Original article

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

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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*.

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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 has 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

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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 L 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) (Fig. 1). The different doses of pesticide used in different treatments unit were assigned as T_C 0 mgL^{-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 for histological analysis.

2.4. Tools for growth biometrics

Different growth biometrics were calculated by using the below formulae.

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

Specific growth rate SGR (%) = $(lnW_2 − lnW_1 /T_2 − T_1) \times 100$, Where W_1 = the initial weight (g) at a time, W_2 = the final weight (g) at a time, T_2-T_1 = Duration in days.
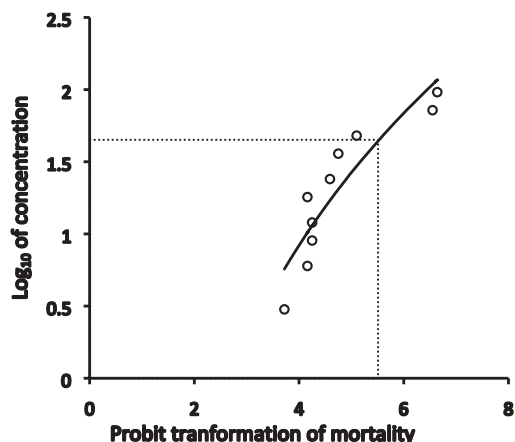

Fig. 1. A 96-h LC_{50} logarithmic curve for *L. marginalis* exposed to Chlorpyrifos 20EC.

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 h 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 µl 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 µm 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 was 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. 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

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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	Malathion	(Keller and Ruessler, 1997)
Elliptio icterina	32		
Villosa lienosa	111		
Villosa villosa	180		

regression model (P < 0.05) (Fig. 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 1). A moderately high estimate of LC$_{50}$ had already been reported for Dimethoate, Malathion, Atrazine, and Glyphosate in several bivalves.

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 in Bangladesh 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 in dissolved O$_2$ values was reported in all the experimental units from day 15 to the end of the trial (P < 0.05) (Table 2).

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 ± 6.7 g and the lowest was 36.67 ± 4.37 g 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, and the lowest SGR was −0.52 ± 0.38 at T$_3$, while the highest was 0.104 ± 0.412 in the control group. 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). The value of Fulton's condition factor and condition index (CI) follows a similar course of declination after being in peak at 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 (Fig. 3). 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, and length gain. The mortality rate was statistically highest in T$_3$ and lowest in Tc (P < 0.05) (Fig. 2).

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) (Fig. 4). The hemocytes counts between T$_1$ (3.2 × 10^6 cells/ml) and T$_2$ (2.7 × 10^6 cells/ml) also confirmed moderate statistical variation (P < 0.05) (Fig. 4).

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 (Fig. 5A). Degeneration of epithelial cells, swelling, and the vacuolated and necrotic epithelium were seen in all treatment groups (Fig. 5B-D). The secondary gill lamellae were fused together, and the gill filament bases widened. Following the exposure of Chlorpyrifos 20 EC, increased the intercellular junction, necrosis occurred in the cell, and tissue rupture and hyperplasia were observed (Fig. 5B-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 histomicrograph of adductor muscles of *L. marginalis* from different treatment groups are shown in Fig. 6. The control group showed well-organized muscular fibers that were connected perfectly (Fig. 6A). 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 (Fig. 6B-D). The T$_3$ treatment group showed a higher level of deformities in all cases. Fig. 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 Fig. 7B-D.

4. 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℃ to 36℃ 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 & Singh, (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}

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
	T_1	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 ± 0.12^b
NH_3 (mg/L)	T_C	0.34 ± 0.21^a	0.71 ± 0.35^a	0.30 ± 0.22^a
	T_1	0.40 ± 0.149^a	0.48 ± 0.15^a	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	0.08 ± 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.

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)	$3.87 \pm 0.20a$	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
Maximum 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
Dry 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% (weight)	0.104 ± 0.412^b	-0.19 ± 0.48^{ab}	-0.40 ± 0.83^a	-0.52 ± 0.38^a
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.
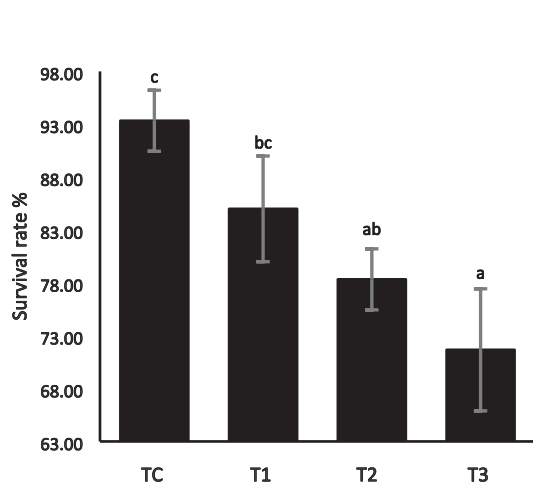
Fig. 2. Survival rate and mortality rate of freshwater mussel *L. marginalis* exposed to Chlorpyrifos 20 EC for 35 days.
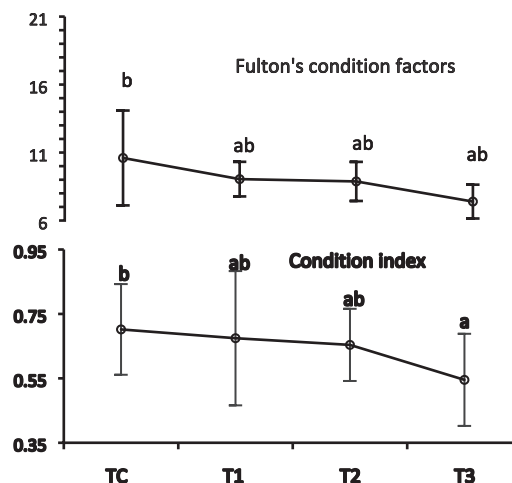
Fig. 3. Fulton's condition factors and condition index of freshwater mussel *L. marginalis* exposed to Chlorpyrifos 20 EC for 35 days.
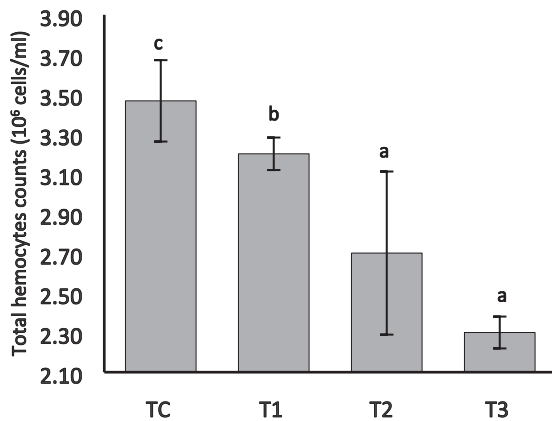
Fig. 4. Total hemocytes counts of freshwater mussel *L. marginalis* exposed to Chlorpyrifos 20 EC for 35 days.

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 5 ppm 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
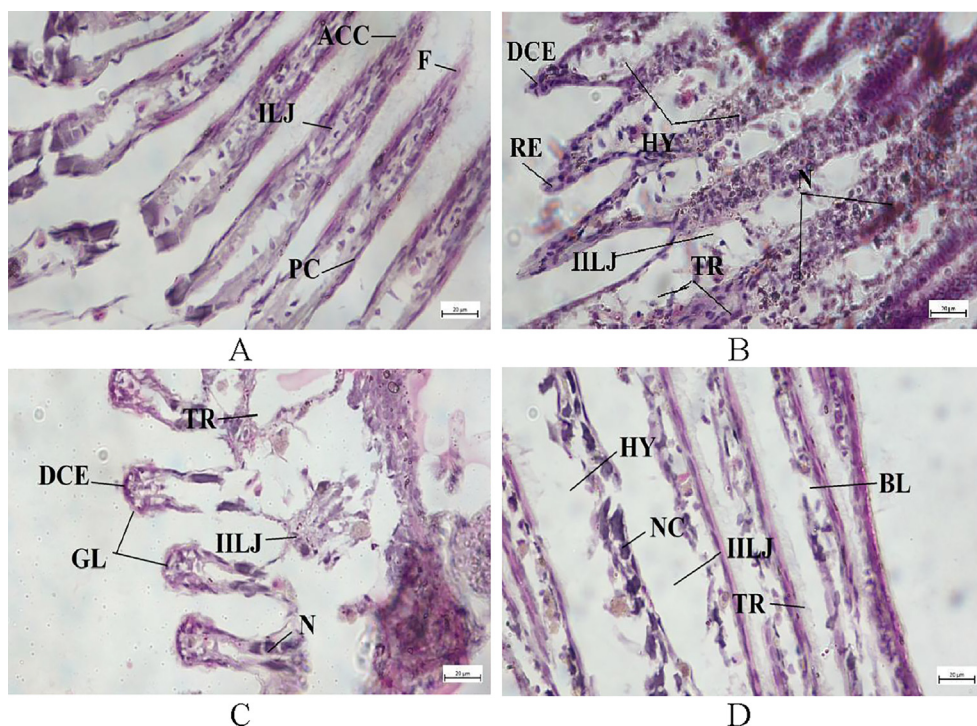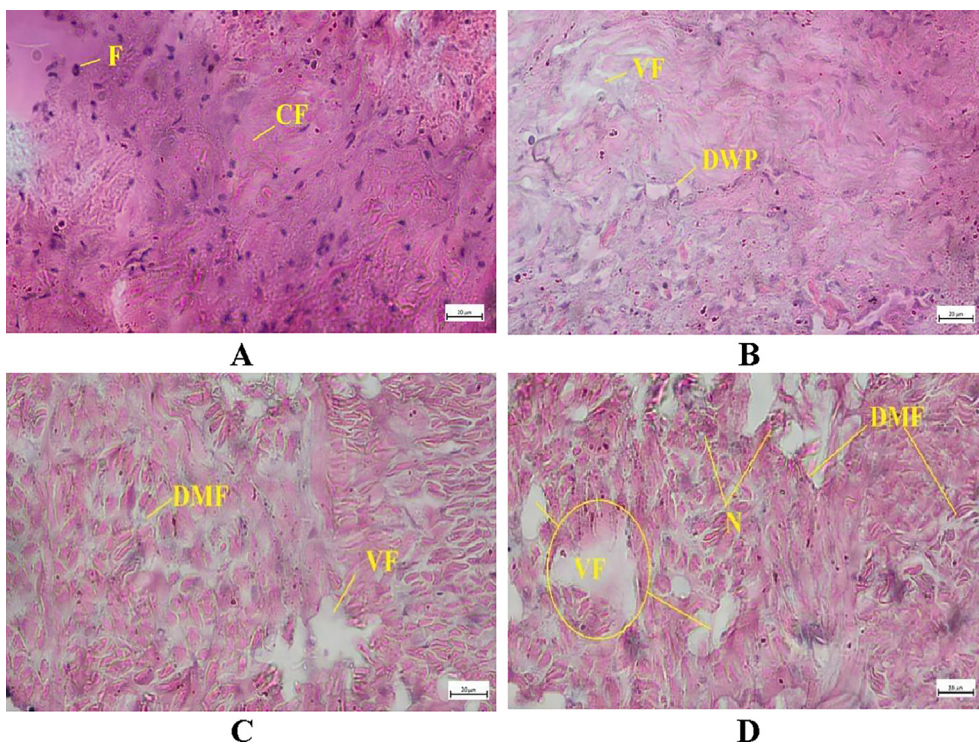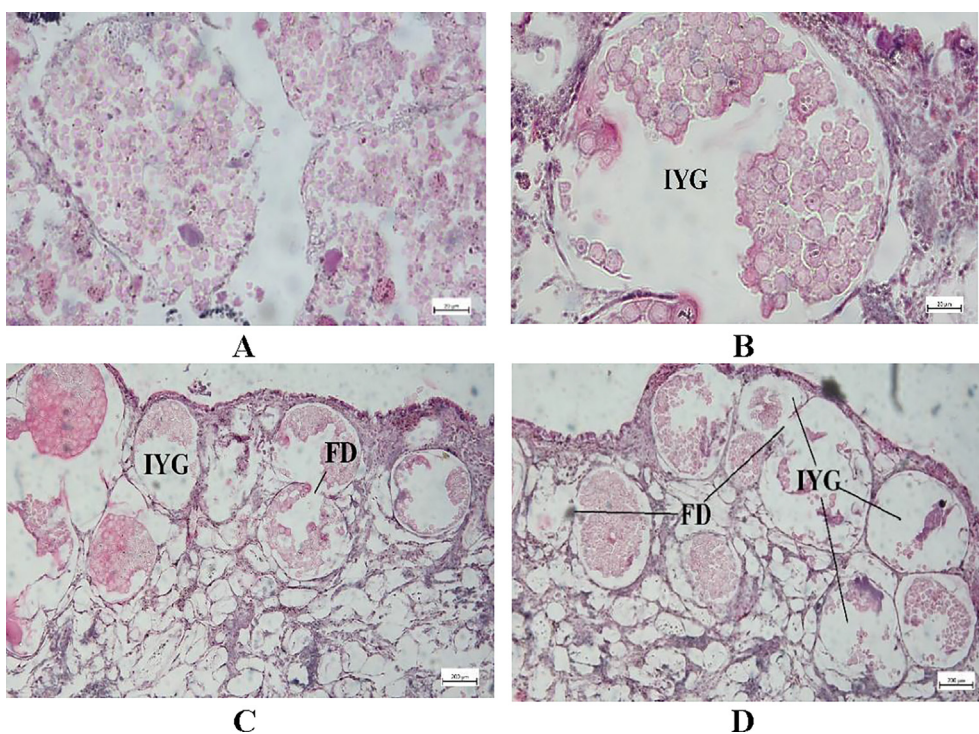

Fig. 5. Gill transverse section photomicrograph of *L. marginalis*. A. T_C, B.T_1, C. T_2 D. T_3. (GL-gill lamellae, ACC-abfrontal ciliated cell, F-frontal, PC-Pillar cells, ILJ-interlamellar junction, DCE-damaged cilia epithelium, TR-tissue rupture, HY-hyperplasia, BL-breakdown of lamellae, NC-necrosis,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

Fig. 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).

Fig. 7. Ovarian transverse section photomicrograph of *L. marginalis*. A. T$_C$, B.T$_1$, C. T$_2$, D. T$_3$. (FD-follicle degradation, GD-gonadal degeneration, IYD-yolk globule disruption).

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.

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CRediT authorship contribution statement

Mohammad Amzad Hossain: Methodology, Software, Visualization, Validation, Writing – review & editing, Funding acquisition. **Tumpa Rani Sarker:** Methodology, Software, Visualization, Validation, Writing – review & editing, Funding acquisition. **Lipi Sutradhar:** Writing – original draft. **Monayem Hussain:** Writing – review & editing, Supervision. **Mohammed Mahbub Iqbal:** Conceptualization, Methodology, Funding acquisition.

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.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2022.102482.

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