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Evaluation of acute toxicity of copper sulphate on the gills of fresh water mussel, *Lamellidens marginalis*

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Abstract
A moderate amount of copper in the ecosystem is vital for the metabolism and normal growth of living organisms. However, elevated levels of copper and its compounds in the environment, particularly in water bodies, can become toxicants. The high levels of copper in water bodies, impose a significant hazard to the wellbeing of underwater plant life and animal populations, given its toxic properties and tendency to accumulate. Copper and their compounds are used in many industries for many purposes. Through precipitation, copper enters water bodies from sources such as mining, fossil fuel combustion, and direct industrial waste disposal. Molluscs have been extensively utilized in assessing toxicity and implementing water quality management programs. Freshwater mussels serve as significant bioindicators of environmental contaminants. Monitoring behavioural and histopathological alterations in various body parts of freshwater mussels can provide sensitive indicators of stress induced by toxicants. Exposure of the freshwater mussel, *Lamellidens marginalis* to acute concentrations of copper sulphate at different time intervals resulted in histological changes in the gills. Histopathological investigations aid in evaluating tissue damage in the gills caused by the toxic effects of copper sulphate.

Keywords: Copper sulphate, toxicant, heavy metal, Bio indicator, *Lamellidens marginalis*

Introduction

Heavy metals represent a significant class of pollutants in the environment. These elements occur naturally and possess high atomic weights and densities, typically at least five times greater than water (Tchonwou *et al*., 2012). Recently, there has been increased focus on the effects of heavy metal pollution on the aquatic environment due to their tendency to undergo biomagnification and their environmental persistence, even at extremely low concentrations. According to Thurberg *et al.* (1973) [25], an animal's capacity to adapt to shifting environmental conditions may be altered by a pollutant, this would ultimately reduce the animal's chances of surviving. It is essential to carry out thorough research on aquatic bodies due to the growing concerns regarding heavy metal contamination, especially copper, and its detrimental impacts on aquatic ecosystems and human health. In aquatic animals, gills are closely involved in osmoregulation and respiration. According to research by Hodson *et al*. (1979) [11], copper interferes with fish osmoregulation and can induce gill injury in aquatic invertebrates at high concentrations. Following acute and subacute exposure to copper, the effects of copper on crustaceans also have been investigated by Shukla S. *et al*. (2019) [21]. High metal uptake by gill tissue during the initial period has been documented by Eisler *et al*., (1972) [8] and Hutcheson (1974) [12]. In numerous studies on the toxicity of heavy metals to freshwater mussels, it has been observed that the highest concentration of toxicants is found in the gill tissue. V. R. Chavan and D.V. Muley (2014) investigated the effects of heavy metals on gills, revealing lamellar degenerations, epithelial lifting, and necrotic changes in intercellular epithelial cells. Freshwater mussels serve as both bioindicators of toxin levels and purifiers, contributing to the preservation of environmental quality. Therefore, they have been extensively utilized in toxicity assessments and water quality management programs (Reddy, 1984) [19]. Addressing these critical research gaps can enhance our understanding and management of the issues arising from heavy metal contamination, promoting sustainable aquatic environments and the well-being of communities. The objective of this research paper is to assess the current state of knowledge regarding the effects of copper on the freshwater mussel, *Lamellidens marginalis* and explore its potential for aquaculture.

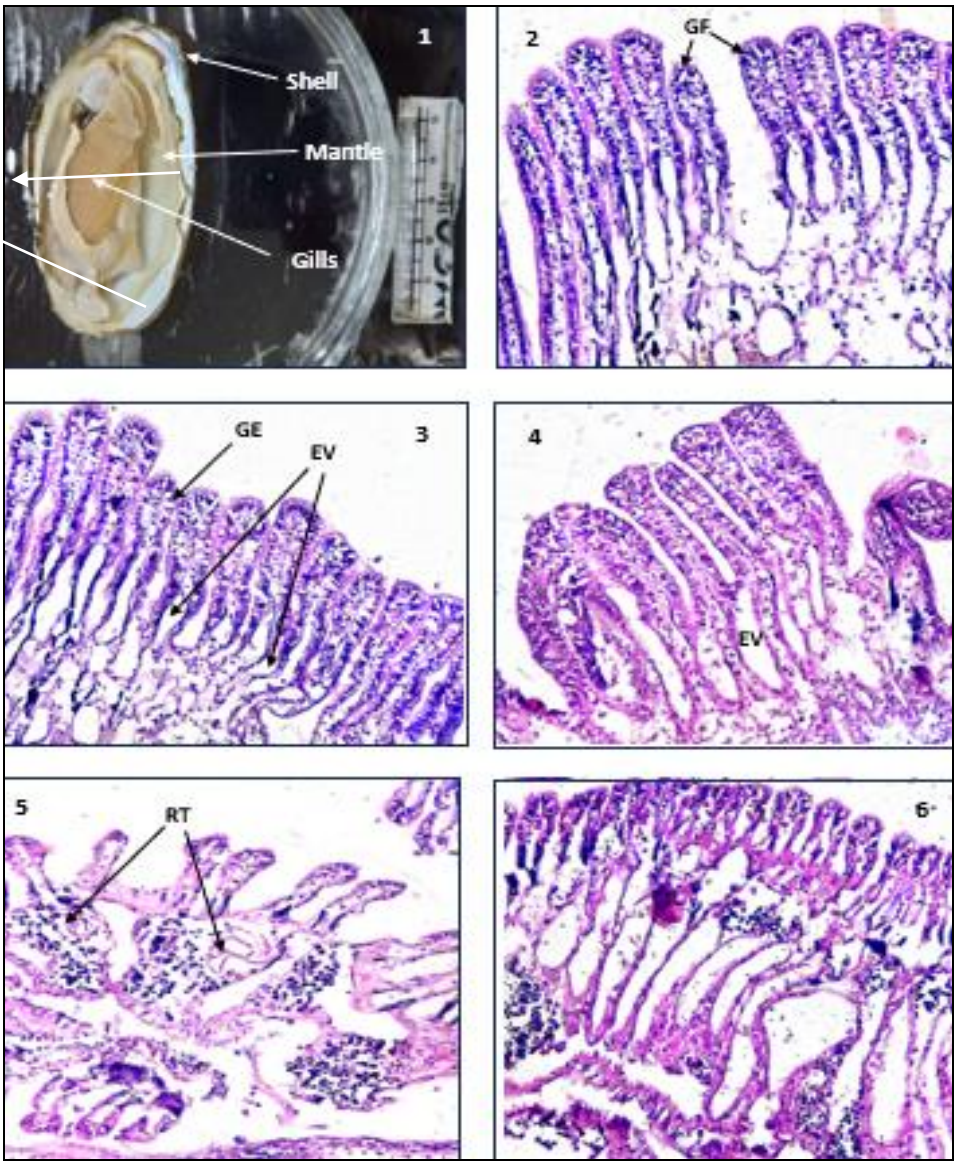
Material and methods

Freshwater mussels, *Lamellidens marginalis*, were sourced from the Gomti River in Lucknow, Uttar Pradesh, India, with the assistance of local fishermen and transported to the departmental laboratory. They were then acclimated to laboratory conditions in glass aquariums for 5-7 days. The animals were housed in glass aquariums containing dechlorinated water with optimal physicochemical parameters, including pH, temperature, partial alkalinity, total hardness, and dissolved oxygen levels, following the guidelines outlined by APHA *et al*., (1998). The water's physiochemical properties were analysed according to the standards set by APHA *et al*., (1985). Proper aeration was ensured using air pumps, with one aquarium containing 10 litres of diluent water serving as the control. All experiments were conducted for up to 96 hours following standard protocols (APHA *et al*., 1985). Percent mortality was recorded in each aquarium at 24, 48, 72, and 96 hours, with experiments replicated thrice. To determine the LC_{50} value, the animals were exposed to varying concentrations (37, 42, 49, 65, and 75 mg/litre) of copper sulphate up to 96 hours, and the value was calculated using the Trimmed Spearman Karber method (Hamilton *et al*., 1977) [10]. Acute exposure was conducted at the 96-hour LC_{50} concentration (50.40 mg/litre) for durations of 24, 48, 72, and 96 hours. For evaluating acute toxicity, gill tissues from control and experimental groups were dissected after 96 hours of exposure (Shukla *et al*., 2021) [22]. For histological

examination, gills were carefully removed from living animals, washed in normal saline, and fixed in Bouin's fluid for 24 hours. Tissue blocks were embedded in paraffin wax at 60°C, and sections of 5-6 microns were cut using a rotary microtome. These sections were then stained with Harris Haematoxylin and Eosin using standard procedures (Rawat, R. S., & Singh, A., 2023). Stained slides were examined, compared with controls, and photographed using an Olympus trinocular microscope.

Results and Discussion

In the freshwater mussel, *L. marginalis*, gill plates or demibranches are situated on both sides of the muscular foot, referred to as ctenidia or gills. Each gill plate consists of two lamellae or comparable flaps, with numerous gill filaments containing ostia or holes. Various types of cilia cover the gill filaments, supported by two chitinous rods. Blood vessels run through the space between the lamellae of a gill plate. In Figure 1 below, the dissected animal displays two flaps of gills protruding outward. Figure 2 depicts the histological condition of the gills in the control group of bivalves. In slides from the control group, the epithelium of the gill lamellae appeared intact, with uniform and normal lengths. The connective tissue at the base of the gill lamellae was observed to be merged. In Figure 3 the slide depicted alterations following a 24-hour acute exposure to copper sulphate: notably, changes in gill lamellae lengths, occurrences of epithelial cell hypoplasia, and damaged gill filaments were observed. The cellular structure of the connective tissue and epithelial cells has been lost and distended, necrotic, and vacuolated epithelium developed. In Figure 4, slides of animals exposed for up to 48 hours displayed longer gill filaments with enlarged lumens. The disruption in shape was primarily attributed to necrosis, accompanied by significant epithelium loss. Despite extensive denudation and tissue ruptures in the connective tissue, small tufts of cilia were still observable on the lateral surfaces. Figure 5 illustrated slides of animals exposed for up to 78 hours, showcasing alterations in the length and morphology of their gill lamellae, along with noted hypoplasia of their epithelial cells. Expansion and hyperproliferation of the epithelium surrounding the haemolymph channels were observed in the inner sections. Figure 6 depicted slides of organisms exposed to copper sulphate for up to 96 hours, revealing compromised inter-lamellar junctions and the complete absence of gill structure. Tissue rupture and the breakdown of regular cell organization in connective tissue were seen. Cytoplasm displayed fragmentation as a result of respiratory epithelial enlargement due to necrosis.



Explanation of figures

Figure 1 presents a photomicrograph of a dissected animal. Figure 2 shows a T.S of the gill from the control group of *L. marginalis*. Figure 3 displays a T.S of the gill from *L. marginalis* exposed to copper sulphate for 24 hours. Figure 4 exhibits a T.S of the gill from *L. marginalis* exposed to copper sulphate for 48 hours. Figure 5 depicts a T.S of the gill from *L. marginalis* exposed to copper sulphate for 72 hours. Figure 6 illustrates a T.S of the gill from *L. marginalis* exposed to copper sulphate for 96 hours. In these figures, GF- Gill Filament, GE- Gill Epithelium, EV- Extended Vessel, and RT- Rupture Tissue.

The metal-exposed animal's gill histopathology revealed distinct indications of injury that were not seen in the control group. Gulbhile (2006) [9], work on *Lamellidens corrianus* exposed to mercuric chloride, their gill lamellae displayed a number of alterations, including the rupture of the ciliated epithelium, an increase in lamella size, an increase in the gap between the inter lamellar junction, and an increase in the space between the inner lamellar junction and the water tube. In aquatic animals, gills play a vital role in respiration. Several researchers have reported damage to gills caused by various heavy metals and pesticides (Khangarot, 1982 [14]; Pawar and Katdare, 1983 [16]; Nilkant and Sawant, 1993). Consequently, it appears that anoxia may be a significant factor contributing to the mortality of organisms exposed to pollutants (Skidmore, 1964 [23]; Burton *et al*., 1972). The lethal effects of heavy metals such as Hg, Cu, Cd, Zn, and Pb have been linked to the secretion of mucus on the gill surface, damage to gill tissues, and subsequent respiratory failure (Dandroff and Katz, 1953) [6]. Similar findings have been reported by various researchers using different heavy metals on various test animals (Pundir and Saxena, 1992 [17]; Wandkhede and Dhande, 1999 [26]; Shrivastava and Shrivastava, 2002 [20]; Bhamre *et al*., 1996 [3]; Drastichova *et al*., 2004 [7]; Ksherwani *et al*., 2009).

Conclusion

Present studies indicates that the mortality of the animals for any given period of time rises with an increase in concentration of toxicant. Hence, based on the current studies, it can be inferred that the toxicity of the tested heavy metal copper sulphate significantly impacts the physiological systems of *Lamellidens marginalis*, leading to severe damage to the gills and ultimately resulting in mortality. Additionally, the present investigations underscore the high sensitivity of molluscs to copper sulphate. Therefore, the histopathological alterations in gill structure resulting from heavy metal exposure can serve as biomarkers in aquatic ecosystems, with *Lamellidens marginalis* proving to be a significant bioindicator aiding in water quality assessment. This study further underscores the potential of a freshwater bivalve such as *Lamellidens marginalis* as a valuable laboratory model for conducting aquatic toxicological investigations.

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