

Review Article

A review on toxic effects of pesticides on various physiological aspects of fish

Abstract

Pesticides are notorious for staying and spreading in the environment, and they can accumulate and disturb the food web, which endangers both animals and humans. In addition to being particularly damaging to fish, pesticides are toxic to other species that are part of the food chain. In this review, an attempt has been made to critically review the harmful effects of different pesticides on different fish species. Their entry into aquatic ecosystems causes disastrous effects on flora and fauna. In this review, we will find various alternations or changes in fish behavior, biochemical characteristics (antioxidant defense enzymes), blood parameters and histopathological parameter changes caused by harmful pesticides. Higher concentrations of pesticides in the water bodies can lead to the mortality of fish species while sub-lethal concentrations have long-lasting effects. An examination of the regulations in India governing the use of pesticides reveals the urgent need for their appropriate execution.

Keywords: pesticides, behavior, hematology, antioxidant defense, histology

Introduction

Pesticides are synthetic chemicals that humans create to protect plants from pests and diseases [1]. Pesticides are part of a large group of chemicals that farmers use for different purposes, such as protecting crops from pests, reducing crop losses, and preventing diseases spread by vectors (e.g., malaria, dengue) [2, 3]. They come in different types depending on what they target or what they are made of, such as insecticides. When they are widely used, pesticide residues often end up in water bodies through farm and city runoff [4], wind drift, or direct spraying, and they can harm aquatic animals that are not meant to be affected, especially in densely populated areas where the levels are too high. Insecticides are the main source of contamination for many water habitats. They enter the water systems through rain, runoff, and wind. They end up in ponds, lakes, and rivers [5] and harm various non target aquatic organisms. The health of fish and other aquatic animals is endangered by the reduced water quality caused by harmful substances such as pesticides. Fish is the most vital vertebrate that serves as food for humans. They have significant economic, aesthetic, nutritional, industrial, religious and medicinal value. Fish provides livelihood for millions of people around the world. The high usage of pesticides shows that more than one active ingredient threatens 64% of the world's farmland with pesticide contamination, and 31% faces a high risk. Also, pesticide pollution is likely to grow soon [6].

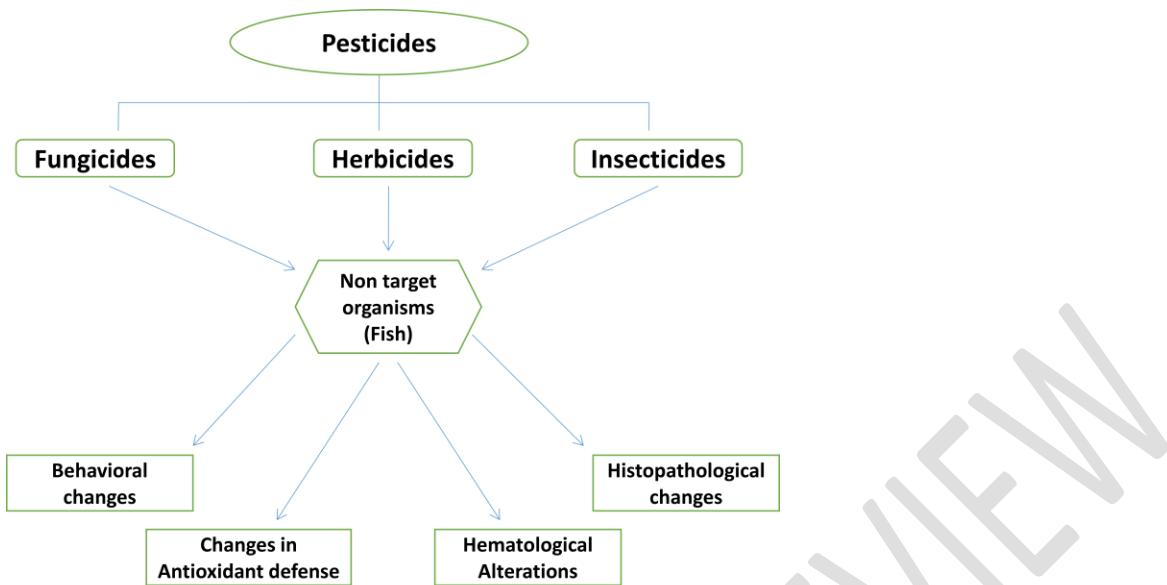


Fig 1: Effects of different pesticides on behavior, hematology, histology and antioxidant defense system of fish.

Examples of pesticides include fungicides, herbicides and insecticides. Fungicides are a type of pesticide that destroys harmful fungi and their spores. They are usually made of chemicals, but some may use biological agents or substances that inhibit fungal growth. Fungi can harm crops and lead to significant reductions in quantity, quality, and income [7]. According to the literature, fungicides have serious effects on fish, ecosystems and, aquatic invertebrates that are not lethal, but affect various biological aspects, such as zooplankton and fish reproduction, fish immunity, zooplankton diversity, enzyme activity, and ecosystem functions, like leaf breakdown in streams [8]. Insecticides differ from other organic pollutants in that they are made to be toxic to specific organisms. Because many of the places they affect are similar across different groups of living things, they can also harm non target organisms like fish. These chemicals stayed in the environment for a long time, built up in living things, and harmed fish a lot. Pyrethroids are more favored than other kinds of insecticides, as Lu *et al.*, [9] stated because they are very effective, less toxic to mammals, more selective to pests, and degrade quickly in the environment. Organophosphates (OPs) are poisonous to insects because they inhibit the enzyme acetylcholinesterase (AChE) and stop acetylcholine from being broken down in nerve junctions, which results in nerve damage and death according to [10]. Pesticides have various effects on the fish including behavioral changes, biochemical changes, hematological changes and various histopathological alterations.

Behavioral changes in fish caused by pesticides

Behavior is the physical manifestation of the animal's internal neural, hormonal and metabolic processes necessary for survival [11] working together with both central and peripheral nervous

systems [12]. It makes it possible for a living thing to adjust to stimuli from within as well as outside to survive in a changing environment. Behavioral disruption may be detected in aquatic organisms at the concentration of pollutants present in the field. The majority of pesticides disrupt fish neurological systems as well as sensory receptors, which can alter fish behavior and cause abnormalities in how fish react to environmental cues. Fish with reduced mobility may also be affected by the way certain pesticides affect acetylcholinesterase (AChE) activity [13]. Enzyme activity variations are frequently linked to changes in a wide range of other metabolic processes, and as a result, they encompass all physiological changes in an organism. According to Banaee [13] and Üner *et al.*, [14], fish that had their AChE inhibited also had higher acetylcholine levels. This can be harmful since it affects the fish's ability to feed, swim, identify itself, and navigate its environment [15]. Thus, it is thought that AChE inhibition is a particular biomarker of exposure to pesticides that are carbamate and organophosphates. In the brain of rainbow trout subjected to sub-lethal levels of lindane, shows disruption in the γ -aminobutyrate (GABA) pathway [16]. Overstimulation of the nervous system can also result from disruptions in GABA receptors, which block the passage of nerve impulses. Fish's close interaction with the aquatic environment makes them perfect sentinels for behavioral tests of chemical toxins and stressors [17, 18]. Different fish species and exposure settings cause significant differences in behavioral patterns. When *C. batrachus* was subjected to propiconazole and mancozeb, Srivastava and Singh [19] observed that the toxicant disrupted the acetylcholinesterase activity of the fish which resulted in death or mortality. According to them substances that prevent cholinesterase from acting upsettingly also cause fish's normal motions to be disrupted. Fish exposed to glyphosate showed increased operculum movement (ventilatory frequency) and swimming activity [20]. The tertiary level of physiological reactions to a toxicant, and behavior responses (like swimming ability) might be utilized as a biomarker of stress. According to Yalsuyi *et al.* [21], the fish (*C. carpio*) preferred to swim close to the water outflow when the level of glyphosate was raised; on the other hand, when the glyphosate level was lowered, the fish swam closer to the water introduction. According to Singh and Narain [22], when *C. carpio* was exposed to acute concentration of dimethoate it displayed inconsistent swimming, increased surfacing, declined opercular movement rate, excess mucus secretion, decreased agility, and an inability to maintain normal posture and balance over time. When *C. carpio* is administered with different concentrations of 2,4-D, it might exhibit aberrant behaviors such as nervousness, jerky movements, loss of balance, inverted or vertical posture, difficulty breathing, profuse mucous secretions, and fading of the body color [23]. *Clarias batrachus* showed significant behavioral alterations after treatment with chlorpyrifos and cypermethrin which included altered schooling behavior, positioning at the bottom, aberrant and uneven swimming patterns, frequent surface visits, caudal bending and a delayed reaction to touch [24]. The morphological alterations included thinness of the body, increased mucus discharge, and body decolorization. Exposure to two organophosphate compounds (dimethoate and chlorpyrifos) to *C. carpio* resulted in various alterations in behavior patterns [25]. These changes were observed using different behavioral indices such as tail and opercular beat frequency, fin and eye deformities and swimming velocity

and activity index. We can say that different pesticides influence differently on behavioral parameters in fish. These behavioral alternations include excess mucus secretion, loss of body balance, altered opercular movements, loss of movement directions, fast and sometimes backward swimming and higher concentrations of pesticides leading to hemorrhage and ultimately lead to death.

Changes in the antioxidant defense system of fish caused by pesticides

Variations in the antioxidant defense system of fish act as useful and crucial markers of pesticidal toxicity. One of the most important biological acceptors of electrons for cellular processes is oxygen. Notwithstanding its advantageous characteristics, it is responsible for the unfavorable generation of various kinds of reactive oxygen species (ROS). Cells rely on a series of antioxidant enzymes—including glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), xanthine oxidase (XOD), catalase (CAT), and superoxide dismutase (SOD)—to combat reactive oxygen species (ROS) as their initial defense mechanism. Cellular injury and oxidative stress are caused by an imbalance between the activity of cellular enzymes that fight oxidative stress and ROS generation. There's a greater chance of oxidative damage if the antioxidant system cannot get rid of or balance off the surplus ROS [26].

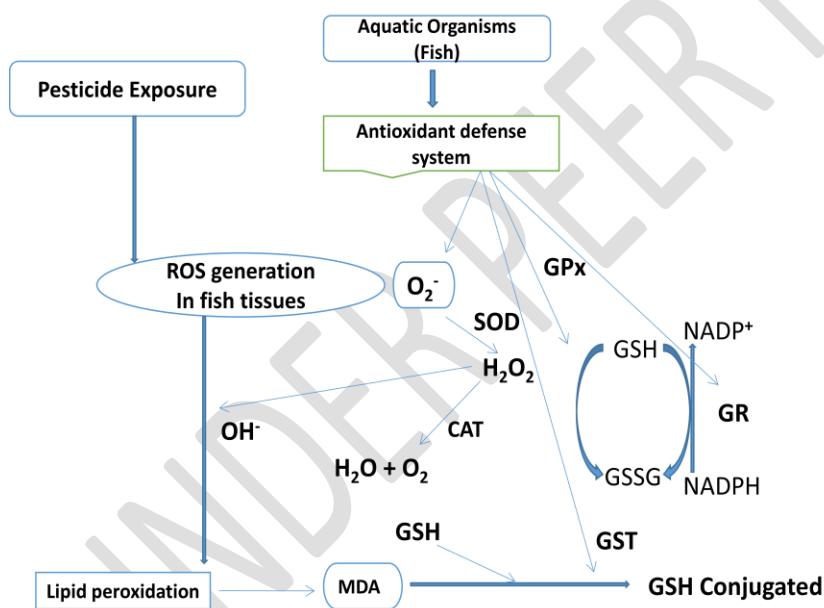


Fig 2: Role of antioxidant defense enzymes to combat the oxidative stress produced due to over-production of ROS after pesticide exposure.

Fishes collected from pesticide-contaminated Ganga River showed an increased amount of CAT, SOD and GST activities [27]. According to Tiwari and Singh [28], there was a noticeable decrease in the quantity of protein in the fish's blood, gills, liver, muscle and intestine following the administration of oleandrin. This is due to the hazardous and sub-lethal adverse effects of oleandrin on the biochemical properties of freshwater air-breathing murrels, *Channa punctatus*.

Juvenile carp (*C. carpio*) exposed to endosulfan at a dose below the fatal threshold for a sub-chronic time showed notable alterations in liver somatic indicators, activation of the first phase biotransformation system as well as oxidative stress [29]. An experiment conducted by Kaviraj and Gupta [30] on freshwater fish Nile tilapia (*Oreochromis niloticus*) showed a decrease in CAT activities in the liver when administered with pyrethroids. *Mugil capito* exposure to different concentrations of meothrin, lambda-cyhalothrin, permethrin, fenpropathrin, and esfenvalerate to *Mugil capito* showed increased levels of MDA [31]. Impact of Persistent Application of Methomyl Pesticide on Antioxidant Defense System in Tilapia Testis (*Oreochromis niloticus*) showed a significant increase in the GPx, CAT, GST, GR, SOD, GSH and GSSG at low concentrations but a significant decrease at higher concentrations [32]. Oxyfluorfen (herbicide) exposure to Catfish, *C. gariepinus*, there was a notable decline in the activities of SOD, GSH, GPx, and CAT along with the rise in MDA levels [33]. Treatment of juveniles (*Astyanax altiparanae*) with herbicide atrazine showed elevated malondialdehyde (MDA) and protein levels indicators in the liver, muscle and gills, indicating an elevated oxidative unbalance [34]. Additionally, they noted that the antioxidant enzyme GST was more active in the liver and SOD and CAT were more active in the muscles and gills. The activities of CAT, GPx and SOD in juvenile fish, *Oreochromis mossambicus* were suppressed by carbaryl and dimethoate [35]. The glyphosate exposure to *Galatea paradoxa* resulted in increased activities of GST, LDH, CAT and SOD [36]. Additionally, the data showed reduced GSH concentration and elevated GPx activity in fish *Galatea paradoxa*. The brains of the zebra fish exposed to the highest quantity of glyphosate showed a marked increase in CAT and SOD activity [37]. Additionally, glyphosate-treated fish showed a concentration-dependent suppression of γ -GCL in their brain, which was accompanied by a marked drop in GPx levels. The brains of the animals exposed to the greatest dose of glyphosate showed a marked rise in lipid peroxidation, which was ultimately indicative of this changed profile of the antioxidant defense system. Therefore, we can conclude that pesticide exposure to fish leads to oxidative stress and there is a significant increase in some oxidative enzymes which can lead to oxidative stress in fish.

Hematological alternations in fish caused by pesticides

It has been established that hematological indicators are useful instruments for tracking fish health and identifying pesticide toxicity. Both internal and external variables may have an impact on the primary hematological parameters in fish, such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hemoglobin (Hb), and red blood cell (RBC) counts. White blood cell (WBC) counts, red blood cell counts and blood cell histology are all altered by fatal and sub-lethal amounts of different pesticides. An essential diagnostic technique for identifying anomalies in the liver and other tissues is the measurement of blood biochemical markers. An investigation of the impact of dimethoate on the hematological characteristics of *Labeo rohita*, a freshwater fish, was carried

out by Binukumari and Vasanthi [38]. *Cirrhinus mrigala* subjected to diazinon, demonstrated increased WBC count whereas RBCs, hematocrit (Ht), MCV, Hb and MCH substantially decreased [39]. *Channa punctatus* subjected to chlorpyrifos and *Oncorhynchus mykiss* exposed to diazinon [40] both showed a considerable drop in erythrocyte count. Significant changes in Differential Leucocyte Count (DLC) in *Channa punctatus* have been seen as a result of endosulfan and dimethoate over 96 hours [41]. They also saw a large rise in neutrophils brought on by endosulfan and a marked decrease in lymphocytes, monocytes, basophils and eosinophils. Freshwater catfish *Mystus keletius* exposed to Ekalux (EC-25%), Impala (EC-55%), and Neemstar (EC-15%) were found to exhibit decreased values for hematological parameters such as RBC, Hb, and Ht packed cell volume as the duration of pesticide exposure increased [42]. Conversely, values for parameters such as WBC, MCHC, MCV and MCH substantially increased. Anemia symptoms included reduced and malformed erythrocytes along with a decline in hemoglobin levels and packed cell volume (PCV). Anemia symptoms were also noted in the form of malformed erythrocytes. Fish *Clarias Batrachus* treated with imidacloprid showed hematological changes in their blood; the concentrations of Hb, RBCs, packed cell volume (PCV), MCV and MCH were all decreased, while the amounts of WBCs were elevated [43]. According to Das *et al.* [44] hematological measures such as total erythrocyte count (TEC) and Hb count were observed to be lowered while TLC was elevated in freshwater fish, *Labeo rohita* intoxicated with malathion. Zeinab *et al.*, [45] observed changes in hematological indices of *Ctenopharyngodon idella* treated with insecticide profenofos including a rise in TLC and monocytosis, lymphocytosis, and moderate neutrophilia. Major observations that were noted after the exposure were extreme microcytic hypochromic anemia. African catfish (*Clarias gariepinus*) are exposed to sublethal concentrations of pesticides that are frequently used in agriculture, including abamectin, carbofuran, chlorpyrifos, cypermethrin, deltamethrin, dichlorvos, dimethoate, fipronil, lambda-cyhalothrin, and paraquat [46]. Significant variations were found between the mean hematological parameters of the treated and control catfish, including WBC, RBC, Hb, Hct, MCH, and MCHC. Malathion exposure in silver barb fish *Barbomyrus gonionotus* was found to cause a concentration and time-dependent increase in WBC, a significant decline in Hb, PCV, and RBC levels, as well as a marked rise in the prevalence and severity of micronucleus during the whole experimental period whereas MCV, MCH, and MCHC readings displayed inconsistent patterns [47].

Histopathological alternation in fish caused by pesticides

Toxicological research and the observation of water pollution might benefit from histopathological examinations of various fish tissues that have been subjected. Tissue changes in fish exposed to different pesticide doses are a functional response of the organisms and describe the specific type of contaminant. We may learn about the condition and operation of organs through histology. Organ tissue harm and trauma can lead to decreased growth, survival,

and physical fitness, poor reproductive outcomes, or increased vulnerability to pathogenic agents.

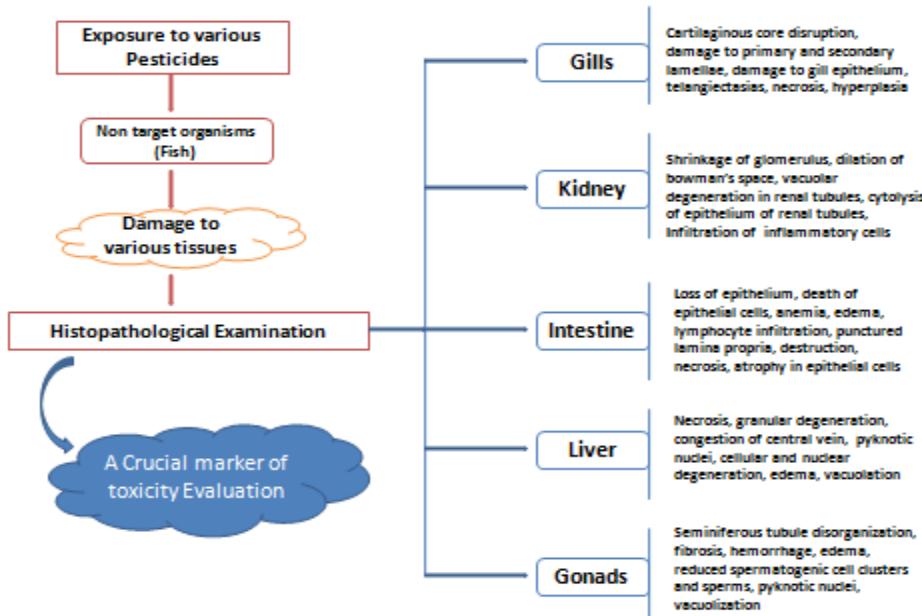


Fig 3: Effects of pesticides on histology of gills, kidney, intestine, liver and gonads offish

Gills

With secondary lamellae on either side, each gill is made up of primary lamellae. In fish under control conditions, normal blood vessels and epithelial cell linings with isolated pillar cells are observed in the major gill filament. Majumder and Kaviraj [48] observed changes in the histology of the gills of *O. niloticus* after the treatment with cypermethrin. Gill lamellae exhibited intralamellar space, necrosis on both sides of the secondary lamellae, and clustered epithelial cells at their base. Along with increased intralamellar space on primary gill lamellae, the higher concentration of this pesticide caused tissue death within primary and secondary gill lamellae, degeneration of the secondary lamellae's epithelium, and constricting of secondary lamellae. Similar histopathological alterations were observed by different researchers after the treatment of different concentrations of cypermethrin on the gills of *C. carpio* [49] and freshwater crayfish *Procambarus clarkia* [50]. Freshwater fish *C. mrigala* treated with pesticides (Chlorfenapyr, Dimethoate, and Acetamiprid) showed several histological changes in gills such as secondary gill lamellae fusion, secondary gill lamellae epithelium raising, hypertrophy of the gill epithelium, and secondary gill lamellae curving [51]. With the increase in the time of exposure, the extent of the histological changes which included telangiectasias, secondary gill lamellae shortening, cartilaginous core disruption, lamellar disorganization, and lamellar atrophy was also increased in all treated groups. Impacts of synthetic pyrethroid on the histology of gills of common carp showed several alterations like fusion of secondary gill lamellae, degenerated

lamellae, vacuolization, necrosis, hyperplasia, ruptured secondary lamellae, vacuolization of gill rakers and vasodilation of central sinus were noticed after the treatment with different concentrations of this pesticide [52]. Research conducted by Shah and Parveen [27] on pesticide-polluted Ganga River on gills of *C. carpio* and *Rita rita* showed sloughing and degradation of lamellar epithelium, vessels dilation, lamellar congestion, gill bridging, inflammatory cell infiltrate, necrosis and hypertrophy in smooth muscles of the gills. Vali *et al.*, [53] studied alterations in the gills of Grass Carp (*Ctenopharyngodon idella*) administered with different amounts of diazinon. Fish gills showed primary lamellar edema, hyperplasia of secondary lamellae, hypertrophy, hemorrhage, shortening and sloughing of secondary lamellae and hypertrophy. Gills of fish exposed to dimethoate exhibited alterations like hyperplasia in the primary lamellae epithelium, shortening, fusion and curling of secondary gill lamellae fusion, infiltration of inflammatory cells hemorrhage and epithelial edema and telangiectasis [54].

Kidney

The kidney of the control fish is constructed from numerous renal tubules and fully formed glomeruli. Majumder and Kaviraj [48] observed changes in the histology of the kidney of *Oreochromis niloticus*. Cypermethrin caused hyaline atrophy in the renal tubular epithelium, glomerulus compression, widening of the gap between the glomerulus and Bowman's capsule, dilatation of the tubular lumen and vacuolation in the epithelium of renal tubules. In the kidney tissues of *Mystustengara* as a result of cypermethrin exposure, Haque *et al.* [55] reported histological alterations such as vacuolation, necrosis, cellular breakdown, karyolysis, and degeneration of renal tubules. Nile Tilapia (*Oreochromis niloticus*), carbofuran sub-lethal dosages resulted in glomerular capsule separation, proximal and distal tubule necrosis, and lack of intercellular space [56]. Nile tilapia treated with sumithion showed a notable morphological alteration in the kidney tissue; glomerular enlargement, dilatation of Bowman's space, pyknotic nuclei, patch atrophy, vacuolation, and a rise in the diameter of the renal tubules were identified at low and high concentrations [57]. Hassan *et al.* [54] investigated changes in the histology of the kidney of Nile tilapia after exposure to dimethoate. Pesticide-exposed kidney showed shrinkage of the glomerulus, dilation of Bowman's space, vacuolar degeneration and cytolysis of renal tubule epithelium and infiltration of inflammatory cells.

Intestine

Numerous contaminants are absorbed and metabolized in large part via the intestines [58]. Vacuolation, tissue necrosis, hemorrhage, disintegration of the epithelium, loss of the intestinal villi, aberration, and death of epithelial cells are the common signs that can be seen in the intestinal tissue after the treatment with different pesticides. Shamloofar *et al.* [59] observed anemia, edema, and swelling in the intestinal tissue of yearling Caspian kutum (*Rutilus kutum*) exposed to Carbaryl. Butachlor-induced histopathological changes in the intestinal tissue of *Clarias batrachus* showed vascularization in the submucosal areas, hemorrhagic areas, upsurge in goblet cells and lymphocyte infiltration in the absorptive region of the intestine in the section

of the intestine [60]. Significant degradation of the villi's brush border, punctured lamina proportion, high degree of lymphocytic infiltration in the mucosa layer, damaged basal lamina, and the existence of many vacuoles in the sub-mucosal and muscular layer are all signs of the significant deterioration in the brush border region. Histological effects of lindane, an organochlorine pesticide, on the intestinal tissue of the teleost fish *C. punctatus* were observed by Bhattacharjee and Das [61]. According to this study, lindane exposure resulted in edema in epithelial cells, expansion in Goblet cells, invasion of inflammatory cells into intestinal tissues, and necrosis. Yön Ertuğ *et al.* [62] noticed various histopathological alterations like hyperplasia in goblet cells, destructed villi structures, edema, necrosis and atrophy in epithelial cells caused by 2, 4-dichloro phenoxy acetic acid in intestinal tissue of zebrafish. Using histological techniques, Das and Gupta [63] investigated the effects of mancozeb on the intestinal tissues of *Esomus danicus*. They saw impacts like ulceration and vacuolization along with chronic inflammatory cell proliferation in the mucosa layer. The toxicity of chlorpyrifos impacted the diversity of the gut microbiota and promoted the growth of pathogenic microorganisms that compromise the intestinal immune system and lead to infection [64].

Liver

The liver shields the body against substances ingested through the gastrointestinal system, gills, and skin that may be hazardous. Necrosis, hemorrhage, degeneration of cytoplasm and nuclei, infiltration of blood cells, hypertrophy, congestion of nuclei and heterochromatin distribution are some of the major histopathological anomalies which can be observed by histopathological investigation after the treatment of different pesticides. Cattaneo *et al.* [65] documented disruption in the connections of hepatocytes, disruption of the cellular membrane, and vacuolated cytosol in the hepatic tissue of *Rhamdia quelen*, following exposure to 2,4-D herbicide. Experiments conducted by Norhan *et al.* [66] on paraquat-induced histopathological changes in liver tissues of *Anabas testudineus*. Treated exhibited many alterations like necrosis, and cell degeneration following a 96-hour exposure to this pesticide. Paraquat exposure at 15.0 mg/L resulted in hemorrhage, necrosis, hydropic degeneration, and pyknosis. The study by Velmurugan *et al.* [67] was done to investigate the toxicity of cypermethrin on the histology of the liver of *Anabas testudineus*. The study revealed that exposure to this pesticide caused several histological changes like pycnotic nucleus, dilatation of sinusoids, hypertrophy of liver cells, necrosis, granular degeneration and congestion. Hassan *et al.* [54] noticed various histopathological changes in the liver of dimethoate-exposed tilapia fish like congestion of the central vein, vacuolar degeneration of the liver cells, dilatation and congestion of sinusoid, aggregation of inflammatory cells, necrosis and hemorrhage. Shah and Praveen [27] researched pesticide-polluted Ganga River. Liver tissues of the fish *Rita rita* collected from the Narora site showed various necrotic changes in hepatocytes and distorted arterial walls. Natraj *et al.*, [68] observed pyknosis in the nucleus, vacuolation, nuclear degradation and cellular edema on the liver tissue of freshwater fish, *Labeo rohita* exposed to profenofos.

Gonads

The tiny diameters of the impacted cells make it challenging to examine some insecticide-induced histopathological alterations in the testes under light microscopy. The research was done by Marutirao [69] to study the histopathological alterations after dimethoate exposure on ovarian tissue of *Puntius ticto*. Following fatal exposure, the ovary displayed notable alterations. Ovarian follicles were partially disrupted, and vacuolation was observed in the cytoplasm of germinal cells. There was an injury to the interfollicular connective tissue. The normal layout of ovarian follicles was lost. There was evidence of injury to the yolk vesicles of developing oocytes as well as necrosis and fibrosis in the connective tissue. Biju *et al.*, [70] conducted research to study exposure-dependent changes in the ovary of Freshwater Fish, *Oreochromis niloticus* after treatment with Malathion. Various alterations like reduced size of mature oocytes, degradation of tissue cells, destruction of follicular epithelium, fractured ova, necrosis, and degradation of the ovarian structure were seen. Ezenwosu *et al.* [71] investigated the harmful effects of lambda-cyhalothrin on the histo-architecture of gonads (testes and ovaries) of *Clarias gariepinus*. Testes of the treated animals showed some damaging alterations like clusters of necrotic spermatogenic cells exhibiting nuclear pyknosis and cytoplasmic swellings. Various changes like degenerative matured flabby and vacuolated oocytes and nuclear pyknosis were seen in the ovaries. A study aimed to assess mancozeb's acute toxicity on the testis of zebrafish (*Danio rerio*) was performed by Gürol *et al.* [72]. Spermatogenic cell degeneration, seminiferous tubule disorganizations, fibrosis, hemorrhage, vacuolization, spermatocyte hypertrophy, edema, reduced spermatogenic cell clusters and sperms, and pyknotic and karyolytic nuclei were all brought on by mancozeb. These findings show that mancozeb might stop zebrafish from reproducing and lower their fertilization ratio. Numerous histological alterations were seen in the ovary of female gangetic fish *Mystus cavasius* by Uddin *et al.* [73] after exposure to cypermethrin. With increasing cypermethrin concentration and exposure period, wrinkle oocyte, cytoplasmic clumping, atretic follicle, degraded granulose layer, distorted oocyte wall, increased inter follicular space, adhesion, cyst, and necrosis were seen.

Conclusion

To summarize, the analysis of the available research indicates that exposure to pesticides triggers antioxidant defenses and detoxification pathways by causing the activation of antioxidant enzymes. However, the use of pesticides, whether they were acute or long-term, changed hematological, biochemical, and immunological parameters in the fish. Histopathological studies revealed that pesticides have dangerous effects on the liver, kidney, intestine, gills and gonads which lead to tissue degeneration, necrosis, degeneration of cells, bleeding in some tissues, vacuolization and many more harmful effects. Pesticides have harmful effects on gonadal activity and the development stages of fish which can lead to juvenile mortality. Pesticide effects on behavior include irregular movements, skin irritation, bleeding from gills, backward swimming and lack of orientation. Furthermore, these pesticides accumulate in the food chain and go to higher trophic levels which include humans through bio-magnification which results in

serious and long-term effects. These changes may affect the survival or life of fish in water bodies. So we can conclude that we need to find alternatives for these toxic substances.

References

1. Tharmavaram M, Pandey G, Bhatt P, Prajapati P, Rawtani D, Sooraj KP, Ranjan M. Chitosan functionalized Halloysite Nanotubes as a receptive surface for laccase and copper to perform degradation of chlorpyrifos in aqueous environment. International Journal of Biological Macromolecules. 2021;191:1046-1055.
<https://doi.org/10.1016/j.ijbiomac.2021.09.098>
2. Agarwal A, Prajapati R, Singh OP, Raza SK, Thakur LK. Pesticide residue in water—a challenging task in India. Environmental monitoring and assessment. 2015;187:1-21. DOI: [10.1007/s10661-015-4287-y](https://doi.org/10.1007/s10661-015-4287-y)
3. Naughton SX, Terry Jr AV. Neurotoxicity in acute and repeated organophosphate exposure. Toxicology. 2018;408:101-112. DOI: [10.1016/j.tox.2018.08.011](https://doi.org/10.1016/j.tox.2018.08.011)
4. Somlyai I, Berta C, Nagy SA, Dévai G, Ács É, Szabó LJ, Nagy J, Grigorszky I. Heterogeneity and anthropogenic impacts on a small lowland stream. Water. 2019 Sep 26;11(10):2002. DOI: [10.3390/w11102002](https://doi.org/10.3390/w11102002)
5. Arjmandi R, Tavakol M, Shayeghi M. Determination of organophosphorus insecticide residues in the rice paddies. International journal of environmental science & technology. 2010;7:175-182. DOI: [10.1007/BF03326129](https://doi.org/10.1007/BF03326129)
6. Tang FH, Lenzen M, McBratney A, Maggi F. Risk of pesticide pollution at the global scale. Nature geoscience. 2021;14(4):206-210. DOI: [10.1038/s41561-021-00712-5](https://doi.org/10.1038/s41561-021-00712-5)
7. Haverkate F, Tempel A, Den Held AJ. Interaction of 2, 4, 5-trichlorophenylsulphonylmethyl thiocyanate with fungal spores. Netherlands journal of plant pathology. 1969;75:308-315. doi: [10.3389/fpls.2017.01715](https://doi.org/10.3389/fpls.2017.01715)
8. Elskus AA. Toxicity, sublethal effects, and potential modes of action of select fungicides on freshwater fish and invertebrates. US Geological Survey; 2012. <https://doi.org/10.3133/ofr20121213>
9. Lu Z, Gan J, Cui X, Delgado-Moreno L, Lin K. Understanding the bioavailability of pyrethroids in the aquatic environment using chemical approaches. Environment international. 2019;129:194-207 <https://doi.org/10.1016/j.envint.2019.05.035>
10. Aldridge WN. Some properties of specific cholinesterase with particular reference to the mechanism of inhibition by diethyl p-nitrophenyl thiophosphate (E 605) and analogues. Biochemical Journal. 1950;46(4):451. doi: [10.1042/bj0460451](https://doi.org/10.1042/bj0460451)
11. Perez MH, Wallace WG. Differences in prey capture in grass shrimp, *Palaemonetes pugio*, collected along an environmental impact gradient. Archives of Environmental Contamination and Toxicology. 2004;46:81-90. DOI: [10.1007/s00244-002-0249-9](https://doi.org/10.1007/s00244-002-0249-9)
12. Baatrup E. Measuring complex behavior patterns in fish—effects of endocrine disruptors on the guppy reproductive behavior. Human and Ecological Risk Assessment. 2009;15(1):53-62. DOI: [10.1080/10807030802615097](https://doi.org/10.1080/10807030802615097).

13. Banaee M, Sureda A, Mirvaghefi AR, Ahmadi K. Biochemical and histological changes in the liver tissue of rainbow trout (*Oncorhynchus mykiss*) exposed to sub-lethal concentrations of diazinon. *Fish physiology and biochemistry*. 2013;39:489-501. doi: [10.1007/s10695-012-9714-1](https://doi.org/10.1007/s10695-012-9714-1).
14. Üner N, Oruç EÖ, Sevgiler Y, Şahin N, Durmaz H, Usta D. Effects of diazinon on acetylcholinesterase activity and lipid peroxidation in the brain of *Oreochromis niloticus*. *Environmental toxicology and pharmacology*. 2006;21(3):241-245. DOI: [10.1016/j.etap.2005.08.007](https://doi.org/10.1016/j.etap.2005.08.007).
15. Branch TN. Sub-lethal toxicity impacts of endosulfan on some biochemical parameters of the freshwater crayfish (*Astacusleptodactylus*). *Research Journal of Environmental Sciences*. 2011;5(11):827-835. DOI: [10.3923/rjes.2011.827.835](https://doi.org/10.3923/rjes.2011.827.835).
16. Aldeguende M, Soengas JL, Ruibal C, Andrés MD. Effects of chronic exposure to γ -HCH (lindane) on brain serotonergic and gabaergic systems, and serum cortisol and thyroxine levels of rainbow trout, *Oncorhynchus mykiss*. *Fish Physiology and Biochemistry*. 1999;20:325-330. <https://doi.org/10.1023/A:1007795121340>.
17. Little EE, Dwyer FJ, Fairchild JF, Delonay AJ, Zajicejk JL. Survival of bluegill and their behavioral responses during continuous and pulsed exposures to esfenvalerate, a pyrethroid insecticide. *Environmental Toxicology and Chemistry: An International Journal*. 1993;12(5):871-878. DOI: 10.1002/etc.5620120511
18. Omitoyin BO. Introduction to fish farming in Nigeria. Ibadan university press; 2007.
19. Srivastava P, Singh A. Study on some neural and behavioral changes induced by carbamate (mancozeb) fungicide on freshwater fish *Clarias batrachus*. *World Journal of Zoology*. 2013;8(4):376-380.
20. Sinhorin VD, Sinhorin AP, Teixeira JM, Miléski KM, Hansen PC, Moeller PR, Moreira PS, Baviera AM, Loro VL. Metabolic and behavior changes in surubim acutely exposed to a glyphosate-based herbicide. *Archives of environmental contamination and toxicology*. 2014;67:659-67.
21. 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;8(10):218. <https://doi.org/10.3390/vetsci8100218>.
22. Singh BB, Narain AS. Acute toxicity of Thiodan to catfish (*Heteropneustesfossilis*). *Bulletin of Environmental Contamination and Toxicology*. 1982;28(1):122-127. DOI: [10.1007/BF01608424](https://doi.org/10.1007/BF01608424)
23. Sarikaya R, Yılmaz M. 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*. 2003;52(1):195-201. DOI: [10.1016/S0045-6535\(03\)00106-1](https://doi.org/10.1016/S0045-6535(03)00106-1).
24. Kumar R, Kumari R, Mishra BK. Behavioural and morphological changes induced in the freshwater fish, *Clarias batrachus* exposed to chlorpyrifos 50% + cypermethrin 5% EC.

Journal of Entomology and Zoology Studies. 2020; 8(5): 1742-1745. DOI: [10.1016/S0045-6535\(03\)00106-1](https://doi.org/10.1016/S0045-6535(03)00106-1).

25. Khan S, Qayoom I, Balkhi MH, Abubakr A, Rashid S, Alsaffar RM, Rehman MU. Behavioural incongruities in juvenile *Cyprinus carpio* exposed to organophosphate compounds. *Heliyon*. 2022 Nov 1;8(11). <https://doi.org/10.1016/j.heliyon.2022.e11227>
26. Üner N, Oruç EÖ, Sevgiler Y, Şahin N, Durmaz H, Usta D. Effects of diazinon on acetylcholinesterase activity and lipid peroxidation in the brain of *Oreochromis niloticus*. *Environmental toxicology and pharmacology*. 2006 May 1;21(3):241-245. DOI: [10.1016/j.etap.2005.08.007](https://doi.org/10.1016/j.etap.2005.08.007).
27. Shah ZU, Parveen S. Oxidative, biochemical and histopathological alterations in fishes from pesticide contaminated river Ganga, India. *Scientific Reports*. 2022 Mar 7;12(1):3628. DOI: [10.1038/s41598-022-07506-8](https://doi.org/10.1038/s41598-022-07506-8)
28. Tiwari S, Singh A. Toxic and sub-lethal effects of oleandrin on biochemical parameters of fresh water air breathing murrel, *Channa punctatus* (Bloch.). *Indian J of Experimental Biology* 2004 42: 413-418.
29. Banaee M. Physiological dysfunction in fish after insecticides exposure. *Insecticides—Development of Safer and More Effective Technologies*. 2013;30:103-143.
30. Kaviraj A, Gupta A. Biomarkers of type II synthetic pyrethroid pesticides in freshwater fish. *BioMed research international*. 2014;2014(1):928063. <https://doi.org/10.1155/2014/928063>.
31. Abd El Megid A, Abd Al Fatah ME, El Asely A, El Senosi Y, Moustafa MM, Dawood MA. Impact of pyrethroids and organochlorine pesticides residue on IGF-1 and CYP1A genes expression and muscle protein patterns of cultured *Mugil capito*. *Ecotoxicology and environmental safety*. 2020;188:109876. DOI: [10.1016/j.ecoenv.2019.109876](https://doi.org/10.1016/j.ecoenv.2019.109876).
32. Meng S, Chen X, Song C, Fan L, Qiu L, Zheng Y, Chen J, Xu P. Effect of Chronic Exposure to Pesticide Methomyl on Antioxidant Defense System in Testis of Tilapia (*Oreochromis niloticus*) and Its Recovery Pattern. *Applied Sciences*. 2021; 11(8):3332. <https://doi.org/10.3390/app11083332>
33. El-Houseiny W, Anter RGA, Arisha AH, Mansour AT, Safhi FA, Alwutayd KM, Elshopakey GE, Abd El-Hakim YM, Mohamed EMM. Growth Retardation, Oxidative Stress, Immunosuppression, and Inflammatory Disturbances Induced by Herbicide Exposure of Catfish, *Clarias gariepinus*, and the Alleviation Effect of Dietary Wormwood, *Artemisia cina*. *Fishes*. 2023; 8(6):297. <https://doi.org/10.3390/fishes8060297>
34. Silva SB, Gregório KP, de Freitas RM, Destro AL, Salaro AL, Zuanon JA, Gonçalves RV, Freitas MB. EXPOSURE TO THE HERBICIDE ATRAZINE INDUCES OXIDATIVE IMBALANCE, MORPHOLOGICAL DAMAGE AND DECREASED SURVIVAL IN JUVENILE FISH. *Bioscience Journal*. 2023;39. <https://doi.org/10.14393/BJ-v39n0a2023-65784>

35. Ndlovu R, Basopo N. Pesticide-Induced Alterations of Esterase and Antioxidant Enzymes of Aquatic Organisms *Oreochromis mossambicus* and *Xenopus laevis*. *Advances in Biological Chemistry*. 2022;12(6):292-305. DOI: [10.4236/abc.2022.126023](https://doi.org/10.4236/abc.2022.126023).

36. Osioma E, Ezugworie TT. Comparative evaluation of antioxidant enzymes, lipid peroxidation, nitric oxide, and non-microsomal oxidases in *Galatea paradoxa* exposed to varying concentrations of 'uproot'a glyphosate-based herbicide. *Egyptian Journal of Aquatic Biology and Fisheries*. 2022;26(3):213-228. DOI: [10.21608/EJABF.2022.239713](https://doi.org/10.21608/EJABF.2022.239713)

37. Faria M, Bedrossian J, Ramírez JR, Mayol M, García GH, Bellot M, Prats E, García-Reyero N, Gómez-Canela C, Gómez-Oliván LM, Raldúa D. Glyphosate targets fish monoaminergic systems leading to oxidative stress and anxiety. *Environment international*. 2021;146:106253. <https://doi.org/10.1016/j.envint.2020.106253>.

38. Binukumari S, Vasanthi J. Impact of the Pesticide Dimethoate 30% EC on the Haematological Parameters of Fresh water fish (*Labeorohita*). *IOSR Journal of Environmental Science, Toxicology and food technology*. 2013;7:2319-402. DOI: [10.9790/2402-0726365](https://doi.org/10.9790/2402-0726365)

39. Haider MJ, Rauf A. Sub-lethal effects of diazinon on hematological indices and blood biochemical parameters in Indian carp, *Cirrhinusmrigala* (Hamilton). *Brazilian Archives of Biology and Technology*. 2014;57(6):947-953. DOI: [10.1590/S1516-8913201402086](https://doi.org/10.1590/S1516-8913201402086)

40. Far MS, Roodsari HV, Zamini A, Mirrasooli E, Kazemi R. The effects of diazinon on behavior and some hematological parameters of fry rainbow trout (*Oncorhynchus mykiss*). *World J Fish Mar Sci*. 2012;4:369-375.

41. Parkash J. Effect of Endosulphan and Dimethoate Pesticides on Haematological Parameters of Fresh Water Fish *Channa punctatus*. *RRJZS*. 2016;4:8-33.

42. Barathinivas A, Ramya S, Neethirajan K, Jayakumararaj R, Pothiraj C, Balaji P, Faggio C. Ecotoxicological effects of pesticides on hematological parameters and oxidative enzymes in freshwater Catfish, *Mystuskeletalius*. *Sustainability*. 2022;14(15):9529. <https://doi.org/10.3390/su14159529>.

43. Tokriya RK, Billore K. HEMATOLOGICAL CHANGES INDUCED BY IMIDACLOPRID IN *CLARIAS BATRACHUS* (MAGUR). *Int. J. Adv. Res.* 2024; 12(01), 546-555. <http://dx.doi.org/10.21474/IJAR01/18153>

44. Das BK, Nayak KK, Kumar V. Alteration of haematological and biochemical biomarkers after sub-lethal chronic malathion (Elathion®) intoxication in freshwater fish, *Labeorohita* (Hamilton, 1822). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 2024;277:109844. <https://doi.org/10.1016/j.cbpc.2024.109844>.

45. El-Bouhy ZM, Mohamed FA, Elashhab MW, El-Houseiny W. Toxicity bioassay and sub-lethal effects of profenofos-based insecticide on behavior, biochemical, hematological, and histopathological responses in Grass carp (*Ctenopharyngodonidella*). *Ecotoxicology*. 2023;32(2):196-210. <https://doi.org/10.1007/s10646-023-02628-9>.

46. Amaeze NH, Komolafe BO, Salako AF, Akagha KK, Briggs TM, Olatinwo OO, Femi MA. Comparative assessment of the acute toxicity, haematological and genotoxic effects of ten commonly used pesticides on the African Catfish, *Clarias gariepinus* Burchell 1822. *Heliyon*. 2020;6(8). <https://doi.org/10.1016/j.heliyon.2020.e04768>.

47. Mrong CE, Islam MR, Kole K, Neepa NN, Alam MJ, Haque MR, Rahman UO, Mostakim GM. Malathion-Induced Hemotoxicity and Its Recovery Pattern in *Barbomyusgonionotus*. *Journal of Toxicology*. 2021;2021(1):9417380. <https://doi.org/10.1155/2021/9417380>

48. Majumder R, Kaviraj A. Histopathological alterations of gills, liver and kidney of freshwater fish, *Oreochromis niloticus*, exposed to Cypermethrin. *Journal of Aquatic Biology & Fisheries*. 2022;10(1):1-5.

49. Arslan H, Özdemir S, Altun S. Cypermethrin toxication leads to histopathological lesions and induces inflammation and apoptosis in common carp (*Cyprinus carpio* L.). *Chemosphere*. 2017;180:491-499. DOI: [10.1016/j.chemosphere.2017.04.057](https://doi.org/10.1016/j.chemosphere.2017.04.057)

50. Wei K, Yang J. Oxidative damage induced by copper and beta-cypermethrin in gill of the freshwater crayfish *Procambarusclarkii*. *Ecotoxicology and environmental safety*. 2015;113:446-53. DOI: [10.1016/j.ecoenv.2014.12.032](https://doi.org/10.1016/j.ecoenv.2014.12.032).

51. Ghayyur S, Khan MF, Tabassum S, Ahmad MS, Sajid M, Badshah K, Khan MA, Ghayyur S, Khan NA, Ahmad B, Qamer S. A comparative study on the effects of selected pesticides on hemato-biochemistry and tissue histology of freshwater fish *Cirrhinusmrigala* (Hamilton, 1822). *Saudi Journal of Biological Sciences*. 2021;28(1):603-11. DOI: [10.1016/j.sjbs.2020.10.049](https://doi.org/10.1016/j.sjbs.2020.10.049).

52. Verma J, Sharma S, Thakur DR. Impact Assessment of Cypermethrin (A Pyrethroid) Induced Chronic Toxicity on Vitals of Adult *Cyprinus carpio* L. *UTTAR PRADESH JOURNAL OF ZOOLOGY*. 2023;44(23):46-51. <https://doi.org/10.56557/upjoz/2023/v44i233762>.

53. Vali S, Majidiyan N, Azadikhah D, Varcheh M, Tresnakova N, Faggio C. Effects of Diazinon on the survival, blood parameters, gills, and liver of grass carp (*Ctenopharyngodonidella* Valenciennes, 1844; Teleostei: Cyprinidae). *Water*. 2022;14(9):1357. DOI: [10.3390/w14091357](https://doi.org/10.3390/w14091357).

54. HASSAN SS, ALI MF, IDRISI SK, Khairy M. Histopathological and biochemical studies on the acute toxicity of Dimethoate on Nile tilapia Fish. *Assiut Veterinary Medical Journal*. 2024;70(180):187-202. <https://doi.org/10.21608/avmj.2023.232748.1183>.

55. Haque SM, Sarkar CC, Khatun S, Sumon KA. Toxic effects of agro-pesticide cypermethrin on histological changes of kidney in Tengra, *Mystustengara*. *Asian Journal of Medical and Biological Research*. 2017;3(4):494-8. DOI: [10.3329/ajmbr. v3i4.35340](https://doi.org/10.3329/ajmbr. v3i4.35340).

56. Américo-Pinheiro JH, Machado AA, da Cruz C, Aguiar MM, Ferreira LF, Torres NH, Machado-Neto JG. Histological changes in targeted organs of Nile tilapia (*Oreochromis*

niloticus) exposed to sublethal concentrations of the pesticide carbofuran. Water, Air, & Soil Pollution. 2020;231(5):228. DOI: [10.1007/s11270-020-04628-5](https://doi.org/10.1007/s11270-020-04628-5).

57. Sharmin S, Islam MT, Sadat MA, Jannat R, Alam MR, Shahjahan M. Sumithion induced structural erythrocyte alteration and damage to the liver and kidney of Nile tilapia. *Environ Sci Pollut Res Int.* 2021;28(27):36695-36706. DOI: [10.1007/s11356-021-13263-4](https://doi.org/10.1007/s11356-021-13263-4)

58. Yuen BB, Wong CK, Woo NY, Au DW. Induction and recovery of morphofunctional changes in the intestine of juvenile carnivorous fish (*Epinepheluscoioides*) upon exposure to foodborne benzo [a] pyrene. *Aquatic toxicology.* 2007;82(3):181-94. DOI: [10.1016/j.aquatox.2007.02.010](https://doi.org/10.1016/j.aquatox.2007.02.010).

59. Shamloofar M, Jorjani S, Ghelichi A. Determination of LC50 and evaluation of tissue lesions caused by Swine toxin in juvenile Caspian kutum (*Rutilus frisiikutum*). *J Aquac Dev.* 2015;9:43-52. DOI: [10.2495/WP100181](https://doi.org/10.2495/WP100181).

60. Chand GB, Verma P, Singh P, Prasad S. Butachlor induced serum biochemical anomalies correlated with histopathological changes in intestinal tissue of *Clarias batrachus* (Linn.): in-vivo study. *UTTAR PRADESH JOURNAL OF ZOOLOGY.* 2021;42(8):18-28.

61. Bhattacharjee D, Das S. Intestinal histopathology as a biomarker for lindane toxicity in teleost fish, *Channa punctata*. *Global Journal of Biology Agriculture and Health Sciences.* 2015;4(3):16-18.

62. 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;74:27021-4.

63. Das S, Gupta A. Histopathological changes in the intestine of Indian flying barb (*Esomusdanricus*) exposed to organophosphate pesticide, malathion (EC 50). *Global Journal of Biology, Agriculture and Health Sciences.* 2013;2(2):90-93.

64. Fang B, Li JW, Zhang M, Ren FZ, Pang GF. Chronic chlorpyrifos exposure elicits diet-specific effects on metabolism and the gut microbiome in rats. *Food and chemical toxicology.* 2018;111:144-152. DOI: <https://doi.org/10.1016/j.fct.2017.11.001>

65. Cattaneo R, Loro VL, Spanevello R, Silveira FA, Luz L, Miron DS, Fonseca MB, Moraes BS, Clasen B. Metabolic and histological parameters of silver catfish (*Rhamdiaquelen*) exposed to commercial formulation of 2, 4-dichlorophenoxyacetic acid (2, 4-D) herbicide. *Pesticide Biochemistry and Physiology.* 2008;92(3):133-137. DOI: <http://dx.doi.org/10.1016/j.pestbp.2008.07.004>.

66. Norhan NA, Zakariah MI, Karim NU, Daud HM, Melad AA, Yusoff NA, Hassan MA. Paraquat-induced histopathological changes on the gills, kidney and liver tissues of *Anabas testudineus* (bloch 1792). *J Sustain Sci Manag.* 2022;17:165-174. DOI: <http://doi.org/10.46754/jssm.2022.08.010>.

67. Velmurugan B, Satar EI, Yolcu M, Uysal E. Effects of Cypermethrin on Serum Biochemistry and Liver Histopathology of *Anabas testudineus*. *Journal of Chemical Health Risks*. 2024;14(1). <https://doi.org/10.22034/jchr.2023.1948154.1479>.

68. Nataraj B, Hemalatha D, Rangasamy B, Maharajan K, Ramesh M. Hepatic oxidative stress, genotoxicity and histopathological alteration in fresh water fish *Labeorohita* exposed to organophosphorus pesticide profenofos. *Biocatalysis and Agricultural Biotechnology*. 2017;12:185-190. <http://dx.doi.org/10.1016/j.bcab.2017.09.006>.

69. Marutirao GR. Histopathological changes in the ovary of freshwater fish *Puntius ticto* (Ham) under dimethoate toxicity. *The Bioscan*. 2013;8(3):989-992.

70. Biju A, Remya Devi GS, Sreejai R. Histopathological Impact of Organophosphorus Pesticide, Malathion on the Ovary of Freshwater Fish *Tilapia niloticus* (Linnaeus, 1758). *Journal of Aquatic Biology & Fisheries*. 2020;8:97-100.

71. Ezenwosu SU, Nnamonu EI, Odo GE, Ikele BC, Ani OC. Evaluation of lambda-cyhalothrin oxidative stress and gonad histoarchitecture toxicity potency in *Clarias gariepinus*. *The journal of basic and applied zoology*. 2021;82:1-1. <https://doi.org/10.1186/s41936-020-00201-y>.

72. Gürol MA, Arman S, Yön ND. Effects of mancozeb on the testicular histology of the zebrafish (*Danio rerio*). In *Annales de Limnologie-International Journal of Limnology* 2020 (Vol. 56, p. 10). EDP Sciences. <http://dx.doi.org/10.1051/limn/2020009>.

73. Uddin MH, Ali MH, Sumon KA, Shahjahan M, Rashid H. Effects of pyrethroid pesticide cypermethrin on the gonad and hemato-biochemical parameters of female gangeticmystus (*Mystuscavassius*). *Aquaculture Studies*. 2022;22(3). DOI : [10.4194/AQUAST819](https://doi.org/10.4194/AQUAST819).