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Effect of heavy metals, cadmium & copper on freshwater prawn, *Macrobrachium lamarrei lamarrei* (Crustacea- Decapoda)

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Abstract
Aquatic environments face a significant threat from heavy metal pollution, which originates from natural and anthropogenic sources. Present study investigates the impact of Cadmium Chloride ($CdCl_2$) and Copper Sulphate ($CuSO_4$) exposure on the behavior of freshwater prawns, *Macrobrachium lamarrei*, and assesses their respective lethal concentration (LC_{50}) values. The study reveals time-dependent toxicity, with LC_{50} values decreasing as exposure duration increases. Initially, $CdCl_2$ is slightly more toxic than$CuSO_4$, but with extended exposure, both metals become equally harmful. The research emphasizes the vulnerability of *Macrobrachium lamarrei* to even low concentrations of these heavy metals, potentially disrupting aquatic ecosystems. Behavioral changes, including avoidance responses and aggression, serve as biomarkers of heavy metal pollution, while increased mucus production and gill darkening are potential defense mechanisms. This study underscores the importance of mitigating Cadmium and Copper pollution to protect ecosystem health and highlights the need for further investigation into toxicological mechanisms. *Macrobrachium lamarrei* emerges as a valuable bio-indicator for assessing freshwater ecosystem health due to its sensitivity to environmental pollutants. The included images depict the behavioral changes observed in the prawns after heavy metal exposure.

Keywords: Copper sulphate, cadmium chloride, heavy metal, freshwater prawn, behaviour, *M. lamarrei*

Introduction
Aquatic pollution caused by heavy metals (HMs) is a significant problem due to their potential harm and increasing concentrations in water habitats [1]. These are generated from various natural and anthropogenic sources. In freshwater ecosystems, metal pollution can arise from direct atmospheric deposition, geological erosion, or through the release of agricultural, municipal, domestic, or industrial waste materials [2]. Cadmium (Cd), along with arsenic, lead, mercury, and chromium, is a heavy metal that lacks a biological role and is often viewed as a harmful substance [3]. High levels of Cd are found in crustaceans, bivalve molluscs, oysters, cephalopods, and crabs [4]. Copper (Cu) is a vital metallic element for all living organisms and primarily exists in Cu^{2+} and Cu^{+} states. It plays a role in numerous physiological processes, including electron transport, mitochondrial function, and antioxidant activity [5]. It's a crucial component of crustaceans' respiratory pigment haemocyanin [6]. Nevertheless, excessive Cu becomes harmful and harms cellular structures [7]. Freshwater Prawns are members of the Palaemonidae family of Decapod-Crustacea. These animals have economic significance and could be used in freshwater aquaculture, as well as serving as valuable subjects for fundamental and toxicological investigations [8]. Changes in behavior represent organisms' earliest reactions to toxicants [9], and crustaceans might function as useful bioindicators [10]. Therefore, this study aims to evaluate LC_{50} value of $CdCl_2$ and $CuSO_4$ and document behavioral changes induced by stress from Cadmium Chloride ($CdCl_2.2^{1/2}$ H20) and Copper Sulphate ($CuSO_4.5H_2O$) in a freshwater prawn, *Macrobrachium lamarrei*.

Material and Method
Fresh water prawns were collected with the help of local fisherman from the river Gomti, a tributary of river Ganga at Lucknow, (U.P.) India. The prawns were brought to laboratory (N-26°5'59E-80°56'17") and transferred into glass aquaria where these prawns were subjected to acclimation for the laboratory conditions.

The collected prawns were kept in glass aquaria of 10liter capacity. These prawns were maintained in laboratory condition according [11]. For the maintenance of the prawns the aquaria were filled with dechlorinated tap water having physico-chemical characteristics analyzed as according to [12] were as follows:

pH-7.1± 0.1; D.O- 7.2 ± 0.1mg/L; Total hardness- 290 ± 2.0mg/L; Temp- 27 ± 2ºC.

In order to prepare stock solution of Cadmium chloride ($CdCl_2 2½\ H_2O$) and Copper sulphate ($CuSO_{4.}5H_2O$), weighed amount of both the salts were dissolved in double distilled water. To prevent the precipitation two drops of glacial acetic acid was added in the stock solution of Copper Sulphate.

Healthy and average sized prawns (4.88 ± 0.54cm; 1.11 ± 0.27 gm) were sorted out and considered for various experiments.

A set of six different concentrations of the test medium was prepared, determine through Pilot test to cover the toxic range. These concentrations were placed in aquaria, filled with 10 liters of diluent water. In each tank with the test medium, 10 healthy prawns were introduced. To serve as a control, one aquarium having10 liters of diluent water only. Proper aeration was ensured using air pumps and tests were conducted following the standard procedure as outlined [12].

Percentage of mortality in each aquarium were recorded at the intervals of 24, 48, 72 and 96 hrs. Prawns that did not respond to gentle prodding were considered dead.

Behavioural observations were also made in each test aquaria on alive animals only, comparing them to the controls.

Experiments were replicated three times and LC50 and their 95% confidence intervals were calculated by Trimmed Spearmann- Kaber method [13] with a software on PC.

Result

Results of LC_{50} – Cadmium is slightly more toxic than copper. Cadmium and copper both were found toxic to freshwater Prawn, *M. lamarrei*. Bioassay results are summarized in table-1.

Table 1: LC_{50} values of Cadmium Chloride and Copper Sulphate to freshwater prawn, *Macrobrachium lamarrei*

Toxicant	Exposure Time (Hrs.)	LC_{50} (mg/L)	95% Confidence limits (mg/l)	
			Lower	Upper
Cadmium Chloride	24	0.34	0.31	0.38
	48	0.30	0.25	0.36
	72	0.26	0.18	0.37
	96	0.18	0.13	0.25
Copper Sulphate	24	0.36	0.27	0.48
	48	0.32	0.24	0.42
	72	0.28	0.21	0.36
	96	0.25	0.17	0.37

LC_{50} values of Cadmium Chloride were 0.34, 0.30, 0.26 and 0.18 mg/l for 24,48,72 & 96 hrs respectively while of Copper Sulphate were 0.36, 0.32, 0.28 & 0.25 mg/l for 24,48.72 &96 hrs respectively. LC_{50} values were inversely proportional to duration of exposure. Considering 96 hrs LC_{50} values Cadmium Chloride was more toxic than Copper Sulphate to freshwater prawn *M. lamarrei*.

Cadmium chloride induced significant changes in the behavior of experimental prawns. Gross behavioural changes after Cd and Cu exposure are summarized in table 2 & 3. Shortly after introducing the prawns into the aquariums, those in the test group displayed irregular swimming and restlessness. Over time, their activity decreased, and they settled at the bottom of the aquarium. After 24 hours of exposure to Cadmium Chloride, the prawns exhibited hyperactivity. Some of them came to the water's surface and remained there for extended periods, while others swam rapidly (Plate- 1, Fig-2). Irritation caused by the toxicant became evident as 70% of the prawns scraped their bodies with their second pincers (cheleped II). Some prawns also surfaced and mounted on the backs of others, with a thin film of mucus noticeable on the lateral sides of their carapaces. Additionally, aggressive behavior was observed in 50% of the prawns, and most of them (50%) gathered at the corners of the aquarium (Plate-1, Fig-3).

Table 2: Impact of Copper Sulphate on behaviour of freshwater prawn, *Macrobrachium lamrarrei*

Time (Hrs)	Swimming		Aggression		Mucous Secretion		Surfacing		Aggregation		Scrapping	
	C	E	C	E	C	E	C	E	C	E	C	E
24	++	++	-	++	-	-	++	+++	-	++	-	+++
48	++	+	-	+	-	+	++	++	-	+	-	++
72	+	-	-	+	-	++	+	+	-	-	-	+
96	+	-	-	-	-	+++	+	-	-	-	-	-

Table 3: Impact of Cadmium Chloride on behaviour of freshwater prawn, *Macrobrachium lamrarrei*

Time (Hrs)	Swimming		Aggression		Mucous Secretion		Surfacing		Aggregation		Scrapping		Blackening of Gill	
	C	E	C	E	C	E	C	E	C	E	C	E	C	E
24	++	+++	++	+++	-	+	++	+++	-	+++	-	+++	-	-
48	++	++	+	++	-	++	++	+	-	++	-	++	-	-
72	+	+	+	+	-	++	+	-	-	+	-	+	-	+
96	+	-	+	-	-	+++	+	-	-	-	-	-	-	+++

C- Control; E- Exposed

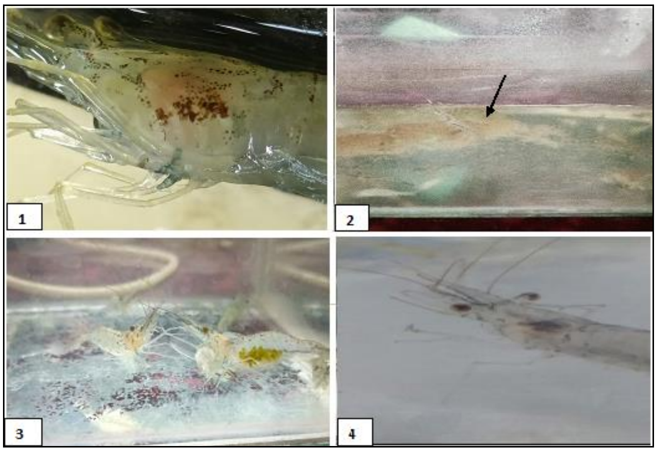
(Nil) + (Less) ++(More) +++ (Prominent)

After 48 hours of exposure to Cadmium Chloride, the prawns displayed slightly sluggish activity. There were instances of fighting encounters in 30% of the prawns, and most of them appeared clumsy, tending to stay at the corners of the aquarium as their swimming activity significantly decreased. When the aquarium walls were gently paddled, the prawns exhibited a feeble response compared to the control group. Vigorous scraping of the body and gills was observed in 50% of the animals, with 10% still surfacing occasionally (Plate-1, Fig-4). More mucus accumulated near the gills and over the carapace. About 30% of the prawns preferred to aggregate at the corners of the aquarium and around the air diffusers. Mounting behavior was also observed in 15% of the prawns. Feeding was reduced.

After 72 hours of exposure to Cadmium Chloride, the prawns appeared feeble and weak, with declining swimming activity as they preferred to stay at the bottom. Aggression along with cannibalistic nature and hyperactivity were seldom noticed, occurring in only 10% of the prawns. Loss of equilibrium was observed in 15% of the prawns when they attempted to walk. Scratching of gills with the help of cheleped II was observed in 15% of the prawns. A thick coat of mucus accumulated over the gills of nearly all the prawns. Surfacing was not observed, and aggregation decreased, with no mounting behavior observed. Blackening over the dorsal abdominal region was relatively apparent in 20% of the animals.

After 96 hours of exposure to Cadmium Chloride, total lethargy and the absence of aggressive behavior were observed in the prawns. Aggregation was absent as they remained dispersed, with a few staying at the sides of the aquarium walls and around air diffusers. When gently prodded, they showed little response, and 20% of the prawns experienced a loss of equilibrium, while 10% crawled using their walking legs. A thick layer of mucus was observed on the carapace, making it difficult to handle them for minor observations. Blackening was most pronounced on the gills, carapace, and dorsal abdominal region in 25% of the prawns (Plate-1, Fig-1). Surfacing and swimming activity declined considerably as they preferred to stay at the bottom.

Plate 1



Explanation of fig: Photographs of freshwater prawn, *Macrobrachium lamarrei* after exposure of cadmium
1. Blackening of gill
2. Surfacing
3. Fighting encounter
4. Scrapping

The presence of copper sulphate led to significant changes in the behavior of the experimental prawns. (Plate-2).

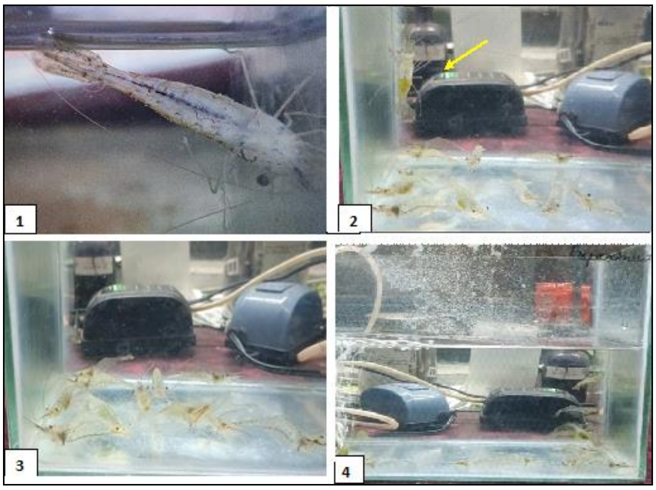
Upon introduction into the aquarium, the prawns in the test tanks initially exhibited erratic swimming and restlessness. Over time, their activity decreased, and they settled at the bottom of the aquarium. After 24 hours of exposure to copper sulphate, the prawns displayed heightened activity like striking at the wall of aquaria (Plate-2, Fig-4) and surfacing (Plate-2, Fig-2). Approximately 70% of the prawns scraped their bodies using their chelipeds.

A thin layer of mucus was observed on the lateral sides of their carapaces. Additionally, aggressive behavior was witnessed in 50% of the prawns, while about half of them congregated in the corners of the aquaria (Plate-2, Fig-3).

After 48 hours of exposure, the prawns displayed somewhat sluggish activity. However, encounters involving fighting were observed in 30% of the prawns. Most of them continued to stay near the corners of the aquaria, as their swimming activity had considerably decreased. When the aquaria walls were gently paddled, the prawns exhibited a feeble response compared to the control group. Approximately 50% of the animals vigorously scraped their bodies and gills. A few of them, around 10%, still surfaced occasionally. More mucus accumulated near the gills and on the carapace. Roughly 30% of the prawns preferred to aggregate near the corners of the aquarium and around the air diffusers.

After 72 hours of exposure, the prawns appeared feeble and weak. Their swimming activity declined, and they preferred to remain at the bottom. Aggressiveness and hyperactivity were seldom observed, occurring in only 10% of the prawns. Loss of equilibrium was noted in 15% of the prawns when they attempted to walk (Plate-2, Fig-1). Approximately 15% of the prawns scraped their gills with the help of their chelipeds II. A thick layer of mucus accumulated over the gills of nearly all the prawns. Surfacing and swimming were not observed, and aggregation was reduced. Blackening over the dorsal abdominal region was observed in approximately 20% of the animals.

Plate 2



Explanation of Fig: - Photograph of freshwater prawn *Macrobrachium lamarrei* after Exposure of Copper
Fig 1: Loss of balance
Fig 2: Surfacing
Fig 3: Aggregation
Fig 4: Striking at the wall of aquaria

After 96 hours of exposure to copper sulphate, total lethargy and a loss of aggressive behavior were observed in the prawns. A few prawns remained near the sides of the aquaria wall and around the air diffusers. When gently paddled, they showed minimal response, and loss of equilibrium was evident in 20% of the prawns. Only 10% of the prawns were capable of crawling with the help of progressive movement of their walking legs. A thick layer of mucus was observed on their carapaces, with the intensity of blackening being most pronounced in these prawns.

Discussion

Both Cadmium and Copper exhibit time-dependent toxicity in *Macrobrachium lamarrei*, with decreasing LC_{50} values as the exposure duration increases. This suggests that both heavy metals become more toxic to the prawn species with prolonged exposure.

At 24 hours of exposure, Cadmium has an LC_{50} value of 0.34 at 96 hrs, while Copper has an LC_{50} value of 0.36 at

96hrs. This indicates that initially, Cadmium is slightly more toxic than Copper to *Macrobrachium lamarrei*.

At 96 hours of exposure, Cadmium exhibits an LC_{50} value of 0.18, while Copper also has an LC_{50} value of 0.25. This suggests that, Cadmium is more toxic than Copper to the prawn species.

The data highlights the vulnerability of *Macrobrachium lamarrei* to the toxic effects of both Cadmium and Copper. The low LC_{50} values indicate that even relatively low concentrations of these heavy metals can have adverse effects on the prawn species.

Prolonged exposure to both Cadmium and Copper can have increasingly detrimental effects on *Macrobrachium lamarrei* populations, which can disrupt the ecological balance of aquatic ecosystems where this species resides. The LC_{50} values for copper and cadmium were found to be slightly lower than the previously reported values [14-15] which may be due to different lab conditions as well as physico- chemical characteristics of water.

The present study highlights the importance of implementing measures to mitigate Cadmium and Copper contamination in freshwater habitats to protect the health and sustainability of aquatic ecosystems.

The data set suggests the need for further research to investigate the underlying mechanisms of Cadmium and Copper toxicity in *Macrobrachium lamarrei*.

Generally, crustaceans tend to exhibit greater sensitivity to metals, making them preferred bio-indicators for assessing the health of freshwater reservoirs. This is because the behavior of any living organism encompasses a range of observable, recordable, and measurable activities, including integrated movements, which serves as a valuable indicator of aquatic organism toxicity [16-17]. Behavioural alterations have been observed after exposure of heavy metal on fishes [18-19] and crab [20] and molluscs [21].

The initial increase in activity observed in *M. lamarrei* when exposed to $CdCl_2$ and $CuSO_4$ could be attributed to their avoidance response to the toxic substance. Similar avoidance behaviors have been documented in *Macrobrachium lamarrei* following exposure to detergents and heavy metals [11-15]. Avoidance response has also been reported in Zebra fish, *Danio rerio*, after exposure of heavy metal [22]. The reason behind this response might stem from changes in the sensitivity of chemoreceptors and the sense of smell [23]. Freshwater prawn *M. lamarrei* showed aggressive behaviour after acute exposure of Cadmium Chloride. Similar observations have also been made in *M. dayanum* [24], in *M. lamarrei* [15] and in *M. dayanum* [25]. Cadmium is a known neuroendocrine disruptor [26] hence it may be the reason behind increased aggressions, loss of balance and erratic swimming. The aggressiveness observed in crustaceans has been associated with changes in the levels of specific neurotransmitters unique to each species. For instance, in case of the hermit crab *Pagurus bernhardus*, it was reported that serotonin (5-HT) levels increased following instances of combat [27].

The enhanced production of mucus in the gills, carapace, abdominal area, and across the body's surface when exposed to cadmium is a general response after exposure to heavy metal. This response could serve as a defense mechanism to prevent the absorption of the heavy metal through the gills and other epithelial linings [11-14-25]. Elevated levels of heavy metals induce fish gills to produce an excess of mucus, leading to death through suffocation due to the coagulation and precipitation of mucous with metals on the gill surface [28].

Heavy metals have the potential to become ensnared within a mucous layer constructed from barrier glycoproteins [29]. Similar enhanced mucus production was reported in *Heteropneustes fossilis* [19].

Food detection was found to have declined after exposure to copper sulphate and cadmium chloride, as reported in *Simocephalus vetulus* [30] and in *Heteropneustes fossilis* [19], possibly due to damaged chemoreception [31].

In the present study, blackening of gills was observed after cadmium exposure. Similar findings were reported in *M. lamarrei* after Hg, Cd, Pb, Ni and Cu exposures [11-14-24-32-33-34]. Gill darkening can result from the death of cells within the gill tissue, accompanied by the accumulation of melanin. In crustaceans, gill discoloration may also be attributed to the presence of metallic sulphides following exposure to substances like mercury (Hg) and cadmium [25].

Conclusion

The present study demonstrates that both Cadmium chloride and Copper sulphate exhibit increasing toxicity to *Macrobrachium lamarrei* over time. Cadmium is slightly more toxic than Copper. These findings emphasize the vulnerability of these prawns to heavy metal contamination, even at low concentrations, which can disrupt aquatic ecosystems.

The observed behavioral changes, such as avoidance responses and aggression may serve as a biomarker of heavy metal pollution and provide insights into how heavy metal exposure affects aquatic organisms. Increased mucus production and gill darkening serve as potential defense mechanisms against heavy metal stress.

This research highlights the importance of mitigating Cadmium and Copper pollution in freshwater habitats to protect ecosystem health. Copper is a micronutrient and widely used as algaecide and weedicide in aquaculture ponds. Present study will also be helpful in standardizing proper dose of copper. Additionally, it highlights the need for further investigation into the mechanisms underlying these toxicological responses. Crustaceans like *Macrobrachium lamarrei* prove to be valuable bio-indicators for assessing freshwater ecosystem health due to their sensitivity to environmental pollutants.

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Conflict of Interest of Statement

The authors declare that they have no conflicts of interest.

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