Hindawi Publishing Corporation
Applied and Environmental Soil Science
Volume 2010, Article ID 138019, 8 pages
doi:10.1155/2010/138019

Research Article

Histopathological Effects of Gammalin 20 on African Catfish (*Clarias gariepinus*)

Lawrence Ezemonye[1] and Temiotan Emmanuel Ogbomida[2]

[1] *Department of Animal and Environmental Biology, Faculty of Life Science, University of Benin, P.M.B 1154 Benin City, Edo State, Nigeria*
[2] *Department of Environmental Forensic and Ecotoxicology, National Centre for Energy and Environment, Energy Commission of Nigeria, University of Benin, P.M.B 1154 Benin City, Edo State, Nigeria*

Correspondence should be addressed to Temiotan Emmanuel Ogbomida, ogbomida2000@justice.com

Received 5 October 2010; Revised 24 November 2010; Accepted 21 December 2010

Academic Editor: Alessandro Piccolo

Clarias gariepinus fingerlings exposed to lethal and sublethal concentrations of Gammalin 20 were investigated in a renewal static bioassay with particular reference to behaviour, survival, and histopathological changes. Early symptoms of gammalin 20 lethal poisoning were, respiratory distress, increased physical activity, convulsions, erratic swimming, loss of equilibrium, and increased breathing activity. Behavioural response was dose dependent and decreased with decreased concentration. The 96-hour lethal concentration (LC_{50}) value was 30 ppb. Histopathological changes of the gill, liver, and intestinal tissues of fish treated with sublethal concentration of gammalin 20 for twelve weeks showed gill distortion and fusion of adjacent secondary lamella as a result of hyperplasia and excessive mucus accumulation. The liver showed swelling of hepatocytes with mild necrosis, pyknosis, and vacuolation, while the intestine showed yellow bodies of the lamina propria at the tip of the mucosal fold.

1. Introduction

Environmental factors of both natural and anthropogenic origins have been known to induce alteration of different magnitudes in the physiological and biochemical status of animals [1, 2]. Therefore, biomarker parameter assessment is a means of environmental monitoring, with the advantage of providing quantitative response as valuable information on ecological relevance as well as on the acute/chronic adverse effects caused by water pollution [3].

Alteration in the chemical composition of a natural aquatic environment, due to contact with hazardous substances like heavy metals, pesticides, and effluents from industries usually affect the behaviours, biochemistry, and physiology of the fauna including fish [4]. Water is one of the most precious natural resources on earth, and it creates a wide range of benefits to humans, including fisheries, wildlife, agriculture, urban, industrial, and social development [5].

However, the unregulated release of agricultural chemicals especially pesticides into water bodies have caused environmental problems to all classes of organisms in the aquatic habitat. The aquatic ecosystem is faced with the threat of biodiversity loss due to indiscriminate use of pesticides [6].

Gammalin 20 is a widely used organochlorine pesticide employed in veterinary and human medicine to treat ectoparasites and pediculosis. It is also used in the control of a broad spectrum of phytophagous and soil-inhabiting insects, public health pests, and animal ectoparasites. It is used in fishing industries for fish kill and on a wide range of crops to control Aphididae larvae of coleoptera, diptera., and so forth in stored product warehouses and storerooms, public health treatments, and seed treatments [7].

The application of environmental toxicology studies on nonmammalian vertebrates is rapidly expanding, and for aquatic system, fish have become an indication for the evaluation of the effects of noxious compounds [8]. Pesticides occupy a unique position among many chemicals which are encountered daily by man. They are deliberately added to the environment for the purpose of killing, injuring, or at times enhancing the development of some forms of life. Water pollution by pesticides is a serious problem to all aquatic fauna and flora. In aquatic environment, pesticides may also cause several physiological and biochemical defects in fishes [9]. Contamination of water with these recalcitrant chemicals often results in bioaccumulation in fish and other biota, sometimes to biologically active levels. These chemicals have been suspected to be cancer-causing agents in fish and other aquatic organism [10]. Residues of these toxic chemicals found in water, sediments, fish, and other aquatic biota can pose risk to organisms, predators, and humans. Pesticides at high concentrations are known to reduce the survival, growth, and reproduction of fish and produce many visible effects on fish [6]. Water pollution also is recognized globally as a potential threat to both human and other animal population, which interact with the aquatic environment [11, 12]. Due to the residual effects of pesticides, important organisms are damaged [6].

Thus, the objective of this study was to investigate the lethal and sublethal effects of Gammalin 20 to African catfish (*Clarias gariepinus*) using mortality, behavioural and histopathological changes as end points. Changes in these parameters are being investigated as potential diagnostic tools in assessing the effects of gammalin 20 on fish with a view of setting up standards for safe disposal of wastes.

2. Materials and Methods

Clarias gariepinus fingerlings of average length (7.8 ± 0.2 cm) and weight (12.0 ± 2.0 g) were purchased from Oyo state and Rayak fish culture in Ibadan, Nigeria. The fish were disinfected with 0.1% potassium permanganate as described by Joshi et al. [13] and acclimated for 14 days in the laboratory in a plastic tank. Ten fish were randomly distributed into each aquarium and fed with 40% crude protein commercial feed twice daily prior to the commencement of the experiment. The tanks were aerated throughout the acclimation period. Water quality parameters such as temperature, dissolved oxygen and pH of the experimental setup were monitored using standard methods APHA [14].

A range-finding test was conducted to determine the concentrations to be used in the actual experiment using standard procedure following the methods of APHA [14]. Based on the results of the range-finding test, nine concentrations (22, 25, 28, 33, 40, 50, 66, 100, 110 ppb) for acute test and two concentrations (22 ppb and 25 ppb) for chronic test of the gammalin 20 and a control (0.00 ppb) were prepared in glass tanks in duplicates. Ten fish were randomly introduced into each of the aquaria. Test solutions and water in the control were renewed daily. Behavioural and cumulative mortalities were recorded and dead fish were removed with an aquarium net. A fish was considered dead when they failed to respond to simple prodding with a glass rod. Presence of mucus on the skin and gills of test fish was also checked by feeling with the fingers.

2.1. Test Chemical. The organochlorine compound, gammalin 20 hexachlorocyclohexane ($C_6H_6C_{l6}$), was obtained from agrochemical shop at Iwo road, Ibadan. The gammalin 20 was stored in the laboratory at room temperature.

2.2. Acute Exposure Studies. A range-finding test was conducted to determine the concentrations to be used in the actual experiment using standard procedure following the methods of APHA [14]. Based on the results of the range-finding test nine concentrations (22, 25, 28, 33, 40, 50, 66, 100 and 110 ppb) of the gammalin 20 and a control (0.00 ppb) were prepared in glass aquaria in duplicates. Ten fish were randomly introduced into each of the aquaria. Test solutions and water in the control were renewed daily.

2.3. Chronic Exposure Studies. Two sublethal concentrations (22 ppb and 25 ppb) were selected for chronic exposure study, and two replicates of each concentration and two groups of control fish were maintained for exposure. Fish were fed with pellet feed at 5% of body mass once a day and water exchange was made at three-day intervals with fresh test solutions in each experimental tank. Test media were kept well aerated. Ten fish were maintained in each tank. After twelve weeks test fish were sacrificed for histopathological studies. Parameters such as temperature, dissolved oxygen, and pH of the test solutions were measured at intervals.

2.4. Replicates. Two replicates per test concentration were used to avoid test repetition due to system failure and to provide a stronger statistical baseline. Each test chamber contains an equal volume of test solution and equal numbers of fish (10). Replicate test chambers were physically separated.

2.5. Histopathology Examination. Liver, gill, and intestinal tissues collected in 10% neutral buffered formalin were processed for paraffin blocks (56–58°C) and sectioning at 3–5 μm using a microtome. Stained sections were examined under a Zeiss compound binocular microscope (Axiophot, Germany) fitted with a photo micrographic attachment.

3. Results

3.1. Toxicity and Behavioural Effects. The mean physicochemical parameters of the test solutions during 96-hour exposure of *C. gariepinus* to various concentrations of Gammalin 20 are presented in Figures 1, 2, and 3 . There was a significant relationship ($P < .05$) between the temperature, pH, and dissolved oxygen with gammalin 20 concentrations.

Clarias gariepinus fingerlings exposed to various concentrations of gammalin 20 showed an initial rapid movements such as opercular and tail movements, accompanied by incessant gulping for air, loss of balance, restlessness, sudden quick movement, excessive secretion of mucus, rolling movement, and swimming on the back. The intensity of the behavioural activities of the fish decreased with increasing concentration and duration of exposure. However, fish in the control maintained normal behaviour within the 96 hours of the experiment. The fish became very weak, settled at the bottom, and died. Mortality at the exposure concentrations

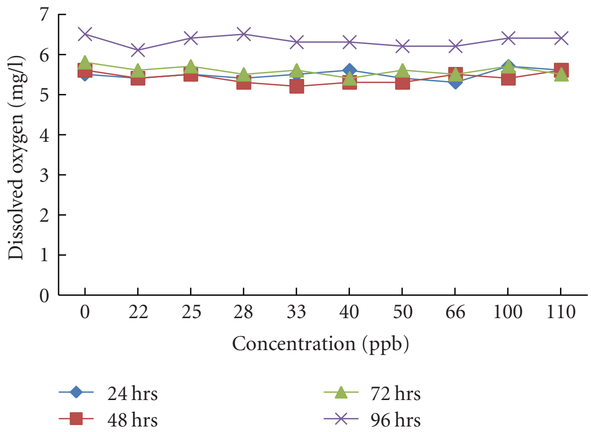

FIGURE 1: Dissolved oxygen of test solution during 96-hour exposure of *Clarias gariepinus* of various concentrations of gammalin 20.

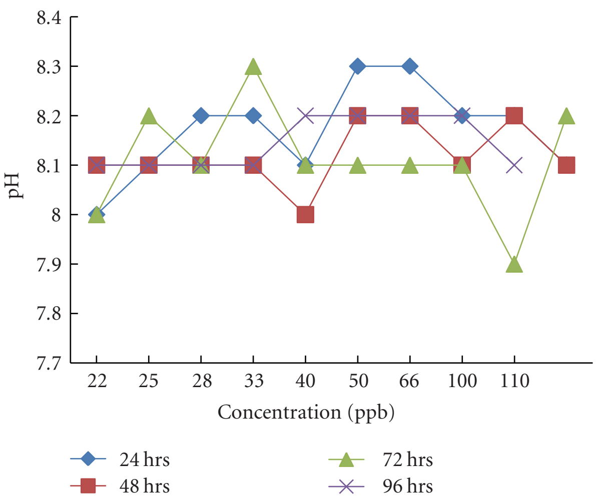

FIGURE 2: pH of test solution during 96-hour exposure of *Clarias gariepinus* of various concentrations of gammalin 20.

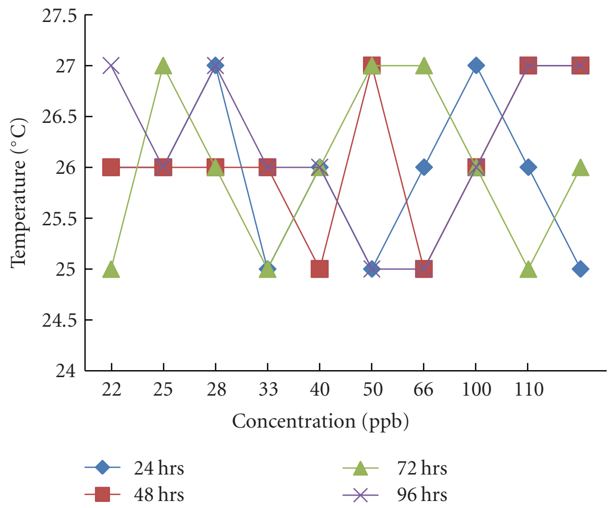

FIGURE 3: Temperature of test solution during 96-hour exposure of *Clarias gariepinus* of various concentrations of gammalin 20.

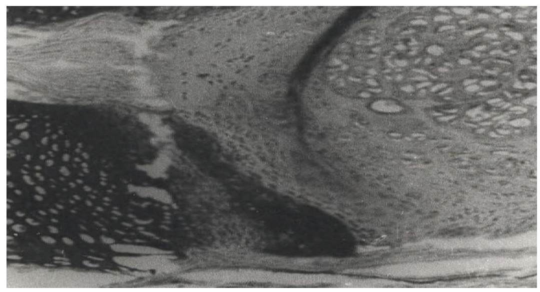

FIGURE 4: Plate of photomicrograph of normal gill (control) *C. gariepinus* after 12 weeks.

increased with duration becoming more variable with time (Table 1). The colour of the skin of *C. gariepinus* changed from the normal dark pigmentation to a very light pigmentation in the dorsal and lateral parts. No adverse behavioral changes or any mortality was recorded in the chronic test throughout the period of the bioassay. The behaviour of the control fishes and their colour were normal.

3.2. Histopathological Studies. Summary of histopathological changes observed in the gill, liver, and intestinal tissues of *C. gariepinus* fingerlings subjected to sublethal concentrations of 22 ppb and 25 ppb of gammalin 20 for 12 weeks are presented in Figures 4 to 12.

3.3. Histopathological Effects. Histopathological changes were pronounced in the gills, liver, and intestinal tissues of *C. gariepinus* fingerlings exposed to different concentrations of gammalin 20. No recognizable changes were observed in the gills of the control fish (Figure 4). Each gill consisted of a primary filament and secondary lamellae. At different concentrations of 22 ppb and 25 ppb of Gammalin 20 there were cellular infiltration, distortion, numerous mucus cells, swollen tip of the gill filament, and congestion (Figures 5 and 6). All the lessons observed indicate impending liver damaged prior to death of the test fish. Photomicrograph of liver of control Clarias gariepinus after 12 weeks keeping in clean water showing hepatocytes arranged in grandular pattern and other cells normal and systematically arranged (Figure 7). At 22 ppb and 25 ppb exposure of *C. gariepinus* to gammalin 20, there was mild necrosis, swelling of blood vessels, pyknosis, and vacuolation (Figures 8 and 9). Photomicrograph of intestine of control Clarias gariepinus after 12 weeks keeping in clean water showed no yellow bodies (Figure 10). Fingerlings treated with 22 ppb and 25 ppb sublethal concentrations showed yellow bodies at the lamina propria of the mucosal fold (Figures 11 and 12).

4. Discussion

The three physicochemical parameters of the test media were fluctuated slightly during the toxicity test. The values were normal for toxicity test [15]. There was a significant negative correlation between pH and dissolved oxygen values. Since

Table 1: Number of surviving *Clarias gariepinus* fingerlings treated with gammalin 20.

Conc. (ppb)	Time (Hours)								
	1	2	4	8	16	24	48	72	96
Control	20	20	20	20	20	20	20	20	20
22	20	20	20	20	20	18	18	18	17
25	20	20	20	20	20	18	18	17	15
28	20	20	20	18	18	16	14	12	11
33	20	20	17	17	15	14	12	10	8
40	20	20	16	16	14	14	12	9	4
50	20	20	14	14	12	12	10	5	3
66	20	20	13	12	9	6	2	1	1
100	19	15	10	7	0	0	0	0	0
110	17	14	6	1	0	0	0	0	0

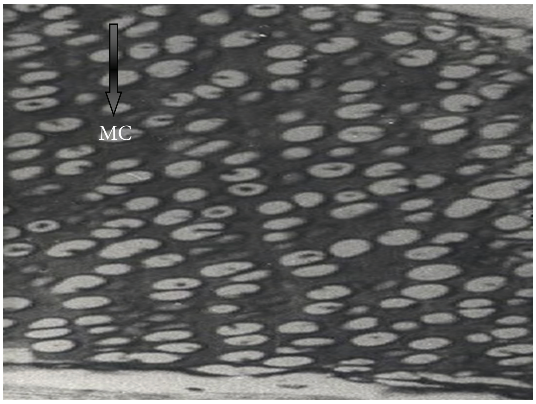

Figure 5: Plate of photomicrograph of gill of *Clarias gariepinus* after 12 weeks of exposure to 22 ppb of gammalin 20 showed numerous mucus cells, distortion, separation of the layers and sloughing.

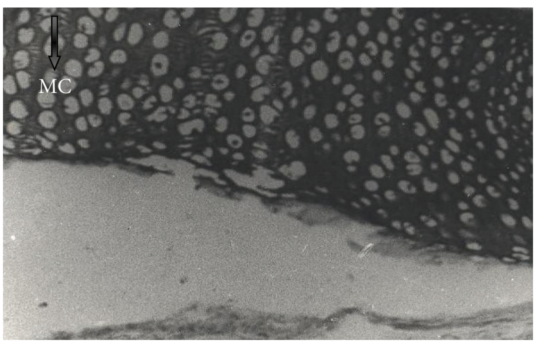

Figure 6: Plate of photomicrograph of gill of *Clarias gariepinus* after 12 weeks of exposure to 25 ppb of gammalin 20 showed numerous mucus cells (MCs), distortion, separation of the layers, and sloughing.

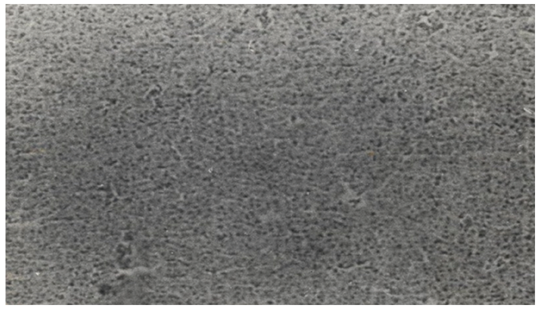

Figure 7: Plate of photomicrograph of normal liver of *Clarias gariepinus* after 12 weeks showing hepatocytes arranged in grandular pattern and other cells normal and systematically arranged.

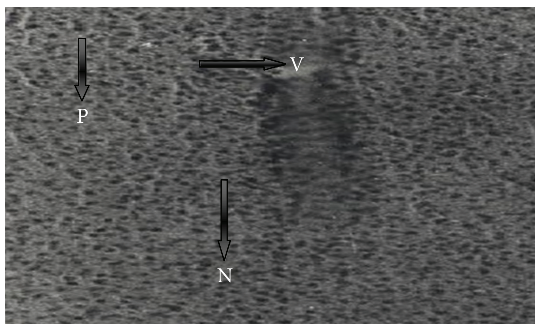

Figure 8: Plate of photomicrograph of liver of *Clarias gariepinus* after 12 weeks of exposure to 22 ppb of gammalin 20 showed mild necrosis, swelling of blood vessels, pyknosis, and vacuolation.

most fish breathe in water in which they live, changes in the chemical properties thereof may be reflected in the animal's respiratory activity, particularly if the environment factors affect respiratory gas exchanges [16]. In case of dissolved oxygen, the treatments did not only show a dose-dependent decline in concentration, but also rapid depletion of dissolved oxygen with time. Meletev et al. [17] and Holden [18] had earlier reported that the introduction of a toxicant into an aquatic system might decrease dissolved oxygen concentration, which will impair respiration leading to asphyxiation. Variation in the oxygen consumption in gammalin- 20- treated fish is probably due to impaired oxidative metabolism and pesticide- induced respiratory stress. Hence, dysfunction of behaviour and respiration can serve as an index of gammalin 20 toxicity.

Several abnormal behaviours such as incessant jumping and gulping of air, restlessness, loss of equilibrium, increased

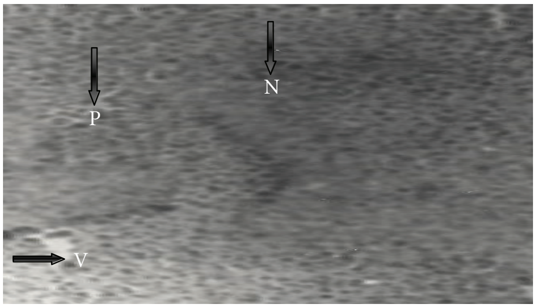

Figure 9: Plate of photomicrograph of liver of *Clarias gariepinus* after 12 weeks of exposure to 25 ppb of gammalin 20 showed mild necrosis (N), swelling of blood vessels, pyknosis (P), and vacuolation (V).

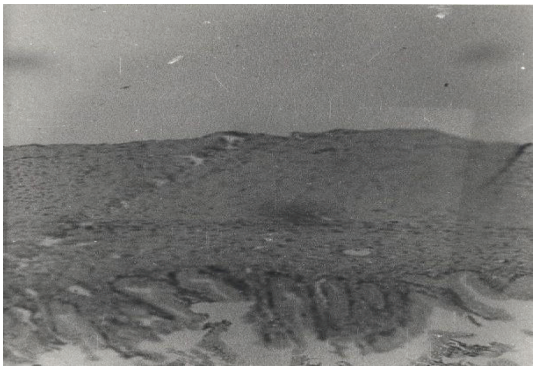

Figure 10: Plate of photomicrograph of intestine of *Clarias gariepinus* after 12 weeks showed no yellow bodies.

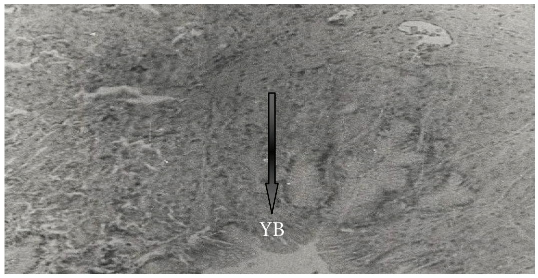

Figure 11: Plate of photomicrograph of intestine of *Clarias gariepinus* after 12 weeks exposure to 22 ppb of gammalin 20 showed yellow bodies (YB) at the lamina propria of the mucosal fold.

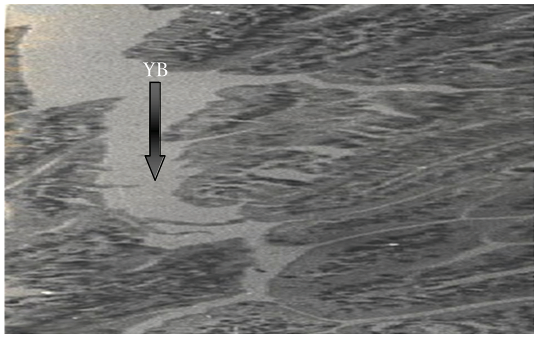

Figure 12: Plate of photomicrograph of intestine of *Clarias gariepinus* after 12 weeks exposure to 25 ppb of gammalin 20 showed yellow bodies (YB) at the lamina propria of the mucosal fold.

opercular activities, surface to bottom movement, sudden quick movement, and resting at the bottom were similar to the observations of Omoniyi et al. [19], Rahman et al. [6] and Aguigwo [20]. The stressful and erratic behaviour of *C. gariepinus* fingerlings in the experiment indicates respiratory impairment, probably due to the effect of the toxicant gammalin 20 on the gills. The stressful behaviour of respiratory impairment due to the toxic effect of gammalin 20 on the gills was similar with the report of Omitoyin et al. [21] and Aguigwo [20] that pesticide impairs respiratory organs.

A change in respiration rate is one of the common physiological responses to toxicants and is easily detectable through changes in oxygen consumption rate, which is frequently used to evaluate the changes in metabolism under environmental deterioration. Opercular movements increased initially in all exposure periods but decreased further steadily in lethal exposure compared to sublethal exposure periods. Increased gill opercular movements observed initially may possibly compensate the increased physiological activities under stressful conditions [22].

Gulping air at the surface, swimming at the water surface and disrupted shoaling behaviour were seen on the first day in lethal and sublethal exposure period and continued the same further intensely which is in accordance with the observations made by Ural and Simsek [23] Gulping of air may help to avoid contact of toxic medium. Surfacing phenomenon, that is, significant preference of upper layers in exposed group might be a demand of higher oxygen level during the exposure period [24]. Fish sunk to the bottom with the least opercular movements and died with their mouth opened. In sublethal exposure, fish body became lean towards abdomen position compared to control fish and was found under stress, but that was not fatal. Leaning of fish indicates reduced amount of dietary protein consumed by the fish at pesticide stress, which was immediately utilized and was not stored in the body weight [25]. Gammalin 20 toxicity is shown to increase with increased concentration. The behavioural effect observed in this study is in consonance with earlier reports of Quist [26], Murty et al. [27],Omregie and Ufodike [28], and Gesamp [10].

It was also observed that the higher the concentration of the toxicant, the higher the mortality rate. This demonstrates the observation of Fryer [29], that in all toxicant, a threshold is reached above which there is no drastic survival of animal. Below the threshold, animal is in a tolerance zone, above the tolerance zone is the zone of resistance. The time of toxicity disappearance and mortality was observed from the record of the relative mortality time in different concentrations of gammalin 20 for 96 hours.

C. gariepinus fingerlings were stressed progressively with time before death. There were mucus secretion in fish which forms a barrier between body and toxic media and thereby probably reduces contact of toxicant so as to minimize its irritating effect, or eliminates it through epidermal mucus. Similar observations were made by Rao et al. [30] and Parma de Croux et al. [31]. The analysis of data from the present investigation evidenced that gammalin 20 is toxic and had profound impact on behaviour and respiration in *C. gariepinus* in both lethal and sublethal concentrations.

The histopathological examination of the liver, gill, and intestinal tissues of the exposed fish indicated that the liver and gills were the organs most affected. In fish, gills are critical organs for their respiratory, osmoregulatory, and excretory functions.

Gills are generally considered good indicator of water quality [32], being models for studies of environmental impact [33–35], since they are the primary route for the entry of pesticide. Gills are the major respiratory organs and all metabolic pathways depend upon the efficiency of the gills for their energy supply, and damage to these vital organs causes a chain of destructive events, which ultimately lead to respiratory distress [36]. If gills would be destroyed due to xenobiotic chemicals [37] or the membrane functions are disturbed by a changed permeability [38], oxygen uptake rate would even rapidly decreased.

The fluctuated response in respiration may be attributed to respiratory distress as a consequence of the impairment of oxidative metabolism. Disturbance in oxidative metabolism was reported earlier under cypermethrin toxicity in *Tilapia mossambica* [39]. Pronounced secretion of mucus layer over the gill lamellae has been observed during gammalin 20 stresses. Secretion of mucus over the gill curtails the diffusion of oxygen [40], which may ultimately reduce the oxygen uptake by the fish.

Damages of the gills, indicated that the sublethal concentrations of insecticide caused impairment in gaseous exchange efficiency of the gills and this is similar to the observation of Rahman et al. [6], Omitoyin et al.[21] and Aguigwo [20]. Histopathological results indicated that gill was the primary target tissue affected by gammalin 20.

The liver is the main organ for detoxification (Dutta et al.,1993) that suffers serious morphological alterations in fish exposed to pesticides (Rodrigues and Fanta, 1998). Alterations in the liver may be useful as markers that indicate prior exposure to environmental stressors. The liver of the exposed fish had vacuolated cells showing evidence of fatty degeneration. Necrosis of some portions of the liver tissue that were observed resulted from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification, and this is similar to the observation of Rahmn et al. [6]. The inability of the fish to regenerate new liver cells might also have led to necrosis. The developmental stages of the fish under study were generally more sensitive to toxic pollutant than adults. This result conforms to reports of the study in Mance [41] and Kelly [42].

The intestinal tract is not easily divided into small and large intestine as it is in mammal. In treated fish the lamina propria at the tip of the mucosal fold of the intestine shows yellow bodies. This suggested that the yellow bodies could be a result of the effect of gammalin 20.

Hoque et al. [43] and Lovely [44] reported similar effect on *Puntius gonionotus, Barbodes gonionotus,* and *C. gariepinus* treated with diazinon and sumithion. According to Bhatnagar et al. [45], the observed irritation and destruction of the mucosa membrane of the intestine hamper absorption. The pathological alterations in the intestine of the studied fish are in agreement with those observed by many investigators about the effects of different toxicants on fish intestine [46, 47]. Epithelial degeneration, and inflammatory cells infiltration in the submucosal as well as submucosal edema were seen in the intestine of tilapia fish exposed to carbofuran [48]. It could be concluded that the environmental contamination can induce several histopathological alterations in the tissues of *C. gariepinus.*

References

[1] I. A. Shilov, "Stress as an ecological phenomenon," *Journal of Zoology*, vol. 58, no. 6, pp. 805–812, 1981.

[2] M. Z. Vosyliene and N. Kazlauskiene, "Alterations in fish health status parameters after exposure to different stressors," *Acta Zoologica Lituanica Hydrobiology*, vol. 9, no. 2, pp. 82–95, 1999.

[3] F. R. De la Torre, A. Saliba, and L. Ferrair, "Biomarkers of native fish species (*Cnesterodon decemaculatus*) application to the water toxicity assessment of a per-urban polluted river of Argentina," *Chemosphere*, vol. 59, no. 4, pp. 577–583, 2005.

[4] V. Radhaiah, M. Girija, and K. J. Rao, "Changes in selected biochemical parameters in the kidney and blood of the fish, *Tilapia mossambica*, exposed to heptachlor," *Bulletin of Environmental Contamination and Toxicology*, vol. 39, no. 6, pp. 1006–1011, 1987.

[5] J. D. Allan and A. S. Flecker, "Biodiversity Conservation in Running Waters," *BioScience*, vol. 43, no. 1, pp. 32–43, 1993.

[6] M. Z. Rahman, Z. Hossain, M. F. A. Mollah, and G. U. Ahmed, "Effect of diazinum 60 EC on Anabas testudineus, *Channa punctatus* and *Barbodes gonionotus'Naga'*," *The ICLARM Quarterly*, vol. 25, pp. 8–12, 2002.

[7] C. D. S. Tomlin, "British crop protection countil British Library *cataloguina publication data*," The pesticide Manual, 1606p, 1997.

[8] H. Ervnest, *A Textbook of Modern Toxicology*, John Wiley and Sons, Hoboken, NJ, USA, 3rd edition, 2004.

[9] R. Vasanbhi, P. Baskaran, S. Palanchyiny, and S. Aruna Chalam, "Impact of carbofuran on feeding evergetice in some fresh water fishes," *Environmental Econology*, vol. 8, pp. 40–45, 1989.

[10] GESAMP (INO/FAO/UNESCO/WHO/IAEA/UN/UNDP), "Joint group of experts in the scientific aspects of marine pollution (1991): review of potential harmful substance carcinogen," Report study 40, GESAMP, 1991.

[11] C. Biney, D. Calamari, T. W. Membe et al., "Scientific bases for pollution control in African inland waters," *FAO Fisheries Report*, vol. 369, pp. 9–23, 1987.

[12] B. G. Svensson, A. Nilsson, E. Jonsson, A. Schutz, B. Akesson, and L. Hagmar, "Fish consumption and exposure to persistent organochlorine compounds, mercury, selenium and methylamines among Swedish fishermen," *Scandinavian Journal of Work, Environment and Health*, vol. 21, no. 2, pp. 96–105, 1995.

[13] P. Joshi, D. Harish, and M. Bose, "Effect of lindane and Malathion exposure to certain Blood Parameters in a freshwater teleost fish *Clarias batrachus*," *Pollution Resources*, vol. 21, pp. 55–57, 2002.

[14] APHA, *Standard Methods for the Examination of Water and Waste Water*, American Public Health Association, New York, NY, USA, 20th edition, 1998.

[15] FAO, "Manual of methods in aquatic environment research part 4. Bases for selecting biological test to evaluate marine pollution," FAO Fish tech. paper 104, 1977.

[16] S. B. Mushigeri, *Effect of fenvalerate on the metabolism of Indian major carp, Cirrhinus mrigala*, Ph.D. thesis, Karnatak University, Karnataka, India, 2003.

[17] V. V. Meletev, A. S. Kanaev, and N. G. Dzasokhova, *Water Toxicology*, American Publishing, 1971.

[18] A. V. Holden, "Effects of pesticides on fish," in *Environmental Pollution by Pesticides*, C. A. Edwards, Ed., pp. 213–253, Plenum, New York, NY, USA, 1973.

[19] I. A. Omoniyi and S. A. Sodunke, "Effects of lethal and sublethal concentrations of Tobacco (Nicotiana tobaccum), Leaf dust extraction on weight and haematological changes in *Clarias gariepinus* (Burchell)," *Journal of Applied Science and Environmental Management*, vol. 6, pp. 37–41, 2002.

[20] J. N. Aguigwo, "The toxic effect of cymbush pesticide on growth and survival of African catfish, Clarias gariepinus (BURCHELL 1822)," *Journal of Aquatic Sciences*, vol. 17, no. 2, pp. 81–84, 2002.

[21] B. O. Omitoyin, E. K. Ajani, B. T. Adesina, and C. N. F. Okuagu, "Toxicity of lindane (gamma hexachlorocyclohexane) to *Clarias gariepinus* (Burchell 1822)," *World Journal of Zoology*, vol. 1, pp. 57–63, 2006.

[22] R. Shivakumar and M. David, "Toxicity of endosulfan to the freshwater fish, *Cyprinus carpio*," *Indian Journal of Ecology*, vol. 31, no. 1, pp. 27–29, 2004.

[23] M. Ş. Ural and S. Şimşek Köprücü, "Acute toxicity of dichlorvos on fingerling European catfish, Silurus glanis," *Bulletin of Environmental Contamination and Toxicology*, vol. 76, no. 5, pp. 871–876, 2006.

[24] S. Katja, B. O. S. Georg, P. Stephan, and E. W. S. Christian, "Impact of PCB mixture (Aroclor 1254) and TBT and a mixture of both on swimming behaviour, body growth and enzymatic biotransformation activities (GST) of young carp, *Cyprinus carpio*," *Aquatic Toxicology*, vol. 71, pp. 49–59, 2005.

[25] K. Kalavathy, A. A. Sivakumar, and R. Chandran, "Toxic effects of the pesticide dimethoate on the fish, *Sarotherodon mossambicus*," *Journal of Ecological Research and Biology*, vol. 2, pp. 27–32, 2001.

[26] C. Quist, *Effect of Piper guineensis on blood, gill, testis and gut of Oreochromis niloticus juvenile*, M.S. thesis, Department of Zoology, University of Ibadan, 1992.

[27] A. S. Murty, B. R. Rajabhushanam, A. V. Ramani, and K. Christopher, "Toxicity of fenitrothion to the fish Mystus cavasius and *Labeo rohita*," *Environmental Pollution Series A*, vol. 30, no. 3, pp. 225–232, 1983.

[28] E. Omoregie and E. B. C. Ufodike, "Histopathology of O.niloticus exposed to Actellic 25EC," *Journal of Aquatic Sciences*, vol. 6, pp. 12–17, 1991.

[29] J. D. Fryer, *Weed Control Handbook*, vol. 1 of *edited by Make Peace*, 1977.

[30] J. V. Rao, C. H. S. Rani, P. Kavitha, R. N. Rao, and S. S. Madhavendra, "Toxicity of chlorpyrifos to the fish *Oreochromis mossambicus*," *Bulletin of Environmental Contamination and Toxicology*, vol. 70, no. 5, pp. 985–992, 2003.

[31] M. J. Parma de Croux, A. Loteste, and J. Cazenave, "Inhibition of plasma cholinesterase and acute toxicity of monocrotophos in a neotropical fish Prochilodus lineatus (pisces, curimatidae)," *Bulletin of Environmental Contamination and Toxicology*, vol. 69, no. 3, pp. 356–363, 2002.

[32] J. C. Rankin, R. M. Atagg, and L. Bolis, "Effects of pollutants on gills," in *Gills*, D. F. Houlihan, J. C. Rankin, and T. J. Shuttleworth, Eds., pp. 207–220, Cambridge University Press, New York, NY, USA, 1982.

[33] J. Mallatt, "Fish gill structural changes induced by toxicants and other irritants: a statistical review," *Canadian Journal of Fisheries and Aquatic Sciences*, vol. 42, no. 4, pp. 630–648, 1985.

[34] J. M. McKim and R. J. Erickson, "Environmental impacts on the physiological mechanisms controlling xenobiotic transfer across fish gills," *Physiological Zoology*, vol. 64, pp. 39–67, 1991.

[35] S. E. Wendelaar Bonga and R. A. C. Lock, "Toxicants and osmoregulation in fish," *Netherlands Journal of Zoology*, vol. 42, no. 2-3, pp. 478–493, 1992.

[36] S. R. Magare and H. T. Patil, "Effect of pesticides on oxygen consumption, red blood cell count and metabolites of a fish, *Puntius ticto*," *Environmental Ecology*, vol. 18, pp. 891–894, 2000.

[37] G. C. M. Grinwis, A. Boonstra, E. J. Vandenbrandhof et al., "Short-term toxicity of bis (tri-n-butyltin) oxide in flounder, *Platichthys flesus*, pathology and immune funktion," *Aquatic Toxicology*, vol. 42, pp. 15–36, 1988.

[38] M. G.J. Hartl, S. Hutchinson, and L. E. Hawkins, "Organotin and osmoregulation: quantifying the effects of environmental concentrations of sediment-associated TBT and TPhT on the freshwater-adapted European flounder, *Platichthys flesus L.*," *Journal of Experimental Marine Biology and Ecology*, vol. 256, no. 2, pp. 267–278, 2001.

[39] M. David, H. B. Shivakumar, R. Shivakumar, S. B. Mushigeri, and B. H. Ganti, "Toxicity evaluation of cypermethrin and its effect on oxygen consumption of the freshwater fish, *Tilapia mossambica*," *Indian Journal of Environment and Toxicology*, vol. 13, no. 2, pp. 99–102, 2003.

[40] M. David, S. B. Mushigeri, and M. S. Prashanth, "Toxicity of fenvalerate to the freshwater fish, *Labeo rohita*," *Geobios*, vol. 29, pp. 25–28, 2002.

[41] G. Mance, *Pollution Threat by Heavy Metals in Aquatic Environment*, Elsevier, London, UK, 1987.

[42] M. Kelly, *Mining and the Freshwater Environment*, Elsevier, London, UK, 1988.

[43] M. M. Hoque, M. J. A. Miraja, and M. S. Maih, "Toxicity of diazinon and sumithion to *Puntuis genionotus*," *Bangladesh Journal of Transactions Development*, vol. 6, no. 1, pp. 19–26, 1993.

[44] F. Lovely, *Toxicity of three commonly used organophosphate insecticides to Thai Sharputi (Barbodes gonionotus) and African catfish (C. gariepinus) fry*, M.S. thesis, Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh, Bangladesh, 1998.

[45] C. Bhatnagar, M. Bhatnagar, and B. Regar, "Fluoride-induced histopathological changes in gill, kidney, and intestine of fresh water teleost, *Labeo rohita*," *Research Report Fluoride*, vol. 40, no. 1, pp. 55–61, 2007.

[46] M. Hanna, I. Shaheed, and N. Elias, "A contribution on chromium and lead toxicity in cultured *Oreochromis niloticus*," *Egyptian Journal of Aquatic Biology and Fish*, vol. 9, pp. 177–209, 2005.

[47] E. I. Cengiz and E. Unlu, "Sublethal effects of commercial deltamethrin on the structure of the gill, liver and gut tissues of mosquitofish, *Gambusia affinis*: a microscopic study," *Environmental Toxicology and Pharmacology*, vol. 21, no. 3, pp. 246–253, 2006.

[48] H. Soufy, M. Soliman, E. El-Manakhly, and A. Gaafa, "Some biochemical and pathological investigations on monosex Tilapia following chronic exposure to carbofuran pesticides," *Global Veterinaria*, vol. 1, pp. 45–52, 2007.