IntroductIon

Glyphosate, N-(phosphonomethyl) glycine, a nonselective, postemergence herbicide, is extensively used worldwide to control broad-leaved annual and perennial weeds in agricultural fields, forestry, and aquatic systems.[1,2] Glyphosate is soluble in water (12 g/L at 25°C) but insoluble in most organic solvents. In addition, it binds tightly to organic matter and sediment/soil within six-inch depth and therefore becomes unavailable to plants or other aquatic organisms, and finally, its activity reduced significantly.[3] Moreover, the half-life of glyphosate in soil ranged between 2 and 197 days, in water ranged from 4 to 91 days, while in vegetation was <24 days.[4] Glyphosate is readily degraded both in water and soil by soil microbes to aminomethylphosphonic acid and carbon dioxide (CO₂).[5] Due to strong adsorptive characteristics, glyphosate are not likely to move to groundwater but have the potency to contaminate surface water.[4] In addition, it is under the toxicity class of III (on I–IV scale, where IV is least dangerous) for oral and inhalation exposure.[6] Its high efficacy and cost-effectiveness favor its repeated applications in commercial formulations.[7] In addition, commercial glyphosate formulations are more toxic because it contains nonionic polyethylene amine surfactant, which is toxic to aquatic organisms, especially...
Materials and Methods

Fish collection and maintenance

O. niloticus of both the sexes with an average weight of 38.57 ± 2.47 g and total length of 13.59 ± 0.496 cm, respectively, were procured from local fish farm market. After that, fishes were brought to the laboratory and were acclimatized for at least 15 days in aquarium (250 L). Fishes were continuously aerated and maintained at natural photoperiod (12-h light/12-h dark). During acclimatization period, the average value of water parameters were as follows: temperature, 26.49 ± 0.13°C; pH, 7.94 ± 0.04; electrical conductivity, 392.22 ± 0.62 μS/cm; total dissolved solids, 279.33 ± 0.69 mg/L; dissolved oxygen, 6.44 ± 0.05 mg/L; total alkalinity, 204.73 ± 7.00 mg/L as CaCO₃; total hardness, 180.44 ± 3.74 mg/L as CaCO₃; orthophosphate, 0.03 ± 0.001 mg/L; ammoniacal nitrogen, 1.66 ± 0.21 mg/L; and nitrate nitrogen, 0.21 ± 0.03 mg/L. After acclimatization, fishes were divided into two groups: one group was transferred to field ponds situated at Crop Research and Seed Multiplication Farm in the premises of the University of Burdwan and other group was transferred to the laboratory aquarium. During acclimatization and experimentation periods, fishes were fed commercial fish pellets (32% crude protein, Tokyu) once a day.

Field experimental design

For field experiments, fish specimens of field group were again divided into two sets: one set of fish specimens was transferred to treatment pond and another set was transferred to control pond. Both ponds are free of contamination. A total of six cages (three for treatment pond and three for control pond) were prepared and installed at experimental ponds. Each cage contains 10 fish species. The dose (750 g/acre) recommended for rice cultivation was dissolved in water and applied to the treatment pond. It was sprayed on the 1st day of the experiment. Duration of the experiment was 30 days. For field experiments, a special type of cage was prepared. Cages were prepared based on Chattopadhyay et al. with some modifications. All cages were square in shape having an area of 2.5 m × 1.22 m and cage height was 1.83 m (submerged height was 0.83 m). Cages were framed by light strong bamboo. Four-sided wall, cage floor, and top of the cage cover were fabricated with nylon net and were embraced by two PVC nets: the inner and outer net-bearing mesh sizes of 1.0 mm × 1.0 mm and 3.0 mm × 3.0 mm, respectively. During the experimentation period, pond water showed the following average values: temperature, 24.03°C ± 0.20°C; pH, 6.56 ± 0.09; electrical conductivity, 347.00 ± 1.15 μS/cm; total dissolved solids, 247.67 ± 1.45 mg/L; dissolved oxygen, 7.00 ± 0.157 mg/L; total alkalinity, 221.33 ± 3.53 mg/L as CaCO₃, total hardness, 140 ± 2.31 mg/L as CaCO₃; orthophosphate, 0.24 ± 0.03 mg/L; ammoniacal nitrogen 0.74 ± 0.11 mg/L; and nitrate nitrogen, 1.66 ± 0.04 mg/L.

Laboratory experimental design

Fishes under laboratory condition were again divided into two groups (control and glyphosate-treated) and maintained in six aquaria (three for control and three for treatment) at Ecotoxicology Laboratory, Department of Environmental Science, the University of Burdwan. Each aquarium contains 10 fishes. Fishes were exposed to sublethal dose of glyphosate, i.e., 17.20 mg/L, for 30 days. Dose was applied on every alternate day.

Experiments were carried out according to the guidelines prescribed by the University of Burdwan and were approved by Ethical Committee. During exposure period, control and glyphosate-treated aquaria were subjected to the same environmental conditions. During experimentation period, water parameters showed the following average values: temperature, 26.63°C ± 0.12°C; pH 7.93 ± 0.06; electrical conductivity, 426.00 ± 5.93 μS/cm; total dissolved solids, 302.89 ± 4.69 mg/L; dissolved oxygen, 5.06 ± 0.43 mg/L; total alkalinity, 209.80 ± 1.45 mg/L as CaCO₃; orthophosphate, 0.24 ± 0.03 mg/L; ammoniacal nitrogen 0.74 ± 0.11 mg/L; and nitrate nitrogen, 1.66 ± 0.04 mg/L.

O. niloticus, belongs to the family Cichlidae, and is extensively used as protein source. Therefore, the present study was aimed to investigate the toxic effects of Excel Mera 71 on O. niloticus both under the laboratory and field conditions on comparative basis through histological and ultrastructural observations in the gill, liver, and kidney.

In the present study, Oreoichromis niloticus (Linnaeus) was considered as model test organism for toxicity study because they grow fast, mature quickly, breed easily without inducement, and finally have good potentiality for cultivation. In addition, it is surface-feeding omnivorous fish, belongs to the family Cichlidae, and is extensively used as protein source. Therefore, the present study was aimed to investigate the toxic effects of Excel Mera 71 on O. niloticus both under the laboratory and field conditions on comparative basis through histological and ultrastructural observations in the gill, liver, and kidney.
Sampling
During experimentation period, water-quality parameters were measured as per the APHA.\textsuperscript{[26]} At the end of the experiment, after 30 days, fishes were collected both from aquaria and field ponds. After collection, fishes were anesthetized with tricaine methanesulfonate (MS 222), then desired organs namely gill, liver, and kidney, were dissected out, and tissues were fixed in respective fixatives accordingly and finally proceeded for histological, scanning, and transmission electron microscopic (TEM) observations.

Histological analysis
For histological observation, fish tissues were fixed in aqueous Bouin’s fluid solution overnight. After fixation, tissues were dehydrated through graded series of ethanol and finally embedded in paraffin. Paraffin sections were then cut at 3–4 $\mu$m using microtome (Leica RM2125). Finally, sections were stained with hematoxylin-eosin (H and E) solution and examined under a light microscope (Leica DM2000).

Ultrastructural analysis
For scanning electron microscopic (SEM) study, tissues were fixed in 2.5% glutaraldehyde solution (prepared in phosphate buffer, 0.2 M and pH 7.4) for 24 h at 4°C and then postfixed with 1% osmium tetroxide solution (prepared in phosphate buffer, 0.2 M and pH 7.4) for 2 h at 4°C. After fixation, tissues were dehydrated through graded series of acetone, subsequently followed by amyl acetate. After that, tissues were dried using liquid CO$_2$ at critical point drier. Tissues were then mounted on metal stubs and sputter-coated with gold (thickness approximately 20 nm). Finally, tissues were examined under SEM (Hitachi S-530) at University Science Instrumentation Centre, the University of Burdwan, West Bengal, India.

For TEM study, fish tissues were fixed in Karnovsky fixative (mixture of 2% paraformaldehyde and 2.5% glutaraldehyde prepared in 0.1 M phosphate buffer, pH 7.4) for 12 h at 4°C and then postfixed with 1% osmium tetroxide solution (prepared in phosphate buffer, 0.2 M and pH 7.4) for 2 h at 4°C. After fixation, tissues were dehydrated through graded series of acetone, infiltrated, and finally embedded in epoxy resin (Araldite CY212). Ultrathin sections were then cut (thickness 70 nm) and collected on naked copper-meshed grids. Grids were then stained with uranyl acetate and lead citrate. Finally, grids were examined under TECNAI G2 high-resolution TEM at Electron Microscope Facility, Department of Anatomy, AIIMS, New Delhi, India.

Results
Gill
Gill of the control fish consists of primary gill lamellae which are composed of cartilaginous skeletal structure, multilayered epithelium, and vascular system. Between secondary epithelium, primary lamella is lined by stratified epithelium and numerous chloride cells in the basement. Secondary gill lamella consists of epithelial cells supported by pillar cells [Figure 1a]. Gills of fishes under glyphosate-treated laboratory condition showed degenerative changes in pillar cells, curling of secondary lamellae, blood congestion, lamellar disarrangement, and appearance of globular structure under light microscopic observations [Figure 1b], but lesions were not so much prominent in field condition [Figure 1c].

Topographical study observed under SEM depicted that each control gill filament is composed of primary and secondary gill lamellae and is embraced by stratified epithelial cells and horizontal flat filaments [Figure 1d]. Ultrastructural alterations in the gill were also supporting the light microscopic lesions as excessive mucus secretion over gill epithelium, loss of microridges structure, disappearance of normal microridges array, and damage in stratified epithelial cells [Figure 1e], while under field condition, stratified epithelial cells and microridges structures showed almost normal appearance [Figure 1f].

TEM observations of the primary epithelium of gills showed general appearance of chloride cells supported by tightly packed pavement cells under control condition [Figure 1g]. Gills of fishes under treated laboratory condition showed severe cytoplasmic vacuolation, degeneration in tubular vascular structures, necrosis, dilation in tight junction, abnormal-shaped nucleus [Figure 1h], while comparatively less pathological lesions were observed under field condition which included dilated mitochondria and rough endoplasmic reticulum (ER) and vacuolation in some places [Figure 1i].

Liver
Histologically, liver of the control fish is generally made up of hepatocytes with centrally placed nucleus and densely stained nucleolus. In addition, acinar cells of hepatopancreas are polyhedral and compactly arranged. Moreover, acinar cells are arranged in two/three rows encircling blood capillaries and apical part contains zymogen granules [Figure 2a]. Most notable lesions observed in the liver of *O. niloticus* under light microscopic study were severe degeneration in hepatocytes, excessive fat deposition, pyknotic nuclei, acenric nuclei, and degenerative hepatopancreas under laboratory condition [Figure 2b]. The extent of damage in field condition was comparatively less than laboratory study which included enlarged acenric nuclei and dilated hepatocytes [Figure 2c].

Under SEM observation, liver of the control fish showed normal palisade arrangement of hepatocytes, hepatic cords, and mucus mass over hepatocytes [Figure 2d]. Topological observation of the liver from laboratory condition under SEM study confirmed the pathological lesions of light microscopy showing severe damage in hepatocytes and hepatic cords and excessive mucus secretion [Figure 2e], but in field condition, lesions were comparatively less [Figure 2f].

TEM study of the control liver showed normal appearance of hepatocytes with centrally placed prominent nucleus and nucleolus [Figure 2g]. In addition, cytoplasm contains large amount of mitochondria, rough ER, and glycogen [Figure 2g]. Degenerative changes, such as necrosis in mitochondria,
cytoplasmic vacuolation, dilation in ER, and reduced number of glycogen droplets, were prominent under laboratory study [Figure 2h]. On the other hand, in the field condition, hepatocytes showed almost normal appearance of mitochondria and dilation in some places and fragmented and vesiculated ER [Figure 2i]. Moreover, the lesions were comparatively higher under laboratory condition than the field.

Kidney

Histologically, normal kidney is generally made up of large number of nephrons and hematopoietic tissues. In addition, nephron comprises Bowman’s capsule and renal tubules. Moreover, Bowman’s capsule contains glomeruli or Malpighian body and renal tubule consists of proximal convoluted tubule (PCT), distal convoluted tubule (DCT), and collecting duct. Renal tubules are mainly consisted of columnar and cuboidal epithelial cells [Figure 3a]. The most conspicuous alterations observed in the kidney of O. niloticus were fragmented glomerulus, severe degenerative changes in PCT and DCT such as swelling, excessive fat deposition, and hypertrophied nuclei [Figure 3b], while under field condition, comparatively less damage was observed in the kidney of O. niloticus [Figure 3c].

Under SEM observation, normal kidney showed glomerulus as cell mass, rounded PCT, oval-shaped DCT [Figure 3d]. SEM observations also showed degenerative changes in the kidney, i.e., shrinkage of glomerulus and distortion of PCT and DCT after glyphosate exposure under laboratory condition [Figure 3e], but in field condition, it was insignificant [Figure 3f].

TEM observation revealed that normal kidney contains electron dense mitochondria and nucleus and abundant vesicular structures in capillary epithelial cell cytoplasm [Figure 3g]. TEM study confirmed the necrosis in mitochondria, dilated and fragmented ER, and severe vacuolation in laboratory...
condition endorsed by light microscope [Figure 3h], but no significant alterations were observed under field condition, except dilation of ER [Figure 3i].

**Discussion**

The present study is the maiden attempt to evaluate the comparative toxicity of Excel Mera 71 herbicide under two conditions, i.e., field and laboratory conditions with regard to histopathological alterations through light microscopic, SEM, and TEM observations in Indian freshwater fish, *O. niloticus*. However, Senapati *et al.*[^27^,^28^] recorded some histopathological alterations in the stomach and intestine of *Anabas testudineus* after Almix exposure under laboratory condition only.

Histopathological changes in the gill induced by glyphosate exposure were more prominent under laboratory condition than field observation. Hypertrophy and hyperplasia in the gill epithelium were common responses observed under both the conditions and were demonstrated by Hued *et al.*[^29^] and Ramírez-Duarte *et al.*[^30^] in the gill of *Jenynsia multidentata* and *Piaractus brachypomus* after Roundup exposure, respectively. In addition, mucus secretion and lamellar disarrangement indicated protective mechanism of gill epithelium to glyphosate exposure. The results were similar to the findings of Kossakowski and Ostaszewska[^11^] and Biagini *et al.*[^32^]. Moreover, curling of secondary gill lamellae as observed in the present study under both the conditions was also reported in *Cyprinus carpio* after chlorpyrifos exposure by Pal *et al.*[^33^]. In addition, appearance of globate structure at gill lamellae tip was also reported by Sorour and Harbev[^34^] in *Oreochromis* sp. collected from polluted and unpolluted Wadi Hanifah stream, Riyadh.

The most notable ultrastructural alterations (SEM) in gill stratified epithelium such as thinning and degeneration of microridges, uplifftment of epithelial cells, and reduced number of mucous and chloride cells were also described by Johal *et al.*[^35^] in *C. carpio* after monocrotophos exposure. In addition, loss of microridge structure from pavement cells of gill epithelium.
was also reported by several authors.[32,34,36] Moreover, Mallatt[37] demonstrated that microridges are playing vital role in cellular protection against environmental contaminants by retention of mucus on gill epithelium. On the other hand, transmission electron micrographic observations such as hypersecretion of mucus and necrosis in gill epithelium indicated impaired gill exchange capacity by gill epithelium.[37,38] Therefore, these pathological lesions in gill morphology could lead to functional abnormalities and interference of normal fundamental processes such as maintenance of osmoregulation and antioxidant defense mechanism of gill epithelium.[39]

In liver, severe necrosis in hepatocytes indicated negative impact of herbicide (Excel Mera 71), which caused functional and structural impairments.[40] Similar observations along with cytoplasmic vacuolation and pyknotic nuclei were also reported by Rahman et al.[41] in Corydoras punctatus and A. testudineus after Diazinon 60 EC exposure. Cytoplasmic vacuolization in hepatocytes observed in the present study was also demonstrated by Biagianti-Risbourg and Bastide[42] in Liza ramada exposed to atrazine. In addition, vacuolization of hepatocytes indicates imbalance between rate of synthesis of substances in parenchymal cells and their release into systemic circulation, which ultimately suggest the stress condition of the fish.[43] In another study, Jiraungkoorskul et al.[19] noticed swelling of hepatocytes, pyknotic nuclei, severe cytoplasmic vacuolation, degenerated cell membrane, and severe leukocytes infiltration in the liver of O. niloticus after Roundup exposure. Degenerative changes in hepatopancreas and distortion in acinar cells seen under laboratory study indicated tissue damage particularly in columnar epithelial cells and this might be an adaptive compensatory response by organism itself to neutralize the glyphosate-induced stress.[43]

SEM observation showed severe damage in hepatocytes and hepatic cords and excessive mucus secretion under laboratory condition, glyphosate-treated field condition. (a) Normal proximal convoluted tubule, distal convoluted tubule, Bowman’s capsule, and glomerulus under light microscopy (C, ×1000). (b) Fragmented glomerulus (oval), degenerative and hypertrophied proximal convoluted tubule and distal convoluted tubule (arrow), and vacuolation in the hematopoietic tissues (broken arrow) under light microscopy (GL, ×1000). (c) Light microscopy showing degenerative and hypertrophied proximal convoluted tubule and distal convoluted tubule (arrow), vacuolation in the hematopoietic tissues (broken arrow) (GF, ×1000). (d) Normal kidney with prominent proximal convoluted tubule and distal convoluted tubule under scanning electron microscopic observation (C, ×600). (e) Degenerative changes and shrinkage of glomerulus under scanning electron microscopic observation (GL, ×600). (f) Degeneration under scanning electron microscopic observation (GF, ×800). (g) Normal appearance of the kidney with electron dense mitochondria (M) and nucleus (N) under transmission electron microscopy (C, ×2550). (h) Necrosis in mitochondria (bold arrow), dilation, vesiculation, and fragmentation of rough endoplasmic reticulum (square) and severe vacuolation (broken arrow) under transmission electron microscopy (GF, ×500).

Figure 3: Histopathological photomicrographs of the kidney of Oreochromis niloticus under control condition (C), glyphosate-treated laboratory condition, glyphosate-treated field condition. (a) Normal proximal convoluted tubule, distal convoluted tubule, Bowman’s capsule, and glomerulus under light microscopy (C, ×1000). (b) Fragmented glomerulus (oval), degenerative and hypertrophied proximal convoluted tubule and distal convoluted tubule (arrow), and vacuolation in the hematopoietic tissues (broken arrow) under light microscopy (GL, ×1000). (c) Light microscopy showing degenerative and hypertrophied proximal convoluted tubule and distal convoluted tubule (arrow), vacuolation in the hematopoietic tissues (broken arrow) (GF, ×1000). (d) Normal kidney with prominent proximal convoluted tubule and distal convoluted tubule under scanning electron microscopic observation (C, ×600). (e) Degenerative changes and shrinkage of glomerulus under scanning electron microscopic observation (GL, ×600). (f) Degeneration under scanning electron microscopic observation (GF, ×800). (g) Normal appearance of the kidney with electron dense mitochondria (M) and nucleus (N) under transmission electron microscopy (C, ×2550). (h) Necrosis in mitochondria (bold arrow), dilation, vesiculation, and fragmentation of rough endoplasmic reticulum (square) and severe vacuolation (broken arrow) under transmission electron microscopy (GF, ×500).
study. The results were corroborated with the findings of Uguz et al.,[44] who reported similar findings and indicated that this might be due to increase in DNA/RNA ratio. Due to higher sensitivity of the hepatocytic ultrastructure, higher glyphosate concentration in the aquatic environment resulted in more extensive degenerative responses under laboratory condition. Most conspicuous alterations in hepatocytes such as nectrotic mitochondria, vacuolated nuclear membrane, dilated and degenerative ER, severe cytoplasmic vacuolations, and reduced number of glycogen droplets were seen under laboratory study. Nectrotic mitochondria observed in the present study was also reported by Bozzola and Russell[45] and indicated that this might be due to inhibition of large number of respiratory enzymes which oxidizes substrates to form ATP during phospholipid metabolism and fatty acid synthesis in mitochondria. In addition, dilation and swelling of rough ER was another most important lesion observed in the present study could be interpreted as morphological counterpart of ethoxyoumarin-O-deethylase and ethoxyresorufin-O-deethylase induction.[46] Moreover, the cytoplasmic vacuolation in hepatocytes indicated decreased protein synthesis due to reduced utilization of lipid-protein conjugation that accompanied hepatocyte injury.[47] However, reduced glycogen content in hepatocytes of A. testudineus under laboratory condition might be due to increased glycolytic activity to meet enhanced energy demand as compensatory mechanism.[48,49] Higher pathological lesions in hepatocytes under laboratory condition compared with field observation might be attributed to availability of natural food under field condition from natural water body.[50] Therefore, less cytopathological alterations were observed in field than laboratory fish.

In the present study, kidney showed fragmentation of glomerulus, severe degenerative changes in PCT such as swelling, excess fat deposition, and hypertrophied nuclei under laboratory condition. However, comparatively less pathological lesions in PCT, DCT, and glomerulus were observed in field condition. Similar results of excessive fat deposition and hypertrophied and pynotic nuclei were also revealed by Jiraungkoorskul et al.[19] in the kidney of O. niloticus after Roundup exposure under laboratory study. In another study, Oulmi et al.[51] reported small cytoplasmic vacuoles and nuclear deformation in Oncorhynchus mykiss to linuron exposure and explained these nephro-histopathological alterations due to herbicidal stress as compensatory response. The nephro-histopathological alterations in the present investigation indicated disruption of several biochemical and physiological pathways including endocrine disruption and these are correlated with other findings.[52,53]

Ultrastructural alterations such as shrinkage of glomerulus and damage in PCT and DCT observed under SEM study were also reported by Fischer-Schlerl et al.[54] On the other hand, TEM observation showed cytopathological alterations such as necrosis in mitochondria, dilation and fragmentation of ER, and appearance of severe vacuolation under laboratory condition in the kidney of Carassius auratus exposed to hexachlorobutadiene reported by Reimschüssel et al.[55] and Bravo et al.[56] in two Venezuelan cultured fishes, Caquetaia kraussii and Colossoma macropomum after triazine exposure. Fischer-Schlerl et al.[54] also reported considerable ultrastructural changes in the kidney of rainbow trout to atrazine exposure and these responses resembled the symptoms of the present study under laboratory condition. However, the presence of hyaline droplets in renal tubules indicated occurrence of renal toxicity after herbicide exposure.[57]

**Conclusions**

Histopathological and ultrastructural responses of Excel Mera 71 in gill, liver, and kidney of O. niloticus were studied and represented as an integrated cumulative effect of physiological and biochemical contaminants. Generally, these responses were more pronounced in laboratory-exposed fish than field exposed and indicating higher disturbances of cellular metabolism as well as stronger structural and functional alterations under laboratory condition. Therefore, these histopathological and ultrastructural responses could be considered as bioindicators to analyze the fish health status under contaminated aquatic environment.

**Acknowledgment**

We like to thank the Department of Science and Technology, Govt. of India, for the financial assistance through DST INSPIRE Fellowship Program (DST/INSPIRE Fellowship/2011/164, Dt. 29.09.2011) to Dr. Palas Samanta. We also like to thank the Head, Department of Environmental Science, the University of Burdwan, West Bengal, India, for providing laboratory and library facilities during the research work. We are also thankful to the respective reviewers of this paper for improving the quality of this paper.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Steinrücken HC, Amrhein N. The herbicide glyphosate is a potent inhibitor of 5-enolpyruvylshikimic acid-3-phosphate synthase. Biochem Biophys Res Commun 1980;94:1207-12.

2. Cavas T, Könen S. Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (Carassius auratus) exposed to a glyphosate formulation using micronucleus test and comet assay. Mutagenesis 2007;22:263-8.

3. Duke SO. Glyphosate. In: Kearney PC, Kaufman DD, editors. Herbicides: Chemistry, Degradation and Mode of Action. New York: Marcel Dekker; 1988. p. 1-70.

4. Giess JP, Dobson S, Solomon KR. Ecotoxicological risk assessment for Roundup herbicide. Rev Environ Contam Toxicol 2000;167:35-120.

5. Ruppel ML, Brightwell BB, Schaefer J, Marvel JT. Metabolism and degradation of glyphosate in soil and water. J Agric Food Chem 1977;25:517-28.

6. United States Environmental Protection Agency. EPA Reregistration Eligibility Decision (RED) Facts Glyphosate (EPA-738-F-93-011). Washington DC: United States Environmental Protection Agency; 1993.

7. Reuters. Roundup: Cancer Cause or Crucial for Food Production?
Samanta, et al.: Excel Mera 71 toxicity on Oreochromis niloticus

Journal of Microscopy and Ultrastructure ¦ Volume 6 ¦ Issue 1 ¦ January-March 2018

8. Peixoto F. Comparative effects of the roundup and glyphosate on mitochondrial oxidative phosphorylation. Chemosphere 2005;61:1115-22.

9. Malty L, Naylor C. Preliminary observations on the ecological relevance of the Gammaurus ‘scope for growth’ assay: Effect of zinc on reproduction-functional ecology. New Horizons Ecotoxicology 1990;4:393-7.

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

11. Sancho E, Cerón JJ, Ferrando MD. Cholinesterase activity and hematological parameters as biomarkers of sublethal molinate exposure in Anguilla anguilla. Ecotoxicol Environ Saf 2000;46:81-6.

12. Crestani M, Menezes C, Glusczak L, Dos Santos Miron D, Lazzari R, Duarte MF, et al. Effects of clomazone herbicide on hematological and some parameters of protein and carbohydrate metabolism of silver carps Rhombus quelen. Ecotoxicol Environ Saf 2006;65:48-55.

13. Glusczak L, dos Santos Miron D, Crestani M, Braga da Fonseca M, de Araújo Pedron F, Duarte MF, et al. Effect of glyphosate herbicide on acetylcholinesterase activity and metabolic and hematological parameters in piava (Leporinus obtusidens). Ecotoxicol Environ Saf 2006;65:237-41.

14. Lushchak OV, Kubrak OL, Storey JM, Storey KB, Lushchak VI. Low toxic herbicide roundup induces mild oxidative stress in goldfish tissues. Chemosphere 2009;76:932-7.

15. Modesto KA, Martinez CB. Roundup causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish Prochilodus lineatus. Chemosphere 2010;78:294-9.

16. 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 Res Int 2014;2014:425159.

17. 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.

18. Nesković NK, Poleksić V, Elezovíc I, Karan V, Budimir M. Biochemical and histopathological effects of glyphosate on carp, Cyprinus carpio L. Bull Environ Contam Toxicol 1996;56:295-302.

19. Jirurngkoorskul W, Upatham ES, Krutachrue M, Sahaphong S, Vichasri-Grams S, Pokhethitiyook P. Histopathological effects of roundup, a glyphosate herbicide, on Nile tilapia (Oreochromis niloticus). Sci Asia 2002;28:121-7.

20. Sepci-Dincel A, Karasu BC, Selvi M, Sarikaya R, Sahin D, Ayhan Ozkul I, et al. Sublethal cyfluthrin to carp Cyprinus carpio fingerlings: Biochemical, hematological, histopathological alterations. Ecotoxicol Environ Saf 2007;69:1433-9.

21. Fagbenro OA, Adefidre CO, Owoseni EA, Ayotunde EO. Studies on the biology and aquacultural potential of feral catfish. Heterobranchus bidorsalis (Geoffroy Saint Hilaire 1809) (Clariidae). Trop Zool 1993;6:67-79.

22. Duhama AK. Organic Farming for Sustainable Agriculture. India: Agro Botanical Publishers; 1996.

23. Samanta P, Pal S, Mukherjee AK, Kole D, Ghosh AR. Toxic effects of glyphosate-based herbicide, Excel Mera 71 on gill, liver and kidney of Heteropneustes fossilis under laboratory and field conditions. J Microsc Ultrastruct 2016a;4:147-55.

24. Samanta P, Pal S, Mukherjee AK, Senapati T, Ghosh AR. Histopathological and ultrastructural alterations in Anabas testudineus exposed to glyphosate-based herbicide, excel mera 71 under field and laboratory conditions. J Aquac Res Dev 2016b;7:436.

25. Chattopadhyay DN, Mohapatra BC, Adhikari S, Paní 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.

26. APHA, AWWA, WPCF. Standard Methods for the Examination of Water and Wastewater. Washington, DC: APHA, AWWA, WPCF; 2005.

27. 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 Agricult Vet Sci 2012;3:32-9.

28. 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 Agricult Vet Sci 2013;3:100-11.

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

30. Ramírez-Duarte WF, Rondón-Barragán LS, Elava-Mocha PR. Acute toxicity and histopathological alterations of Roundup® herbicide on “Cachama blanca” (Piaractus brachypomus). Pesq Vet Bras 2008;28:547-54.

31. Kossakowski MK, Ostaszewska T. Histopathological changes in the juvenile carp Cyprinus carpio. Arch Pol Fish 2003;11:57-67.

32. Barros JA, de Oliveira SO, Fontoura MSA, Ferreira FL. Effect of histological, histochemical and ultramorphological techniques to detect gill alterations in Oreochromis niloticus reared in treated polluted waters. Micron 2009;40:839-44.

33. Pal S, Kokushi E, Koyama J, Uno S, Ghosh AR. Histopathological alterations in gill, liver and kidney of common carp exposed to chlorpyrifos. J Environ Sci Health B 2012;47:180-95.

34. Sorour JM, Harbea DA. Histological and ultrastructural changes in gills of tilapia fish from Wadi Hanifah Stream, Riyadh, Saudi Arabia. J Am Sci 2012;8:180-6.

35. Jothai MS, Sharmal ML, Ravneet K. Impact of low dose of organophosphate, monocrotophos on the epithelial cells of gills of Cyprinus carpio communis Linn. — SEM study. J Environ Biol 2007;28:663-7.

36. 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.

37. Mallatt J. Fish gill structural changes induced by toxicants and other irritants: A statistical review. Can J Fish Aquat Sci 1985;42:630-48.

38. Pawert M, Müller E, Triebkorn R. Ultrastructural changes in fish gills as biomarker to assess small stream pollution. Tissue Cell 1998;30:617-26.

39. 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. Biochem. Chem Biol Interact 2008;174:183-92.

40. Stentford GD, Longshaw M, Lyons BP, Jones G, Green M, Feist SW, et al. Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. Mar Environ Res 2003;55:137-59.

41. Rahman MZ, Hossain Z, Mollah MF, Ahmed GU. Effect of Diazinon 60 EC on Anabas testudineus, Channa punctatus, Barbodes gonionotus. Naga, The ICLARM Quarterly, 8-12.

42. Biagianti-Risbourg S, Bastide J. Histopathological effects of a herbicide (atrazine) in juvenile rainbow trout (Oncorhynchus mykiss). Environ Res 2003;92:262-70.

43. Bozolla JF, Russell LD. Electron Microscopy: Principles and Techniques for Biologists. Boston: Jones and Bartlett Publishers; 1992.

44. Braunbeck T, Storch V, Nagel R. Sex-specific reaction of liver ultrastructure in zebrafish Brachydanio rerio after prolonged sublethal exposure to 4-nitrophenol. Aquat Toxicol 1989;14:185-202.

45. Ceceville NF. Ultrastructural Pathology. Ames: Iowa State University Press; 1994.

46. Hanke W, Gluth G, Müller R. Physiological changes in carp induced by pollution. Ecotoxicol Environ Saf 1983;7:229-41.

47. 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.

50. Diaz JP, Mani-Ponset L, Guyot E, Connes R. Hepatic cholestasis during the post-embryonic development of fish larvae. J Exp Zool 1998;280:277-87.

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

52. 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.

53. 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. Rijeka, Croatia: InTech Publisher; 2012. p. 163-4.

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

55. 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.

56. 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 Suppl 1:55-60.

57. 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.