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Impact of MCPA on *Oreochromis mossambicus*: A Histopathological Assessment

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Abstract:
Background*: The extensive use of 2-methyl-4-chlorophenoxyacetic acid (MCPA) in Sri Lankan agriculture raises concerns about its ecological impact on aquatic ecosystems. Despite its widespread application, there is limited information regarding the toxicity of MCPA on Oreochromis mossambicus, an iconic and commercially important fish species in freshwater ecosystems. This study seeks to address this gap by investigating the toxicity of MCPA in Oreochromis mossambicus, focusing on determining its 96h lethal concentration 50% (LC50) and examining its sub-lethal effects on gill, muscle, and intestine histology over a 21-day period.*
Materials and methods: *Oreochromis mossambicus juveniles were acclimated and were exposed to various concentrations of MCPA ranging from 4 to 8 mg L⁻¹ to determine the LC50 value and were monitored for clinical indicators and mortality throughout the exposure period. The LC50 value was determined using Probit analysis. Sub-lethal study was conducted with fish exposed to a concentration of 1.5 mg L⁻¹, equivalent to 1/4th of the LC50 value, for 21 days. Histopathological examinations were then conducted on gill, intestine, and muscle tissues to assess the sub-lethal effects of MCPA exposure.*
Results: *Results unveiled severe alterations in all tissues. The gills exhibited fusion, distortion, and abnormal curling of secondary lamellae, clubbing of lamellae tips, epithelial lifting, pillar cell degeneration, hyperplasia, aneurysm, and degeneration followed by vacuolation. Similarly, muscles displayed focal degeneration, necrosis, broken fibers, muscle thickening, hyaline degeneration, myocyte losses, sarcoplasm fragmentation, and atrophic myocytes. Intestines exhibited villus atrophy, edema, fusion, branching, edema, degeneration, mucosal disruption, and necrosis.*
Conclusion: *These findings underscore MCPA's significant impact on multiple organ systems in Oreochromis mossambicus, highlighting potential environmental repercussions of herbicide exposure in aquatic ecosystems.*
Keyword: *Herbicide exposure; Histopathological effects; MCPA; Oreochromis mossambicus*

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I. Introduction

The extensive utilization of synthetic herbicides in agriculture has become a common practice aimed at increasing crop yields and combating weed infestations worldwide[1,2]. Among these herbicides, 2-methyl-4-chlorophenoxyacetic acid (MCPA), belonging to the Chlorophenoxy herbicide family, has gained significant attention due to its widespread use in controlling broadleaf weeds across various crops[3]. However, the application of synthetic herbicides like MCPA in agricultural fields can contaminate natural water systems through runoff. This contamination poses a notable hazard to aquatic life, including fish, as these chemicals have the ability to accumulate within fish tissues[4,5,6,7].

In the Sri Lankan context, the utilization of the MCPA is of particular concern, given its implications for the country's agricultural practices and environmental health. Reflecting broader global trends, Sri Lanka relies heavily on MCPA to manage weeds and enhance agricultural productivity[8,9,10,11]. However, the country's intricate network of rivers, streams, and irrigation canals serves as conduits for MCPA runoff from agricultural fields into freshwater ecosystems, exacerbating the chemical's adverse effects on aquatic life[12,13]. This contamination is particularly concerning for fish populations like *Oreochromis mossambicus* (Peters 1852), commonly known as Mozambique tilapia, which inhabit these freshwater habitats. As one of the most widely cultivated and ecologically significant fish species in the country, *Oreochromis mossambicus* plays a crucial role in local aquaculture and freshwater ecosystems[14,15,16]. Consequently, understanding the toxicological effects of MCPA on *Oreochromis mossambicus* is essential for assessing the broader ecological consequences of herbicide usage in Sri Lanka.

Therefore, the present study aims to investigate the toxicity of MCPA on *Oreochromis mossambicus*, specifically determining its lethal concentration 50% (LC50) value, and examining its sub-lethal effects on the histology of the gills, muscle, and intestine of *Oreochromis mossambicus* over a 21-day period. Tissue histology is considered an indicator of exposure to pollutants and serves as a valuable tool to assess the degree of pollution, especially for sub-lethal concentrations and chronic effects[17]. The detailed microscopic examination of tissues allows for the detection of subtle changes at the cellular and structural levels, providing insights into the long-term and often less evident impacts of pollutants on organisms[18]. Histopathological analysis is most commonly used in fish toxicological studies and aids in identifying abnormalities, such as cellular damage, inflammation, and changes in organ morphology, that may not be immediately apparent through other assessment methods[19]. Through comprehensive toxicological assessments and histopathological examinations, we seek to discern the sub-lethal effects of MCPA on *Oreochromis mossambicus*, shedding light on the potential long-term ramifications for freshwater ecosystems and fisheries management in Sri Lanka.

II. Materials And Methods

The acclimation tanks and the conducted experiments adhered to the guidelines set by the European Council Directive 86/609/EEC[20] and followed the guidelines for the care and use of fish in research by DeTolla et al.[21]. All experimental protocols received approval from the Departmental Research Committee of the Department of Zoology at Eastern University, Sri Lanka.

Fish Collection, Acclimation, and Stock Tank Maintenance

Juveniles of *Oreochromis mossambicus*, weighing 6.7 ± 1.0 g and measuring 7.2 ± 1.0 cm, were taken from fish culture tanks in the Department of Zoology at Eastern University of Sri Lanka. Prior to the experiment, fish were acclimatized to laboratory conditions for 10 days in a 300L capacity tank. They were kept in continuously aerated de-chlorinated water and water was renewed every 24h. During the acclimation period the average of water parameters were as follow: temperature 28.0 ± 2.0 °C, pH 6.8 ± 0.2 units, dissolved oxygen 6.6± 2.0 mg L^{-1}. During the acclimatization period, the fish were fed twice a day with commercially available fish pellets[22].

Experimental tanks setup and management

To eliminate any potential contaminants that could interfere with the results, aquarium tanks, each with a capacity of 50L, were cleaned using potassium permanganate solution before and after the experiment[23]. Upon cleaning, the aquarium tanks were filled with de-chlorinated water, ensuring optimal conditions for the experimental setup. The aquariums were also set up with continuous system of water aeration. Water quality was assessed according to the American Public Health Association guidelines[24]. Temperature was regulated to 28.0 ± 2.0 °C, pH levels were maintained at 6.8 ± 0.2 units and dissolved oxygen levels were targeted at 6.6± 2.0 mg L^{-1}. The water parameters were carefully monitored and maintained throughout the experiment to simulate a controlled aquatic environment. To prevent fish from escaping and to minimize mosquito breeding, each tank was covered with a small mesh net[22].

Determination of 96h LC50

Fish were divided into six groups, each comprising ten individuals, and placed in separate glass tanks filled with 50L of de-chlorinated water. Prior to commencing the 96h LC50 test, the fish were starved for 24h to ensure uniform conditions across all groups. The concentrations of MCPA herbicide administered to these groups were 4 mg L^{-1}, 5 mg L^{-1}, 6 mg L^{-1}, 7 mg L^{-1} and 8 mg L^{-1}, respectively, with the 6th group serving as the control, receiving no MCPA. Throughout the 96h observation period, careful monitoring was conducted on various clinical indicators such as alterations in skin colour, response to external stimuli, swimming patterns, and occurrences of mortality. Fish mortalities were recorded during the 96h exposure period, and dead fish were promptly removed from the test environment. The data collected were analyzed using the Probit analysis technique, as described by Finney[25], and SPSS version 20 statistical software was utilized to determine the 96h LC50 value.

Experimental Setup and Methodology for Histopathological Examination

For the histopathological study, a sub-lethal concentration of 1.5 mg L^{-1} of MCPA, equivalent to 1/4th of the determined LC50 value, was selected. Ten *Oreochromis mossambicus* juveniles without any structural, behavioral and clinical symptoms were chosen for experiment, after careful observation and subjected to a 24h starvation period before the initiation of the experiment. The experimental group, consisting of ten individuals, was exposed to the sub-lethal dose for 21 days, while a control group of another ten individuals was simultaneously maintained. The control group resided in experimental water without the addition of MCPA, with all other conditions held constant. Fish in both groups were fed twice a day throughout the experimental

period. To prevent degradation, the experimental water was regularly replaced with water containing the necessary quantity of the MCPA. Daily recordings of water quality parameters were conducted to ensure a constant and suitable aquatic environment.

After the completion of 21 days of the experimental period, the fish were humanely sacrificed. The process involved initially immobilizing the fish in ice and subsequently conducting careful dissection. Specifically, the gill, intestine, and muscles were removed from each fish. These extracted tissues were then fixed in Bouin's solution for a duration of 24h. After fixation, the tissue samples were dehydrated by transferring them sequentially into alcohol solutions with increasing concentrations (70%, 90%, and 100%) to remove water from the tissues. Subsequently, the dehydrated tissue samples were cleared by immersion in xylene to remove the alcohol and render the tissues transparent. Following clearing, the tissue samples were embedded in paraffin wax to provide support and facilitate sectioning. Tissue sections of 5µm thickness were obtained using a thermo scientific shandon finesse 325 manual microtome. These tissue sections were then stained with hematoxylin and eosin (H&E) to visualize cellular structures[26]. The stained slides were examined under a light microscope, and photomicrographs were captured to assess any histopathological effects present in the tissue samples.

III. Result

Table no 1 shows the MCPA concentrations and corresponding fish mortality rates after 96h of exposure for the LC50 experiment. The 96h LC50 value of MCPA obtained for the fish *Oreochromis mossambicus* is 6mg L^{-1} and 1/4th of the LC50 value which is 1.5 mg L^{-1} has been taken as sub lethal concentration for observing the histopathological changes in gill, muscle and intestine after the exposure 21 days and the results were compared with control.

Table no 1: MCPA concentrations and fish mortality rates in the 96h LC50 experiment

Concentration of MCPA (mg L^{-1})	Number of fish exposed	Number of fish dead	Morality (%)
0	10	0	0
4	10	1	10
5	10	2	20
6	10	5	50
7	10	8	80
8	10	9	90

The histopathological examinations of the gill, muscle, and intestine tissues of fish subjected to MCPA treatment displayed structural alterations compared to those of the control group fish.

Gill

Normal structure of the gills of *Oreochromis mossambicus* are composed of two sets of four holobranchs. Each holobranch comprises two hemibranchs extending from the posterior edge of the branchial arch in a manner where the free edges diverge and touch those of the neighboring holobranchs. Upon close examination of a fresh gill, the hemibranchs reveal a series of long, thin filaments known as primary lamellae, projecting from the arch. To enhance the surface area of each primary lamella, regular semilunar folds form across its dorsal and ventral surfaces, creating secondary lamellae. Secondary lamellae are arranged in rows along the gill filaments, resembling the structure of a comb. The surface of secondary lamellae is covered by a single layer of epithelial cells. Beneath the epithelial layer of the secondary lamellae lies a dense network of capillaries. In addition to the epithelial cells, secondary lamellae also contain pillar cells which provide structural support.

The gills of *Oreochromis mossambicus* in the control group exhibited a typical arrangement of primary and secondary lamellae, indicating normal histological structure (Figure 1. A). After 21 days of exposure to MCPA, significant histopathological changes were observed in the gills of *Oreochromis mossambicus* (Figure 1. B, C, D). Distortion of gill lamellae, characterized by irregularities or alterations in the shape and structure of the lamellae, was evident. Additionally, there was clubbing of secondary lamellae tips, where the tips of the lamellae appeared enlarged or swollen. Fusion of adjacent gill lamellae was observed, indicating the abnormal merging or joining together of neighboring lamellae. Furthermore, epithelial lifting, which refers to the separation or detachment of the epithelial layer from the underlying tissue, was noted. Abnormal curling of secondary lamellae was observed, with the lamellae exhibiting unusual twisting or bending patterns. Vacuole formation, characterized by the presence of fluid-filled spaces within the tissue, was also evident. Degeneration of pillar cells, which are essential structural components supporting the gill lamellae, was observed. This degeneration indicates damage or deterioration of these supportive cells. Additionally, aneurysm, defined as the localized dilation or swelling of blood vessels, was noted within the gill tissues. Moreover, degeneration

followed by vacuolation was observed, suggesting progressive tissue damage followed by the formation of vacuoles or empty spaces within the tissue. Finally, thickening of gill filaments due to hyperplasia, indicating an increase in the size of the filamentous structures within the gills, was noted. These histopathological alterations collectively indicate severe damage and structural changes in the gill tissues of *Oreochromis mossambicus* exposed to MCPA, highlighting the toxic effects of the herbicide on the respiratory and osmoregulatory functions of the gills.

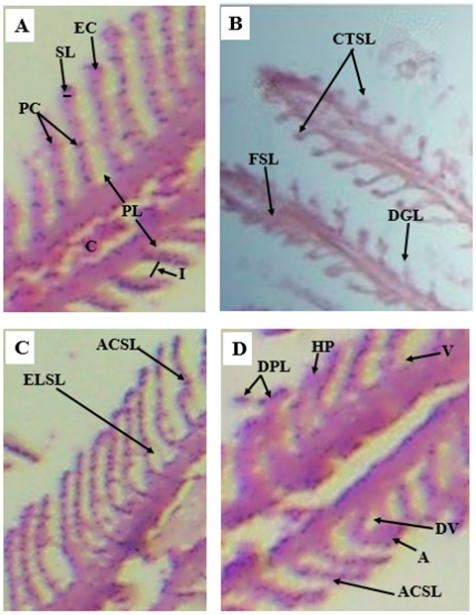

Figure no 1: Histological sections of the gill tissue of *Oreochromis mossambicus*. **(A)** Normal Histology of gills of the control group; PL: Primary Lamellae, SL: Secondary Lamellae, EC: Epithelial cell, PC: Pillar cell, I: Inter-filament lamellae space, C: Central venous of primary lamellae (erythrocytes). **(B), (C), (D)** Histopathological alterations of the gill tissue of *Oreochromis mossambicus* exposed to 1.5mg/L MCPA for 21 days; FSL: Fusion of secondary lamellae, CTSL: Clubbing tip of secondary lamellae, DGL: Distortion of gill lamellae, ELSL: Epithelial lifting of secondary lamellae, ACSL: Abnormal curling of secondary lamellae, V: Vacuole, DPL: Degeneration of pillar cells, HP: Hyperplasia, A: Aneurysm, DV: Degeneration followed by vacuolation (Stain: H&E; Magnification: ×400)

Muscle

In the control group, histological examination of *Oreochromis mossambicus* muscle tissues revealed a typical and well-organized structure (Figure 2. A). Longitudinal sections stained with H&E displayed clearly defined muscle fibers with characteristic alignment, showing evident arrangements of myofibrils and myofilaments. However, longitudinal sections through the muscles of *Oreochromis mossambicus* exposed to MCPA, exhibited distinct alterations (Figure 2. B, C, D). These alterations included necrosis, indicating areas of cell death within the muscle tissue, along with a reduction in the size of muscle cells, suggestive of muscle atrophy. Additionally, broken muscle fibers were observed, indicating disruptions or fractures in individual muscle fibers. Furthermore, myocyte losses indicated a notable decrease in the number of muscle cells. Moreover, focal degeneration, characterized by localized areas of tissue damage or breakdown, and thickening of muscle fibers, which suggests an increase in muscle cell size, were evident. Furthermore, fragmentation of sarcoplasm, where sarcoplasm becomes fragmented or disrupted, was also noticed. Additionally, hyaline degeneration, characterized by the accumulation of eosinophilic hyaline material within the muscle tissue, was observed. These observations collectively underscore the detrimental effects of MCPA exposure on muscle tissue integrity and function in *Oreochromis mossambicus*, compared to the control group.

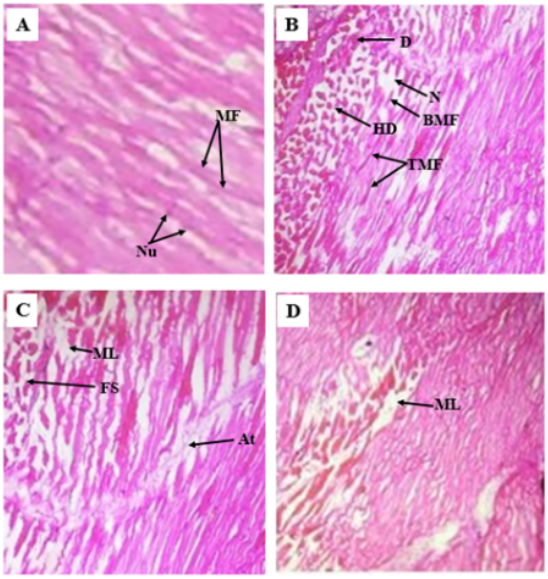

Figure no 2: Longitudinal histological sections of the skeletal muscle tissue of *Oreochromis mossambicus*. **(A)** Normal Histology of muscle tissue of the control group; MF: Muscle fibers, Nu: Nucleus. **(B), (C), (D)** Histopathological alterations of the muscle tissue of *Oreochromis mossambicus* exposed to 1.5mg/L MCPA for 21 days: D: Focal Degeneration, N: Necrosis, BMF: Broken muscle fibers, TMF: Thickening of muscle fibers, HD: Hyaline degeneration, ML: Myocyte loses, FS: Fragmentation of sarcoplasm, At: Atrophic myocytes (Stain: H&E; Magnification: ×400)

Intestine

Normal intestine of *Oreochromis mossambicus* the wall of the intestine consists of inner mucosa, the middle submucosa, muscularis and outer serosa layer. The mucosa is lined by a single layer of epithelial cells, interspersed with goblet cells that secrete protective mucus. In the intestine, finger-like villi project into the lumen, increasing the surface area for nutrient absorption, while microvilli on the surface of epithelial cells further enhance absorptive capabilities.

In the control group, histological examination of the intestine in *Oreochromis mossambicus* revealed a typical and well-organized structure (Figure 3. A). Longitudinal sections stained with H&E displayed intact villi with characteristic architecture, showing clear separation between villi and a regular pattern of epithelial cells lining the mucosal surface. However, longitudinal sections through the intestines of *Oreochromis mossambicus* exposed to MCPA, exhibited distinct alterations (Figure 3. B, C, D, E). These alterations included villus atrophy, characterized by a reduction in the size and height of villi, indicative of tissue shrinkage and loss of structural integrity. Additionally, villus edema was observed, indicating the accumulation of fluid within the villi, leading to swelling and distortion of their normal morphology. Villus fusion was noted, involving the merging or adhesion of adjacent villi, resulting in the formation of larger, fused structures. Furthermore, villus branching was evident, describing the division or splitting of individual villi into two or more branches, leading to an increase in the number of villi. Intestinal edema affected the entire intestinal wall, resulting in swelling and distortion of tissue layers. Hydropic degeneration was observed, characterized by the abnormal accumulation of fluid within intestinal cells, leading to cellular swelling and disruption of cellular architecture. Finally, mucosal disruption involved the breakdown or rupture of the mucosal layer within the villi, leading to the loss of epithelial integrity and exposure of underlying tissue layers. These observations collectively underscore the detrimental effects of MCPA exposure on the integrity and function of the intestinal tissue in *Oreochromis mossambicus*, compared to the control group. They reflect various histopathological changes, including structural alterations, fluid accumulation, and tissue damage, highlighting the severity of the toxicological impact on the intestinal mucosa.

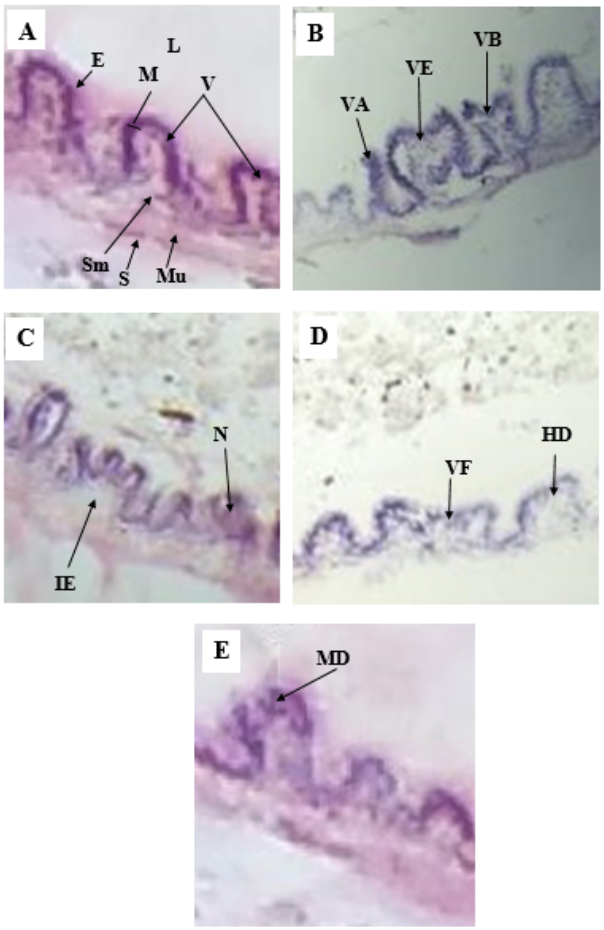

Figure no 3: Longitudinal histological sections of the intestine of *Oreochromis mossambicus*. **(A)** Normal Histology of intestine of the control group; M; Mucosa, Sm: Submucosa, Mu: Muscularis, S: Serosa, L: Lumen, E: Epithelium, V: Villi. **(B), (C), (D)** Histopathological alterations of the intestine of *Oreochromis mossambicus* exposed to 1.5mg/L MCPA for 21 days; VA: Villus atrophy, VE: Villus edema, VF: Villus fusion, VB: Villus branching, IE: intestinal edema, HD: Hydropic degeneration, MD: Mucosal disruption, N: Necrosis (Stain: H&E; Magnification: ×400)

IV. Discussion

In the present study the toxicity test showed that the weedicide MCPA is toxic to the *Oreochromis mossambicus*, causing large number of changes in the gill, muscle and intestine.

Fish gills have many important functions involving gaseous exchange, ion transport, nitrogenous waste excretion, acid-base balance[27]. The gill surfaces are protected by epithelial cells, including pavement cells, chloride cells, and mucous cells. These cells are sustained by a complex system of blood vessels[28]. As a result, the gill epithelium provides a broad interface for engagement with the surroundings, this epithelium is notably vulnerable to waterborne pollutants[29].

The exposure of *Oreochromis mossambicus* to MCPA resulted in notable histological alterations in gills, encompassing distortion and deformation of gill lamellae, epithelial lifting, clubbing of secondary lamellae tips, hyperplasia, fusion of adjacent gill lamellae, and degeneration of pillar cells. These observations coincide with findings from prior studies examining the effects of various pesticides and herbicides. Ahmad et al. documented analogous alterations in the gills of *Ctenopharyngodon idella* subsequent to exposure to atrazine[30]. Similarly, Jayachandran and Pugazhendy observed comparable effects of glyphosate based herbicide in *Labeo rohita*[31], while Samanta et al. reported analogous histological changes *in Heteropneustes fossilis* under similar exposure conditions[32]. Yalsuyi et al. documented analogous histological alterations in *Cyprinus carpio* exposed to atrazine[33]. Likewise, Kumar et al. observed hyperplasia, filament thickening, and lamellar fusion in *Oreochromis mossambicus* exposed to endosulfan, indicating consistent responses across different toxic substances[34]. Additionally, our study observed abnormal curling of secondary lamellae, vacuole formation, degeneration of pillar cells, aneurysm, and degeneration followed by vacuolation, which aligns with findings reported by Subburaj et al.[35]. They observed similar histological changes in gills following exposure to

Malathion, underscoring common histopathological responses among various pesticides. Bojarski et al. further supported these findings through their investigation of the physiological and histological effects of herbicides in fish, emphasizing the necessity of understanding the broader implications of chemical exposures on aquatic organisms[29]. These findings underscore the profound impact of herbicide exposure on fish gill tissues and emphasize the need for continued research to understand the broader implications of chemical pollutants on aquatic ecosystems and organismal health.

Exposure to MCPA induced notable histological changes in the muscle tissue of *Oreochromis mossambicus*, characterized by muscle atrophy, myocyte losses, focal degeneration, Hyaline degeneration, broken muscle fibers, necrosis, fragmentation of sarcoplasm and thickening of muscle fibers. Comparing our results with previous studies reveals similar findings regarding the histopathological effects of herbicide exposure on fish skeletal muscles. Abd-Algadir et al. investigated the effect of pendimethalin herbicide on *Tilapia nilotica* skeletal muscles, reporting histopathological alterations including fragmentation of sarcoplasm and necrosis[36]. Ramah studied the impact of rice herbicides on *Ctenopharyngodon idella*, observing similar histopathological changes such as thickening of muscle fibers and necrosis[37]. Aly et al. examined the effects of saturn herbicide on *Oreachromis niloticus*, noting histological alterations in muscle tissues, including hyaline degeneration and necrosis[38]. Furthermore, Sayed et al. investigated the histopathological effects of silver nanoparticles on *Clarias garepinus* muscles, observing changes such as muscle fiber degeneration, broken muscle fibers, myocyte loses, atrophy and thickening of muscle fibers, which are consistent with our findings[39]. These studies collectively support the notion that exposure to herbicides and nanoparticles can induce significant histopathological alterations in fish muscle tissues, underscoring the importance of assessing the environmental impact of chemical pollutants on aquatic organisms.

The pathological changes observed in the intestinal tissues of *Oreochromis mossambicus* exposed to MCPA in our study are consistent with findings reported in previous research involving exposure to various herbicides and pollutants. Villus atrophy, edema, fusion, branching, intestinal edema, hydropic degeneration, and mucosal disruption are indicative of severe damage to the intestinal mucosa, resulting from toxicant exposure. These alterations disrupt the normal structure and function of the intestine, potentially compromising nutrient absorption and overall health in fish. Our findings align with those of Samanta et al. observed similar pathological changes in the intestine of *Heteropneustes fossilis* treated with glyphosate based herbicide[32]. Similarly, Ertug et al. reported comparable effects on the intestine of zebrafish exposed to 2,4-Dichlorophenoxyacetic acid[40], while Stalin et al. documented analogous alterations in the intestine of *Channa punctatus* exposed to chlorpyrifos[41]. Furthermore, the observations reported by Bhatnagar et al. on *Labeo rohita* exposed to fluoride revealed fusion of villi and flattened villi, indicative of structural damage to the intestinal mucosa[42]. Ravanaiah and Murthy reported vacuolization, damage of villi and serosa layer, necrotized mucous epithelium, and hyperemia of mucous cells in *Tilapia mossambica* exposed to industrial pollutants, highlighting extensive tissue damage and mucosal dysfunction[43]. These findings underscore the vulnerability of fish intestines to a wide range of environmental contaminants and emphasize the urgent need for stringent monitoring and regulation to mitigate their detrimental effects on aquatic ecosystems and organismal health.

V. Conclusion

The findings of the present study underscore the importance of histopathology as a reliable indicator of environmental contamination, particularly evident in the severe tissue damage caused by MCPA exposure in *Oreochromis mossambicus*. Despite pollutants like MCPA often persisting at sub-lethal levels without causing immediate mortality, they can significantly impact fish health, leading to illness and reduced fitness for survival.

This investigation highlights the necessity of understanding MCPA's toxic effects on freshwater fish histology, such as *Oreochromis mossambicus*, to implement effective measures for environmental protection and fish population sustainability.

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References

[1] Hossain Mm. Recent Perspective Of Herbicide: Review Of Demand And Adoption In World Agriculture. Journal Of The Bangladesh Agricultural University. 2015 Jun 30;13(1):13-24.

[2] Liu Y, Pan X, Li J. Current Agricultural Practices Threaten Future Global Food Production. Journal Of Agricultural And Environmental Ethics. 2015 Apr;28:203-16.

[3] Mcghee I, Burns Rg. Biodegradation Of 2, 4-Dichlorophenoxyacetic Acid (2, 4-D) And 2-Methyl-4-Chlorophenoxyacetic Acid (Mcpa) In Contaminated Soil. Applied Soil Ecology. 1995 Sep 1;2(3):143-54.

[4] Caux Py, Kent Ra, Bergeron V, Fan Gt, Macdonald Dd. Environmental Fate And Effects Of Mcpa: A Canadian Perspective. Critical Reviews In Environmental Science And Technology. 1995 Nov 1;25(4):313-76.

[5] Bach M, Frede Hg. Trend Of Herbicide Loads In The River Rhine And Its Tributaries. Integrated Environmental Assessment And Management. 2012 Jul;8(3):543-52.

[6] Palma P, Köck-Schulmeyer M, Alvarenga P, Ledo L, Barbosa Ir, De Alda Ml, Barceló D. Risk Assessment Of Pesticides Detected In Surface Water Of The Alqueva Reservoir (Guadiana Basin, Southern Of Portugal). Science Of The Total Environment. 2014 Aug 1;488:208-219.

[7] Loos R, Tavazzi S, Mariani G, Suurkuusk G, Paracchini B, Umlauf G. Analysis Of Emerging Organic Contaminants In Water, Fish And Suspended Particulate Matter (Spm) In The Joint Danube Survey Using Solid-Phase Extraction Followed By Uhplc-Ms-Ms And Gc–Ms Analysis. Science Of The Total Environment. 2017 Dec 31;607:1201-12.

[8] Bandara Rm, Abeysekara As, Wickrama Wm, Dissanayaka Hm, De Silva Ym, Dissanayake Dm. Herbicides For Rice Cultivation Under Limited Irrigation. In Paper Proceedings Of The Second Asia-Pacific Conference On Multidisciplinary Research 2017 (Apmr2017) 2017 Jul 29 (P. 130).

[9] Kuruppu Iv. Restructuring Extension On Herbicide Usage Of Paddy Farmers In Anuradhapura District. Applied Economics & Business. 2018 Dec;2(2):51.

[10] Malkanthi Sh, Sandareka Ug, Wijerathne Aw, Sivashankar P. Banning Of Glyphosate And Its Impact On Paddy Cultivation: A Study In Ratnapura District In Sri Lanka. Journal Of Agricultural Sciences (Sri Lanka). 2019 May 1;14(2):129-144.

[11] Marambe B, Herath S. Banning Of Herbicides And The Impact On Agriculture: The Case Of Glyphosate In Sri Lanka. Weed Science. 2020 May;68(3):246-52.

[12] Steele P, Konradsen F, Imbulana Ka. Irrigation, Health And The Environment: A Literature Review With Examples From Sri Lanka. Colombo, Sri Lanka: International Irrigation Management Institute; 1997.

[13] Padmajani Mt, Aheeyar Mm, Bandara Ma. Assessment Of Pesticide Usage In Up-Country Vegetable Farming In Sri Lanka. Colombo: Hector Kobbekaduwa Agrarian Research And Training Institute; 2014.

[14] De Silva Ss. Body Condition And Nutritional Ecology Of Oreochromis Mossambicus (Pisces, Cichlidae) Populations Of Man Made Lakes In Sri Lanka. Journal Of Fish Biology. 1985 Nov;27(5):621-633.

[15] Jayathilaka Br, Wathsala Rh, Fernando Ap. Views Of Fishermen And Aquaculture Authorities On The Stocking Of Tilapia And Carp Species In The Dry Zone Reservoirs, Sri Lanka.In Proceedings Of Fifth Annual Science Research Sessions 2016 On "Enriching The Novel Scientific Research For The Development Of The Nation". South Eastern University: Faculty Of Applied Sciences, South Eastern University Of Sri Lanka 2016 (P. 30-37).

[16] Athukorala Da, Amarasinghe Us. Population Dynamics Of Commercially Important Fish Species In Two Reservoirs Of The Walawe River Basin, Sri Lanka. Asian Fisheries Science. 2010;23(1):71-90.

[17] Cengiz Ei, Ünlü E. Histopathology Of Gills In Mosquitofish, Gambusia Affinis After Long Term Exposure To Sublethal Concentrations Of Malathion. Journal Of Environmental Science And Health, Part B. 2003 Aug 1;38(5):581-589.

[18] Poole Jj, Mostaço-Guidolin Lb. Optical Microscopy And The Extracellular Matrix Structure: A Review. Cells. 2021 Jul 12;10(7):1760.

[19] Hinton De, Segner H, Braunbeck T. Toxic Responses Of The Liver. In Target Organ Toxicity In Marine And Freshwater Teleosts. 2017 Mar 27 (Pp. 224-268). Crc Press.

[20] Council Of The European Communities. Council Directive 86/609/Eec Of 24 November 1986 On The Approximation Of Laws, Regulations And Administrative Provisions Of The Member States Regarding The Protection Of Animals Used For Experimental And Other Scientific Purposes. Official Journal Of The European Union. 1986;L358:1-28.

[21] Detolla Lj, Srinivas S, Whitaker Br, Andrews C, Hecker B, Kane As, Reimschuessel R. Guidelines For The Care And Use Of Fish In Research. Ilar Journal. 1995 Jan 1;37(4):159-73.

[22] Selvi Rt, Ilavazhahan M. Histopathological Changes In Gill Tissue Of The Fish Catla Catla Exposed To Sublethal Concentration Of Pesticide Methyl Parathion And A Heavy Metal Ferous Sulphate. Biomedical And Pharmacology Journal. 2015 Apr 26;5(2):305-12.

[23] Kumar Bg, Nandan Sb, Kumari Ta, Sajeevan K. Curative Effect Of Vitamin C On The Variation In Biochemical And Histopathological Parameters Induced By Copper Exposure In The Teleost Fish, Anabas Testudineus (Bloch, 1792). Iosr Journal Of Environmental Science, Toxicology And Food Technology. 2014;8:28-35.

[24] American Public Health Association (Apha). Standard Methods For The Examination Of Water And Wastewater. 21st Centennial Edition. Washington, Dc: Apha, Awwa, Wpcf; 2005.

[25] Finney Dj. Probit Analysis. Cambridge University Press, Cambridge, Uk. 1971;333.

[26] Samanta P, Kumari P, Pal S, Mukherjee Ak, Ghosh Ar. Histopathological And Ultrastructural Alterations In Some Organs Of Oreochromis Niloticus Exposed To Glyphosate-Based Herbicide, Excel Mera 71. Journal Of Microscopy And Ultrastructure. 2018 Jan 1;6(1):35-43.

[27] Perry Sf, Laurent P. Environmental Effects On Fish Gill Structure And Function. In Fish Ecophysiology 1993 (Pp. 231-264). Dordrecht: Springer Netherlands.

[28] Wilson Jm, Laurent P. Fish Gill Morphology: Inside Out. Journal Of Experimental Zoology. 2002 Aug 1;293(3):192-213.

[29] Bojarski Ba, Jakubiak M, Witeska M. Physiological And Histological Effects Of Herbicides In Fish. Annals Of Warsaw University Of Life Sciences-Sggw. Animal Science. 2018;57(3):207-217.

[30] Ahmad W, Sultan A, Jan Sm, Ali A, Rahman Iu, Usman H, Sher A, Khan N, Khan Z, Khan A. Environmental Risk Of Atrazine (Herbicide) To Brain, Gills And Liver Tissues Of Fish Ctenopharyngodon Idella (Valenciennes, 1844). Journal Of Geoscience And Environment Protection. 2021 Feb 4;9(2):1-6.

[31] Jayachandran K, Pugazhendy K. Histopathological Changes In The Gill Of Labeo Rohita (Hamilton) Fingerlings Exposed To Atrazine. American-Eurasian Journal Of Scientific Research. 2009 Dec 9;4(3):219-21.

[32] Samanta P, Mukherjee Ak, Pal S, 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. Journal Of Microscopy And Ultrastructure. 2016 Sep 1;4(3):147-55.

[33] Yalsuyi Am, Vajargah Mf, Hajimoradloo A, Galangash Mm, Prokić Md, Faggio C. Evaluation Of Behavioral Changes And Tissue Damages In Common Carp (Cyprinus Carpio) After Exposure To The Herbicide Glyphosate. Veterinary Sciences. 2021 Oct 5;8(10):218.

[34] Kumar N, Sharma R, Tripathi G, Kumar K, Dalvi Rs, Krishna G. Cellular Metabolic, Stress, And Histological Response On Exposure To Acute Toxicity Of Endosulfan In Tilapia (Oreochromis Mossambicus). Environmental Toxicology. 2016 Jan;31(1):106-15.

[35] Subburaj A, Jawahar P, Jayakumar N, Srinivasan A, Ahilan B. Acute Toxicity Bioassay Of Malathion (Ec 50%) On The Fish, Oreochromis Mossambicus (Tilapia) And Associated Histological Alterations In Gills. Journal Of Entomology And Zoology Studies. 2018;6(1):103-7.

[36] Abd-Algadir Mi, Idris Of, Elkhier Mk. Effect Of Pendimethalin Herbicide On Fish (Tilapia Nilotica) Skeletal Muscles, Gills And Its Influence On Human. World Journal Of Life Sciences And Medical Research. 2011;1(5):5-10.
[37] Ramah K. Histopathological Study On The Effect Of Rice Herbicides On Grass Carp (Ctenopharyngodan Idella). African Journal Of Biotechnology. 2011;10(7):1112-1116.
[38] Aly Sm, Hasanin Si, Hssab-Alla Az. Impact Of Saturn Herbicide On Histological Structures And Residual Aspects Of Nile Tilapia (Oreachromis Niloticus). Egypt. J. Comp. Path. & Clinic. Path. 2008;21:259-274
[39] Sayed Ae, Mekkawy Ia, Mahmoud Um, Nagiub M. Histopathological And Histochemical Effects Of Silver Nanoparticles On The Gills And Muscles Of African Catfish (Clarias Garepinus). Scientific African. 2020 Mar 1;7:E00230.
[40] Ertug Nd, Akbulut C, Abar M, Günes S. The Histopathological Effects Of 2, 4-Dichlorophenoxyacetic Acid On Intestine Tissue Of Zebrafish (Danio Rerio). Elixir Pollution. 2014 Aug 21;74:27021-27024.
[41] Stalin A, Suganthi P, Mathivani S, Paray Ba, Al-Sadoon Mk, Gokula V, Musthafa Ms. Impact Of Chlorpyrifos On Behavior And Histopathological Indices In Different Tissues Of Freshwater Fish Channa Punctatus (Bloch). Environmental Science And Pollution Research. 2019 Jun 1;26:17623-31.
[42] Bhatnagar C, Bhatnagar M, Regar Bc. Fluoride-Induced Histopathological Changes In Gill, Kidney, And Intestine Of Fresh Water Teleost, Labeo Rohita. Fluoride. 2007 Mar;40(1):55-61.
[43] Ravanaiah G, Murthy Cv. Impact Of Aquaculture And Industrial Pollutants Of Nellore District On The Histopathological Changes In The Liver And Intestine Tissues Of Fish, Tilapia Mossambica. National Journal Of Life Sciences. 2010;7(2):117-22.

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