Hossain et al, 2022_091022.docx

by Mohammad Amzad Hossain

Submission date: 01-Sep-2022 09:36PM (UTC+1000)

Submission ID: 1890596795

File name: 127456_Mohammad_Amzad_Hossain_Hossain_et_al__2022_091022_420048_941658112.docx (931.51K)

Word count: 4949

Character count: 29969

Toxic effects of chlorpyrifos on the growth, hemocytes count, and vital organ's histopathology of freshwater mussel, *Lamellidens marginalis*

Abstract

The Chlorpyrifos 20 EC (emulsifying concentration) is one of the extensively used agro-pesticide in Bangladesh, most of which are residual in nearby natural water reservoirs. The freshwater mussel, *Lamellidens marginalis* have been exposed to Chlorpyrifos 20 EC at varying concentrations of T_c 0 mgL^{-1}, T_1 2.53 mgL^{-1}, T_2 5.07 mgL^{-1} and T_3 10.15 mgL^{-1} for 35 days to investigate its toxic effects on growth biometrics, hemocyte counts and histopathology of gill, muscle, and ovary. The 96-hour Chlorpyrifos 20 EC LC_{50} for *L. marginalis* has been computed as 25.37 mgL^{-1} from PROBIT analysis. Major water regulating parameters were recorded and analyzed for each treatment group. The specific growth rate (%) was reported statistically highest in T_C (0.104±0.412) and lowest in T_3 (-0.52±0.38). Similarly, the condition index (CI) and Fulton's condition factor were decent at T_C and downed at T_3 (P<0.05). A high mortality rate occurred in T_3 and the lowest mortality in T_C. The value of total hemocytes count were also reported to be highest for T_C and gradually decreased as the dosage increased (P<0.05). The histopathology of gill, muscle, and ovary also revealed moderate to severe pathological signs in treatment groups in comparison to control. Administration of Chlorpyrifos 20 EC results in detrimental growth, cytological and hematological alternation in *L. marginalis*.

Keywords: Chlorpyrifos 20 EC, *Lamellidens marginalis,* hemocytes, histopathology, growth biometrics.

1. Introduction

The pearl mussel, *Lamellidens marginalis,* is the most common and abundant freshwater bivalve species in the tropical climate of southeast Asia (Mishra et al., 2008; Siddique et al., 2020). They serve as excellent natural water filters and therefore, are used as biological agents in water quality monitoring (Hussain et al., 2022; Sicuro, 2015). Being a filter feeder, freshwater mussels are highly influenced by anthropogenic factors such as the internment of rivers, flow modification, pollution, and climatic change. (Bolotov et al., 2019). The freshwater mussel *L. marginalis* is the primary bivalve species used for commercial pearl production across the Indian subcontinent (Sicuro, 2015). They also contributed to ecological importance by providing a source of food, and shelter for a wide range of terrestrial and aquatic life (Carboni et al., 2019).

Organophosphorus has been among the most widely applied group of insect repellents in the global pest control industries (Cacciatore et al., 2013) and it had successfully replaced the old organochlorine derivatives in Agri farm practices (Zahran et al., 2018). Chlorpyrifos is labeled as a priority element following the directive of the European Commission aquatic ecosystems protection policy (Rouillard et al., 2018). This group represents low environmental persistence with a moderately little half-life in aquatic habitats ranging from 29 to 74 days (Bondarenko et al., 2004). Histopathology, hematology, and growth evaluation of mollusks could be evidence of their overall health status (Zahran et al., 2018). The changes in the abundance of mussels in a particular water body would be a truthful indicator of its physicochemical properties (Traiger et al., 2022). The histological assays are more widely used methods in evaluating the impact of toxicants at the cellular or tissue level (Ait-Ayad et al., 2011; Hussain et al, 2022). The present study is a baseline effort to expand information concerning the risks of existing pesticides usages to native freshwater mussels in Bangladesh. Very little data exist pertaining to the toxicity of pesticides in invertebrates and other aquatic biotas in Bangladesh.

2. Material and Methods

2.1 Collection of animals and rearing

Live samples of freshwater mussel, *L. marginalis* were collected from the nearby river water areas and transported in a large, aerated plastic drum to the wet laboratory facilities of Sylhet Agricultural University, Bangladesh. The collected mussels were initially checked for health status and acclimatized in separate aquariums with proper temperature and aeration. There was no provision for feeding during acclimatization. After acclimatizing, 20 healthy and uniformed-sized mussels were stocked into each of the 50-liter treatment aquariums to rear for up to 35 days. A control and three different treatment groups were assigned following the 96-hour LC_{50} value of chlorpyrifos 20 EC. The mussels were fed with dried and formulated green algae three times a day at 1.5-1.25 % of their body weight and the amount of food was sequentially decreased after two weeks to prevent over-primary productivity in the aquarium system. Uneaten feed and the feces materials were siphoned out once per two days and partially water was added to prevent loss via evaporation.

2.2 LC_{50} test and planning the dose of exposure

A 96-hour LC_{50} test has been done with 20 animals in each of 0, 3.0, 6.0, 9.0, 12, 18, 24, 36, 48, 72, and 96 mgL^{-1} of chlorpyrifos 20 EC treatment units. Each aquarium contained 25 liters of water, a well aeration system and 20 animals were employed in each unit following the standard OECD guidelines (OECD, 2009). The resulting mortality rates were put in the SPSS for the PROBIT regression model to analyze LC_{50} (Finney, 1971) (Figure 1). The different dose of pesticide used in different treatment assigned as T_C $0mgL^{-1}$ (0% of LC_{50}), T_1 2.53 mgL^{-1} (10% of LC_{50}), T_2 5.07 mgL^{-1} (20% of LC_{50}) and T_3 10.15 mgL^{-1} (40% of LC_{50}) following the LC_{50} values.

2.3 Monitoring hydrological parameters and sampling

Various hydrological parameters such as temperature, dissolved oxygen, salinity, TDS, and pH were measured each 7 days interval by using YSI Professional Plus Handheld Multiparameter Meter (Model 6050000), and ammonia levels were detected by employing a commercially manufactured ammonia test kit (Model HI 3824, Japan). At the same interval, three animals from each replication unit were sampled randomly for all the treatment groups, and length, weight, and body depth data were acquired immediately. At the end of the trial, all animals were sacrificed to measure their soft tissue wet weight, and a section from muscle tissue, gonad, and gill was preserved in neutral buffered formalin (NBF) for histological analysis.

2.4 Tools for growth biometrics

Different growth biometrics were calculated by using formulae.

Percentage of weight gain = $(W_f - W_i / W_1) \times 100$, Where W_f-Mean final weight and W_I-Mean initial weight.

Specific growth rate SGR (%) = $(\ln W_2 - \ln W_1 / T_2 - T_1) \times 100$, Where W_I= the initial weight (g) at a time, W_2 = the final weight (g) at a time, T_2-T_I=Duration in days.

Percentages of length gain = $(L_f - L_i / L_i) \times 100$; Where, L_f-Mean final length and L_I-Mean initial length.

The Fulton's condition factors were computed from the length-weight equation of Htun-Han, (1978). The condition index (CI) has been calculated by using following the methodology adopted from Uddin et al., (2010). The shells were sundried for 2.5 hours before taking their weight in the ratio between wet tissue wet and dry weight of the shell.

2.5 Collection of hemolymphs and counting total hemocytes

At the end of 25 days of rearing, the hemolymph samples were collected from fleshy abductor muscle by using a 0.8 mm needle and 1 ml syringe. About 300-500 microliter of samples were collected from each animal and placed in the centrifuge tube. The samples were then mixed as a 1:2 ratio of Alsevier solution (MP Biomedicals, Ohio) prepared with the same volume of 3%

formaldehyde solution following the description of Juhel et al., (2015). The concentration of the cell was counted by using a conventional hemocytometer process.

2.6 Histology of gill, muscle, and gonad tissues

Previously NBF preserved samples were washed under tap water and underwent repeated alcoholic dehydration and xylene cleaning process before infiltrating them on paraffin. Paraffin-embedded tissue was sliced at 3-5 micrometers and stained with Hematoxylin-Eosin stain to visualize the cell under the light microscope. Photographs are taken by using Zeiss software version 2.3 on Windows 10 installation connected to Carl ZEISS Primostar 3 Microscope.

2.7 Statistical analysis and visual constructions

A comparison of the mean between different parameters were conducted by using IBM SPSS v27 and Duncan's Multiple Range test (DMRT) at $p<0.05$. Graph and visualization were performed in office 365 tools by using data from SPSS analysis.

3.0 Results

3.1 Toxicity tests

The 96-hour LC_{50} of Chlorpyrifos 20 EC for *L. marginalis* in the current trial was computed as 25.37 mgL^{-1} from the PROBIT regression model ($P<0.05$) (figure 1). A reference table has been constructed based on previous trials in 96-hour LC_{50} of different formulations of organophosphate pesticides in bivalves (table 2). A moderately high estimate of LC_{50} had already been reported for Dimethoate, Malathion, Atrazine, and Glyphosate in several bivalves (table 2).

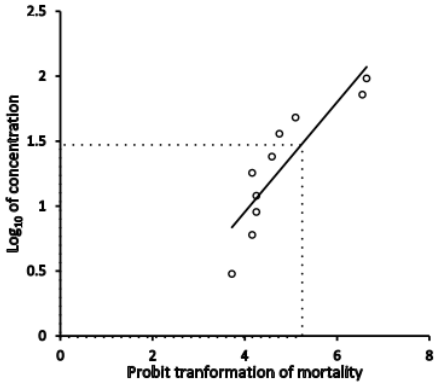


Figure 1. A 96-hour LC_{50} regression curve for *L. marginalis* exposed to Chlorpyrifos 20EC.

Table 1. 96-h LC_{50} for commonly used pesticide formulation in mussels.

Species	Value of 96-hour LC_{50} (mgL^{-1})	Formulation of Pesticides	References
Mytilus galloprovincialis	7700	Neonicotinoid Calypso 480 SC (CAL)	(Stara et al., 2020)
Unio tigridis	324	Chlorpyrifos 48 EC	(Al-Fanharawi et al., 2019)
L. marginalis	36.36	Dimethoate	(Kumar et al., 2012)
Utterbackia imbecillis	7.9	Carbaryl	(Conners and Black, 2004)
	19.4	Atrazine	
	18.3	Glyphosate	
	241.3	Diazinon	
	215		
Elliptio icterina	32	Malathion	(Keller and Ruessler, 1997)
Villosa lienosa	111		
Villosa villosa	180		

3.2 Hydrological parameters

The environmental data obtained in table 2 demonstrates that the temperature value was stable for all treatments and fluctuation took place as the duration of rearing increased. It occurred due to seasonal changes and entering the winter season in the last 15 days of the trial. The salinity contents remained relatively the same for different time slots and treatment units and no statistically significant changes were reported ($P<0.05$). The ammonia and pH levels were not significantly varied throughout the whole experiment period. However, a significant increase ($P<0.05$) in dissolved O_2 values was reported in all the experimental units from day 15 to the end (table 2).

Table 2. Water quality parameters for different treatment groups during the 35 days experiment.

Parameter	Treatments	DAY-0	DAY-15	DAY-35
Temperature (°C)	T_C	25.22±0.60^b	24.2±0.6^b	20.5±1.29^a
		24.87±0.51^b	23.25±0.369^b	20.12±1.31^a
	T_2	25.02±0.61^b	23.62±0.59^b	20.87±0.62^a
	T_3	25.02±0.67^b	23.95±0.64^b	21.12±0.25^a
pH	T_C	7.29±0.45^a	7.87±0.10^a	7.65±0.23^a
	T_1	7.47±0.12^a	7.60±0.12^a	7.55±0.05^a
	T_2	7.64±0.10^a	7.66±0.08^a	7.61±0.06^a
	T_3	7.49±0.17^a	7.71±0.08^a	7.6±0.18^a
Dissolved O_2	T_C	6.59±0.10^a	7.27±0.15^b	6.97±0.33^b
	T_1	6.48±0.25^a	7.06±0.48^b	7.22±0.12^b
	T_2	6.62±0.32^a	6.91±0.44^b	7.32±0.35^b
	T_3	6.83±0.24^a	7.21±0.23^b	7.15±0.[illegible]^b
NH_3 (mg/L)	T_C	0.34±0.21^a	0.71±0.35^a	0.30±0.22^a
		0.40±0.149^a	0.48±0	0.35±0.06^a
	T_2	0.57±0.17^a	0.54±0.14^a	0.37±0.09^a
	T_3	0.46±0.17^a	0.54±0.08^a	0.40±0.15^a
Salinity (mg/L)	T_C	[illegible]±0.017^a	0.08±0.01^a	0.08±0.01^a
	T_1	0.08±0.00^a	0.08±0.00^a	0.07±0.01^a
	T_2	0.08±0.00^a	0.08±0.01^a	0.07±0.01^a
	T_3	0.08±0.01^a	0.09±0.01^a	0.08±0.01^a
Total dissolved solids (mg/L)	T_C	109.12±22.06^a	101.87±16.12^a	107±8.86^a
	T_1	100.12±15.45^a	93.00±6.58^a	93.0±4.69^a
	T_2	109.75±18.30^a	103.12±1.65^a	101.25±2.5^a
	T_3	109.00±17.59^a	102.25±6.65^a	97.50±4.12^a

The raw with different superscripts indicate significant differences at $P < 0.05$; values are means ± SD.

3.3 Growth biometrics, survival rate, and condition indices

The obtained data in table 3 confirmed that the value of final total body weight differed statistically between the different treatment groups (P<0.05). The highest value of the final total body weight was observed in T_C as 49.24±6.7g and the lowest was 36.67±4.37g in T_3. The least value of weight gain was reported in the T_3 (-7.37±5.0 g), while maximized at T_c (49.24±6.7 g) (P<0.05). Specific growth rate (SGR %) among the treatment varied statistically; the lowest SGR was -0.52±0.38 at T_3, while the highest was 0.104±0.412 in the control group. However, statistically insignificant differences in SGR between T_2 and T_3 are observed (table 3). Similarly, the soft tissue wet weight was highest in T_c (12.20±2.12 g) and lowest in T_3 (8.82±1.40 g). Condition index (CI) value was at a peak (0.70±0.14) in T_C as and the lowest value was 0.54±0.14 in T_3 (P<0.05) (table 3). The value of Fulton's condition factor also followed the same trends (P<0.05). There were no significant changes over other parameters

such as initial shell length, initial total body weight initial, maximum shell depth, final shell length final, dry shell weight, length gain, etc. The mortality rate was statistically highest in T_3 and lowest in this treatment T_C ($P<0.05$) (figure 2).

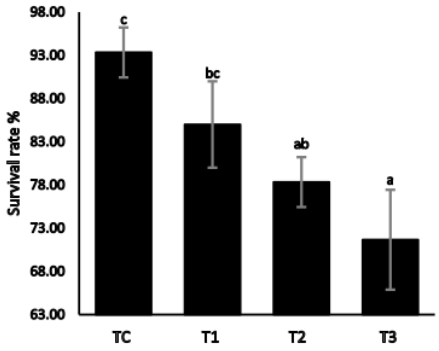


Figure 2. Survival rate and mortality rate of freshwater mussel *L. marginalis* exposed to Chlorpyrifos 20 EC for 35 days.

Table 3. Changes in growth parameters of *L. marginalis* after 35 days of exposure to Chlorpyrifos 20 EC.

Parameters	T_C	T_1	T_2	T_3
Shell length initial (cm)	7.85±0.56^a	7.93±0.52^a	7.53±0.49^a	7.93±0.42^a
Total body weight initial (g)	47.79±9.44^a	49.05±10.04^a	45.32±14.97^a	44.04±5.61^a
Maximum shell depth (cm)	3.87±0.20a	4.05±0.48^a	3.85±0.48^a	3.98±0.18^a
Shell length Final (cm)	7.86±0.70^a	7.95±0.37^a	7.57±0.61^a	7.94±0.41^a
Total body weight Final (g)	49.24±6.7^b	46.08±10.37^b	38.82±9.67^a	36.67±4.37^a
ximum shell depth Final	3.83±0.38^a	3.91±0.22^a	3.72±0.27^a	3.78±0.23^a
Soft tissue wet weight (g)	12.20±2.12^b	11.53±1.83^b	8.97±1.47^a	8.82±1.40^a
y shell weight (g)	18.09±5.39^b	18.62±5.89^b	13.95±2.44^a	16.75±3.104^{ab}
Length gain (cm)	0.008±1.06^a	0.025±0.36^a	0.041±0.60^a	0.0083±0.45^a
Weight gain (g)	1.44±8.21^b	-2.97±7.30^{ab}	-6.5±13.30^a	-7.37±5.0^a
Length gain (%)	0.83±106.46^a	2.5±36.46^a	4.16±60.06^a	0.83±45.41^a
Weight gain (%)	144.91±821.72^b	-297.33±730.7^{ab}	-650±1330.07^a	-737.25±500.47^a
Specific growth rate SGR%	0.104±0.412^b	-0.19±0.48^{ab}	-0.40±0.83^a	-0.52±0.38^a
eight)				
Average daily length gain (cm)	0.0002±0.030^a	0.0007±0.01^a	0.0012±0.01^a	0.00023±0.01^a
Average daily weight gain (g)	0.041±0.23^b	-0.085±0.20^{ab}	-0.186±0.38^a	-0.21±0.14^a

The column with different superscripts indicates significant differences at $P < 0.05$; values are means ± SD.

The value of Fulton's condition factor and condition index follows a similar course of declination after the control unit. The lowest values for both indices were allocated for the T3 treatment group, while the highest was for the control T_c group (Figure 3).

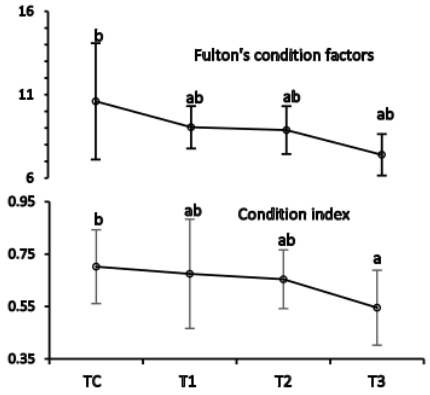


Figure 3. Fulton's condition factors and condition index of freshwater mussel *L. marginalis* exposed to Chlorpyrifos 20 EC for 35 days.

3.4 Total hemocytes count

The highest hemocytes count has been noted for the control group T_c (3.47×10^6 cells/ml) while the lowest was for the T_3 (2.3×10^6 cells/ml) group ($P<0.05$) (figure 4). The hemocytes counts between T_1 (3.2×10^6 cellml^{-1}) and T_2 (2.7×10^6 cells/ml) also confirmed moderate statistical variation ($P<0.05$) (figure 4).

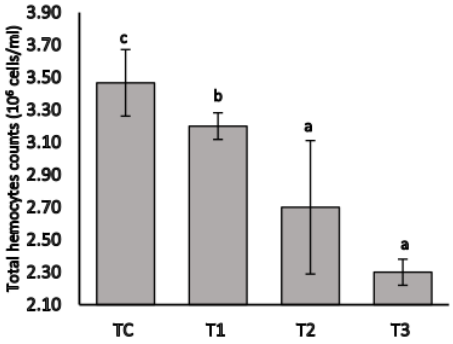
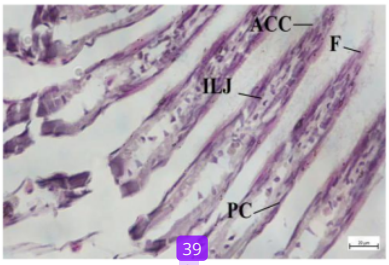


Figure 4. Total hemocytes counts of freshwater mussel *L. marginalis* exposed to Chlorpyrifos 20 EC for 35 days.

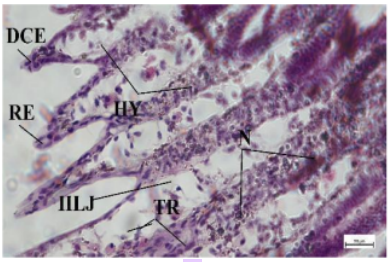
3.5 Histopathology of gill, muscle, and ovary

The bivalves exposed to higher doses of Chlorpyrifos 20 EC endured more damage to their gill architecture. Gill lamellae in bivalves kept as controls are made up of a large number of closely

spaced, thin, vertical gill filaments that have a porous structure punctured by minute openings and bound filaments (figure 5. A). Degeneration of epithelial cells, swelling, and the vacuolated and necrotic epithelium were seen in all treatment groups (figure 5. B-D). The secondary gill lamellae were fused together, and the gill filament bases widened (figure 3). Following the exposure of Chlorpyrifos 20 EC, increased the intercellular junction, necrosis occurred in the cell, and tissue rupture and hyperplasia were observed (figure 5. B-D). The epithelium of the gill filaments and all forms of the cilia were disrupted, and the epithelium showed considerable oedema formation and necrotic bodies. Epithelial separation, lamellar distortion, epithelia loss, and necrosis were increased at the gill with the intensified concentration of chlorpyrifos 20 EC. The histopathology of pesticide-exposed bivalves' gills indicated clear signs of damage not seen in the control group. The histo-micrograph of adductor muscles of *L. marginalis* from different treatment groups are shown in figure 6. The control group showed well-organizd muscular fibers that were connected perfectly (figure 6. A). In the case of T_1, T_2, and T_3 treatment groups, degeneration of muscle fiber (DMF), vacuoles (V), degenerative waving pattern, and necrosis were common pathologies. was seen after pesticide exposure (figure 6. B-D). The T_3 treatment group showed a higher level of deformities in all cases. Figure 7. A displays the normal structure of the mussel's ovary at vitellogenic stage. After pesticide administration, the normal structure of gonad cells was disorganized, follicle degradation occurred, and yolk globules started to disrupt at different intensities as observed in Figure 7. B-D.



A



B

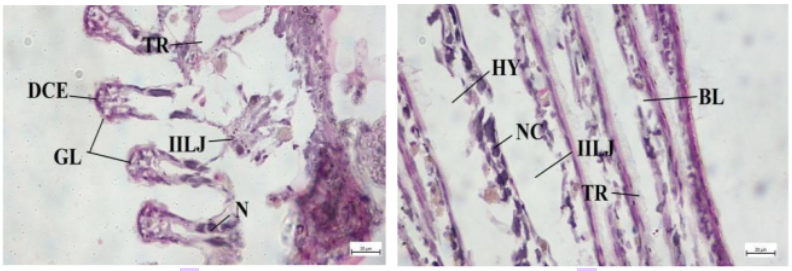


Figure 5. Gill transverse section photomicrograph of *L. marginalis*. **A**. T_C, **B**.T_1, **C**. T_2 **D**. T_3. (GL-gill lamellae, ACC-abfrontal ciliated cell, F-frontal, PC-Pillar cells, ILJ-interlamellar junction, DCE-damaged cilia epithelium, TR-tissue rupture, HY-hyperplasia, BL-breakdown of lamellae, NC-necrosis, NL-necrotic lamellae, IILJ-Increased interlamellar junction).

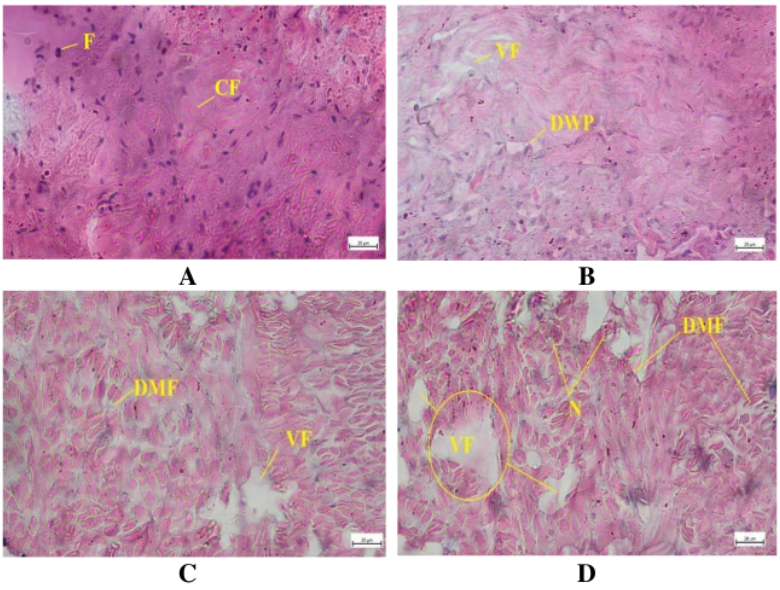


Figure 6. Muscle transverse section photomicrograph of *L. marginalis*. **A. T_C, B.T_1, C. T_2, D. T_3**. (DMF-degeneration of muscle fiber; VF-vacuole formation; DWP-disruption of waving pattern, N-necrosis; F-healthy fiber, CF-collagen fiber).

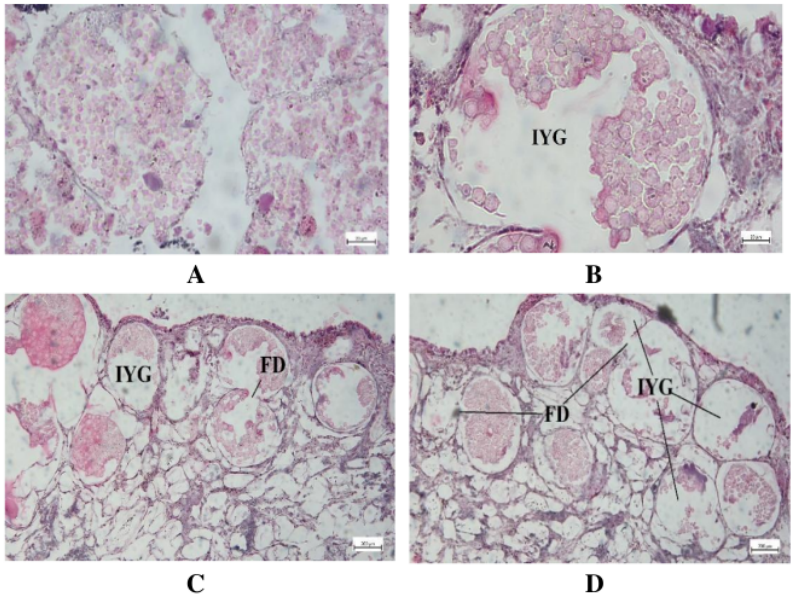


Figure 7. Ovarian transverse section photomicrograph of *L. marginalis*. **A**. Tc, **B**.T1, **C**. T2, **D**. T3. (FD-follicle degradation, GD-gonadal degeneration, IYD-yolk globule disruption).

4.0 Discussions

The growth, development, and propagation of aquatic animals are solely associated with water quality parameters of surroundings (Ahmed et al., 2020). The thermal constitution varied between 20°C to 36°C and seems to be optimal for supporting life in tropical aquatic systems (Kua et al., 2020). A value of DO> 5 ppm is vital to sustaining aquatic animals' existence and development (Bhatnagar and Singh, 2010). Water quality features acquired in current research remained quite optimal for the growth and reproduction of aquatic animals following the hydrology assessment criteria of Bhatnagar & Devi, (2010) and Chapman et al., (2016).

The LC_{50} data from previous studies exemplifies that aquatic mollusks are moderate to highly tolerant to acute toxicity of different organophosphate formulations (Becker et al., 2020; Conners and Black, 2004). The LC_{50} values for different mollusks ranged from least 7.9 mgL^{-1} Carbaryl (Kumar et al., 2012) to 7700 mgL^{-1} Neonicotinoid Calypso 480 SC (CAL) (Stara et

al., 2020). The animals in the control group of the current trial demonstrate 100 % survival rates, which fall within the regulation and description code of APHA, (1999) for testing rapid and acute toxicity trials in aquatic animals.

The substantial biochemical and cellular changes in animals are reported to be initiated by organophosphate toxicants (Stalin et al., 2017). Intensification of toxicant in living water cause behavioral, physiological, and cellular changes in the animal which eventually lead to death (Kumar et al., 2012). Cypermethrin was reported to disrupt normal valve activity and survival rate of marine mussel *Mytilus galloprovincialis (*Ait-Ayad et al., 2011). Similar fixation has been recounted in current research and mortality rates were higher as the concentration of Chlorpyrifos 20 EC increased. The intrusion of the municipal effluent causes reduced growth in *Elliptio complanate* (Gagné et al., 2007). The accumulation of Pb was addressed to alter soft tissue weight, filtration behavior, and mortality of *Dreissena polymorpha* (Rahnama et al., 2010). Chlorpyrifos had been registered to prevent growth, reproduction, and development in *Unio tigridis* and *Viviparous benglensis* (Al-Fanharawi et al., 2019).

The Fulton's condition factor and condition index are widely used morphometric tools in assessing the animal's fitness, nutritive status, and associated ecological condition (Siddique et al., 2020). The alternation in CIs and Fulton's condition was described to be correlated with food availability, parasitic loads, and water-born stressors in several invertebrates (Marwaha et al., 2019). Intensifying the pesticide toxicant in current research also revealed reduced values for both condition factors in the treatment group in comparison with the control group. This would happen due to decreased food intake in treatment groups and earning poor soft tissue weight as well. Hemocytes are treated as a vital regulatory component of the molluscan immune system (Uddin et al., 2010) and their concentrations are related to being impacted by different forms of aquatic toxicants (Lu et al., 2021). The reduced concentration of hemocytes was noted by 21 days of exposure to neonicotinoid Calypso 480 SC in *Mytilus galloprovincialis*

(Stara et al., 2020) and with polystyrene microplastics treatment in oyster *Crassostrea gigas* (Sussarellu et al., 2016). The current trial also resulted in reduced hemocytes counts for highly concentrated treatment groups in comparison to the control.

The gill is a vital organ in bivalve mollusk, which is engaged in the organism's respiration and food-sieving activities (Andreyeva et al., 2021). Analysis of histological malformations is a widely used method to assess the effects of toxicants and the environment (Hussain et al., 2022). Increased concentration of toxicants like pesticides, metals, etc. inhibits the metabolic processes of cells inside the tissues and leads to cell death, hyperplasia, or cellular inequality (Joshy et al., 2022). Swollen gill filament, lamellar fusing, necrosis, and structural modification were noted in *L. marginalis* exposed to 5ppm 30 days chlorpyrifos toxicant (Stalin et al., 2017). Similar histological alterations in gill were documented by Katalay et al., (2016). The epithelium oedema formation and necrotic gill were observed in mussels associated with agro-pesticides (Rane et al., 2019). Histological alterations in muscle tissue due to bisphenol-A exposure were reported in soft tissues of *Corbicula fluminea* (Benjamin et al., 2019). Increased vacuole formation and necrotic cell death are prominent pathologies of muscle tissue in response to toxicants (Alonso et al., 2019). The current research also acts as a supportive trail to the previous finding and extensive histopathology was photographed for the animals in a higher concentration of Chlorpyrifos 20 EC treatment.

Current results concluded that exposure to sublethal concentrations of the Chlorpyrifos 20 EC leads to the significant manifestation of growth, fitness, and survival of bivalve mollusks. The study also noticed major alterations in cellular architecture. Chlorpyrifos 20 EC is also documented to impact the reproduction and growth of mussels as indicated by condition indices and histopathology of gonads.

Authors contribution

Mohammad Amzad Hossain and Tumpa Rani Sarker designed the methodology, performed assays, data analysis, software and visualization, validation, editing, fund acquisition, and preparation of the manuscript. Lipi Sutradhar performed rearing and laboratory activities, writing, and preparation of the original report. Monayem Hussain performed sampling, data reviewing, and editing. Mohammed Mahbub Iqbal was involved in conceptualization, methodology development, fund administration and administration, and supervision.

Funding

This research has been funded by UGC (University Grant Commission), Bangladesh through Sylhet Agricultural University Research System (SAURES), Bangladesh (budget reference code 3632104). The research student stipend was supported by the National Science and Technology Fellowship scheme (NST) from the Ministry of Science and Technology, Bangladesh.

Data Availability

Raw data will be provided based on reasonable demand.

References

Ahmed, I., Reshi, Q.M., Fazio, F., 2020. The influence of the endogenous and exogenous factors on hematological parameters in different fish species: a review. Aquac. Int. 28, 869–899. https://doi.org/10.1007/s10499-019-00501-3

Ait-Ayad, M., Ait Fdil, M., Mouabad, A., 2011. Effects of cypermethrin (pyrethroid insecticide) on the valve activity behavior, byssal thread formation, and survival in air of the marine mussel *Mytilus galloprovincialis*. Arch. Environ. Contam. Toxicol. 60, 462–470. https://doi.org/10.1007/s00244-010-9549-7

Al-Fanharawi, A.A., Rabee, A.M., Al-Mamoori, A.M.J., 2019. Multi-biomarker responses after exposure to organophosphates chlorpyrifos in the freshwater mussels *Unio tigridis*

and snails *Viviparous benglensis*. Hum. Ecol. Risk Assess. An Int. J. 25, 1137–1156. https://doi.org/10.1080/10807039.2018.1460800

Alonso, A., Suárez, P., Ruiz, Y., Dobal, V., San Juan, F., 2019. Gonadal histopathological disorders in *Mytilus galloprovincialis* male exposed to tars used in mussel farms. Front. Mar. Sci. 6, 1–20. https://doi.org/10.3389/fmars.2019.00577

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.

Andreyeva, A.Y., Gostyukhina, O.L., Kladchenko, E.S., Vodiasova, E.A., Chelebieva, E.S., 2021. Acute hypoxic exposure: effect on hemocyte functional parameters and antioxidant potential in gills of the pacific oyster, *Crassostrea gigas*. Mar. Environ. Res. 169, 105389. https://doi.org/10.1016/j.marenvres.2021.105389

Becker, J.M., Ganatra, A.A., Kandie, F., Mühlbauer, L., Ahlheim, J., Brack, W., Torto, B., Agola, E.L., McOdimba, F., Hollert, H., Fillinger, U., Liess, M., 2020. Pesticide pollution in freshwater paves the way for schistosomiasis transmission. Sci. Rep. 10, 3650. https://doi.org/10.1038/s41598-020-60654-7

Benjamin, K.B., Co, E.L., Competente, J.L., de Guzman, D.G.H., 2019. Histopathological effects of bisphenol a on soft tissues of *Corbicula fluminea* Mull. Toxicol. Environ. Health Sci. 11, 36–44. https://doi.org/10.1007/s13530-019-0386-4

Bhatnagar, A., Singh, G., 2010. Culture fisheries in village ponds: a multi-location study in Haryana, India. Agric. Biol. J. North Am. 1, 961–968. https://doi.org/10.5251/abjna.2010.1.5.961.968

Bolotov, I.N., Klass, A.L., Kondakov, A. V, Vikhrev, I. V, Bespalaya, Y. V, Gofarov, M.Y., Filippov, B.Y., Bogan, A.E., Lopes-Lima, M., Lunn, Z., Chan, N., Aksenova, O. V, Dvoryankin, G.A., Chapurina, Y.E., Kim, S.K., Kolosova, Y.S., Konopleva, E.S., Lee,

J.H., Makhrov, A.A., Palatov, D.M., Sayenko, E.M., Spitsyn, V.M., Sokolova, S.E., Tomilova, A.A., Win, T., Zubrii, N.A., Vinarski, M. V, 2019. Freshwater mussels house a diverse mussel-associated leech assemblage. Sci. Rep. 9, 16449. https://doi.org/10.1038/s41598-019-52688-3

Bondarenko, S., Gan, J., Haver, D.L., Kabashima, J.N., 2004. Persistence of selected organophosphate and carbamate insecticides in waters from a coastal watershed. Environ. Toxicol. Chem. 23, 2649. https://doi.org/10.1897/03-436

Cacciatore, L.C., Verrengia Guerrero, N., Cochón, A.C., 2013. Cholinesterase and carboxylesterase inhibition in *Planorbarius corneus* exposed to binary mixtures of azinphos-methyl and chlorpyrifos. Aquat. Toxicol. 128–129, 124–134. https://doi.org/10.1016/j.aquatox.2012.12.005

Carboni, S., Kaur, G., Pryce, A., McKee, K., Desbois, A.P., Dick, J.R., Galloway, S.D.R., Hamilton, D.L., 2019. Mussel consumption as a "food first" approach to improve Omega-3 status. Nutrients 11, 1381. https://doi.org/10.3390/nu11061381

Chapman, D. V., Bradley, C., Gettel, G.M., Hatvani, I.G., Hein, T., Kovács, J., Liska, I., Oliver, D.M., Tanos, P., Trásy, B., Várbíró, 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

Conners, D.E., Black, M.C., 2004. Evaluation of lethality and genotoxicity in the freshwater Mussel *Utterbackia imbecillis* (Bivalvia: Unionidae) exposed singly and in combination to chemicals used in lawn care. Arch. Environ. Contam. Toxicol. 46, 362–371. https://doi.org/10.1007/s00244-003-3003-z

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

Gagné, F., Gagnon, C., Turcotte, P., Blaise, C., 2007. Changes in metallothionein levels in

freshwater mussels exposed to urban wastewaters: effects from exposure to heavy metals? Biomark. Insights 2, 107–116. https://doi.org/10.1177/117727190700200012

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, 351–359. https://doi.org/10.1111/j.1095-8649.1978.tb03443.x

Hussain, M., Hossain, M.A., Begum, M., Roy, N.C., 2022. Freshwater mussel (*Lamelliedens marginalis*) to reduce the lead (Pb) bioaccumulation in aquaculture of stinging catfish, *Heteropneustes fossilis*. J. Appl. Aquac. 1–17. https://doi.org/10.1080/10454438.2021.2010630

Joshy, A., Sharma, S.R.K., Mini, K.G., Gangadharan, S., Pranav, P., 2022. Histopathological evaluation of bivalves from the southwest coast of India as an indicator of environmental quality. Aquat. Toxicol. 243, 106076. https://doi.org/10.1016/j.aquatox.2022.106076

Juhel, G., Ramsay, R.M., Davenport, J., O'Halloran, J.&, Culloty, S.C., 2015. Effect of the microcystin-producing cyanobacterium, microcystis aeruginosa, on immune functions of the zebra mussel *Dreissena polymorpha*. J. Shellfish Res. 34, 433–442. https://doi.org/10.2983/035.034.0227

Katalay, S., Yavaşoğlu, A., Yiğittürk, G., Oltulu, F., Sarı, G., Karabay, N., 2016. Histological Effects of pollution on gill and hepatopancreas tissues of black mussels (*M. Galloprovincialis* L.) from izmir Bay of Turkey. Fresenius Environ. Bull. 25, 1461–1467.

Keller, A.E., Ruessler, D.S., 1997. The toxicity of malathion to unionid mussels: Relationship to expected environmental concentrations. Environ. Toxicol. Chem. 16, 1028–1033. https://doi.org/10.1002/etc.5620160524

Kovačić, I., Medić, N., 2016. The effect of chlorpyrifos on protein content and acid DNase activity in the mussel, *Mytilus galloprovincialis*. Mar. Freshw. Behav. Physiol. 49, 265–275. https://doi.org/10.1080/10236244.2016.1190522

Kua, Z.X., Hamilton, I.M., McLaughlin, A.L., Brodnik, R.M., Keitzer, S.C., Gilliland, J., Hoskins, E.A., Ludsin, S.A., 2020. Water warming increases aggression in a tropical fish. Sci. Rep. 10, 20107. https://doi.org/10.1038/s41598-020-76780-1

Kumar, S., Pandey, R.K., Das, S., Das, V.K., 2012. Acute toxicity and behavioural responses of a freshwater mussel *Lamellidens marginalis* (Lamarck) to dimethoate exposure. Recent Res. Sci. Technol. 4, 39–45.

Lu, J., Shi, Y., Yao, T., Bai, C., Jiang, J., Ye, L., 2021. Gender differences in hemocyte immune parameters of hong kong oyster *Crassostrea hongkongensis* during immune stress. Front. Immunol. 12, 1–12. https://doi.org/10.3389/fimmu.2021.659469

Mishra, S., Mishra, R.K., Sahu, B.K., Nayak, L., Senga, Y., 2008. Differential growth of the freshwater mussel, *Lamellidens marginalis* in relation to certain drugs. Environ. Toxicol. 23, 379–386. https://doi.org/10.1002/tox.20351

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

Rahnama, R., Javanshir, A., Mashinchian, A., 2010. The effects of lead bioaccumulation on filtration rate of zebra mussel (*Dreissena polymorpha*) from Anzali wetland – Caspian Sea 2248. https://doi.org/10.1080/02772240902744444

Rane, M.S., Kolhe, B.G., others, 2019. Histopathological alterations in gills of freshwater bivalve, *Lamellidens marginalis* (Lamarck) after acute exposure to thiamethoxam and triazophos. Int. J. Life Sci. 23–28.

Rouillard, J., Lago, M., Abhold, K., Roeschel, L., Kafyeke, T., Klimmek, H., Mattheiß, V., 2018. Protecting and restoring biodiversity across the freshwater, coastal and marine realms: is the existing EU policy framework fit for purpose? Environ. Policy Gov. 28, 114–128. https://doi.org/10.1002/eet.1793

Sicuro, B., 2015. Freshwater bivalves rearing: a brief overview. Int. Aquat. Res. 7, 93–100.

https://doi.org/10.1007/s40071-015-0098-6

Siddique, M.A., Khatun, M.A., Rahman, M.M., Ahmed, G.U., Moniruzzaman, M., Jasim Uddin, M., 2020. Annual gametogenic cycle of the freshwater pearl mussel, *Lamellidens marginalis* (Lamarck, 1819) collected from a perennial lentic habitat of Bangladesh. Molluscan Res. 40, 36–43. https://doi.org/10.1080/13235818.2019.1682954

Stalin, A., Gokula, V., Amanullah, B., 2017. Effect of chlorpyrifos on biochemical changes in freshwater mussel *Lamellidens marginalis*. Int. J. App. Res. 3(8): 157-159

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

Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Le Goïc, N., Quillien, V., Mingant, C., Epelboin, Y., Corporeau, C., Guyomarch, J., Robbens, J., Paul-Pont, I., Soudant, P., Huvet, A., 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. Proc. Natl. Acad. Sci. 113, 2430–2435. https://doi.org/10.1073/pnas.1519019113

Traiger, S.B., Bodkin, J.L., Coletti, H.A., Ballachey, B., Dean, T., Esler, D., Iken, K., Konar, B., Lindeberg, M.R., Monson, D., Robinson, B., Suryan, R.M., Weitzman, B.P., 2022. Evidence of increased mussel abundance related to the Pacific marine heatwave and sea star wasting. Mar. Ecol. 43, e12715. https://doi.org/10.1111/maec.12715

Uddin, M., Yang, H.-S., Choi, K.-S., Kim, H.-J., Hong, J.-S., Cho, M., 2010. Seasonal changes in *Perkinsus olseni* infection and gametogenesis in manila clam, *Ruditapes philippinarum*, from Seonjaedo Island in Incheon, off the West Coast of Korea. J. World Aquac. Soc. 41, 93–101. https://doi.org/10.1111/j.1749-7345.2009.00337.x

Zahran, E., Risha, E., Awadin, W., Palić, D., 2018. Acute exposure to chlorpyrifos induces

reversible changes in health parameters of Nile tilapia (*Oreochromis niloticus*). Aquat. Toxicol. 197, 47–59. https://doi.org/10.1016/j.aquatox.2018.02.001

Hossain et al, 2022_091022.docx

ORIGINALITY REPORT

14%	8%	11%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Mohammad Amzad Hossain, Lipi Sutradhar, Tumpa Rani Sarker, Shuva Saha, Mohammed Mahbub Iqbal. "Toxic effects of chlorpyrifos on the growth, hematology, and different organs histopathology of Nile tilapia, Oreochromis niloticus", Saudi Journal of Biological Sciences, 2022 Publication	3%
2	Monayem Hussain, Mohammad Amzad Hossain, Mohajira Begum, Nirmal Chandra Roy. " Freshwater mussel ( ) to reduce the lead (Pb) bioaccumulation in aquaculture of stinging catfish, ", Journal of Applied Aquaculture, 2022 Publication	1%
3	www.frontiersin.org Internet Source	1%
4	hal.archives-ouvertes.fr Internet Source	1%
5	Submitted to Sparsholt College, Hampshire Student Paper	<1%

6	Noriko Yamamoto, Hideki Okayasu, Satoru Murayama, Sachiko Mori, Kenji Hunahashi, Koji Suzuki. "Measurement of volatile organic compounds in the urban atmosphere of Yokohama, Japan, by an automated gas chromatographic system", Atmospheric Environment, 2000 Publication	<1 %
7	Raue R.. "Reversion of molecularly engineered, partially attenuated, very virulent infectious bursal disease virus during infection of commercial chickens", Avian Pathology, 4/1/2004 Publication	<1 %
8	academicjournals.org Internet Source	<1 %
9	dergipark.org.tr Internet Source	<1 %
10	res.mdpi.com Internet Source	<1 %
11	Rajib Majumder, Anilava Kaviraj. " Acute and sublethal effects of organophosphate insecticide chlorpyrifos on freshwater fish ", Drug and Chemical Toxicology, 2018 Publication	<1 %
12	I. Kovačić, N. Medić. " The effect of chlorpyrifos on protein content and acid	<1 %

DNase activity in the mussel, ", Marine and Freshwater Behaviour and Physiology, 2016
Publication

---

13 T Sultana, MM Haque, MA Salam, MM Alam. "Effect of aeration on growth and production of fish in intensive aquaculture system in earthen ponds", Journal of the Bangladesh Agricultural University, 2017 <1%
Publication

---

14 austinpublishinggroup.com <1%
Internet Source

---

15 www.intechopen.com <1%
Internet Source

---

16 Hyuk-Sang Kwon, Jae-Hyoung Cho, Hee-Soo Kim, Bok-Re Song et al. "Establishment of Blood Glucose Monitoring System Using the Internet", Diabetes Care, 2004 <1%
Publication

---

17 Ka Ming Chan, Siu-Gin Cheung, Zongwei Cai, Jian-Wen Qiu. "Sensitivity of different biological responses to accumulation and depuration of butyltins in the neogastropod Thais clavigera: implications for biomonitoring", Ecotoxicology, 2008 <1%
Publication

---

18 Md. Rashedul Islam, Mohammad Amzad Hossain, Farjana Afrose, Nirmal Chandra Roy, <1%

	Mohammed Mahbub Iqbal. " Effect of temperature on the growth performance, haematological properties and histomorphology of gill, intestine and liver tissues in juvenile butter catfish ", Aquaculture, Fish and Fisheries, 2022 Publication	
19	Mello, Danielle F., Eliza S. de Oliveira, Renato C. Vieira, Erik Simoes, Rafael Trevisan, Alcir Luiz Dafre, and Margherita Anna Barracco. "Cellular and Transcriptional Responses of Crassostrea gigas Hemocytes Exposed in Vitro to Brevetoxin (PbTx-2)", Marine Drugs, 2012. Publication	<1 %
20	aquatres.scientificwebjournals.com Internet Source	<1 %
21	repository.tudelft.nl Internet Source	<1 %
22	www.ncbi.nlm.nih.gov Internet Source	<1 %
23	Banga Medjo Pascale, Bilong Bilong Charles Félix, Zébazé Togouet Serge Hubert. "Spatio-Temporal Distribution of Phytoplankton and Water Quality of Some Rivers of Haute Sanaga Department (Central Africa)", Journal of Biology and Life Science, 2022 Publication	<1 %

24	research.journalspress.com Internet Source	<1 %
25	www.nature.com Internet Source	<1 %
26	www.tandfonline.com Internet Source	<1 %
27	Arumugam Stalin, Palani Suganthi, Subramanian Mathivani, Bilal Ahmad Paray et al. "Impact of chlorpyrifos on behavior and histopathological indices in different tissues of freshwater fish Channa punctatus (Bloch)", Environmental Science and Pollution Research, 2019 Publication	<1 %
28	Julieta S. De Anna, Juan M. Castro, Luis Arias Darraz, Federico D. Elías, Juan G. Cárcamo, Carlos M. Luquet. "Exposure to hydrocarbons and chlorpyrifos alters the expression of nuclear receptors and antioxidant, detoxifying, and immune response proteins in the liver of the rainbow trout, Oncorhynchus mykiss", Ecotoxicology and Environmental Safety, 2021 Publication	<1 %
29	Pattanan Nuchan, Uthaiwan Kovitvadhi, Akkarasiri Sangsawang, Satit Kovitvadhi, Pakasinee Klaimala, Nopparat Srakaew.	<1 %

	"Biochemical and cellular responses of the freshwater mussel, Hyriopsis bialata, to the herbicide atrazine", Environmental Pollution, 2022 Publication	
30	Peien Wang, Joycelyn K. Quansah, Katie B. Pitts, Jinru Chen. "Hygiene status of fresh peach packing lines in Georgia", LWT, 2021 Publication	<1 %
31	air.uniud.it Internet Source	<1 %
32	downloads.hindawi.com Internet Source	<1 %
33	fireant.tamu.edu Internet Source	<1 %
34	mafiadoc.com Internet Source	<1 %
35	mts.intechopen.com Internet Source	<1 %
36	pt.scribd.com Internet Source	<1 %
37	www.allresearchjournal.com Internet Source	<1 %
38	www.limnofish.org Internet Source	<1 %

39	Kimberly B. Benjamin, Elisa L. Co, Jessmine L. Competente, Dyan Gabrielle H. de Guzman. "Histopathological Effects of Bisphenol A on Soft Tissues of Corbicula fluminea Mull", Toxicology and Environmental Health Sciences, 2019 Publication	<1%

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		