



Original article

Toxic effects of chlorpyrifos on the growth, hematology, and different organs histopathology of Nile tilapia, *Oreochromis niloticus*Mohammad Amzad Hossain^{1,*}, Lipi Sutradhar¹, Tumpa Rani Sarker, Shuva Saha, Mohammed Mahbub Iqbal

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ARTICLE INFO

Article history:

Received 13 January 2022

Revised 12 April 2022

Accepted 19 May 2022

Available online 22 May 2022

Keywords:

Pesticide

Organophosphate

Fish

Toxicity

Hemoglobin

SGR

HSI

ABSTRACT

Chlorpyrifos is a widely applied insecticide that permeates on most waterways and affects aquatic organisms. The growth performances, hematological and histological impacts on Nile tilapia, *Oreochromis niloticus* following a 60 day of exposure to varying concentrations of chlorpyrifos 20 EC (T_1 08 μgL^{-1} , T_2 16 μgL^{-1} , and T_3 32 μgL^{-1}) were compared to a control T_c 0 μgL^{-1} . The 96-hour LC50 of chlorpyrifos 20 EC was calculated as 46.80 μgL^{-1} . The water quality parameters were recorded regularly. The value of dissolved O_2 and NH_3 stayed rather steady, although temperature varied considerably. It was revealed that as chlorpyrifos levels go up, the percentage of weight gain (WG %), specific growth rates (SGR), and survival rate decreases. The control group T_c had the highest percentages of SGR weight (1.16 ± 0.58) and the T_3 group had the lowest percentages of SGR weight (0.25 ± 0.77). The hematological assessment showed significant differences of hemoglobin concentration, white blood cell counts and red blood cell numbers between chlorpyrifos treatment and control group ($P < 0.05$). Histological alterations in the liver, gills, and muscle tissues reported to be worse for T_3 as compared to others. There were no statistical differences in GSI, HSI values between control and treatment groups. The chlorpyrifos 20 EC was shown to be highly toxic to *O. niloticus* at sub-lethal dosages.

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1. Introduction

The Nile tilapia, *Oreochromis niloticus*, is one of the highly cultured commercial fish species in Asian territory (Syed et al., 2022). It is considered as a promising tropical aquaculture species, especially for low-income countries and proven to play a vital role in rural nutrition and economy of Afro-Asian realms (Chan et al., 2019). This fish showed excellent capacity to grow well in a wide range of thermal and environmental states (Edwin et al., 2019). As it can withstand physical and chemical changes, and toxins in water, it's been often employed as a bioindicator of water pollution (Ihsan et al., 2019). Tilapia is now one of the most refereed model animals in aquatic toxicology investigation (Yan et al., 2020).

Chlorpyrifos, an organophosphate derivative is widely used insect repellent in global commercial Agro-farming practices (Hasanuzzaman et al., 2018; Silva and Samayawardhena, 2002) and it is counted as India's second most popular means of synthetic pest control (Stalin et al., 2019). It is extensively used in Bangladesh due to low price, availability in different formulations and effectiveness in little amount (Ali et al., 2020; Ihsan et al., 2018). Most of the insecticides used in crops are eventually expelled into nearby water bodies through canal, rains, and farm runoff. While pesticides serve a vital role in increasing land productivity and food quality for the world's rising population, particularly in developing nations, their presence in agricultural drainage poses a major threat to all aquatic ecosystem components (Abhilash and Singh, 2009). The minimal durability of such substances in aquatic ecosystems has prompted worries about their ability to harm non-target organisms, particularly fish (Ali et al., 2009). Different formulations of Chlorpyrifos are mostly used pesticides in Bangladesh and their incursion in aquatic habitat and life forms are still a mystery.

Pesticides are exposed directly to humans and terrestrial animals in a variety of ways, including chronic exposure, domestic exposure, and intake of pesticide-polluted water and food (Ullah et al., 2018). Insecticides penetrate to fish via absorption across

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Peer review under responsibility of King Saud University.



skin, uptake during respiration in gills and importantly through food or drinking contaminated water (Dawood et al., 2020). Insecticides being accumulated in edible aquatic organisms are finally consumed by human beings and then cause public health risk (Petchoy and Pung, 2017). Fish that are exposed to environmental contaminants, would experience functional disturbances in respiration, feeding, growth and reproductive capacities. Therefore, the impacts of chlorpyrifos on fish would be traced by histological, hematological and growth metric analysis. The hematology of fish serves as an appropriate means to assess the stress and associated health issues generated by biotic and abiotic mediators of the environment (Adhikari et al., 2004; Martins et al., 2011). Hematological indices such as red blood cells count, white blood cells count, hemoglobin level, and many other features were being used to diagnose fish health and as indications for alterations during stressful toxic conditions (Kumar et al., 2011). Muñoz et al., (2015) suggested the histopathology of vital organs in fish as a bio-monitoring method for accessing aquatic pollution. Histopathology could be used as a biomarker to track the environment or look at specific cellular response in the key organs. Current study intended to examine the effects of pesticide chlorpyrifos 20 EC on growth, blood counts and different organ levels of Nile tilapia.

2. Materials and methods

2.1. Designing the experiment and acclimatization of fish

The experiment was conducted for a period of 60 days from November 17, 2020, to January 16, 2021. Nile tilapia juveniles were acquired from nearby BRAC hatchery and carried out by oxygenated poly bags. Afterwards, fish were stocked in glass aquaria and reared for 7 days with continuous aeration to acclimatize under laboratory conditions. Then, fish with equal preliminary weight (3.60 ± 0.85 g) were allotted in 12 glass aquaria (60 L). Four groups of triplicate glass aquaria were indiscriminately disseminated with 25 fish in each aquarium. Each glass aquarium was set up with air stones for aeration. The treatment groups were assigned based on the computation of LC_{50} (section 2.2) and presented in Table 1. Fish were fed with commercial formulated diets three times (08:00 h, 14:00 h and 22.00 h) a day at 2–3% of their body weight. Uneaten feed was removed everyday through siphoning and siphoned water was then filtered and added to the treatment tank.

2.2. Toxicity test and preparing dosages for exposure

A prior 96-hour acute toxicity test had been conducted by using differential concentrations (0, 8, 16, 24, 32, 36, 40, 48, 56, 62 μgL^{-1}) of chlorpyrifos 20EC. Each of the treatment units had been employed with 40 L of tap water, well installed aeration system and 20 fish in each following the OECD standard protocol guidelines for aquatic toxicity assessment (EPA, 2021; OECD, 2009). The obtained mortality rates were plotted against 10 base logarithms of chlorpyrifos concentration in SPSS to do PROBIT analysis at $P < 0.05$ (Finney, 1971).

2.3. Acquisition of water quality factors

Temperature, dissolved oxygen, and pH was monitored and determined each week by using automated YSI Professional Plus (Pro Plus) Multiparameter Instrument (Brannum Lane, USA). The concentration of NH_3 level was calculated by using a ready to use commercial Keen kit from USA.

Table 1
Designing the dosages for different treatment units following the 96-hour LC_{50} .

Treatment	Dose of Chlorpyrifos (μgL^{-1})	Stocking density (no./treatment)	Replication
T _C (Control)	0 (0 % of LC_{50})	25	3
T ₁	8 (17 % of LC_{50})	25	3
T ₂	16 (34 % of LC_{50})	25	3
T ₃	32 (68 % of LC_{50})	25	3

2.4. Growth measurement and bleeding

Sampling was done on a weekly basis to define the growth of *O. niloticus* and to correct the feeding rate. At the end of the 60 days trial, body weight and total length of all fishes were recorded for growth analysis. Additionally, three fishes from each treatment unit were randomly selected, anesthetized with MS222 and blood sample was collected by using 1 mL heparinized syringes (Jimi syringes and medical devices Ltd, Bangladesh). Collected 300–500 μL blood were kept in an EDTA tube for further analysis. The complete blood counting was conducted by placing the EDTA tube on automated Hematology analyzer ELite Erba 3.0 (Erba Lachema S.R.O.). Again, the same fishes were sacrificed and, liver and gonads were separated carefully. Finally, weight of above organs was recorded for analysis of hepatosomatic index (HSI) and gonadosomatic index (GSI). A piece of gill, liver and muscle were collected gently and preserved in neutral buffered formalin (NBF) for further histological analysis.

2.5. Tools for growth, hepatosomatic index (HSI) and gonadosomatic index (GSI)

The specific growth fish which was calculated by following formula-

$\text{SGR (weight or Length) \%} = (\log W_f - \log W_i) / T \times 100$; Where, W_i = Initial body weight (g) or length (cm) and W_f = Final body weight (g) or length (cm); T = duration in days.

Fulton's condition factor, $K = \text{Body Weight} / (\text{Total length})^3$ (Htun-Han, 1978).

Hepatosomatic index, $\text{HSI} = (\text{weight of liver} / \text{weight of fish}) \times 100$ (Chellappa et al., 1995).

Gonadosomatic index, $\text{GSI} = (\text{weight of gonad} / \text{weight of fish}) \times 100$ (Brooks et al., 1997).

2.6. Histological analysis

The NBF preserved samples were washed out on tap water and went through xylene cleaning and graded series alcoholic dehydration process. Then tissues were infiltrated in paraffin and resulting blocks were sectioned at 3–5 μm by using the Leica Microtome Machine (Wetzlar, Germany). The standard staining process was conducted by following the protocol from Van-Dyk & Pieterse, (2008). Tissues were visualized under Zeiss Primo Star 3.0 microscope and micrographs were taken by using Zeiss Core 3.0 software on Windows 10 installation.

2.7. Data analysis

The results were evaluated by applying the one-way analysis of variance tools in IBM SPSS v27 and Duncan's Multiple Range test (DMRT) to establish $P < 0.05$. Graph and visualization were performed in office 365 tools based on the data obtained from SPSS output.

Table 2
Value of 96-h Chlorpyrifos LC₅₀ in previous studies with *Oreochromis* genus.

Species	Value of 96-hour LC ₅₀ (µg/L)	Formulation of chlorpyrifos	References
<i>O. niloticus</i>	42.0	Chlorpyrifos 20% EC	(Majumder and Kaviraj, 2019)
	76	Chlorpyrifos 20% EC	(Ihsan et al., 2018)
	70	Not specified	(El-Bouhy et al., 2016)
	1023	Not specified	(Girón-Pérez et al., 2006)
	1500	Chlorpyrifos-methyl	(Gül, 2005)
<i>O. mossambicus</i>	22	Chlorpyrifos 20% EC	(Muttappa et al., 2015)
	25.97	Chlorpyrifos 20% EC	(Rao et al., 2003)

3. Results

3.1. Acute toxicity test

The results from PROBIT analysis exhibited 96-hour LC₅₀ of Chlorpyrifos at 46.80 µg/L-1 ($P < 0.05$). A reference table has been constructed to reinforce the current finding, which described acute toxicity tests for different formulations of Chlorpyrifos on *Oreochromis* genus (Table 2).

3.2. Water quality parameters

The water quality parameters are summarized in Table 3. The concentration of dissolved O₂, NH₃ remained constant for all the

Table 3
Changes in water quality parameters of *O. niloticus* exposed to chlorpyrifos (20% EC) for 60 days.

Parameters	Treatments	DAY_0	DAY-15	DAY-30	DAY-60
Temperature	T _c (0 µg/L)	29.92 ± 0.15 ^d	27.57 ± 2.18 ^c	24.22 ± 0.32 ^b	21.12 ± 0.25 ^a
	T ₁ (8 µg/L)	30.5 ± 0.57 ^d	26.3 ± 1.67 ^c	24.62 ± 1.21 ^b	21 ± 0.00 ^a
	T ₂ (16 µg/L)	30.25 ± 0.5 ^d	28.3 ± 1.13 ^c	25.35 ± 1.17 ^b	21.5 ± 0.57 ^a
	T ₃ (32 µg/L)	30.75 ± 0.5 ^d	27.07 ± 1.45 ^c	25.42 ± 1.02 ^b	21 ± 0.00 ^a
pH	T _c (0 µg/L)	6.27 ± 0.05 ^a	6.3 ± 0.00 ^a	6.08 ± 0.33 ^{ab}	5.97 ± 0.15 ^a
	T ₁ (8 µg/L)	6.25 ± 0.057 ^a	6.25 ± 0.05 ^a	6.13 ± 0.33 ^a	6.15 ± 0.23 ^a
	T ₂ (16 µg/L)	6.32 ± 0.09 ^c	6.27 ± 0.09 ^{bc}	6.06 ± 0.17 ^a	6.1 ± 0.11 ^{ab}
	T ₃ (32 µg/L)	6.3 ± 0.08 ^b	6.32 ± 0.09 ^b	6.03 ± 0.10 ^a	6.15 ± 0.23 ^{ab}
Dissolved O ₂	T _c (0 µg/L)	5.82 ± 0.23 ^a	6.42 ± 0.47 ^b	6.7 ± 0.14 ^b	6.85 ± 0.05 ^b
	T ₁ (8 µg/L)	6.25 ± 0.17 ^a	6.8 ± 0.42 ^b	6.63 ± 0.23 ^{ab}	6.76 ± 0.20 ^b
	T ₂ (16 µg/L)	6.07 ± 0.15 ^a	6.55 ± 0.1 ^a	6.28 ± 0.50 ^a	6.15 ± 0.23 ^a
	T ₃ (32 µg/L)	6.15 ± 0.1 ^a	6.52 ± 0.26 ^{ab}	6.54 ± 0.42 ^{ab}	6.88 ± 0.13 ^b
NH ₃	T _c (0 µg/L)	0.013 ± 0.12 ^a	0.020 ± 0.25 ^a	0.020 ± 0.28 ^a	0.012 ± 0.06 ^a
	T ₁ (8 µg/L)	0.018 ± 0.12 ^a	0.015 ± 0.09 ^a	0.013 ± 0.20 ^a	0.012 ± 0.08 ^a
	T ₂ (16 µg/L)	0.013 ± 0.12 ^a	0.017 ± 0.16 ^a	0.014 ± 0.15 ^a	0.014 ± 0.07 ^a
	T ₃ (32 µg/L)	0.020 ± 0.08 ^a	0.022 ± 0.32 ^a	0.012 ± 0.20 ^a	0.012 ± 0.10 ^a

Table 4
Changes in growth parameters of *O. niloticus* exposed to chlorpyrifos (20% EC) for 60 days. The results showed significant difference at $P < 0.05$.

Parameters	T _c (0 µg/L)	T ₁ (8 µg/L)	T ₂ (16 µg/L)	T ₃ (32 µg/L)
Initial length (cm)	5.82 ± 0.95 ^a	5.84 ± 0.57 ^a	5.85 ± 0.31 ^a	5.87 ± 0.70 ^a
Initial weight (g)	3.77 ± 1.26 ^a	3.73 ± 0.61 ^a	3.32 ± 0.40 ^a	3.58 ± 0.95 ^a
Final length (cm)	7.77 ± 0.61 ^b	7.21 ± 1.23 ^{ab}	6.82 ± 0.80 ^a	6.57 ± 0.76 ^a
Final weight (g)	7.37 ± 1.51 ^b	5.65 ± 2.73 ^{ab}	4.58 ± 1.61 ^a	4.36 ± 1.71 ^a
Length gain (cm)	1.95 ± 0.87 ^b	1.36 ± 0.93 ^{ab}	0.96 ± 1.04 ^a	0.70 ± 1.02 ^a
Weight gain (g)	3.59 ± 1.69 ^b	1.92 ± 2.72 ^{ab}	1.25 ± 1.86 ^a	0.78 ± 1.83 ^a
Length gain %	36.28 ± 21.30 ^b	23.16 ± 15.70 ^{ab}	17.23 ± 18.02 ^a	13.29 ± 18.65 ^a
Weight gain %	111.35 ± 63.64 ^b	53.57 ± 71.63 ^{ab}	42.46 ± 59.93 ^a	28.00 ± 57.90 ^a
Specific growth rate (% weight)	1.16 ± 0.58 ^b	0.56 ± 0.72 ^{ab}	0.44 ± 0.76 ^{ab}	0.25 ± 0.77 ^a
Percentage of Specific growth rate (% Length)	201.93 ± 7.82 ^b	193.34 ± 16.96 ^{ab}	188.43 ± 12.22 ^a	184.82 ± 11.72 ^a
Average daily length gain	0.032 ± 0.014 ^b	0.022 ± 0.015 ^{ab}	0.016 ± 0.017 ^a	0.011 ± 0.017 ^a
Average daily weight gain	0.059 ± 0.028 ^b	0.032 ± 0.045 ^{ab}	0.020 ± 0.031 ^a	0.013 ± 0.039 ^a
Fulton's condition factor	1.55 ± 0.13 ^c	1.46 ± 0.26 ^b	1.39 ± 0.16 ^a	1.47 ± 0.20 ^b

treatments during the different phases of trial. However, temperature and pH were reported as more fluctuated parameters during the trial periods. The value of temperature was decreased from 30.75 ± 0.5 °C to 21 °C as trials progressed. Again, pH values were also oscillated on different time slots. The pH varied slightly between 6.32 ± 0.09 to 5.97 ± 0.15. The values of other water quality parameters were conducted stable at different time slots and treatment units. Dissolved oxygen level also showed a trend of rising after the first 14 days of trial for all treatment groups (Table 3).

3.3. Growth parameters

Growth performances of *O. niloticus* have been organized in Table 4. It showed that the percentage of weight gain (WG %), specific growth rate (SGR), and survival rate gradually declined with the increase in concentration of chlorpyrifos. The highest weight gain (g) found in the control group was 3.59 ± 1.69 g while lowest in T₃ was 0.78 ± 1.83 g followed by T₂, T₃ was 1.92 ± 2.72 g and 1.25 ± 1.86 g respectively ($P < 0.05$). Again, significant differences were observed between control and T₁ groups in terms of final length (cm), final weight (g), length gain %, weight gain %, average daily length gain, and average daily weight gain. The highest percentages of SGR (weight) were demonstrated in control group T_c (1.16 ± 0.58) and the lowest in T₃ group (0.25 ± 0.77), while differences between T₁ and T₂ were statistically non-significant ($P < 0.05$) (Table 4). The SGR (length) percentages also showed a similar pattern of variation between different treatment groups. There was significant variation in mortality rate between the treatments and control group (Fig. 1). Mortality rate was recorded as maximum 53% in T₃ group, followed by 35 % in T₂. It has been well indicated that higher mortality rates are associated with the higher concentration of toxicant.

3.4. Hematological parameters

The hematological properties of *O. niloticus* exposed to different doses of chlorpyrifos 20EC are tabulated in Table 5. The results showed a significant drop in the counts of hemoglobin concentration, WBC, monocytes, RBC, HCT with the increased concentration of chlorpyrifos exposure ($P < 0.05$). In contrast, the Total White blood cells (WBC) were reported as maximum in T_3 (83233.33 ± 11718.93 cells/mm³) and minimum on T_c (45883.33 ± 5150.32 cells/mm³) ($P < 0.05$). Lymphocytes and Monocytes counts were described statistically stable in each treatment group. Total platelet count was lowest in T_1 (150666.7 ± 11547.01 cells/mm³) and highest in T_3 (230666.7 ± 10016.65 cells/mm³). Besides, Procalcitonin (PCT) accounted as highest in T_1 (0.12 ± 0.00 ng/mL) and lowest in T_3 (0.18 ± 0.00 ng/mL). The mean corpuscular hemoglobin concentration was highest in T_2 (34.7 ± 4.203 g/dL) and lowest in control T_c (28.45 ± 0.75 g/dL). Hematocrit concentration also remained statistically highest for control group compared to the treatment groups. A strong tendency of rising procalcitonin has been allocated among the treatment group. However, blood glucose level was reported to be stable for all the treatment and control group.

3.5. Hepatosomatic index (HSI) and gonadosomatic index (GSI)

There was no statistical difference for HSI and GSI values between the treatments and control group. However, an apparent increasing trend of HSI has been reported as the doses of Chlorpyrifos 20 EC increased (Fig. 2).

3.6. Histopathology of gill, liver, and muscle tissues

The structure of the gill of control fish having neat order of primary and secondary lamella, secondary lamella, pillar cell, mucous cell and no abnormalities were detected (Fig. 3.A). Necrosis of epithelial cells, epithelial lifting, lamellar fusion, and ruptured secondary lamellae and swelling were the most prevalent alterations in the treatment units with varied concentrations of Chlorpyrifos 20EC. Fish treated in T_1 exhibited swelling of primary lamellar epithelium, damaged gill filament (Fig. 3.B). In the T_2 group, necrotic secondary lamellae, diffusion of mucous cells and epithelial lifting were noted (Fig. 3.C). Fish in the T_3 group showed fusion and shortening of secondary lamellae, and epithelial necrosis (Fig. 3.D).

The continuous mass of large hexagonal hepatic cells, amid sinusoids and centrally located prominent nuclei were registered in control group (Fig. 4.A), while liver in T_1 group showed focal necrosis, degenerative nuclei, granular degeneration, and vascular dilation (Fig. 4.B). The liver in T_2 also witnessed widespread necrosis and vascular dilation (Fig. 4.C). The T_3 group were characterized with severe nuclei congestion, acute necrosis, vacuoles formation and dilation of sinusoids (Fig. 4.D).

The control fish showed normal muscle fiber as in Fig. 5.A; on the other hand, degeneration of muscle fiber, splitting of muscle fiber and vacuoles in different rates were reported in T_1 and T_2 (Fig. 5.B-C). Extensive necrosis and vacuole formation were detected in T_3 (Fig. 5.D).

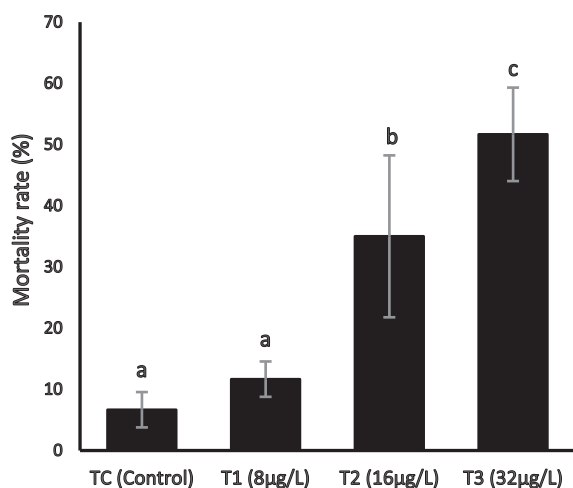


Fig. 1. Mortality rate of *O. niloticus* exposed to chlorpyrifos 20% EC for 60 days.

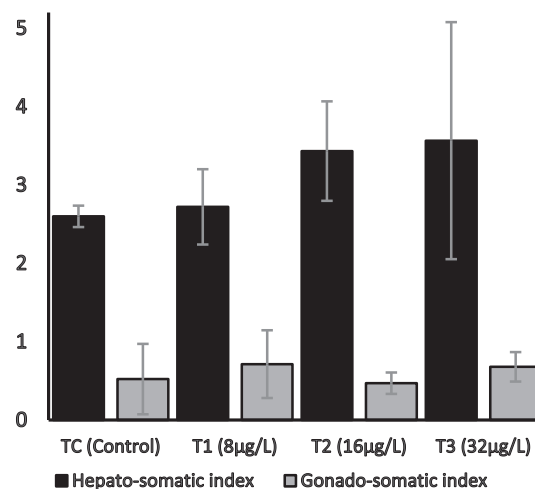


Fig. 2. Hepatosomatic index (HSI) and gonadosomatic (GSI) index of *O. niloticus*.

Table 5
Changes in hematological parameters of *O. niloticus* exposed to chlorpyrifos 20EC for 60 days.

Parameters	T_c ($0 \mu\text{g/L}^{-1}$)	T_1 ($8 \mu\text{g/L}^{-1}$)	T_2 ($16 \mu\text{g/L}^{-1}$)	T_3 ($32 \mu\text{g/L}^{-1}$)
Haemoglobin (Hg) (g/dL)	4.43 ± 0.50^a	2.96 ± 0.11^a	3.36 ± 1.51^a	2.83 ± 0.11^a
Total White blood cells (WBC) (cells /mm ³)	45883.33 ± 5150.32^a	$55966.67 \pm 26633.31^{ab}$	51266.67 ± 6824.46^a	83233.33 ± 11718.93^b
Lymphocytes (%)	94.18 ± 0.59^a	96.06 ± 0.12^a	95.5 ± 2.5^a	96 ± 0^a
Monocytes (%)	1.78 ± 0.71^a	0.95 ± 0.05^a	1.5 ± 0.5^a	1.0 ± 0.0^a
Total Plate counts (cells /mm ³)	219333.3 ± 9073.77^b	150666.7 ± 11547.01^a	$217166.7 \pm 719094.07^{ab}$	$230666.7 \pm 10016.65^{ab}$
Mean platelet volume (MPV) (fL)	7.45 ± 0.25^c	7.85 ± 0.25^a	7.55 ± 0.15^{ab}	7.7 ± 0.1^{ab}
Procalcitonin (PCT) (ng/mL)	0.12 ± 0.00^a	0.16 ± 0.0^b	0.17 ± 0.01^{bc}	0.18 ± 0.00^c
Red Blood Cell Count (cells *10 ³ /mm ³)	1.19 ± 0.15^b	0.74 ± 0.05^a	0.93 ± 0.38^{ab}	0.8 ± 0.02^{ab}
Haematocrit (HCT)	15.45 ± 1.35^b	9.7 ± 0.3^a	9.826 ± 0.551^a	9.3 ± 0.1^a
Mean corpuscular volume (MCV) (fL)	130.5 ± 5.1^a	133.2 ± 5^a	121.1 ± 3^a	$114.96 \pm 0.5.37^a$
Mean corpuscular haemoglobin (MCH) (pg/cell)	37.05 ± 0.45^{ab}	39.9 ± 2.7^b	36.68 ± 1.206^a	36.2 ± 0.8^a
Mean corpuscular haemoglobin concentration (MCHC) (g/dL)	28.45 ± 0.75^a	29.95 ± 0.95^a	34.7 ± 4.203^b	31.7 ± 0.2^{ab}
Red cell distribution width (RDW)(fL)	17 ± 1.4^c	13 ± 1.1^b	12.2 ± 0.5^{ab}	11.15 ± 0.35^a
Glucose (mg/dL)	196.70 ± 5.06^a	194.63 ± 7.0^a	189.70 ± 2.95^a	184.03 ± 4.22^a

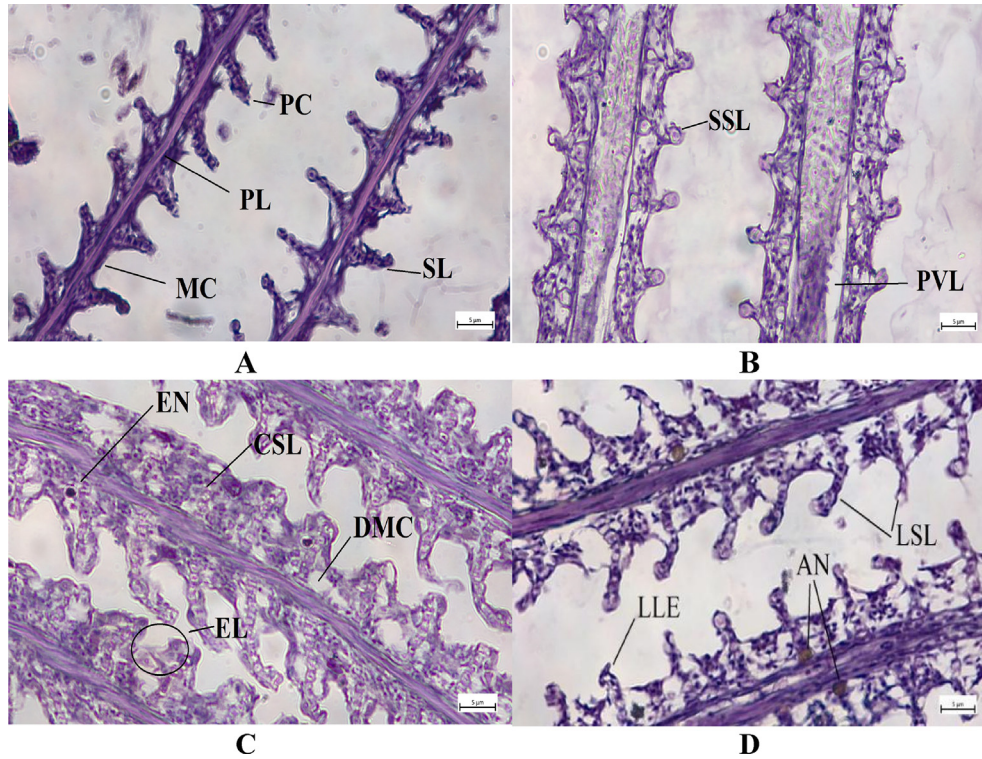


Fig. 3. Longitudinal histological sections through the gill of Nile tilapia, *Oreochromis niloticus* **A.** Control 0 μgL^{-1} Chlorpyrifos (0 % of LC_{50}); **B.** T_1 8 μgL^{-1} Chlorpyrifos (17% of LC_{50}); **C.** T_2 16 μgL^{-1} Chlorpyrifos (34% of LC_{50}); **D.** T_3 32 μgL^{-1} Chlorpyrifos (68% of LC_{50}) exposure for 60 days. (PL-Primary lamella, SL-Secondary lamella, PC-Pillar cell, MC-Mucous cell, SSL-shortening secondary gill lamella, PLE-primary lamellar epithelium, DF- damage of filament, EN-epithelial necrosis, CSL-congestion of secondary gill lamella, DMC-diffusion of mucous cell, EL-epithelial lifting, LLE-lifting of lamellar epithelium, AN-acute necrosis).

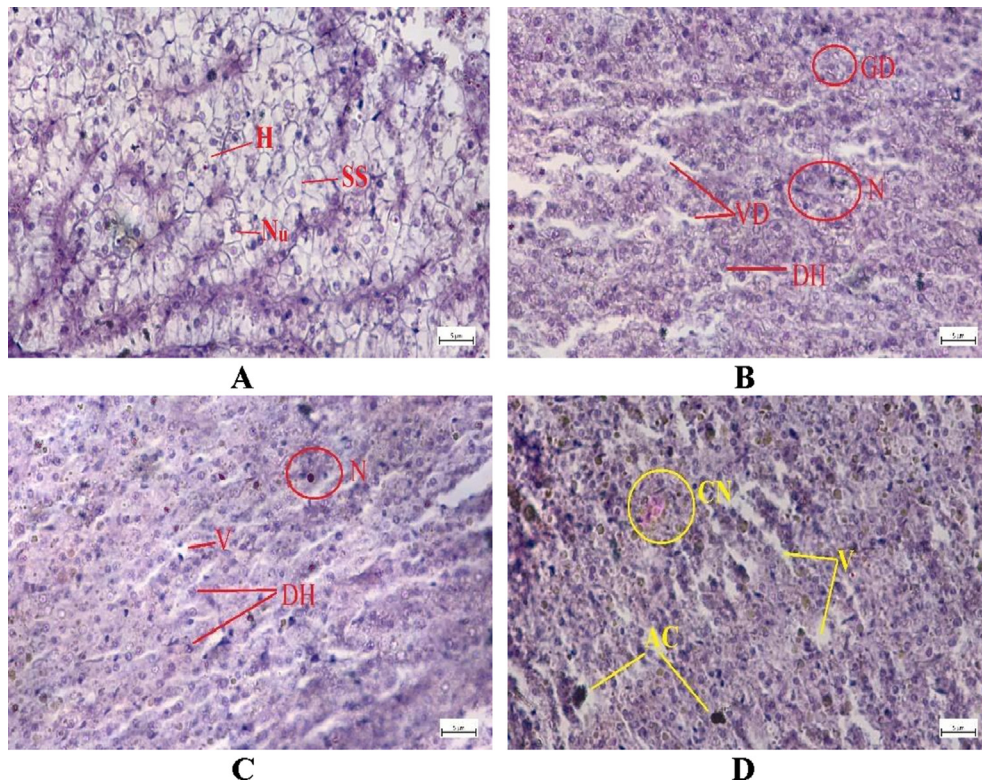


Fig. 4. Histopathological alterations of liver in Nile tilapia, *Oreochromis niloticus* **A.** Control 0 μgL^{-1} Chlorpyrifos (0 % of LC_{50}); **B.** T_1 8 μgL^{-1} Chlorpyrifos (17% of LC_{50}); **C.** T_2 16 μgL^{-1} Chlorpyrifos (34% of LC_{50}); **D.** T_3 32 μgL^{-1} Chlorpyrifos (68% of LC_{50}) exposure for 60 days. (H-hepatocytes, Nu-nuclei, SS-Sinusoids, DH-degenerative hepatocytes, GD-Granular degeneration, VD-vascular dilation, N-necrosis, V-Vacuoles, AN/CN-acute/chronic necrosis).

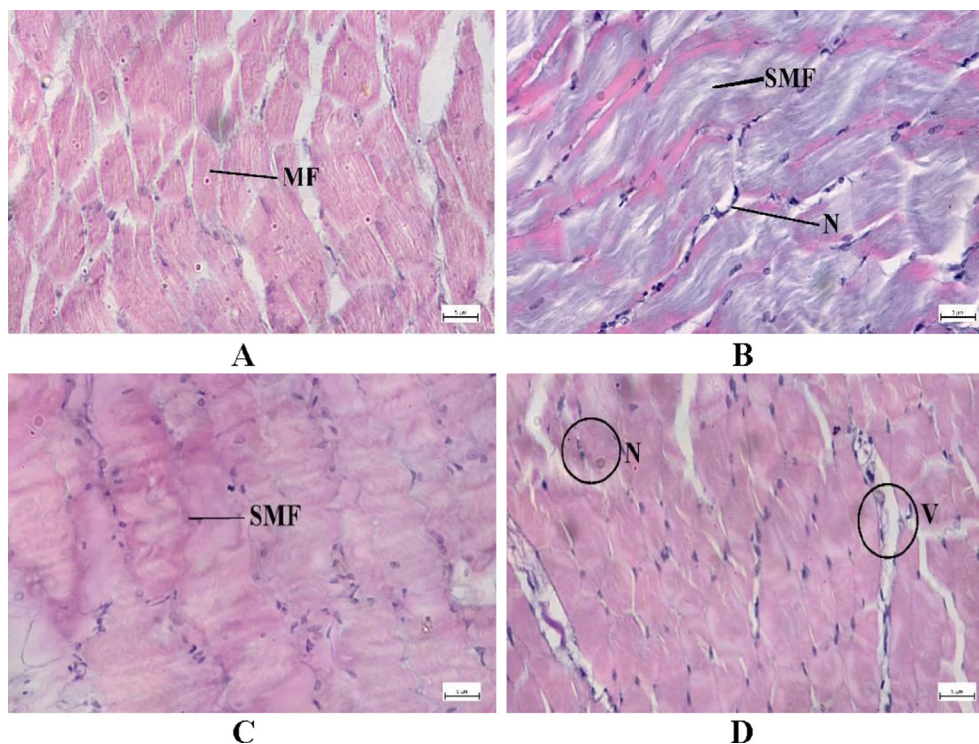


Fig. 5. Muscle tissue section of Nile tilapia, *Oreochromis niloticus* **A.** Control 0 µg⁻¹ Chlorpyrifos (0 % of LC₅₀); **B.** T₁ 8 µg⁻¹ Chlorpyrifos (17% of LC₅₀); **C.** T₂ 16 µg⁻¹ Chlorpyrifos (34% of LC₅₀); **D.** T₃ 32 µg⁻¹ Chlorpyrifos (68% of LC₅₀) exposure for 60 days. (DMF-Degeneration of muscle fiber, V-vacuoles, SMF-splitting of muscle N-necrosis).

4. Discussion

Basic physico-chemical parameters such as conductivity of water, dissolved oxygen level, pH, and water temperature were kept essentially consistent during the time of exposure following the standard parameters of Bhatnagar & Devi, (2013) and Chapman et al., (2016). As the trials progressed, the temperature dropped from 30 °C to 21 °C. This can be explained by the drop in temperature during November to December, accompanied by the cycle of winter in Bangladesh.

The lethal concentration index LC₅₀ is one of the widely used sensitivity tests to quantify chemical toxicants on animals (Sharmin et al., 2021). Current findings suggest that most of the commercial formulations of Chlorpyrifos imposed strong toxicity at very low doses for fish. The 96-hour LC₅₀ for Chlorpyrifos 20% EC ranged between 22 and 76 µg⁻¹ (Ihsan et al., 2018; Muttappa et al., 2015). The highest concentrations were noted as 1023–1500 µg⁻¹, although formulation of pesticides was not specified (Girón-Pérez et al., 2006; Gül, 2005). A stable survival rate of 100 % has been reported for control units which were within the condition for testing toxicity in aquatic organisms according to APHA, (1999). The differences in LC₅₀ are attributed to type of species used, their size and age, physicochemical properties of the rearing environment and formulation of pesticide administered.

Fishes exposed to organophosphate insecticides are reported to experience behavioral abnormalities, and increased death rate due to metabolic disturbances linked with toxicants (Dembélé et al., 2000). *Tilapia guineensis* was subjected to higher mortality rate with increased concentration and treatment duration of chlorpyrifos (Chindah et al., 2005). Mortality of stinging catfish *Heteropneustes fossilis* rose at higher concentrations of Envoy 50 (Akter et al., 2020) and with exposure to sublethal concentration of Pb toxicant (Hussain et al., 2021). Chlorpyrifos has been shown to have considerable impacts on growth of *O. niloticus* (Majumder

and Kaviraj, 2019). Negative growth issues were detected in *Tandanus tandanu* treated with Chlorpyrifos (Huynh and Nugegoda, 2012). Reduced body weight might be caused by poor food intake and liberation of extensive energy to compensate for toxic related stress while rearing (Adel et al., 2017; Majumder and Kaviraj, 2019). Poor body biomass and increased mortality rate in the treatment group compared to the control group also act as supportive to the above findings.

Hematology results corresponds to a better measurement of fish health and environmental status (Eissa and Abou-ElGheit, 2014) and they can be substantially manipulated by animal’s age, size, physiology, diet component and surrounding hydrology (Parrino et al., 2018). The hematology counts in current research demonstrated significant alternation between the animals in control group and treatment groups. A study by Singh and Srivastava, (2010) revealed there was an increased count of total leukocyte in *Labeo rohita* exposed to 28 days of organophosphate pesticide profenofos. In contrast, it had also been noted that hematology remains non affected in Caspian brown trout treated for 20 days with Chlorpyrifos (Adel et al., 2017) and common carp for three-week chlorpyrifos (Jaffer et al., 2017). A noticeable variation was reported in the hemoglobin concentration and white blood cells of *O. niloticus* associated with 30 days exposure to Chlorpyrifos (Abdo et al., 2021). Again, *O. niloticus* treated with Sumithion considerably yields lower red blood cell and hemoglobin levels, with increased white blood cell counts (Sharmin et al., 2021). Reduced mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) were detected in fish treated with Ronster for three weeks (Oluah et al., 2020). Increased level of blood procalcitonin could be regarded as a biomarker for indicating respiratory tract inflammation (Gilbert, 2011).

Increased HSI in *Mullus barbatus* had been documented due to heavy metals treatment and it could be associated with cellular deformities and an increase in hepatic enzyme degradation

activities (Kucuksegin et al., 2011). Majumder and Kaviraj, (2019) also noted elevated HSI value in chlorpyrifos intoxicated *O. niloticus*. As the rate of necrosis in liver cells increased current research following the pesticides exposure, the liver would have gained weight as extra mass in the form of fluid congestion.

Tissue histology, which is used as an indicator of pollution exposure, is a valuable method for determining the extent of pollution, especially for sublethal and chronic effects (Cengiz & Ünlü, 2003). Fish gills are sensitive to contaminants in water because of their placement and huge exterior area (Afshan et al., 2014; Stara et al., 2020). Pesticides have been proven to have comparable effects on fish gills in several studies (Cengiz and Unlu, 2006) as they execute a variety of tasks including breathing and digestion, osmoregulation, and excretion. Metal-contaminated *Oreochromis niloticus* and *Lates niloticus* from Lake Nasser, Egypt, showed shortening or fusion of gill lamellae, lamella destruction, increased vacuolation, and irregular gill lamellae (Younis et al., 2013). The effects of similar histopathology have been conducted while treating guppy *Poecilia reticulata* with chlorpyrifos (Silva and Samayawardhena, 2002). The liver functioned as principal organ in detoxifying metabolites and its histology would act as effective biomarkers in demonstrating the impact of toxicant at cellular level (Cuevas et al., 2016). Hepatic lesions were stated to related with increased toxicant levels (Sadiq et al., 2012). The Cory, *Corydoras paleatus* treated with methyl parathion developed swelling, bile stagnation, necrosis, atrophy, and vacuolization in hepatic cells (Fanta et al., 2003). Sarkar et al., (2005) reported hyperplasia, vacuolation, interrupted hepatocytes and necrosis, in *Labeo rohita* treated with cypermethrin. Enhanced degeneration, necrosis, and localized hemorrhage were detected on liver tissue of the same fish due to a two-week chlorpyrifos treatment (Farhan et al., 2021). Hepatic hypertrophy, displaced nuclei, necrotic cells sites and degenerative cells were reported in the same species in 7 days of carbofuran treatment (Américo-Pinheiro et al., 2020). The current findings are coherent with above investigations. It had been identified that increasing the amount of chlorpyrifos 20EC accelerated the level of degenerative process in liver cells. Muscle cell damage and vacuolation were detected in *Oreochromis* treated with Chromiam (Abbas and Ali, 2007). The exposure to chlorinated pesticides and PCBs also induced histological adjustments in the muscular tissue of freshwater fish *Hoplias alabaricus* (Miranda et al., 2008). In conclusion, the findings of the present histological investigations demonstrated a direct correlation between pesticide exposure and histopathological disorders as observed in several tissues.

5. Conclusion

Results of the current study clearly revealed that chlorpyrifos 20EC is highly toxic to aquatic vertebrates and had a detrimental impact on growth, hematological indicators, and somatic indices. The effect of toxicity is also proven in cellular level through distinctive histopathology in high dose treatment units.

Ethical approval

The Animals Ethics Committee from the Sylhet Agricultural University Research System (SAURES), Sylhet Agricultural University, Sylhet-3100, Bangladesh, approved the design and utilization of animals for the current project.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Thanks to UGC (University Grant Commission) and Sylhet Agricultural University Research System (SAURES), Bangladesh, for providing funds for research and student's allowance in referring to budget allocation code 3632104. Authors acknowledge the NST (National Science and Technology) fellowship scheme, for providing their partial fundings in research and student stipend as well.

Reference

- Abbas, H.H., Ali, F.K., 2007. Study the effect of hexavalent chromium on some biochemical, citotoxicological and histopathological aspects of the *Oreochromis spp.* fish. Pakistan J. Biol. Sci. Pjbs 10 (22), 3973–3982. <https://doi.org/10.3923/pjbs.2007.3973.3982>.
- Abdo, S.E., Gewaily, M.S., Abo-Al-Ela, H.G., Almeer, R., Soliman, A.A., Elkomy, A.H., Dawood, M.A.O., 2021. Vitamin C rescues inflammation, immunosuppression, and histopathological alterations induced by chlorpyrifos in Nile tilapia. Environ. Sci. Pollut. Res. 28 (22), 28750–28763. <https://doi.org/10.1007/s11356-021-12711-5>.
- Abhilash, P.C., Singh, N., 2009. Pesticide use and application: an Indian scenario. J. Hazard. Mater. 165 (1–3), 1–12. <https://doi.org/10.1016/j.jhazmat.2008.10.061>.
- Adel, M., Dadar, M., Khajavi, S.H., Pourgholam, R., Karimi, B., Velisek, J., 2017. Hematological, biochemical and histopathological changes in Caspian brown trout (*Salmo trutta caspius* Kessler, 1877) following exposure to sublethal concentrations of chlorpyrifos. Toxin Rev. 36 (1), 73–79. <https://doi.org/10.1080/15569543.2016.1230631>.
- Adhikari, S., Sarkar, B., Chatterjee, A., Mahapatra, C.T., Ayyappan, S., 2004. Effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recovery in a freshwater teleost, *Labeo rohita* (Hamilton). Ecotoxicol. Environ. Saf. 58, 220–226. <https://doi.org/10.1016/j.ecoenv.2003.12.003>.
- Afshan, S., Ali, S., Ameen, U., Farid, M., Bharwana, S., Hannan, F., Ahmad, R., 2014. Effect of different heavy metal pollution on fish. Res. J. Chem. Environ. Sci. 2, 74–79.
- Akter, R., Pervin, M.A., Jahan, H., Rakhi, S.F., Reza, A.H.M.M., Hossain, Z., 2020. Toxic effects of an organophosphate pesticide, envoy 50 SC on the histopathological, hematological, and brain acetylcholinesterase activities in stinging catfish (*Heteropneustes fossilis*). J. Basic Appl. Zool. 81, 47. <https://doi.org/10.1186/s41936-020-00184-w>.
- Ali, D., Nagpure, N.S., Kumar, S., Kumar, R., Kushwaha, B., Lakra, W.S., 2009. Assessment of genotoxic and mutagenic effects of chlorpyrifos in freshwater fish *Channa punctatus* (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis. Food Chem. Toxicol. 47 (3), 650–656. <https://doi.org/10.1016/j.fct.2008.12.021>.
- Ali, M., Majid, M., Hussain, I., Kali, S., Naz, T., Niazi, M.B.K., Khan, M.R.A., Zafar, M.I., 2020. Chlorpyrifos mediated oxidative damage and histopathological alterations in freshwater fish *Oncorhynchus mykiss* in Northern Pakistan. Aquac. Res. 51 (11), 4583–4594. <https://doi.org/10.1111/are.14804>.
- American Public Health Association, 1999. Standard Methods for Examination of Water and Wastewater. 20th ed. American Public Health Association, American Water Works Association, Water Environment Federation, Washington DC, USA.
- Américo-Pinheiro, J.H.P., Machado, A.A., da Cruz, C., Aguiar, M.M., Ferreira, L.F.R., Torres, N.H., Machado-Neto, J.G., 2020. Histological changes in targeted organs of Nile tilapia (*Oreochromis niloticus*) exposed to sublethal concentrations of the pesticide carbofuran. Water, Air, Soil Pollut. 231, 228. <https://doi.org/10.1007/s11270-020-04628-5>.
- Bhatnagar, A., Devi, P., 2013. Water quality guidelines for the management of pond fish culture. Int. J. Environ. Sci. 3, 1980–2009. <https://doi.org/10.6088/ijes.2013030600019>.
- Brooks, S., Tyler, C.R., Sumpter, J.P., 1997. Egg quality in fish: what makes a good egg? Rev. Fish Biol. Fish. 7, 387–416. <https://doi.org/10.1023/A:1018400130692>.
- Cengiz, E.I., Ünlü, E., 2003. Histopathology of gills in mosquitofish, *Gambusia affinis* after long-term exposure to sublethal concentrations of malathion. J. Environ. Sci. Heal. Part B 38 (5), 581–589. <https://doi.org/10.1081/PFC-120023516>.
- Cengiz, E.I., Unlu, E., 2006. Sublethal effects of commercial deltamethrin on the structure of the gill, liver and gut tissues of mosquitofish, *Gambusia affinis*: a microscopic study. Environ. Toxicol. Pharmacol. 21 (3), 246–253. <https://doi.org/10.1016/j.etap.2005.08.005>.
- Chan, C.Y., Tran, N., Pethiyagoda, S., Crissman, C.C., Sulser, T.B., Phillips, M.J., 2019. Prospects and challenges of fish for food security in Africa. Glob. Food Sec. 20, 17–25. <https://doi.org/10.1016/j.gfs.2018.12.002>.
- Chapman, D.V., Bradley, C., Gettel, G.M., Hatvani, I.G., Hein, T., Kovács, J., Liska, I., Oliver, D.M., Tanos, P., Trásy, B., Várbró, G., 2016. Developments in water quality monitoring and management in large river catchments using the Danube River as an example. Environ. Sci. Policy 64, 141–154. <https://doi.org/10.1016/j.envsci.2016.06.015>.
- Chellappa, S., Huntingford, F.A., Strang, R.H.C., Thomson, R.Y., 1995. Condition factor and hepatosomatic index as estimates of energy status in male three-spined stickleback. J. Fish Biol. 47 (5), 775–787. <https://doi.org/10.1111/j.1095-8649.1995.tb06002.x>.

- Chindah, A.C., Sikoki, F.D., Vincent-Akpu, I., 2005. Toxicity of an organophosphate pesticide (Chlorpyrifos) on a common niger delta wetland fish - *Tilapia guineensis* (Bleeker 1862). *J. Appl. Sci. Environ. Manag.* 8. <https://doi.org/10.4314/jasem.v8i2.17233>.
- Cuevas, N., Zorita, I., Franco, J., Costa, P.M., Larreta, J., 2016. Multi-organ histopathology in gobies for estuarine environmental risk assessment: a case study in the Ibaizabal estuary (Bay of Biscay). *Estuar. Coast. Shelf Sci.* 179, 145–154. <https://doi.org/10.1016/j.ecss.2015.11.023>.
- Dawood, M.A.O., El-Shamaa, I.S., Abdel-Razik, N.I., Elkomy, A.H., Gewaily, M.S., Abdo, S.E., Soliman, A.A., Paray, B.A., Abdelkhalik, N., 2020. The effect of mannanoligosaccharide on the growth performance, histopathology, and the expression of immune and antioxidative related genes in Nile tilapia reared under chlorpyrifos ambient toxicity. *Fish Shellfish Immunol.* 103, 421–429. <https://doi.org/10.1016/j.fsi.2020.05.061>.
- Dembélé, K., Haubruge, E., Gaspar, C., 2000. Concentration effects of selected insecticides on brain acetylcholinesterase in the Common Carp (*Cyprinus carpio* L.). *Ecotoxicol. Environ. Saf.* 45 (1), 49–54. <https://doi.org/10.1006/eesa.1999.1829>.
- Edwin, T., Ihsan, T., Rahmatika, A., Darlis, N., 2019. Impact of chlorpyrifos toxicity on gill damage of two species of freshwater fish in Lake Diatas. *Environ. Heal. Eng. Manag.* 6, 241–246. <https://doi.org/10.15171/ehem.2019.27>.
- Eissa, N., AbouElGheit, E., 2014. Dietary supplementation impacts of potential non-pathogenic isolates on growth performance, hematological parameters, and disease resistance in Nile Tilapia (*Oreochromis niloticus*). *J. Vet. Adv.* 4 (10), 712. <https://doi.org/10.5455/jva.20141025045451>.
- El-Bouhy, Z., El-Nobi, G., Reda, R., Ibrahim, R., 2016. Effect of insecticide "chlorpyrifos" on immune response of *Oreochromis niloticus*. *Zagazig Vet. J.* 44, 196–204. <https://doi.org/10.21608/zvjz.2016.7872>.
- EPA, U., 2021. Guideline No. 497: OECD Guidelines for the Testing of Chemicals, Section 4, OECD Guidelines for the Testing of Chemicals, Section 4. OECD. <https://doi.org/10.1787/b92879a4-en>.
- Fanta, E., Rios, F.S., Romão, S., Vianna, A.C.C., Freiburger, S., 2003. Histopathology of the fish *Corydoras paleatus* contaminated with sublethal levels of organophosphorus in water and food. *Ecotoxicol. Environ. Saf.* 54 (2), 119–130. [https://doi.org/10.1016/S0147-6513\(02\)00044-1](https://doi.org/10.1016/S0147-6513(02)00044-1).
- Farhan, M., Wajid, A., Hussain, T., Jabeen, F., Ishaque, U., Iftikhar, M., Daim, M.A., Nooreen, A., 2021. Investigation of oxidative stress enzymes and histological alterations in tilapia exposed to chlorpyrifos. *Environ. Sci. Pollut. Res.* 28 (11), 13105–13111. <https://doi.org/10.1007/s11356-020-11528-y>.
- Finney, D.J., 1971. *Probit Analysis*, 3rd ed. Cambridge University Press, 32 E. 57th St., New York, NY 10022. <https://doi.org/10.1002/jps.2600600940>.
- Gilbert, D.N., 2011. Procalcitonin as a biomarker in respiratory tract infection. *Clin. Infect. Dis.* 52, S346–S350. <https://doi.org/10.1093/cid/cir050>.
- Girón-Pérez, M.I., Barcelós-García, R., Vidal-Chavez, Z.G., Romero-Bañuelos, C.A., Robledo-Marengo, M.L., 2006. Effect of chlorpyrifos on the hematology and phagocytic activity of Nile tilapia cells (*Oreochromis niloticus*). *Toxicol. Mech. Methods* 16 (9), 495–499. <https://doi.org/10.1080/15376510600751988>.
- Gül, A., 2005. Investigation of acute toxicity of chlorpyrifos-methyl on Nile tilapia (*Oreochromis niloticus* L.) larvae. *Chemosphere* 59 (2), 163–166. <https://doi.org/10.1016/j.chemosphere.2004.10.065>.
- Hasanuzzaman, M., Rahman, M.A., Islam, M.S., Salam, M.A., Nabi, M.R., 2018. Pesticide residues analysis in water samples of Nagarpur and Satura Upazila. *Bangladesh. Appl. Water Sci.* 8, 8. <https://doi.org/10.1007/s13201-018-0655-4>.
- Htun-Han, M., 1978. The reproductive biology of the dab *Limanda limanda* (L.) in the North Sea: Seasonal changes in the ovary. *J. Fish Biol.* 13 (3), 351–359. <https://doi.org/10.1111/j.1095-8649.1978.tb03443.x>.
- Hussain, M., Hossain, M.A., Begum, M., Roy, N.C., 2021. Freshwater mussel, *Lamelliedens marginalis* to reduce the lead (Pb) bioaccumulation in aquaculture of stinging catfish, *Heteropneustes fossilis*. *J. Appl. Aquac.* <https://doi.org/10.1080/10454438.2021.2010630>.
- Huynh, H.P.V., Nugegoda, D., 2012. Effects of chlorpyrifos exposure on growth and food utilization in Australian catfish, *Tandanus tandanus*. *Bull. Environ. Contam. Toxicol.* 88 (1), 25–29. <https://doi.org/10.1007/s00128-011-0431-8>.
- Ihsan, T., Edwin, T., Anggraeni, W., 2018. Behavioral responses of Nile tilapia (*Oreochromis niloticus*) by sublethal exposure to chlorpyrifos: a case study in Twin Lakes of West Sumatra. *Environ. Heal. Eng. Manag.* 5, 205–210. <https://doi.org/10.15171/EHEM.2018.28>.
- Ihsan, T., Edwin, T., Yanti, R.D., 2019. The effect of sublethal exposure of chlorpyrifos to Nile tilapia (*Oreochromis niloticus*): Case study of twin lakes of West Sumatra, Indonesia. *Nat. Environ. Pollut. Technol.* 18, 1399–1403.
- Jaffer, N.S., Rabee, A.M., Al-Chalabi, S.M.M., 2017. Biochemical and hematological parameters and histological alterations in fish *Cyprinus carpio* L. as biomarkers for water pollution with chlorpyrifos. *Hum. Ecol. Risk Assess.* An Int. J. 23 (3), 605–616. <https://doi.org/10.1080/10807039.2016.1261626>.
- Kucuksezgin, F., Kontas, A., Uluturhan, E., 2011. Evaluations of heavy metal pollution in sediment and *Mullus barbatus* from the Izmir Bay (Eastern Aegean) during 1997–2009. *Mar. Pollut. Bull.* 62 (7), 1562–1571. <https://doi.org/10.1016/j.marpolbul.2011.05.012>.
- Kumar, N., Antony Jesu Prabhu, P., Pal, A.K., Remya, S., Aklakur, M.d., Rana, R.S., Gupta, S., Raman, R.P., Jadhao, S.B., 2011. Anti-oxidative and immunohematological status of Tilapia (*Oreochromis mossambicus*) during acute toxicity test of endosulfan. *Pestic. Biochem. Physiol.* 99 (1), 45–52. <https://doi.org/10.1016/j.pestbp.2010.10.003>.
- Majumder, R., Kaviraj, A., 2019. Acute and sublethal effects of organophosphate insecticide chlorpyrifos on freshwater fish *Oreochromis niloticus*. *Drug Chem. Toxicol.* 42 (5), 487–495. <https://doi.org/10.1080/01480545.2018.1425425>.
- Martins, M.L., Xu, D.H., Shoemaker, C.A., Klesius, P.H., 2011. Temperature effects on immune response and hematological parameters of channel catfish *Ictalurus punctatus* vaccinated with live theronts of *Ichthyophthirius multifiliis*. *Fish Shellfish Immunol.* 31 (6), 774–780. <https://doi.org/10.1016/j.fsi.2011.07.015>.
- Miranda, A.L., Roche, H., Randi, M.A.F., Menezes, M.L., Ribeiro, C.A.O., 2008. Bioaccumulation of chlorinated pesticides and PCBs in the tropical freshwater fish *Hoplias malabaricus*: histopathological, physiological, and immunological findings. *Environ. Int.* 34 (7), 939–949. <https://doi.org/10.1016/j.envint.2008.02.004>.
- Muñoz, L., Weber, P., Dressler, V., Baldisserotto, B., Vigliano, F.A., 2015. Histopathological biomarkers in juvenile silver catfish (*Rhamdia quelen*) exposed to a sublethal lead concentration. *Ecotoxicol. Environ. Saf.* 113, 241–247. <https://doi.org/10.1016/j.ecoenv.2014.11.036>.
- Muttappa, K., Reddy, H.R.V., Padmanabha, A., Shridhar, B., Prabhudeva, K.N., Basavaraju, Y., Gangadhar, G., Rajanna, K.B., Chethan, N., 2015. Chlorpyrifos induced histopathological changes in the liver of tilapia (*Oreochromis mossambicus*). *Int. J. Recent Sci. Res.* 6, 5819–5823.
- OECD, 2009. Test No. 403: Acute Inhalation Toxicity, OECD Guidelines for the Testing of Chemicals, Section 4. OECD. <https://doi.org/10.1787/9789264070608-en>.
- Oluah, N.S., Aguzie, I.O., Ekechukwu, N.E., Madu, J.C., Ngene, C.I., Oluah, C., 2020. Hematological and immunological responses in the African catfish *Clarias fahaka* exposed to sublethal concentrations of herbicide Ronstar®. *Ecotoxicol. Environ. Saf.* 201, 110824. <https://doi.org/10.1016/j.ecoenv.2020.110824>.
- Parrino, V., Cappello, T., Costa, G., Cannavà, C., Sanfilippo, M., Fazio, F., Fasulo, S., 2018. Comparative study of haematology of two teleost fish (*Mugil cephalus* and *Carassius auratus*) from different environments and feeding habits. *Eur. Zool. J.* 85, 193–199. <https://doi.org/10.1080/24750263.2018.1460694>.
- Petchoy, T., Pung, T., 2017. Effect of chlorpyrifos and dimethoate spraying doses on residue concentrations in scallion (*Allium cepa* var. aggregatum). *Agric. Sci. J.* 48, 108–117.
- Rao, J.V., Rani, C.H.S., Kavitha, P., Rao, R.N., Madhavendra, S.S., 2003. Toxicity of chlorpyrifos to the fish *Oreochromis mossambicus*. *Bull. Environ. Contam. Toxicol.* 70 (5), 985–992. <https://doi.org/10.1007/s00128-003-0079-0>.
- Sadiq, B.A., Syed Mohamed, H.E., Broos, K.V., Stalin, A., Singhal, R.K., Venubabu, P., 2012. Histological variations in liver of freshwater fish *Oreochromis mossambicus* exposed to 60Co gamma irradiation. *J. Environ. Radioact.* 113, 57–62. <https://doi.org/10.1016/j.jenvrad.2012.04.011>.
- Sarkar, B., Chatterjee, A., Adhikari, S., Ayyappan, S., 2005. Carbofuran- and cypermethrin-induced histopathological alterations in the liver of *Labeo rohita* (Hamilton) and its recovery. *J. Appl. Ichthyol.* 21 (2), 131–135. <https://doi.org/10.1111/j.1439-0426.2004.00590.x>.
- Sharmin, S., Islam, M.T., Sadat, M.A., Jannat, R., Alam, M.R., Shahjahan, M.d., 2021. Sumithion induced structural erythrocyte alteration and damage to the liver and kidney of Nile tilapia. *Environ. Sci. Pollut. Res.* 28 (27), 36695–36706. <https://doi.org/10.1007/s11356-021-13263-4>.
- De Silva, P.M.C.S., Samayawardhena, L.A., 2002. Low concentrations of lorsban in water result in far reaching behavioral and histological effects in early life stages in guppy. *Ecotoxicol. Environ. Saf.* 53 (2), 248–254.
- Singh, N.N., Srivastava, A.K., 2010. Haematological parameters as bioindicators of insecticide exposure in teleosts. *Ecotoxicology* 19 (5), 838–854. <https://doi.org/10.1007/s10646-010-0465-4>.
- Stalin, A., Suganthi, P., Mathivani, S., Paray, B.A., Al-Sadoon, M.K., Gokula, V., Musthafa, M.S., 2019. Impact of chlorpyrifos on behavior and histopathological indices in different tissues of freshwater fish *Channa punctatus* (Bloch). *Environ. Sci. Pollut. Res.* 26 (17), 17623–17631. <https://doi.org/10.1007/s11356-019-05165-3>.
- Stara, A., Pagano, M., Capillo, G., Fabrello, J., Sandova, M., Vazzana, I., Zuskova, E., Velisek, J., Matozzo, V., Faggio, C., 2020. Assessing the effects of neonicotinoid insecticide on the bivalve mollusc *Mytilus galloprovincialis*. *Sci. Total Environ.* 700, 134914. <https://doi.org/10.1016/j.scitotenv.2019.134914>.
- Syed, R., Masood, Z., Ul Hassan, H., Khan, W., Mushtaq, S., Ali, A., Gul, Y., Jafari, H., Habib, A., Ishaq Ali Shah, M., Gabol, K., Gul, H., Ullah, A., 2022. Growth performance, haematological assessment and chemical composition of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) fed different levels of Aloe vera extract as feed additives in a closed aquaculture system. *Saudi J. Biol. Sci.* 29 (1), 296–303. <https://doi.org/10.1016/j.sjbs.2021.08.098>.
- Ullah, S., Zuberi, A., Alagawany, M., Farag, M.R., Dadar, M., Karthik, K., Tiwari, R., Dhama, K., Iqbal, H.M.N., 2018. Cypermethrin induced toxicities in fish and adverse health outcomes: Its prevention and control measure adaptation. *J. Environ. Manage.* 206, 863–871. <https://doi.org/10.1016/j.jenvman.2017.11.076>.
- Van-Dyk, J.C., Pieterse, G.M., 2008. A histo-morphological study of the testis of the sharpthroat catfish (*Clarias gariepinus*) as reference for future toxicological assessments. *J. Appl. Ichthyol.* 24, 415–422. <https://doi.org/10.1111/j.1439-0426.2008.01127.x>.
- Yan, W., Hamid, N., Deng, S., Jia, P.-P., Pei, D.-S., 2020. Individual and combined toxicogenetic effects of microplastics and heavy metals (Cd, Pb, and Zn) perturb gut microbiota homeostasis and gonadal development in marine medaka (*Oryzias latipes*). *J. Hazard. Mater.* 397, 122795. <https://doi.org/10.1016/j.jhazmat.2020.122795>.
- Younis, E.S.M., Abdel-Warith, A.W.A.M., Al-Asgah, N.A., Ebaid, H., Mubarak, M., 2013. Histological changes in the liver and intestine of Nile tilapia, *Oreochromis niloticus*, exposed to sublethal concentrations of cadmium. *Pak. J. Zool.* 45, 833–841.