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TOXICOLOGICAL EFFECT OF MARBLE WATER ON GILLS OF GRASS CARP (CTENOPHARYNGODAN IDELLA)

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ABSTRACT

The given study was formulated to analyze toxicological effect Marble water on gills Grass Carp (Ctenophryngodon idella), during this study we calculated and Grass Carp (Ctenophryngodon idella) was cultured in aquarium and four different aquaria was established, one was named as control group which was remain untreated, and remaining three was named as experimental group1, 2 and 3, in each three different concentration 1mg\L (group 1), 1.5mg\L (Group 2) and 2mg\L (Group 3) was used. The purpose of the study was to assess histopathological findings, and samples of the gills were collected on days 14 and 28. Histopathological lesions, including severe lamella fusion, necrotic changes, diluted tubule formation, and cellular damage in the cell caused by marble water, were found in the gills of grass carp (Ctenophryngodon idella). The purpose of the study was to assess histopathological findings, and samples of the gills were collected on days 14 and 28. Histopathological lesions, including severe lamella fusion, necrotic changes, diluted tubule formation, and cellular damage in the cell caused by marble water, were found in the gills of grass carp (Ctenophryngodon idella).

Keywords: *Marble wastewater,* Ctenopharyngodon idella, *Grass carp, Gills histopathology, Aquatic toxicology*

INTRODUCTION

The purpose of the study was to assess histopathological findings, and samples of the gills were collected on days 14 and 28. Histopathological lesions, including severe lamella fusion, necrotic changes, diluted tubule formation, and cellular damage in the cell caused by marble water, were found in the gills of grass carp (Ctenophryngodon idella). Normal temperature needed for their survival is about 15-25c (Khan et al., 2022). Grass carp is also cultured in suitable water of Pakistan especially in warm water and all over the world for control of aquatic vegetation and also used as a source of food (Lane et al., 2023).

Grass carp possess an elongated, flabby mouth and exhibit a streamlined body form. The anterior region of the oral cavity is somewhat oblique, characterized by firm, fleshless lips devoid of bulges. The lips are relatively short, and an elongated upper jaw is present. The grass carp
1 exhibits a globular belly and possesses a lateral line comprising 40-42 lateral scales (Stechkina,

2019). Grass carp possess various fin types, including dorsal fins located above the head, which feature 8-10 soft rays, and anal fins situated near the anal region. Notably, both the dorsal and anal fins are devoid of spikes or spines. The diploid chromosome count is 48, whereas biochemical analysis has indicated the presence of a total of 49 loci. The species typically achieve a weight range of approximately 30-50 kg and can also reach a length of 1 meter (Britvin et al., 2023).

Marble is a metamorphic rock composed mainly of carbonate minerals such as calcite ($CaCO_3$) or dolomite ($CaMg(CO_3)_2$), which recrystallize under heat and pressure to form a crystalline, typically non-foliated texture. Various methods are used for marble cutting, but two major techniques require water, and the resulting wastewater is referred to as marble water. The first method, **Waterjet technology,** involves the use of a high-pressure waterjet machine to cut marble precisely on all sides and create intricate designs without generating heat or pressure, thus preserving the stone's appearance. It is widely used for engineered stone, thick granite, and marble cutting. The second, more **archaic method,** involves continuous watering and the use of a large-diameter diamond saw blade to cut marble blocks vertically and horizontally to achieve the desired thickness (Günkaya, Karacasulu, Evliyaoğlu, & Çiftçi, 2018).

However, the use of water in marble processing leads to environmental issues. The **side effects of marble water** include the deposition of solidified waste residues on the surface as water evaporates, seepage of marble waste into the soil after rainfall, and airborne dust from improperly dumped waste. Fine marble particles containing $CaCO_3$ contribute to air and visual pollution, while marble slurry can cause soil clogging, increased alkalinity, disruption of photosynthesis, and reduced soil fertility, ultimately harming plant and animal life. Many factories **discharge marble wastewater** into nearby streams, canals, and rivers, causing serious ecological impacts (Hammouda, Ghienne, Dion, & Ben Yahia, 2022).

When **marble water reaches aquatic ecosystems,** it introduces various chemicals harmful to fish and other organisms. **Heavy metals** present in the wastewater are particularly toxic, as they generate reactive oxygen species that damage fish physiology, affect hemato-biochemical and immunological functions, and reduce growth and survival rates. Furthermore**, tin oxide,** used in marble polishing, enters water bodies through wastewater; although not acutely toxic, tin oxide nanoparticles can accumulate in fish organs through gills and intestines. Similarly, high concentrations of **calcium carbonate** discharged with marble water, though beneficial in trace amounts, can become detrimental to fish health when excessive, disrupting aquatic balance and threatening biodiversity in affected ecosystems(Khan et al., 2024).

The rapid expansion of marble processing industries in regions such as Khyber Pakhtunkhwa, Pakistan, has resulted in the excessive discharge of marble wastewater into nearby aquatic ecosystems, posing serious ecological and toxicological threats. The marble effluents, rich in suspended solids, heavy metals, calcium carbonate, and polishing agents such as tin oxide, may alter water chemistry, reduce oxygen availability, and induce physiological and histopathological stress in aquatic fauna. Despite growing concern, limited scientific data exist on how marble wastewater affects the gill architecture and respiratory efficiency of freshwater fish species that serve as key bio indicators of aquatic pollution. Therefore, the present study aims to evaluate the toxicological effects of marble wastewater on the gills of Ctenopharyngodon idella (grass carp) under controlled laboratory conditions. The research specifically focuses on identifying histopathological alterations caused by exposure to marble water, thereby providing critical insight into the ecological risks associated with industrial marble effluents and contributing to the formulation of effective environmental management strategies.

Materials and Methodology

Study Design and Sampling

The experiment was conducted to assess the histopathological effects of marble wastewater on the gills of Ctenopharyngodon idella (grass carp). Fish specimens were collected during two seasons—winter (January) and summer (August)—from the Peshawar region, where average ambient temperatures ranged between 11–42 °C, while aquarium water temperatures during experimental trials ranged from 8–31 °C. All procedures were carried out in accordance with the European Union Directive No. 2010/63 on the protection of animals used for scientific purposes (Morosan & Coman, 2020).

Materials

The following reagents and chemicals were used: 10% phosphate-buffered saline (PBS), clove oil, 10% formalin, graded series of ethanol (60%, 70%, 80%, 95%, 100%), acetone, xylene I & II, and paraffin wax for tissue embedding.

Collection and Acclimatization of Fish

Healthy fingerlings of grass carp (C. idella) were procured from the Carp Hatchery and Training Center, Sherabad (Peshawar), and transferred to the Zoology Laboratory, University of Peshawar. Fish were rinsed in 0.05% $KMnO_4$ solution to remove surface pathogens and acclimatized for 10 days in glass aquaria (50 L capacity) under controlled aeration and temperature. After acclimatization, fish were randomly distributed into four groups: one control (C) and three experimental groups (G_1, G_2, G_3), each containing eight individuals(Naguib, Mahmoud, Mekkawy, & Sayed, 2020).

Determination of Sub-Lethal Concentration

The sub-lethal concentration (LC_{50}) of marble wastewater was determined using the arithmetic method of Kerber (Dede & Kaglo, 2001). Three different exposure concentrations were tested using eight fish per aquarium. The LC_{50} value was established to determine safe sub-lethal doses for histopathological evaluation. Data were analyzed statistically using SPSS software (IBM Corp., 2020).

Experimental Design

The experimental setup consisted of four aerated aquaria (C, G_1, G_2, G_3), each containing eight fish. Fish were fed a commercial diet at 3% of body weight daily for 28 days. Water renewal was performed daily to maintain quality and remove marble wastewater residues. Throughout the experiment, fish behavior and feeding responses were recorded to monitor signs of toxicity or stress.

Histopathological Analysis of Gills

After the exposure period, fish were anesthetized using clove oil, and gills were dissected for histological examination. Samples were fixed in 10% PBS-buffered formalin for morphometric and histopathological analysis. The fixed tissues were processed following standard histological procedures as described by (Ismail, Wahdan, Yusuf, Metwally, & Mabrok, 2019).

Tissue Processing

The gill tissues underwent sequential dehydration through graded alcohol concentrations (30–100%), followed by clearing in xylene I and II, and infiltration with molten paraffin wax at 60–62 °C. Samples were embedded in paraffin blocks, cooled at –10 °C, and stored at –20 °C prior to sectioning (Amin et al., 2021).

Sectioning and Staining

Paraffin blocks were trimmed and sectioned at 4–5 µm thickness using a rotary microtome. Sections were floated on a 50–52 °C water bath to remove folds, mounted on glass slides, and air-dried. Deparaffinization was performed using xylene, followed by rehydration through descending grades of alcohol. Slides were stained with hematoxylin and eosin (H&E) to differentiate cellular structures. Nuclei stained blue with hematoxylin, while the cytoplasm appeared pink to dark red with eosin. Finally, slides were mounted with cover slips using a neutral resin and examined under a compound light microscope for histopathological alterations (Ahirwar & Tripathi, 2021).

Result

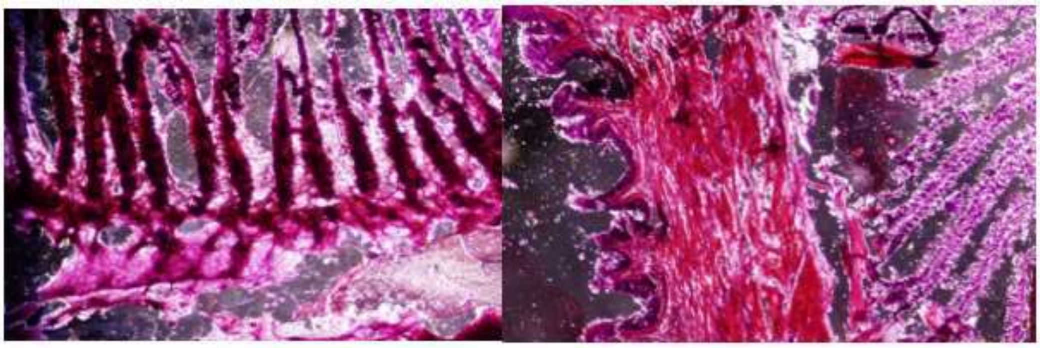
After 14 days of exposure, the histopathological examination of gill tissues in Ctenopharyngodon idella revealed varying degrees of lesions across different treatment concentrations of marble effluent. In the control group, no structural alterations were observed, and all parameters, including secondary lamellae damage (SDL), clubbing of filament lamellae (CFL), disorganization of cellular boundaries (DCB), and necrosis of cells (NC), scored zero, indicating normal gill architecture. In Group 1 (1 mg/L concentration), mild lesions were recorded, with SDL showing a score of 3, while CFL, DCB, and NC remained unaffected. Group 2 (3 mg/L concentration) exhibited moderate damage, as SDL, CFL, DCB, and NC recorded scores of 3, 5, 3, and 3, respectively. The highest exposure concentration (Group 3; 5 mg/L) resulted in severe histopathological alterations, where all lesion types—SDL, CFL, DCB, and NC—recorded high scores (5, 5, 5, and 3, respectively), indicating pronounced tissue damage and necrosis.

TABLE 1
14th Days Histopathological Analysis

Gills lesion	14th Days Histopathological Effects On Gills Tissue				
	Concentration Mg/L	SDL	CFL	DCB	NC
	Control	0	0	0	0
	G1	1	3	0	0
	G2	3	5	3	3
	G3	5	5	5	3

Terms: SDS=sever diluted tubules; CFL=complete fusion of lamella; DCB= Degeneration of cartilaginous core; NC= necrotic changes

FIGURE 1
Histopathological Lesion in Grass Carp at 14th Day



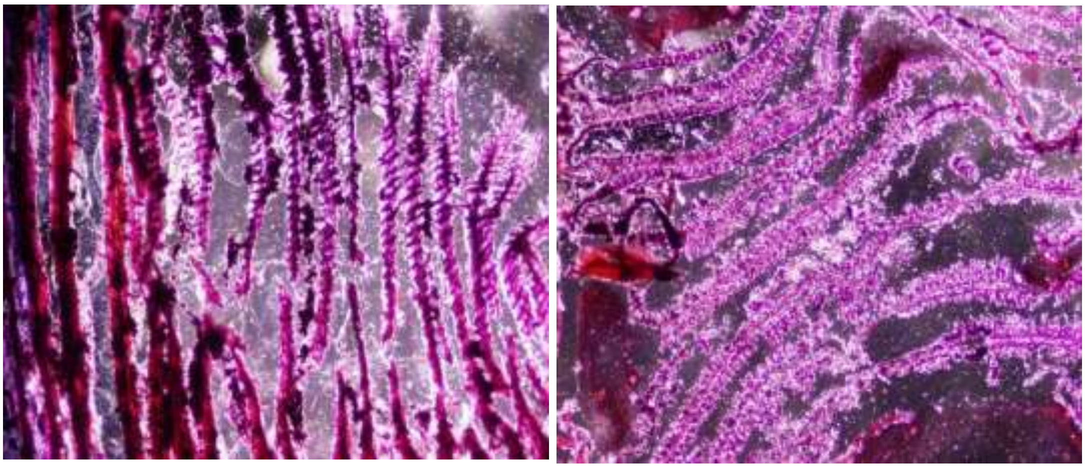
After 28 days of exposure, marked histopathological alterations were observed in the gill tissues of Ctenopharyngodon idella exposed to different concentrations of marble effluent. In the control group, no lesions were detected, and all parameters—including secondary lamellae damage (SDL), clubbing of filament lamellae (CFL), disorganization of cellular boundaries (DCB), necrosis of cells (NC), and lifting of epithelium (LF)—remained at a score of zero, indicating normal gill structure. In Group 1 (1 mg/L concentration), severe damage to SDL and CFL was recorded (score 5 each), while mild alterations were noted in DCB and NC (score 1 each) and moderate lifting of epithelium (score 3). Group 2 (3 mg/L concentration) exhibited severe SDL and CFL (score 5 each), moderate DCB and NC (score 3 each), and moderate LF (score 3). In Group 3 (5 mg/L concentration), the gill tissues showed the most extensive damage, with all parameters—SDL, CFL, DCB, and LF—recording high lesion scores (5, 5, 5, and 5, respectively) and NC showing a moderate score (3), indicating significant tissue degeneration and necrosis due to prolonged exposure.

TABLE 2
28th Days Histopathological Assessment

Gills lesion	28th Days Histopathological Effects on Gills Tissue					
	Concentration Mg/L	SDL	CFL	DCB	NC	LF
	Control	0	0	0	0	0
	G1	5	5	1	1	3
	G2	5	5	3	3	3
	G3	5	5	5	3	5

Terms: SDS=sever diluted tubules; CFL=complete fusion of lamella; DCB= Degeneration of cartilaginous core; NC= necrotic changes; (0= None; 1= Moderate; 3= Mild, 5= Severe)

FIGURE 2
Histopathological lesion in Grass Carp at 28th day



The results derived from the histopathological analysis indicate that an increase in the concentration of marble water and the duration of exposure leads to significant damage. This damage is characterized by severe dilution of tubules, complete fusion of the lamella in most areas, degeneration of the cartilaginous core, and the formation of lacunae. It also results in the lifting of epithelial tissue and the curling of lamellae; in rare instances, it affects the lifting of epithelial cells and the infiltration of inflammatory cells.

Discussion

Grass carp fish gills are performing different function and mostly work as a homeostatic organ of the body which consist of gill filaments that is composed of primary lamellae (non-respiratory lamellae) and secondary lamellae (respiratory lamellae), the secondary lamellae placed perpendicularly on the surface of primary lamellae that are separated peculiar intracellular spaces, gill are mostly easily accessible to aquatic environment so it's easily effected because exchange of chemical and water are done through gills so due to aquatic toxicity and industrial effluents easily cause lesion in the structure of the gills, and its mostly due to direct exposure towards aquatic toxicity (Shah, Khisroon, & Shah, 2021).

During the experiment, the exposure to the marble water caused a severe dilution of tubules within the gills tissue of the Grass Carp (Ctenophryngodon idella). These tubules were moderate at the beginning of the experiment, but as the experiment progressed and the experimenter was exposed to more and more marble water, the dilution became more severe. In excess amounts, the gills lamellae of the Grass Carp (Ctenophryngodon idella) degenerate and complete fusion of the gills lamellae of the Grass Carp (Ctenophryngodon idella) occurred. Primary gill lamellae are the primary necessary part of the gills, and they increase the oxygen uptake and its concentration within the blood. This allows the Grass Carp (Ctenophryngodon idella) to maintain their body function and provide oxygen to their blood. While the experiment is being conducted, many findings are also reviewed. These findings include cellular damage, the creation of lacunae, and damage to the cartilaginous core. This damage in the gills of Grass Carp (Ctenophryngodon idella) is caused by the hematoxylin dye effect, which causes the distance between the blood and the water to increase. As a result, the biological oxygen demand (BOD) decreases, which leads to difficulties in body function. Moreover, due to hematoxylin dye epithelial lifting, which is the most prominent type of cellular damage in the gills of Grass Carp (Ctenophryngodon idella), which has a negative impact on the ionic exchange in the body, the Grass Carp (Ctenophryngodon idella) suffers a great deal of damage. As a result of marble water's excessive usage in industries and their unchecked effluents to aquatic fauna, the Marble Carp (Ctenophryngodon idella) suffers a great deal of damage (de Almeida Rodrigues, Ferrari, Dos Santos, & Junior, 2019) where fish exposed to varying lethal concentrations of heavy metals exhibit severe edema and epithelial lifting, indicating that their supply chain was destroyed due to tissue damage and epithelial lifting, as well as a decrease in ventilation rate and an increase in biological oxygen needs. In the given process mild to severe lamellar disorganization and its followed by necrotic changes occur in the gill of Grass Carp (Ctenophryngodon idella), and disorientation of gills lamella occur, during this experiment the marble water none of the orientation of lamella were found but as the concentration of trial were increased then effect were found from mild moderate to severe, at mid time of experiment during 14th days of observation moderate type of lamellar effect were found but at 28th Days of observation it become mild type but at the end the effect were somewhat unexpected and at the start of the trial the severe type of orientation were observed (Farag et al., 2021).

Conclusion

The findings of this study clearly indicate that Grass Carp (Ctenopharyngodon idella) act as a biological indicator for identifying and assessing the toxicological effects of marble water. This study investigates the genotoxic, cytotoxic, and histopathological effects of marble water on Grass Carp (Ctenopharyngodon idella). The trial illustrates that the magnitude of the impact is affected by the duration of the trial as well as the length of exposure to marble water. The findings of this experiment clearly demonstrated the impact of marble water on the gills of Grass Carp (Ctenopharyngodon idella).

Recommendations

- Aquatic toxicity is reaching critical levels in our environment as aquatic pollution escalates daily. This pollution severely impacts aquatic fauna, particularly fish. Consequently, water quality is regularly monitored, and its parameters are rigorously assessed. It is essential for the government to establish dedicated agencies and laboratories for water protection.
- I also promoted the need for further research into the water quality issues within the textile industry and the impact of aquatic waste, while emphasizing the importance of educating the public about the risks associated with marble water.
- This research presents a novel approach to exploring and understanding various aspects of behavioral and systematic cell damage. It offers clear recommendations for future studies, paving the way for a new era of investigation into marble water discharge and its potential to reduce aquatic pollutants in our environment.

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