Toxicity assessment of agrochemical Almix in *Heteropneustes fossilis* through histopathological alterations

Palas SAMANTA 1,2, Rituparna DAS 1, Sandipan PAL 3, Aloke Kumar MUKHERJEE 4, Tarakeshwar SENAPATI 5, Debraj KOLE 3, Apurba Ratan GHOSH 1

1 Ecotoxicology Lab, Department of Environmental Science, The University of Burdwan, Golapbag, Burdwan 713104, West Bengal, India
2 Division of Environmental Science and Ecological Engineering, Korea University, Anam-dong, Sungbuk-gu, Seoul 136-713, Republic of Korea
3 Department of Environmental Science, Aghorekamini Prakashchandra Mahavidyalaya, Subhoanagar, Bengar, Hooghly 712611, West Bengal, India
4 P.G. Department of Conservation Biology, Durgapur Govt. College, Durgapur 713214, West Bengal, India
5 School of Basic and Applied Sciences, Poornima University, Jaipur 302022, Rajasthan, India

ABSTRACT
The present study was designed to assess the adverse effects of the agrochemical Almix on comparative basis in gill, liver and kidney of *Heteropneustes fossilis* through histological and ultrastructural observations under field (8 g/acre) and laboratory (66.67 mg/L) conditions. Exposure duration of both experiments was 30 days. Gill showed atrophy in secondary lamellae, hypertrophied gill epithelium, damage in chloride and pillar cells, and detachment of chloride cells from gill epithelium under laboratory condition, but hypertrophy in gill epithelium and fusion in secondary lamellae were seen under field condition. In gill, scanning electron microscopy (SEM) showed fragmentation in microridges, hyper-secretion of mucus and loss of normal array in microridges, while transmission electron microscopy (TEM) displayed dilated mitochondria and rough endoplasmic reticulum (RER), abnormal sized vacuolation in chloride cells under laboratory condition but in field condition the liver showed little alterations. TEM study showed severe degeneration in RER and mitochondria and cytoplasmic vacuolation under laboratory condition but dilated mitochondria were prominent in field observation. Kidney showed severe nephropathic effects including degenerative changes in proximal and distal convoluted tubule, damage in glomerulus under light microscopy, while deformity in nucleus, fragmentation in RER, severe vacuolation and necrosis in kidney were prominent under TEM study. The results clearly demonstrated that responses were more prominent in laboratory than field study. Thus the responses displayed by different tissues of concerned fish species exposed to Almix could be considered as indications of herbicide toxicity in aquatic ecosystem.

KEY WORDS: Almix; scanning electron microscopy; transmission electron microscopy; *Heteropneustes fossilis*

Introduction

In modern agricultural practices, the introduction of new technology for crop production and protection has several times increased the use of herbicides. Herbicides play an important role in controlling the annual grasses, broad leaved weeds and sedges from various agricultural fields. Indiscriminate uses, careless handling, accidental spillage, or discharge of untreated effluents on herbicidal uses into natural waterways, including fish farms, can cause damage in fish population and other aquatic animals or plants (Sarikaya & Yilmaz, 2003; Fonseca *et al.*, 2008). The application of environmental toxicological studies on non-mammalian vertebrates has been rapidly expanding in recent times, and for aquatic systems, fish have become indicators for the evaluation of the toxic effects of these noxious compounds. In aquatic toxicological studies, laboratory experiments are performed to estimate the potential hazards of these chemicals to establish “safe” levels of these xenobiotics (Anto’n *et al.*, 1994).

Almix’ 20 WP is the new fourth generation herbicide. It is widely used to control broad-leaf weeds and sedges both in terrestrial and aquatic systems. Almix is a selective contact as well as systematic and both pre-emergent and post-emergent herbicide of the sulfonylurea group. It is composed of 10.1% metsulfuron methyl (C\textsubscript{14}H\textsubscript{15}N\textsubscript{2}O\textsubscript{5}S) [methyl 2-((4-methoxy-6-methyl-1,3,5-triazin-2-yl-
carbamoyl-sulfamoyl) benzoate], 10.1% chlorimuron ethyl (C13H15ClN4O5S) [ethyl 2-(4-chloro-6-methoxy-pyrimidine-2yl-carbamoyl-sulfamoyl) benzoate] and 79.8% adjuvants (DuPont Safety Data Sheet, 2012).

Documentation of Almix herbicide toxicosis to freshwater teleostean catfish, *Heteropneustes fossilis* has been started recently (Samanta *et al.*, 2014a, b; Samanta *et al.*, 2015a, b). Low concentrations of Almix, such as those used in rice fields, might cause changes in metabolic and enzymatic parameters of catfish, *H. fossilis* (Samanta *et al.*, 2014a, b) and other fish species such as *Anabas testudineus* and *Oreochromis niloticus* concerning reduction of protein level and glutathione S-transferase (GST), and enhancement of acetylcholinesterase (AChE), lipid peroxidation (LPO) and catalase (CAT) activities in different tissues of *A. testudineus* and *O. niloticus* (Senapati *et al.*, 2012; Samanta *et al.*, 2014a, b; Samanta *et al.*, 2015a, b). However, only metabolic and physiological activities alone do not satisfy the complete understanding of pathological alterations of the tissues under toxic stress. In order to know the extent of tissue damage it is thus useful to have an insight into the analysis of cellular and subcellular orientations, although the severity of damage depends on toxic potentiality of the particular toxic effect (Tilak *et al.*, 2001; Srivastava *et al.*, 2008). The great advantages of using histopathological biomarkers in environmental monitoring is that it allows an easy examination of specific target organs including gills, liver and kidney, which are responsible for vital physiological functions, such as respiration, accumulation and biotransformation, and excretion of xenobiotics (Gernhöfer *et al.*, 2001; Camargo & Martinez, 2007). A number of studies have been reported by several authors to understand the biochemical, physiological and metabolic alterations caused by exposure to different pesticides and/or herbicides on animals and fishes (Geetha *et al.*, 1999; Sambasiva Rao, 1999; Aruna *et al.*, 2000; Sornaraj *et al.*, 2005). However, studies regarding histology and ultrastructural effects of Almix herbicide on fish tissues and other aquatic invertebrates are relatively scanty (Senapati *et al.*, 2012) and still need to be evaluated when compared with mammals and was carried out only in laboratory study. Nevertheless, field studies using histopathology and ultramicroscopic observations of fish tissues as biomarkers of aquatic contamination by Almix herbicide have not so far been reported. Thus the present study was aimed to investigate the marked changes in the histological and ultrastructural architectures in gills, liver and kidney of *H. fossilis* to Almix intoxication on comparative basis under laboratory and field conditions (*i.e.*, higher vs lower).

**Materials and methods**

**Chemicals**

Commercial formulation of the Almix herbicide (Almix® 20 WP, DuPont India Pvt. Ltd., Gurgaon, Haryana, India) was used in both the experiments. Delafield’s hematoxylin stain, eosin yellow, xylene, DPX, amyl acetate, acetone, glutaraldehyde solution, sodium hydroxide, tricaine methanesulphonate, 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. Osmium tetroxide was purchased from Spectrochem Pvt. Ltd., Mumbai, India.

**Fish**

Freshwater teleostean fish, *Heteropneustes fossilis* (Bloch) of both the sexes with an average weight of 37.91±5.43 g and total length of 18.58±0.959 cm were procured from a local fish farm and brought to the laboratory. Fish were acclimatized under congenial conditions for 15 days in aquarium (250 L). Fish were kept in continuously aerated water with a static-renewal system and experiments were conducted under natural photoperiod (12-h light/12-h dark). During the acclimatization, the average value of water parameters were as follows: temperature, 18.61±0.808°C; pH, 7.23±0.082; electrical conductivity, 413.67±0.90 μS/cm; total dissolved solids, 295.11±1.16 mg/L; dissolved oxygen, 6.46±0.215 mg/L; total alkalinity, 260.00±16.90 mg/L as CaCO₃; total hardness, 177.33±5.50 mg/L as CaCO₃; ammoniacal-nitrogen, 2.31±0.43 mg/L; and nitrate-nitrogen, 0.30±0.058 mg/L. After acclimatization, fish were divided into two groups: one group was transferred to field ponds situated at Crop Research Farm premises of the University of Burdwan and the other group was transferred to laboratory aquarium. Fish were fed once a day with commercial fish pellets (32% crude protein, Tokyu) during both acclimation and exposure periods. Therefore, the study was carried out under two different experimental conditions: field and laboratory, both for the duration of 30 days.

**Field experiment**

Fish were again divided into two groups as follows: control groups (triplicate cages), each cage contained 10 fish species, and Almix-exposure group with 10 fish species in three separate cages (Figure 1). The desired dose (8 g/acre) corresponds to the concentration recommended for rice culture was dissolved in water and applied once (Samanta *et al.*, 2014a; Samanta *et al.*, 2015b). Duration of the exposure period was 30 days. It was sprayed on the first day of the experiment on the surface of each Almix-treated cage. For these field experiments, special type of cage was prepared and installed separately at two different ponds of Burdwan University Crop Research Farm, University of Burdwan. Cages were prepared for the culture of experimental fish species as per Chattopadhyay *et al.* (2012) with some modifications. All the cages were square in shape having an area of 2.5×1.22 m and cage height was 1.83 m (submerged height was 0.83 m). Cages were framed by light strong bamboo. The four-sided wall, cage floor and top of the cage cover were fabricated with nylon net and embraced by two PVC nets: the inner and outer nets bearing mesh sizes of 1.0×1.0 mm² and 3.0×3.0 mm², respectively. During the experimentation
period, pond water had the following average values: temperature 15.67±0.145 °C; pH 7.89±0.033; electrical conductivity 390.33±2.19 µS/cm; total dissolved solids 276.33±1.45 mg/L; dissolved oxygen 7.47±0.088 mg/L; total alkalinity 101.33±0.67 mg/L as CaCO₃; total hardness 152.00±0.31 mg/L as CaCO₃; ammoniacal-nitrogen 6.06±0.875 mg/L; and nitrate-nitrogen 0.58±0.016 mg/L.

Laboratory experiment
Fish were divided again into two groups (control and Almix-treated) and maintained in six aquaria (three for control and three for treatment), containing 10 fishes in each aquarium in the Ecotoxicology Lab, Department of Environmental Science, the University of Burdwan. Fish were exposed to sub-lethal dose of Almix, i.e., 66.67 mg/L (40 L) for a period of 30 days (Samanta et al., 2015a, b). Doses were applied every alternate day. During experimentation, Almix-treated and control were subjected to the same environmental conditions. During experimentation period, the average water parameters were as follows: temperature 19.67±0.293 °C; pH 7.48±0.052; electrical conductivity 478.33±9.70 µS/cm; total dissolved solids 341.44±6.56 mg/L; dissolved oxygen 5.82±0.394 mg/L; total alkalinity 317.30±15.60 mg/L as CaCO₃; total hardness 188.89±8.58 mg/L as CaCO₃; ammoniacal-nitrogen 6.63±1.15 mg/L; and nitrate-nitrogen 0.46±0.108 mg/L.

Sampling
During the experimentation period, water quality parameters were analyzed as per APHA (2005). After completion of the experiment, i.e., 30 days, fish were collected both from aquarium and pond and were anesthetized with tricaine methanesulphonate (MS 222). After that, gill, liver and kidney were taken immediately after dissection and proceeded in specific ways for histological, scanning and transmission electron microscopic study.

Histological analysis
Gill, liver and kidney from control and treatment fish were collected and fixed in aqueous Bouin’s fluid solution for overnight. After fixation, tissues were dehydrated through graded series of ethanol and finally embedded in paraffin. Paraffin sections were then cut at 3–4 μm using Leica RM2125 microtome. Finally, sections were stained with hematoxylin-eosin (H&E) solution and pathological lesions were examined under Leica DM2000 light microscope. Additionally, semi-quantitative analysis was also carried out by observing the frequency of pathological lesions based on Pal et al. (2012) with some modifications.

Ultrastructural analysis
For scanning electron microscopic study, tissues were fixed in 2.5% glutaraldehyde solution prepared in phosphate buffer (0.2 M, pH 7.4) for 24 h at 4 °C and then post-fixed with 1% osmium tetroxide prepared in phosphate buffer (0.2 M, pH 7.4) for 2 h at 4 °C. After fixation, tissues were dehydrated through graded series of acetone, followed by amyl acetate and subjected to critical point drying with liquid carbon dioxide. Tissues were then mounted on metal stubs and sputter-coated with gold with thickness of approximately 20 nm. Finally, tissues were examined with a scanning electron microscope (Hitachi S-530) at the University Science Instrumentation Centre of the University of Burdwan.

For transmission electron microscopic study, tissues were fixed in Karnovsky fixative (mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer) for 12 h at 4 °C and then post-fixed with 1% osmium tetroxide in phosphate buffer (0.2 M, pH 7.4) for 2 h at 4 °C. After fixation, tissues were dehydrated through graded acetone, infiltrated and embedded in epoxy resin (araldite CY212). Ultrathin sections (70 nm) were then cut using glass knife on an “Ultracut E Reichart – Jung” and collected on naked copper-meshed grids. After air-drying, grids were stained with uranyl acetate and lead citrate. Finally, tissues were examined under TECHNAI G2 high resolution transmission electron microscope at Electron Microscope Facility, Department of Anatomy, AIIMS, New Delhi.

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

Results
Gill
Histologically, gill is composed of primary and secondary gill lamellae. Free edges of the lamellae are extremely thin, covered with stratified epithelium and contain a vast network of capillaries supported by pilaster cells. Primary gill lamella was supported by gill rays which were bony in nature (Figure 2.1).

Semi-quantitative evaluation of frequency of pathological lesions in gill of laboratory and field condition
fish compared to control fish is given in Table 1. Atrophy in secondary lamellae, hypertrophied gill epithelium and damage in chloride as well as pillar cells, detachment of chloride cells from gill epithelium and stunted growth of gill lamellae are the most common histological lesions observed under laboratory study (Figure 2.2). Contrary to laboratory findings, slight hypertrophy in gill epithelium and fusion in secondary gill lamellae are the only pathological lesions observed under field condition (Figure 2.3).

Under scanning electron microscopic study, gill epithelium showed fragmentation of microridges, swelling of microridges and loss of normal array of microridges (Figure 2.5), but under field condition hyper-secretion of
 useRouter(['Table 1. Semi-quantitative assessment of frequency of pathological lesions in gill, liver and kidney of H. fossilis under laboratory and field conditions.'])

| Pathological Lesion                  | Control | Laboratory condition | Field condition |
|--------------------------------------|---------|----------------------|-----------------|
| Gill                                 |         |                      |                 |
| Histopathological                    |         |                      |                 |
| Proliferated gill epithelum          | –       | ++                   | +               |
| Hypertrophy of the gill epithelium   | –       | +++                  | +               |
| Hyperplasia of the gill epithelium   | –       | +++                  | +               |
| Scanning electron microscopic        |         |                      |                 |
| Damage of microridge structures      | –       |                      | –               |
| Disappearance of normal array of microridges | –       |                      | +               |
| Mucus secretion                      | –       | ++                   | –               |
| Distortion of stratified epithelial cells | –   |                      | +               |
| Swelling of stratified epithelial cells | –       |                      | +               |
| Necrosis                             | –       | ++                   | +               |
| Transmission electron microscopic    |         |                      |                 |
| Chloride cell damage                 | –       | ++                   | –               |
| Dilated mitochondria                 | –       | +                    | ++              |
| Mitochondrial degeneration           | –       | +                    | –               |
| Cytoplasmic vacuolation              | –       |                      | –               |
| Nuclear distortion                   | –       | ++                   | +               |
| Liver                                |         |                      |                 |
| Histopathological                    |         |                      |                 |
| Disoriented hepatic cord             | –       | ++                   | ++              |
| Hypertrophy of hepatocytes           | +       | +++                  | ++              |
| Degeneration of hepatocytes          | –       | +                    | +               |
| Nuclear hypertrophy                  | +       | +++                  | +               |
| Cytoplasmic vacuolation              | –       | +++                  | +               |
| Pyknotic nucleus                     | –       |                      | –               |
| Detachment of hepatopancreatic acinar cells from hepatocytes | – | +++ | + |
| Deformed hepatopancreas              | –       |                      | –               |
| Loss of zymogen granules             | –       | +                    | –               |
| Transmission electron microscopic    |         |                      |                 |
| Cytoplasmic vacuolation              | –       | +++                  | +               |
| Loss of rough endoplasmic reticulum  | –       | +                    | –               |
| Loss of glycogen granules            | –       | ++                   | +++             |
| Dilated mitochondria                 | –       | +                    | ++              |
| Kidney                               |         |                      |                 |
| Histopathological                    |         |                      |                 |
| Shrinkage of glomerulus              | –       | ++                   | +               |
| Lipid vacuoles in epithelial cells   | –       | +                    | +               |
| Swelling in tubular epithelium       | –       | +                    | –               |
| Hypertrophy in tubular epithelium    | +       | +                    | +               |
| Fragmentation of glomerulus          | –       | +                    | +               |
| Tubular degeneration                 | –       | +                    | ++              |
| Loss of hematopoietic tissue         | –       |                      | +               |
| Transmission electron microscopic    |         |                      |                 |
| Vacuolation in epithelial cytoplasm  | –       | +++                  | +               |
| Damage in proximal convoluted tubules | –     |                      | +               |
| Dilated mitochondria                 | –       | +                    | ++              |

mucus, damage of microridges in few places were noticed after Almix exposure (Figure 2.6). Transmission electron microscopy analyses showed dilated mitochondria and endoplasmic reticulum, abnormal sized vacuolation in gill epithelium of H. fossilis under laboratory condition (Figure 2.8); however, in field condition gill epithelium showed almost normal appearance of pavement cells, chloride cells, mitochondria, apical pore except vacuolation in some places (Figure 2.9).

Liver

Semi-quantitative evaluation of frequency of pathological lesions in gill of laboratory and field condition fish compared to control fish is given in Table 1. The most expressive changes after Almix exposure in hepatocytes of the concerned fish species seen under light microscopy were distortion in hepatocytes with clumping of nuclei, hypertrophied and pyknotic nuclei, disarrangement of hepatic cords and cytoplasmatic vacuolation under laboratory condition (Figure 3.2), while under field condition it showed only distended appearance of hepatocytes and short central vein and fat deposition in sinusoidal spaces in some places (Figure 3.3).

Ultrastructural alterations as viewed under TEM study showed severe degeneration in rough endoplasmic reticulum and mitochondria, vacuolation in cytoplasm and reduced amount of glycogen droplets in hepatocytes (Figure 3.5) under laboratory condition as compared to control (Figure 3.4), but in field condition, hepatocytes showed no significant changes in nucleus and rough endoplasmic reticulum but only dilated mitochondria in some places (Figure 3.6).

Kidney

Histologically, the kidney is made up of a large number of nephrons, each consisting of a renal corpuscle or the Malpighian body and renal tubules. Renal tubules consist of columnar epithelial cells and renal tubules which are spherical or oval in shape. Renal tubules are differentiated into proximal convoluted tubule (PCT), distal convoluted tubule (DCT) and collecting ducts (Figure 4.1).

Semi-quantitative evaluation of frequency of pathological lesions in gill of laboratory and field condition fish compared to control fish is given in Table 1. Nephropathic effects due to Almix toxicosis under laboratory condition included degenerative changes in PCT and DCT, distorted glomerulus in certain Bowman’s capsules (Figure 4.2). However, under field condition no such significant alterations in PCT and DCT of H. fossilis were observed but aggregation and fatty deposition in hematopoietic tissues were prominent (Figure 4.3).

After 30 days of Almix exposure in laboratory condition, transmission electron microscopic study showed severe degenerative changes in mitochondria, deformity in nucleus, dilation, fragmentation and vesiculation in rough endoplasmic reticulum, severe vacuolation in cytoplasm and necrosis (Figure 4.5), while dilation in mitochondria, abundance of numerous mitochondria, lower amount of vacuolation were observed under field...
study but damage was comparatively less compared with laboratory condition (Figure 4.6).

**Discussion**

The present study is a maiden attempt to report Almix toxicosis with regard to histological and ultrastructural observations in *H. fossilis*, although Senapati et al. (2012, 2013) reported histopathological alterations in stomach and intestine of *A. testudineus* exposed to Almix herbicide under laboratory condition.

Fish are considered a sentinel organism for ecotoxicological studies and play a significant role in evaluating the risk in aquatic ecosystem (Lakra & Nagpure, 2009). Simultaneously, cellular biomarkers including histological and ultrastructural study in tissues of pollutant-induced organism represent an intermediate level of biological organization between lower-level biochemical effects and higher-level population effects (Adams et al., 2001). This will ultimately provide a better evaluation of the organism’s health than a single biochemical response (Triebskorn et al., 1997). They are now widely used as efficient biomarker of water quality, cellular state and mode of action of the xenobiotic contaminants under microscopic study as well as reflecting the overall health of the entire population in the ecosystem (Schwaiger et al., 1997; Kammenga et al., 2000).

The results of the present study showed that Almix intoxication caused serious pathological alterations in gill, liver and kidney of *H. fossilis* under laboratory study and less in field condition. Gills are considered the most vulnerable organ (Dutta et al., 1996) because they are under direct contact with the surrounding contaminant medium and consequently are the first door of entrance for these contaminants (Machado & Fanta, 2003). Detailed description of each pathological lesion through light and electron microscopic observation in gills of *H. fossilis* helps to evaluate the degree of damage and potential consequences.

Hypertrophy and detachment of chloride cells from gill epithelium are the most profound alterations due
to Almix exposure. Similarly, Mallatt (1985) reported detachment of the gill epithelium as serious alteration. Fusion of secondary lamellae as observed in gills of *H. fossilis* indicated reduction of total respiratory area, which ultimately reduces oxygen uptake capacity (Karan et al., 1998). Similar finding was also reported in *Lepomis macrochirus* after malathion exposure by Richmonds & Dutta (1989). Damage in chloride cells was also prominent under laboratory conditions. Similar results were also reported by van der Heuvel et al. (2000) along with loss of structural integrity of secondary lamellae and accumulation of blood cells. Stunted growth of gill lamellae is another most conspicuous change observed under the present study after Almix exposure. Scanning electron microscopic study showed fragmentation of microridges, swelling of microridges and loss of normal microridge array under laboratory condition. Light microscopic evidence as observed under the present study could be correlated with strands of secreted mucus cells over the gill surface through SEM study. Similar results of hyperplasia, loss of microridge and excess mucus secretion was also reported by Pfeiffe et al. (1997) in gill of juvenile goldfish, *Carassius auratus*, induced by 1-naphthyl-N-methylcarbamate (carbaryl). Excess mucus secretion as observed under field study indicated compensatory mechanism as well as defensive mechanism by the fish species against herbicidal exposure. In the present study, transmission electron micrograph showed double layered nucleus, dilated mitochondria and endoplasmic reticulum, and abnormal vacuolation in the chloride cells of gill epithelium after Almix exposure. Similar observation of chloride cell damage was also reported by Schaiger et al. (2004) and indicated that these alterations might interfere with normal respiratory functions and general fish health status. Additionally, chloride cell damage might increase blood flow inside the lamellae, dilatation of marginal channel, and blood congestion or even aneurism (Rostey-Rodriguez et al., 2002; Camargo & Martinez, 2007). Vacuolations in gill epithelium might impede gas exchange capacity as well as indication of swelling of mitochondria and rough endoplasmic reticulum (Ultsch et al., 1980; Pawert et al.,...
Mitochondrial damage observed under the present study was also reported by Perry & Laurent (1989) and Goss et al. (1995) in their study after exposure to different contaminants. Although significant ultrastructural differences were observed in gill epithelium both under laboratory and field study, pathological responses were more pronounced in laboratory conditions than in field study. These alterations in gill morphology could lead to functional anomalies as well as interfere with the fundamental process such as maintenance of osmoregulation and antioxidant defence mechanism of gill epithelium (Pandey et al., 2008).

Hypertrophied and pyknotic nuclei in hepatocytes of H. fossilis are the most pronounced lesions observed in the present study. Similar observations along with nuclear hypertrophy and cellular atrophy were reported in liver of Cyprinus carpio after chlorpyrifos exposure by Pal et al. (2012). Additionally, vacuolation in cytoplasm, infiltration of leukocytes and pyknotic nuclei were also reported by Jiraungkoorskul et al. (2002) in liver of Oreochromis niloticus after Roundup exposure. In the present study, vacuolization in hepatocytes indicated an imbalance between the rate of synthesis of substances in parenchymal cells and the rate of their release into systemic circulation. Additionally, enhanced glycolytic activity as compensatory response imposed by enhanced metabolic activity or reduction of carbohydrate absorption by intestinal part were reported (Hanke et al., 1983; Gluth & Hanke, 1985; Braunbeck & Appelbaum, 1999). Disarrangement of hepatic cord is another most important hepatic lesion observed under the present study. Cytoplasmic vacuolation in hepatocytes observed under TEM study was also reported by Li et al. (2001). Damage in rough ER is the common response to herbicide exposure. Braunbeck & Volkl (1993) and Au et al. (1999) correlated damage in rough ER with higher biotransformation capacity of hepatocytes, while Ghadially (1988) demonstrated dilation of ER cisternae as enhanced storage of proteins due to reduced secretory activity. Similar findings were reported in rainbow trout after exposure to endosulfan and disulfoton (Arnold et al., 1995), and in demersal fish following intraperitoneal injection of benzo(a)pyrene (Au et al., 1999). Mitochondrial degeneration observed under the present study indicated impaired hepatocyte oxidative capability due to inhibition of respiratory chain enzymes function through oxidation of ATP molecule during phospholipid metabolism and fatty acid synthesis. Marked ultrastructural changes including swollen mitochondria have already been reported in liver of catfish exposed to methyl parathion by Tripathi & Shukla (1990). Reduced glyogen content is another most important cytological change associated with herbicide exposure. Cytopathological responses observed under the present investigation were more pronounced in laboratory condition compared with field study as fish are in natural condition and quickly adapt under herbicide-induced aquatic environment.

In kidney, light microscopic observation showed degenerative changes in PCT and DCT, and damage in glomerulus. The results of the present study were also in agreement with the findings of Fischer-Scherl et al. (1991) and Neskovic et al. (1993). Jiraungkoorskul et al. (2002) in their study also reported damage in PCT, dilation of Bowman’s capsule along with accumulation of hyaline droplets in epithelial cells of renal tubule of Oreochromis niloticus after Roundup exposure. Additionally, alterations observed under the present investigation could be correlated with disruption of several biochemical and physiological pathways including endocrine disruption (Mekkawy et al., 2011; Sayed et al., 2012). Degenerative changes in mitochondria and deformed nucleus observed under the present study indicated impaired metabolic activity, in particular enzyme activity. Cytoplasmic vacuolation observed under both conditions have also been reported in gold fish kidney after hexachlorobutadiene exposure by Reimenschuessel et al. (1989). Similarly, Fischer-Scherl et al. (1991) reported degeneration and vacuolation in epithelial cells of kidney after lethal and sub-lethal atrazine exposure. Additionally, Fischer-Scherl et al. (1991) also reported dilation, fragmentation and vesiculation of RER in kidney of rainbow trout. Moreover, Bucher & Hofer (1993) reported accumulation of hyaline droplets in kidney. Abundance of large number of mitochondria, and lower amount of vacuolation observed under field condition indicated that fish are under stress. Additionally, protection against the stress-imposed conditions was observed; however, severity of damage is more pronounced under laboratory conditions than field study due to dilution capability of the natural environment.

Conclusion

In conclusion, cytopathological responses observed due to Almix intoxication indicated that laboratory study displayed higher impacts than did field study. Therefore, marked histological and ultrastructural alterations observed in gill, liver and kidney of H. fossilis could be considered as biomarkers of herbicidal toxicosis and might be helpful to characterize the health status of the entire aquatic ecosystem.

Acknowledgements

The authors would like to thank the INSPIRE Program Division (DST/INSPIRE Fellowship/2011/164, Dt. 29.09.2011), Department of Science & Technology, Govt. of India for the financial assistance to Dr Palas Samanta. We’d also like to thank the Head, Department of Environmental Science, the University of Burdwan, Burdwan, West Bengal, India for providing the laboratory facilities during the course of research. The authors are also thankful to the respective reviewers for improving the quality of this paper. Presently, Dr Samanta joined Korea University as Research Professor through BK21 Plus fellowship program funded by the Ministry of Education of Korea.
REFERENCES

Adams SM, Giess JP, Tremblay LA, Eason CT. (2001). The use of biomarkers in ecological risk assessment: recommendations from the Christchurch conference on Biomarkers in Ecotoxicology. Biomarkers 6: 1–6.

Anto&#x00e7;o FA, Laborda E, DeAriz M. (1994). Acute toxicity of the herbicide glyphosate to fish. Chemosphere 28: 745–753.

APHA, AWWA, WPCF. (2005). Standard Methods for the Examination of Water and Wastewater. Washington, DC.

Arnold H, Plutab HJ, Braunbeck T. (1995). 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 33: 17–43.

Au DWT, Wu RSS, Zhou BS, Lam PKS. (1999). Relationship between ultrastructural changes and EROD activities in liver of fish exposed to Benzo(a)pyrene. Environ Pollut 104: 235–247

Braunbeck T, Appelbaum S. (1999). Ultrastructural alterations in the liver and Intestine of carp Cyprinus carpio induced orally by ultra-low doses of endosulfan. Dix Aquat Organ 36: 183–200.

Braunbeck T, Voikl A. (1998). Toxicant-induced cytological alterations in fish liver as biomarkers of environmental pollution? A case study on hepato-cellular effects of dinofluoro-cresol in golden ide (Leuciscus idus melanotus) (Braunbeck), Hanké, Segner H edp. pp. 55–80, VCH Verlagsgesellschaft, Weinheim.

Bucher F, Hofer R. (1993). The effects of treated domestic sewage on three organs (gill, kidney, liver) of brown trout (Salmo trutta). Water Res 27(2): 255–261.

CamargoMMP, Martinez CBR. (2007). Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. Neotrop Ichthyol 5(3): 327–336.

Chattopadhyay DN, Mohapatra BC, Adhikari S, Pani PC, Jena JK, Eknath AE. (2013). Effects of stocking density of Labeo rohita on Microcystis aeruginosa. Aquat Toxicol 140: 454–461.

Datta HM, Munshi JD, Roy PK, Singh NK, Adhikari S, Kilius AE. (1996). Ultrastructural changes in the respiratory lamellae of the catfish, Heteropneustes fossilis after sub lethal exposure to malathion. Environ Poll 92(3): 329–341.

Fischer-Scherl T, Veeser A, Hoffman RW, Kühnhauser C, Negele RD, Euringer Date 27.07.2012 (Ref. 130000029001)

Gluth G, Hanke W. (1985). A comparison of physiological changes in carp, Cyprinus carpio with those in goldfish, Carassius auratus. Fish Physiol Biochem 3: 121–127.

Kammenga JE, Dallingr R, Donker MH, Kohler HR, Simonsen V, Triebssorn K, Weeks JM. (2000). Biomarkers in terrestrial invertebrates for ecotoxicological soil risks assessment. Rev Environ Contam Toxicol 164: 93–147.

Karan V, Vitosovic S, Tutundzic V, Polekisic V. (1996). Functional enzymes activity and gill histology of carp after copper sulphate exposure and recovery. Ecotoxicol Environ Saf 40: 49–55.

Khare A, Singh S, Shrivastava K. (2000). Malathion induced biochemical changes in the kidney of freshwater fish Clarias batrachus. J Ecotoxicol Environ Monit 10(1): 11–14.

Lakra WS, Nagpure NS. (2009). Genotoxicological studies in fishes: A review. Indian J Anim Sci 79(1): 93–98.

Li XY, Liu YD, Song LR. (2001). Cytological alterations in isolated hepatocytes from common carp (Cyprinus carpio L.) exposed to microcystin-LR. Environ Toxicol 16: 517–522.

Machado MR, Fanta E. (2003). Effects of the organophosphorous methyl parathion on the branchial epithelium of a freshwater fish Metynnis rosettii. Braz Arch Biol Technol 46(3): 361–372.

Mallatt J. (1985). Fish gill structural changes induced by toxicants and other irritants: a statistical review. Can J Fish Aquat Sci 42: 630–648.

Mekkawy IA, Mahmoud UM, Sayed AH. (2011). Effects of 4-nonylphenol on blood cells of the African catfish Clarias gariepinus (Burchell, 1822). Tissue Cell 43(4): 223–229.

Nesković NK, Elezović, I. Karan V, Polekisic V, Budimir M. (1993). Acute and subacute toxicity of atrazine to carp (Cyprinus carpio L). Ecotoxicol Environ Saf 25(2): 173–182.

Pal S, Kokushi E, Koyoma Y, Uno S, Ghosh AR. (2012). Histopathological alterations in gill, liver and kidney of common carp exposed to chlorpyrifos. J Environ Sci Health Part B 47: 180–195.

Pandey S, Parvez S, Ansari RA, Ali M, Kaur M, Hayat F, Ahmad F, Raisuddin S. (2008). 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 174: 183–192.

Pawert M, Miller E, Triebssorn R. (1998). Ultrastructural changes in fish gills as biomarker to assess small stream pollution. Tissue Cell 30(6): 617–626.

Perry SE, Laurent P. (1993).Environmental effects on fish gill structure and function (Rankin JC, Jenseu FB eds) pp. 231–264, Chapman and Hall, London.

Pfeiffer CJ, Qul B, Cho CH. (1997). Electron microscopic perspectives of gill pathology induced by 1-naphthyl-N-methylcarbamate in the goldfish (Carassius auratus Linnaeus). Histol Histopathol 12: 645–653.

Reimschüssel R, Bennet RO, May EB, Lipsky MM. (1989). Renal histopathological changes in the goldfish (Carassius auratus) after sublethal exposure to hexachlorobutadiene. Aquat Toxicol 15: 169–180.

Richmond C, Dutta HL. (1989). Histopathological alterations induced by maitaihion in the gills of blugill (Lepomis macrochirus). Bull Environ Contam Toxicol 43(1): 123–130.

Rosety-Rodriguez M, Ordonez FI, Rosety JM, Rosety L, Ribelles A, Carrasco C. (2002). Morphological and biochemical effects in the gills of turbot, Scophthalmus maximus L, induced by sodium dodecyl sulfate. Ecotoxicol Environ Saf 51: 223–228.

Samanta P, Bandypadhyay H, Pal S, Mukherjee AK, Ghosh AR. (2015b). Histopathological and ultrastructural changes in gill, liver and kidney of Anabas testudineus (Bloch) after chronic intoxication of alimix (metsulfuron methyl 10.1%–chlorimuron ethyl 10.1%) herbicide. Ecotoxicol Environ Saf 122: 360–367.

Samanta P, Pal S, Mukherjee AK, Ghosh AR. (2014a). Biochemical effects of alimix herbicide in three freshwater teleostean fishes (HydroMetrid - 2014, 1st International Congress of Applied Ichthyology & Aquatic Environment, Vois, Greece, November 13–15) pp. 168–174.

Samanta P, Pal S, Mukherjee AK, Senapati T, Ghosh AR. (2015a). Evaluation of enzymatic activities in liver of three teleostean fishes exposed to commercial herbicide, Almix 20 WP. Proc Zool Soc 68(1): 9–13.

Samanta P, Pal S, Mukherjee AK, Senapati T, Cole D, Ghosh AR. (2014b). Effects of Alimix herbicide on alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) of three teleostean fishes in rice field condition. Global J Environ Sci Res 1(1): 1–9.

Sambasiva Rao KRS. (1999). Pesticide Impact on Fish Metabolism. Discovery Publishing House, New Delhi.

Sarikaya R, Yilmaz M. (2003). Investigation of acute toxicity and the effect of 2,4-D (2,4-dichlorophenoxyacetic acid) herbicide on the behavior of the common carp (Cyprinus carpio L., 1758; pisces, cyprinidae). Chemosphere 52(1): 195–201.

Sayed AH, Mekkawy IA, Mahmoud UM. (2012). Histopathological alterations in some organs of adults of Clarias gariepinus (Burchell, 1822) exposed to 4-nonylphenol (Garoto MD ed)pp. 163–164, IntTech Publisher, Rijeka, Croatia.
Schwaiger J, Ferling H, Mallow U, Wintermahr H, Negele RD. (2004). Toxic effects of the non-steroidal anti-inflammatory drug diclofenac. Part I: histopathological alterations and bioaccumulation in rainbow trout. *Aquat Toxicol* 68: 141–150.

Schwaiger J, Wanke R, Adam S, Pawert M, Honnen W, Triebskorn R. (1997). The use of histopathological indicators to evaluate contaminant-related stress in fish. *J Aquat Ecosyst Stress Recovery* 6: 75–86.

Senapati T, Mukherjee AK, Ghosh AR. (2012). Observations on the effect of Almix 20WP herbicide on ultrastructure (SEM) in different regions of alimentary canal of *Anabas testudineus* (Cuvier). *Int J Food Agricul Vet Sci* 2(1): 32–39.

Senapati T, Samanta P, Mandal S, Ghosh AR. (2013). Study on histopathological, histochemical and enzymological alterations in stomach and intestine of *Anabas testudineus* (Cuvier) exposed to Almix 20WP herbicide. *Int J Food Agricul Vet Sci* 3(2): 100–111.

Sornaraj IR, Ranjitsingh AJ, Pushparaj A, Ramathilagam G. (2005). Pesticidal stress influenced respiratory alterations in the fresh water fish, *Mystus vittatus*. *Indian J Environ Ecoplan* 10: 803–806.

Srivastava RK, Yadav KK, Trivedi SP. (2008). Devicyprin induced gonadal impairment in a freshwater food fish, *Channa punctatus* (Bloch). *J Environ Biol* 29: 187–191.

Tilak KS, Veeraiah K, Yacobu K. (2001). Studies on histopathological changes in the gill, liver and kidney of *Ctenopharyngodon idellus* (Valenciennes) exposed to technical fenvalerate and EC 20%. *Poll Res* 20: 387–393.

Triebskorn R, Köhler HR, Honnen W, Schramm M, Adams SM, Müller EF. (1997). Induction of heat shock proteins, changes in liver ultrastructure, and alterations of fish behaviour: Are these biomarkers related and are they useful to reflect the state of pollution in the field? *J Aquat Ecosyst Stress Recovery* 6: 57–73.

Tripathi CL, Shukla SP. (1990). Enzymatic and ultrastructural studies in a freshwater catfish: Impact of methyl parathion. *Biomed Enviro Sci* 3: 166–182.

Ultsch GR, Ott ME, Heisler N. (1980). Standard metabolic rate, critical oxygen tension and aerobic scope for spontaneous activity of trout (*Salmo gairdneri*) in acidified water. *Comp Biochem Physiol* 67A: 329–335.

van den Heuvel MR, Power M, Richards J, Mackinnon M, Dixon DG. (2000). Disease and gill lesions in Yellow Perch (*Perca flavescens*) exposed to oil sands mining-associated waters. *Ecotoxicol Environ Saf* 46: 334–341.