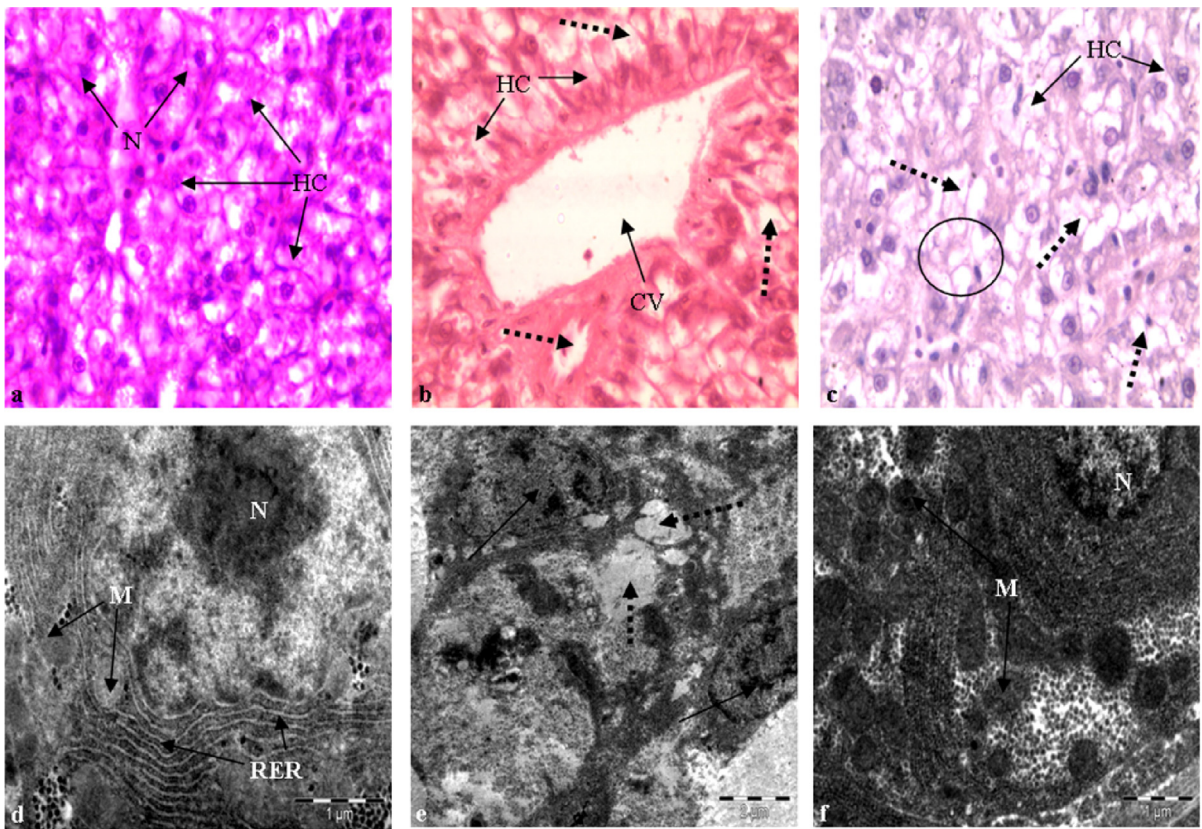
Figure 1. Histopathological photomicrographs of gills of *Heteropneustes fossilis* under control conditions (Co), glyphosate-treated laboratory conditions (GL), and glyphosate-treated field conditions (GF). (A) Normal structure of primary gill lamellae (PGL) and secondary (SGL) lamella under light microscopy (Co, 400×). (B) Hypertrophy (arrow), fusion (white arrow) of SGL and distortion of chloride (oval) and pillar cells (broken arrow) under light microscopy (GL, 400×). (C) Partial fusion of SGL (white arrow) and hypertrophy (arrow) under light microscopy (GF, 400×). (D) SEM showing normal arrangement of gill rackers (GR) with primary gill lamellae (PGL) and stratified epithelial cells (SEC) on the PGL (Co, 200×). (E) Gill epithelium showing loss of Microridge (MR) in SEC (arrow) under SEM (GL, 8000×). (F) Almost normal appearance of MR in SEC and excess mucin (M) droplets under SEM (GF, 5000×). (G) Gill epithelial cell under TEM showing normal chloride cells (CC), pavement cells (PC) with prominent mitochondria (M) with apical pore (square) (Co, 3200×). (H) Degenerative chloride cells (arrow), severe distortion in mitochondria (bold arrow), severe cytoplasmic vacuolation (broken arrow) with dilated endoplasmic reticulum under TEM (GL, 2500×). (I) Vacuolation (broken arrow) and dilated mitochondria (bold arrow) under TEM (GF, 8000×). SEM = scanning electron microscopy; TEM = transmission electron microscopy.

cells, fusion of microridges, dilation of RER and mitochondria, vacuolation of chloride cell cytoplasm (Figure 1H), while damage to the tubular vascular network, dilation of mitochondria, and vacuolation in the cytoplasm were the most significant changes under field conditions (Figure 1I).

3.2. Liver

Fish liver consists of hepatocytes that are polygonal and/or hexagonal cells with a centrally placed spherical nucleus and a densely stained nucleolus and granular

Figure 2. Histopathological photomicrographs of liver of *Heteropneustes fossilis* under control conditions (Co), glyphosate-treated laboratory conditions (GL), and glyphosate-treated field conditions (GF). (A) Normal appearance of hepatocytes (HC) and compact arrangement around central vein (CV) with distinct nucleus (N) under light microscopy (Co, 1000×). (B) Hypertrophied and pyknotic nuclei (white arrow), vacuolation in cytoplasm of hepatocytes (broken arrow) under light microscopy (GL, 1000×). (C) Light microscopy showing vacuolation in cytoplasm (broken arrow) and appearance of sinusoidal space (oval) (GF, 1000×). (D) Normal appearance of hepatocytes with large number of mitochondria (M), rough endoplasmic reticulum (RER) and glycogen droplets (GY) under TEM (Co, 6300×). (E) Hepatocytes showing necrotic nucleus (arrow), vacuolation in cytoplasm (broken arrow) with reduced amount of RER under TEM (GL, 2500×). (F) TEM showing hepatocytes with dilated mitochondria (bold arrow) and cytoplasmic vacuolation (broken arrow; GF, 5000×).
TEM = transmission electron microscopy.

cytoplasm (Figure 2A). The most conspicuous histopathological alterations due to glyphosate toxicity identified by light microscopy in the liver of *H. fossilis* under laboratory conditions were enlarged and pyknotic hepatocytes, vacuolation in the cytoplasm, excess fat deposition, and hepatocytic inflammation (Figure 2B), while vacuolation in the cytoplasm, enlarged acentric nuclei, and increased sinusoidal space were observed under field conditions (Figure 2C).

TEM of the liver showed normal appearance of hepatocytes with centrally placed prominent nucleoli and cytoplasm that contained a large number of mitochondria, RER, and glycogen (Figure 2D). Under laboratory conditions, TEM showed reduced endoplasmic reticulum, presence of necrosis, cytoplasmic vacuolation, and reduced glycogen (Figure 2E), while under field conditions, damage was less than under laboratory conditions, which included dilated mitochondria, large amount of endoplasmic reticulum, fewer glycogen droplets, and less cytoplasmic vacuolation in some regions (Figure 2F).

3.3. Kidneys

Histologically, fish kidneys comprised a large number of nephrons and hematopoietic tissues. Nephrons contained Bowman's capsules and renal tubules. Renal tubules consisted of proximal convoluted tubules (PCTs), distal convoluted tubule (DCTs), and collecting ducts. The renal tubules mainly consisted of columnar and cuboidal epithelial cells but a cross section were either oval or spherical (Figure 3A). The most evident damage noticed in the kidneys of *H. fossilis* under light microscopy under laboratory conditions after glyphosate exposure were loss of hematopoietic tissue, degenerative changes in the glomeruli, PCTs and DCTs, and vacuolation in the epithelial cell lining of the renal tubules (Figure 3B), while mild changes in PCTs and DCTs and fat deposition in some places were noted in kidneys after glyphosate exposure under field conditions (Figure 3C).

TEM of normal kidneys showed electron-dense mitochondria and nuclei and abundant vesicular structures in the capillary epithelial cell cytoplasm (Figure 3D).
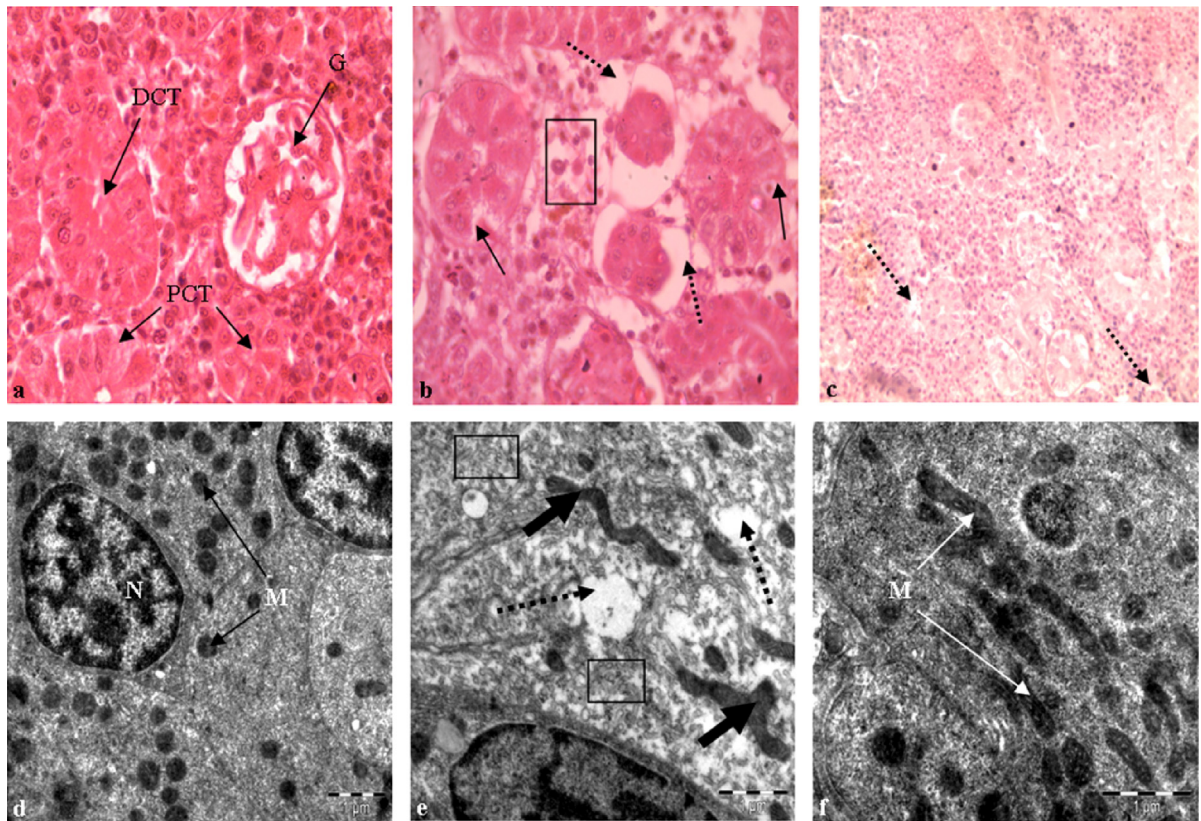
Figure 3. Histopathological photomicrographs of kidney of *Heteropneustes fossilis* under control conditions (Co), glyphosate-treated laboratory conditions (GL), and glyphosate-treated field conditions (GF). (A) Normal proximal convoluted tubule (PCT), distal convoluted tubule (DCT), Bowman's capsule and glomerulus (G) under light microscopy (C, 1000×). (B) Degeneration of PCT and DCT (arrow), vacuolation in hematopoietic tissues (broken arrow) and loss of hematopoietic tissue (square) under light microscopy (GL, 1000×). (C) Light microscopy showing normal structure of PCT and DCT and vacuolation (broken arrow; GF, 400×). (D) Normal appearance of kidney with electron-dense mitochondria (M) and nucleus (N) under TEM (Co, 4000×). (E) Dilation and fragmentation of rough endoplasmic reticulum (square), degeneration in mitochondria (bold arrow) and severe vacuolation (broken arrow) under TEM (GL, 5000×). (F) Normal appearance of kidney under TEM (GF, 6300×).
TEM = transmission electron microscopy.

The kidneys showed epithelial cell necrosis, dilation, and fragmentation of the endoplasmic reticulum, degeneration of mitochondria, and severe vacuolation under laboratory conditions after glyphosate exposure (Figure 3E), but under field conditions, the kidneys showed normal appearance of the nucleus with prominent nucleoli and a large number of mitochondria (Figure 3F).

4. Discussion

The present study is believed to be the first attempt to report the toxicity of the glyphosate-based herbicide Excel Mera 71 by histopathological and ultrastructural observations through SEM and TEM in the freshwater teleostean fish, *H. fossilis*. However, Senapati et al. [18,19] reported histopathological alterations in the stomach and intestine of *Anabas testudineus* after almix herbicide exposure under laboratory conditions.

The major changes were hypertrophy of the epithelial cells, fusion of some secondary lamellae, and severe damage to chloride cells. These alterations may be the early responses in the gills as a defense mechanism against toxic xenobiotic substances. Similar alterations in the gills

have also been reported in the fish exposed to metals [20], and after acute exposure to insecticides [21]. According to Mallatt [8], such alterations are nonspecific and may be induced by different types of xenobiotic contaminant. According to Arellano et al. [22] and Biagini et al. [23], these histological alterations observed in fish gills can be considered as a rapid and valid method to determine damage caused by exposure to different xenobiotic substances. The most conspicuous ultrastructural alterations in the gills of fish after glyphosate exposure under laboratory conditions were the complete loss of microridges in some gill epithelial cells, swelling of microridges, and irregular arrangement of microridges. Similar observations were reported by Schwaiger et al. [24] who suggested that these alterations might interfere with normal respiratory function and ultimately lead to impairment of the general health condition of the fish. Under field conditions, no significant alterations in stratified epithelial cells and microridge structures were observed after glyphosate exposure and this may have been due to natural habitat and dilution effects. TEM showed degenerative changes in chloride cells, fusion of microridges, dilation of RER and mitochondria, and vacuolation in chloride cell cytoplasm

of *H. fossilis* under laboratory conditions. Damage to chloride cells can result in increased blood flow inside the lamellae, causing dilatation of the marginal channel, blood congestion, or even an aneurysm [2,25]. Loss of microridge structures as well as fusion as observed in the present study was also reported by Mazon et al. [26] and Biagini et al. [23]. Mallatt [8] reported that the microridges were related to the retention of mucus on the epithelium to protect gill epithelium against environmental alterations. The appearance of vacuoles in glyphosate-exposed fish as seen in the present study may impede gas exchange capacity as well as indicate swelling of the mitochondria and RER [27,28]. Therefore, these histopathological and ultrastructural lesions in the gill morphology observed in the present study could lead to functional alterations and interference in osmoregulation and the antioxidant defense system of the gills [29].

The present study exhibited severe damage in the liver tissue of *H. fossilis* including enlarged and pyknotic hepatocytes, excess fat deposition, and hepatocytic inflammation along with vacuolation in the cytoplasm. Enhanced and pyknotic hepatocytes observed in the present study were also reported by Uguz et al. [30] who assumed that this may have been due to an increase in the DNA/RNA ratio, which was also observed in carcinogenic cells induced by 4-nonylphenol [31,32]. Jiraungkoorskul et al. [33] also noticed swelling of hepatocytes, nuclear pyknosis, severe vacuolation in the cytoplasm, degenerative changes in the cell membrane, and severe infiltration of leukocytes in the liver of *Orechromis niloticus* after Roundup (Glyphosate) exposure. Rahman et al. [34] reported severe necrosis, a large number of vacuoles in the cytoplasm, and pyknotic nuclei in the liver of *Corydoras punctatus* and *A. testudineus* exposed to Diazinon (IUPAC name: *O,O*-Diethyl *O*-[4-methyl-6-(propan-2-yl)pyrimidin-2-yl] phosphorothioate) 60 EC. Hued et al. [35] reported focal necrosis, infiltration of leukocytes, dilation of blood sinusoids, and vascular congestion in the liver of *Jenynsia multidentata* after Roundup intoxication under laboratory conditions. Among the major cytopathological responses in the hepatocytes of laboratory-exposed fish in the present study was the loss of cellular compartmentation, which might have been due to chemical attack on the cytoskeleton [36]. In addition to this, it has also been assumed that poorly developed cellular compartmentation indicates severe disturbance of cellular metabolism [37]. Hepatocytes of fish exposed to glyphosate under laboratory conditions generally showed greater disturbance of cellular compartmentation than fish exposed under field conditions. Ultrastructural changes in the endoplasmic reticulum of fish exposed to other toxicants have also been described by Braunbeck et al. [38]. Alterations of the RER, including proliferation, fragmentation, and vesiculation, are the common response to xenobiotic stress [39–41]. Although Braunbeck and Völkl [42] and Au et al. [43] correlated the alterations of the RER with higher biotransformation capacity of hepatocytes, Ghadially [44] interpreted the dilation of endoplasmic reticulum cisternae as a result of enhanced storage of proteins due to reduced secretory activity. Alteration of RER also indicates the induction of mixed-function oxidase [45], which can also be interpreted

as the morphological counterpart of ethoxycoumarin-O-deethylase and ethoxyresorufin-O-deethylase induction. Similar observations were also reported in rainbow trout after exposure to endosulfan and disulfoton [46], and in the demersal fish following intraperitoneal injection of benzo(a)pyrene [43]. Enhanced lipid droplets in hepatocytes of exposed fish species observed in the present study were also reported by others [37,47–50]. This enhanced number of lipid droplets indicated a decline of protein synthesis in the cytoplasm, which ultimately blocks the utilization of lipid–protein conjugation [51]. Glycogen content declined in hepatocytes under both conditions but in the present study the maximum reduction occurred in the laboratory. Similar observations were reported in other fish species such as *Channa punctata*, *Oncorhynchus mykiss*, *Danio rerio*, and *Liza ramada*, following exposure to several toxicants [37,47]. This may be due to either increased glycolytic activity to meet the energy demands imposed by enhanced metabolic activity [52,53] or reduced intestinal absorption of carbohydrates [54]. Generally, the lesions displayed in the investigated cells, tissues, and/or organs represent an integration of cumulative effects of physiological and biochemical contaminants, and therefore, can be linked to the exposure and subsequent metabolism of chemical contaminants [55]. Therefore, it can be interpreted that hepatocytes of laboratory-exposed fish showed stronger structural alterations than hepatocytes of field-exposed fish, which ultimately indicates greater disturbance of the cellular metabolism under laboratory conditions.

Due to central role of the kidneys in xenobiotic metabolism and excretion [56], and that like the liver, they receive the largest proportion of the post-branchial blood, renal lesions might be expected to be good indicators of environmental pollution [21]. A large number of studies used histopathological characteristics of the kidneys as indicators of aquatic pollution, especially by herbicides [33,35], but studies on the glyphosate-based herbicide, Excel Mera 71, are rare. The present study demonstrated the same histological changes in the kidneys after exposure to glyphosate. Similar results have also been reported in different fish species after exposure to different xenobiotic substances [21,33,57–59]. Nephrohistopathological alterations in kidneys of *H. fossilis* may have been due to herbicidal stress as a compensatory response, and may also be correlated with disruption of several biochemical and physiological pathways including endocrine disruption [60,61]. Several ultrastructural alterations such as epithelial cell necrosis, dilation, and fragmentation of endoplasmic reticulum, degenerative changes in the mitochondria, and severe vacuolation under laboratory conditions were hard evidence of glyphosate stress. Fischer-Scherl et al. [57] reported major ultrastructural changes such as degeneration and vacuolation in the epithelial cells, and fragmentation in the RER in the kidneys of rainbow trout exposed to atrazine. Cytoplasmic vacuolation has also been reported in the kidneys of gold fish exposed to hexachlorobutadiene by Reimschüssel et al. [62] and Segnini de Bravo et al. [63] in two Venezuelan cultured fish, *Caquetaia kraussii* and *Colossoma macropomum*, after triazine exposure. The presence of hyaline droplets

in renal tubules indicates the occurrence of renal toxicity after herbicide exposure [64].

5. Conclusion

In conclusion, the present study indicates that chronic exposure to glyphosate induces histopathological and ultrastructural changes in gills, liver, and kidneys. The cytopathological lesions in all three tissues recorded under two different exposure conditions demonstrate the cumulative physiological and biochemical effects of glyphosate exposure. The effects were more pronounced in laboratory-exposed than field-exposed fish, which ultimately indicated greater disturbance of cellular metabolism as well as serious structural alterations under laboratory conditions. Therefore, these histopathological including ultrastructural alterations under different environmental conditions for fish could be considered as sensitive biomarkers of xenobiotic exposure.

Acknowledgments

References

[1] Gernhöfer M, Pawert M, Schramm M, Müller E, Triebskorn R. Ultrastructural biomarkers as tools to characterize the health status of fish in contaminated streams. J Aquat Ecosyst Stress Recovery 2001;8:241–60.

[2] Camargo MM, Martinez CB. Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. Neotrop Ichthyol 2007;5:327–36.

[3] Fanta E, Rios FS, Romão S, Vianna ACC, Freiberger S. Histopathology of the fish *Corydoras paleatus* contaminated with sublethal levels of organophosphorus in water and food. Ecotoxicol Environ Saf 2003;54:119–30.

[4] Hinton DE, Lauren DJ. Liver structural alterations accompanying chronic toxicity in fishes: potential biomarkers of exposure. In: McCarthy JF, editor. Biomarkers of environmental contamination. Boca Raton: Lewis; 1990. p. 17–57.

[5] Sorour J. Ultrastructural variations in *Lethocerus niloticum* (Insecta: Hemiptera) caused by pollution in Lake Mariut, Alexandria. Egypt. Ecotoxicol Environ Saf 2001;48:268–74.

[6] Sindermann CJ. Pollution-associated diseases and abnormalities of fish and shellfish: a review. Fish Bull 1979;76:717–49.

[7] Bucke D, Vethaak D, Lang T, Mellegaard S. Common disease and parasites of fish in the North Atlantic: Training guide for identification. International Council for the Exploration of the Sea Techniques in Marine Environmental Sciences, Copenhagen, 1996.

[8] Mallatt J. Fish gill structural changes induced by toxicants and other irritants: a statistical review. Can J Fish Aquat Sci 1985;42:630–48.

[9] Poleksic V, Mitrovic-Tutundzic V. Fish gills as a monitor of sublethal and chronic effects of pollution. In: Müller R, Lloyd R, editors. Sublethal and chronic effects of pollutants on freshwater fish. Oxford: Fishing News Books; 1994. p. 339–52.

[10] Myers SM, Johnson LL, Olson OP, Stehr CM, Lomax DP, Horness BH, et al. Toxicopathic hepatic lesions and other biomarkers of exposure to chemical contaminants in marine bottomfish species from the northeast and Pacific coast, U.S.A. In: Bylund G, Lonnstrom LG, editors. Disease and parasites of flounder (Platichthys flesus) in the Baltic Sea. Turku/Abo: Baltic Marine Biologists Publication; 1994. p. 81–98.

[11] Bucher F, Hofer R. The effects of treated domestic sewage on three organs (gills, kidney, liver) of brown trout (*Salmo trutta*). Water Res 1993;27:255–61.

[12] Skidmore JF, Tovell WA. Toxic effects of zinc sulphate on the gills of rainbow trout. Water Res 1972;6:217–30.

[13] Roberts RJ. Fish pathology. London: Bailliere Tindall London; 1978.

[14] Chattopadhyay DN, Mohapatra BC, Adhikari S, Pani PC, Jena JK, Eknath AE. Effects of stocking density of *Labeo rohita* on survival, growth and production in cages. Aquacult Int 2013;21:19–29.

[15] Samanta P, Pal S, Mukherjee AK, Ghosh AR. Evaluation of metabolic enzymes in response to Excel Mera 71, a glyphosate-based herbicide, and recovery pattern in freshwater teleostean fishes. BioMed Research International 2014;2014:425159. http://dx.doi.org/10.1155/2014/425159.

[16] Samanta P, Pal S, Mukherjee AK, Ghosh AR. Biochemical effects of glyphosate based herbicide, Excel Mera 71 on enzyme activities of acetylcholinesterase (AChE), lipid peroxidation (LPO), catalase (CAT), glutathione-S-transferase (GST) and protein content on teleostean fishes. Ecotoxicol Environ Saf 2014;107:120–5.

[17] American Public Health Association (APHA), American Water Works Association (AWWA), Water Pollution Control Federation (WPCF). Standard methods for the examination of water and wastewater. Washington, DC; 2005.

[18] Senapati T, Mukherjee AK, Ghosh AR. Observations on the effect of Almix 20WP herbicide on ultrastructure (SEM) in different regions of alimentary canal of *Anabas testudineus* (Cuvier). Int J Food, Agri Vet Sci 2012;2:32–9.

[19] Senapati T, Samanta P, Mandal S, Ghosh AR. Study on histopathological, histochemical and enzymological alterations in stomach and intestine of *Anabas testudineus* (Cuvier) exposed to Almix 20WP herbicide. Int J Food, Agri Vet Sci 2013;3:100–11.

[20] Oliveira Ribeiro CA, Pelletier E, Pfeiffer WC, Rouleau C. Comparative uptake, bioaccumulation, and gill damages of inorganic mercury in tropical and Nordic freshwater fish. Environ Res 2000;83:286–92.

[21] Cengiz EI. Gill and kidney histopathology in the freshwater fish *Cyprinus carpio* after acute exposure to deltamethrin. Environ Toxicol Pharmacol 2006;22:200–4.

[22] Arellano JM, Ortiz JB, de Canales KLG, Sarasuete C. Histopathological alterations and induction of cytochrome P-450 1A in the liver and gills of the gilthead seabream (*Sparus aurata*) exposed to 2,3,7,8,-tetrachlorodibenzo-p-dioxin. Histochem J 2001;33:663–74.

[23] Biagini FR, David JAO, Fontanetti CS. The use of histological, histochemical and ultramorphological techniques to detect gill alterations in *Oreochromis niloticus* reared in treated polluted waters. Micron 2009;40:839–44.

[24] Schwaiger J, Ferling H, Mallow U, Wintermagr H, Negele RD. Toxic effects of the non-steroidal anti-inflammatory drug diclofenac. Part I: histopathological alterations and bioaccumulation in rainbow trout. Aquat Toxicol 2004;68:141–50.

[25] Rosety-Rodríguez M, Ordoñez FJ, Rosety M, Rosety JM, Ribelles A, Carrasco C. Morpho-histochemical changes in the gills of turbot, *Scophthalmus maximus* L., induced by sodium dodecyl sulfate. Ecotoxicol Environ Saf 2002;51:223–8.

[26] Mazon AF, Monteiro EA, Pinheiro GH, Fernandes MN. Hematological and physiological changes induced by short-term exposure to copper in the freshwater fish, *Prochilodus scrofa*. Braz J Biol 2002;62:621–31.

[27] Ultsch GR, Ott ME, Heisler N. Standard metabolic rate, critical oxygen tension and aerobic scope for spontaneous activity of trout (*Salmo gairdneri*) in acidified water. Comp Biochem Physiol 1980;67A:329–35.

[28] Pawert M, Miller E, Triebskorn R. Ultrastructural changes in fish gills as biomarker to assess small stream pollution. Tissue Cell 1998;30:617–26.

[29] Pandey S, Parvez S, Ansari RA, Ali M, Kaur M, Hayat F, et al. Effects of exposure to multiple trace metals on biochemical, histological and ultrastructural features of gills of a freshwater fish, *Channa punctata* Bloch. Chem Biol Interact 2008;174:183–92.

[30] Uguz C, Iscan M, Ergu A, Belgin IV, Togan I. The bioaccumulation of nonyphenol and its adverse effect on the liver of rainbow trout (*Onchorynchus mykiss*). Environ Res 2003;92:262–70.

[31] Chiriboga L, Yee H, Diem M. Infrared spectroscopy of human cells and tissue. Part VI: a comparative study of histology and infrared microspectroscopy of normal, cirrhotic, and cancerous liver tissue. Appl Spectrosc 2000;54:1–8.

[32] Chiriboga L, Yee H, Diem M. Infrared spectroscopy of human cells and tissue. Part VII: FT-IR microspectroscopy of DNase- and RNase-treated normal, cirrhotic, and neoplastic liver tissue. Appl Spectrosc 2000;54:480–5.

[33] Jiraungkoorskul W, Upatham ES, Kruatrachue M, Sahaphong S, Vichasri-Grams S, Pokethitiyook P. Histopathological effects of Roundup, a glyphosate herbicide, on Nile tilapia (*Oreochromis niloticus*). Sci Asia 2002;28:121–7.

[34] Rahman MZ, Hossain Z, Mollah MFA, Ahmed GU. Effect of Diazinon 60 EC on *Anabas testudineus*, *Channa punctatus*, *Barbodes goniontus*. NAGA, The ICLARM Quarterly 2002;25:8–11.

[35] Hued AC, Oberhofer S, de los Ángeles Bistoni M. Exposure to a commercial glyphosate formulation (Roundup®) alters normal gill and liver histology and affects male sexual activity of *Jenynsia multidentata* (Anablepidae, Cyprinodontiformes). Arch Environ Contam Toxicol 2012;62:107–17.

[36] Rez G. Electron microscopic approaches to environmental toxicity. Acta Biol Hung 1986;37:31–45.

[37] Braunbeck T. Cytological alterations in fish hepatocytes following *in vivo* and *in vitro* sublethal exposure to xenobiotics – structural biomarkers of environmental contamination. In: Braunbeck T, Hinton DE, Streit B, editors. Fish ecotoxicology. Basel: Birkhauser Verlag; 1998. p. 61–140.

[38] Braunbeck T, Storch V, Nagel R. Sex-specific reaction of liver ultrastructure in zebra fish (*Brachydanio rerio*) after prolonged sublethal exposure to 4-nitrophenol. Aquat Toxicol 1989;14:185–202.

[39] Hacking MA, Budd J, Hodson K. The ultrastructure of the liver of the rainbow trout: normal structure and modifications after chronic administration of a polychlorinated biphenyl Aroclor 1254. Can J Zool 1978;56:477–91.

[40] Burckhardt-Holm P, Oulmi Y, Schroeder A, Storch V, Braunbeck T. Toxicity of 4-chloroaniline in early life stages of zebrafish (*Danio rerio*): II. Cytopathology and regeneration of liver and gills after prolonged exposure to waterborne 4-chloroaniline. Arch Environ Contam Toxicol 1999;37:85–102.

[41] Wu WZ, Zhou BS, Xu Y, Schramm KW, Kettrup A. Cytological and biochemical alterations in *Carassius auratus* hepatocytes from exposure to sediment containing dioxins and related compounds. Arch Environ Contam Toxicol 1999;37:358–63.

[42] Braunbeck T, Völkl A. Toxicant-induced cytological alterations in fish liver as biomarkers of environmental pollution? A case study on hepatocellular effects of dinitro-o-cresol in golden ide (*Leuciscus idus melanotus*). In: Braunbeck T, Hanke W, Segner H, editors. Fish ecotoxicology and ecophysiology. Weinheim: VCH; 1993. p. 55–80.

[43] Au DWT, Wu RSS, Zhou BS, Lam PKS. Relationship between ultrastructural changes and EROD activities in liver of fish exposed to Benzo[a]pyrene. Environ Pollut 1999;104:235–47.

[44] Ghadially FN. Ultrastructural pathology of the cell and matrix. London: Butterworths; 1988.

[45] Hawkes JW. The effects of xenobiotics on fish tissues: morphological studies. Fed Proceed 1980;39:3230–6.

[46] Arnold H, Plutab HJ, Braunbeck T. Simultaneous exposure of fish to endosulfan and disulfoton *in-vivo*: ultrastructural, stereological and biochemical reactions in hepatocytes of male rainbow trout (*Oncorhynchus mykiss*). Aquat Toxicol 1995;33:17–43.

[47] Biagianti-Risbourg S, Bastide J. Hepatic perturbations induced by a herbicide (atrazine) in juvenile grey mullet *Liza ramada* (Mugilidae, Teleostei): an ultrastructural study. Aquat Toxicol 1995;31:217–29.

[48] Biagianti-Risbourg S, Pairault C, Vernet G, Boulekbache H. Effect of lindane on the ultrastructure of the liver of the rainbow trout, *Oncorhynchus mykiss*, sac-fry. Chemosphere 1996;33:2065–79.

[49] Strmac M, Braunbeck T. Cytological and biochemical effects of a mixture of 20 pollutants on isolated rainbow trout (*Oncorhynchus mykiss*) hepatocytes. Ecotoxicol Environ Saf 2002;53:293–304.

[50] Thophon S, Pokethitiyook P, Chalermwat K, Upatham ES, Sahaphong S. Ultrastructural alterations in the liver and kidney of white sea bass, *Lates calcarifer*, in acute and subchronic cadmium exposure. Environ Toxicol 2004;19:11–9.

[51] Cheville NF. Ultrastructural pathology. Ames: Iowa State University Press; 1994.

[52] Hanke W, Gluth G, Mtiller R. Physiological changes in carps induced by pollution. Ecotoxicol Environ Saf 1983;7:229–41.

[53] Gluth G, Hanke W. A comparison of physiological changes in carp, *Cyprinus carpio*, induced by several pollutants at sublethal concentrations. Ecotoxicol Environ Saf 1985;9:179–88.

[54] Braunbeck T, Appelbaum S. Ultrastructural alterations in the liver and intestine of carp *Cyprinus carpio* induced orally by ultra-low doses of endosulfan. Dis Aquat Organ 1999;36:183–200.

[55] Adeyemo OK. Histological alterations observed in the gills and ovaries of *Clarias gariepinus* exposed to environmentally relevant lead concentrations. J Environ Health 2008;70:48–51.

[56] Hinton DE, Baumann PC, Gardner GR, Hawkins WE, Hendricks JD, Murchelano RA, et al. Histopathological biomarkers. In: Huggett RJ, Kimerle RA, Mehrle Jr PM, Bergman HL, editors. Biomarkers: biochemical, physiological, and histological markers of anthropogenic stress. USA: Lewis; 1992. p. 155–96.

[57] Fischer-Scherl T, Veeser A, Hoffman RW, Kühnhauser C, Negele RD, Euringmann T. Morphological effects of acute and chronic atrazine exposure in rainbow trout (*Oncorhynchus mykiss*). Arch Environ Contam Toxicol 1991;20:454–61.

[58] Nesković NK, Elezović I, Karan V, Poleksić V, Budimir M. Acute and subacute toxicity of atrazine to carp (*Cyprinus caprio* L.). Ecotoxicol Environ Saf 1993;25:173–82.

[59] Oulmi Y, Negele RD, Braunbeck T. Cytopathology of liver and kidney in rainbow trout (*Oncorhynchus mykiss*) after long-term exposure to sub-lethal concentrations of linuron. Dis Aquat Org 1995;21:35–52.

[60] Mekkawy IA, Mahmoud UM, Sayed AH. Effects of 4-nonylphenol on blood cells of the African catfish *Clarias gariepinus* (Burchell, 1822). Tissue Cell 2011;43:223–9.

[61] Sayed AH, Mekkawy IA, Mahmoud UM. Histopathological alterations in some organs of adults of *Clarias gariepinus* (Burchell, 1822) exposed to 4-nonylphenol. In: Garcia MD, editor. Zoology. Croatia: InTech Publisher; 2012. p. 163–4.

[62] Reimschüssel R, Bennet RO, May EB, Lipsky MM. Renal histopathological changes in the goldfish (*Carassius auratus*) after sublethal exposure to hexachlorobutadiene. Aquat Toxicol 1989;15:169–80.

[63] Segnini de Bravo MI, Medina J, Marcano S, Finol HJ, Boada-Sucre A. Effects of herbicide on the kidneys of two Venezuelan cultured fish: *Caquetaia kraussii* and *Colossoma macropomum* (Pisces: Ciclidae and Characeae). Rev Biol Trop 2005;53:55–60.

[64] Chaudhuri BN, Kleywegt GJ, Bjorkman J, Lehman-McKeeman LD, Oliver JD, Jones TA. The structures of alpha 2u-globuline and its complex with a hyaline droplet inducer. Acta Crystallogr D Biol Crystallogr 1999;55:753–62.