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Acute toxicity of copper sulphate to fresh water prawns

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Abstract: *Fresh water prawns, Macrobrachium lamarrei and Macrobrachium dayanum (Crustacea-Decapoda) were subjected to static bioassay tests to ascertain the LC_{50} values of copper sulphate. The 24, 48, 72 and 96hr LC_{50} values of copper sulphate for M. lamarrei were 0.38, 0.361, 0.343 and 0.300 mg/l and for M. dayanum were 1.634, 0.988, 0.532 and 0.418 mg/l respectively. Behavioral responses and LC_{50} values indicate that M. lamarrei were more sensitive to copper sulphate than M. dayanum.*

Key words: *Acute toxicity, Copper sulphate, LC_{50}, Crustacea, Macrobrachium.*

Introduction

Increased industrialization, urbanization and man's greed to over exploit nature has led to imbalance of various intrinsic and extrinsic elements of environment causing global problems in the form of different type of pollution. Among pollutants, heavy metals create serious threat to biosphere due to their property of bioaccumulation in different trophic levels of ecosystem (Salunk *et al.*, 1982; Berman and Lal 1994). Accumulation of heavy metals in aquatic environment occurs mainly by two sources: - (i) natural sources such as volcanic eruptions and soil erosion. (ii) by anthropogenic activities. Among these two factors, the later is prime factor of damage in aquatic environment and ultimately to man through food chain. Since most of the metals are the micronutrients for most of the living systems, evolution has provided no effective homeostatic mechanisms in living systems for these metals.

Copper, a "grey listed metal" (Mason, 1996) naturally occurs in rocks, soil, water, sediments and even in air (average concentration in earth crust is about 50ppm). Copper also occurs naturally in plants and animals as essential micronutrient to perform various physiological and biochemical processes, yet the hyper concentration (even a little bit more than needed) causes serious threat to life.

Copper salts (copper hydroxide, copper carbonate and copper sulphate) are widely used in agriculture as fungicide, algaecide and nutritional supplement in fertilizers. They are also used in veterinary practices and industrial applications.

Copper sulphate is released to water as a result of natural weathering of soil and discharge from industries, sewage treatment plants and agricultural run off. Copper sulphate is also intensively introduced in water reservoirs to kill algae. Thus excessive amount of copper accumulates in water bodies and cause toxicity to aquatic fauna and flora and ultimately to man. Copper and its compounds have been designated as priority pollutants by EPA (40 CFR 401.15).

Freshwater prawns (crustaceans), besides being important members of aquatic food chain, having high nutritional, medicinal and economical value for human beings, are greatly affected by these pollutants but are completely neglected (Murti and Shukla, 1984; Shukla and Sharma, 1993) as much emphasis was given to fishes. These aquatic creatures also serve as important biological indicators for aquatic pollution.

Keeping in view, the ecological, nutritive, academic and overall economic value of the freshwater prawns, present study was carried out on the fresh water prawns *Macrobrachium lamarrei* and *Macrobrachium dayanum* (Crustacea - Decapoda) to evaluate the LC_{50} values of copper sulphate to these prawns and to study the behavioral alterations due to heavy metal toxicity.

Materials and Methods

Freshwater prawns, *Macrobrachium lamarrei* and *Macrobrachium dayanum* (crustacea - decapoda) were collected from river Gomti with the help of local fisherman and were brought to laboratory and maintained in glass aquaria (Sharma and Shukla, 1990). The prawns were subjected to 5 to 7 days acclimation prior to experiments.

Stock solution of copper sulphate ($CuSO_4.5H_2O$: AR grade: BDH laboratories, chemical division) was prepared by dissolving 100mg of salt in 100ml double distilled water. Two drops of glacial acetic acid was added to stock solution so as to prevent the precipitation.

Physiological properties of the diluent water was analyzed according to APHA *et al.*, 1985 were as follows :-

pH- 7.0$\pm$0.2; DO- 7.0$\pm$0.1mg/l; Total hardness-268$\pm$ 2.5mg/l; Temp.- 26$\pm$2°C.

A series of six concentrations of test medium were prepared (toxic ranges determined by exploratory tests) in 20liter glass aquaria containing 10liter of diluents water. 10 healthy prawns of average weight (*M. lamarrei* – 1.107$\pm$0.26 gm; *M. dayanum* – 3.261$\pm$0.68 gm) and length (*M. lamarrei* – 4.86$\pm$0.55 cm; *M. dayanum* – 5.64$\pm$0.42 cm) were carefully introduced in each aquaria containing test medium. One aquarium having 10liters of diluent water only, served as

Table – 1: LC_{50} values of copper sulphate to fresh water prawns, *M. lamarrei* and *M. dayanum.*

Time (hr)	*M. lamarrei*			*M .dayanum*		
	LC_{50}	95% confidence limits (mg / l.)		LC_{50} (mg / l.)	95% confidence limits (mg / l.)	
24	0.380	0.304	0.684	1.634	0.836	3.192
48	0.361	0.304	0.418	0.988	0.722	1.330
72	0.343	0.304	0.380	0.532	0.418	0.684
96	0.304	0.304	0.380	0.418	0.343	0.532

Table – 2: LC_{50} values of copper sulphate for different species of crustaceans.

Species	LC_{50}	Duration (hr)	Reference
1. Other crustaceans			
C. rajadhari	$1x10^{-5}$M	96	Chinnayya, 1971
Caridina Sp.	281±14 µg/l	48	Ghate and Mulherkar, 1979
P. indicus	1.72 mg/l	48	Govindrajan *et al.*, 1993
Moina irrasa	3.30 mg/l	96	Zou and Bu, 1994
P. indicus	2.5350 mg/l	96	Chinni and Yallapragada, 2000
Mysis sp.	1.44 mg/l	96	Zyadah and Abdel-Bakey,2000
Paratelphusa hydrodroma	114.0 mg/l	96	Vardhanan and Radhakrishnan,2002
Penaeus monodon	1.20 mg/l	96	Joseph *et al.*, 2002
2. *Macrobrachium species*			
M. kistenensis	279±36 µg/l	48	Ghate and Mulherkar, 1979
M. lamarrei	0.247 mg/l	96	Murti and Shukla, 1984
M. hendersodayanum	1.75 mg/l	96	Patil and Kaliwal, 1986

control. Proper aeration was carried out with the air pumps. All tests were carried out up to 96hr following standard procedure (APHA *et al.*, 1985). Percent mortality in each aquarium was recorded at 24, 48, 72 and 96hr. The animals failing to response the gentle prodding were considered as dead.

Behavioral observations were taken in each test aquaria along with the comparison of control. Experiments were replicated thrice. The LC_{50} values and their 95% confidence limits were calculated according to trimmed Spearman-Karber method (Hamilton *et al.*, 1977) using a PC.

Results and Discussion

The LC_{50} values of copper sulphate at 24, 48, 72 and 96hr for *M. lamarrei* were 0.380, 0.361, 0.342 and 0.304 mg/l and for *M .dayanum* were 1.634, 0.988, 0.532 and 0.418 mg/l respectively. Results of bioassay tests like LC_{50} values and their 95% confidence limits have been summarized in Table 1. The LC_{50} values clearly indicate that *M. lamarrei* was more sensitive to copper sulphate than *M .dayanum*.

The bioassay tests have proved as an essential tool to evaluate chemical toxicity thereby much attention has been drawn on it during last few decades. Acute toxicity is caused by a relatively large dose of a chemical, the onset of symptom is sudden and the intensity of effects rises rapidly and may result to death (Pham-Hun-Chanh, 1965), therefore acute toxicity tests are most widely used methods for determining the toxic range of heavy metals. The animals used in the present study *viz M. lamarrei* and *M. dayanum*, fulfilled almost all major criterion for the test animals laid down by APHA *et al.*, 1985.

The LC_{50} values of copper sulphate found in the present study for both the species of prawns are comparable to earlier studies on the toxicity of copper sulphate to different crustaceans (Table 2).

It is interesting to infer that though major differences among LC_{50} values between marine and fresh water crustaceans does not exist, even then some of the fresh water crustaceans appear more sensitive than marine ones. The LC_{50} values obtained in present study are mainly closure to the findings of Ghate and Mulherkar (1979) and Murti and Shukla (1984). The minor difference in LC_{50} values for *M. lamarrei* may be due to different environmental conditions of the testing places. Furthermore comparing the LC_{50} values obtained in the present study *M. lamarrei* appears more sensitive to copper sulphate than *M. dayanum*, which may be due to the larger size and difference in living habits of the latter.

Peculiar behavioral alterations have been observed in both the species of prawn after exposure to copper sulphate during the acute toxicity tests. A quick response in the form of increased movement and increased chelepede scrapping of body parts was observed just after introduction into the test medium. The increased surface movements were observed in the first few hr of experiment, which normalized within 24hr of exposure, thereafter animals settled on the bottom of aquaria.

Animals became sluggish after 72hr of exposure with decreased chelepede scrapping and responded feebly to gentle paddling.

The mucous production on the gills and inner lining of branchiostegite and carapace started after 24hr of exposure and was maximum covering almost all the body parts after 96hr and was more prominent in the *M. lamarrei* than in the *M. dayanum*. In case of *M. lamarrei* the blackening of the undersurface of the carapace (just above the gill region), abdominal segments and some of the appendages were observed after 48hr of exposure which increased and became quite prominent after 96hr of exposure in 20% of the animals while no such blackening was observed in case of *M. dayanum*.

Acute toxicity induced behavioral alterations observed in the present study like increased swimming, surfacing movements and chelepede scrapping up to 24hr followed by a reduction in all activities up to maximum letharginess are quite comparable to the observations on other crustaceans (Ghate and Mulherkar, 1979; Murti and Shukla, 1984; Patil and Kaliwal, 1986; Sharma and Shukla, 1990), and which may be due to avoidance of animal to the toxicant.

Profused secretion of mucous on whole body parts, more pronounced in gill region was a protection device of the animals as it is well known that crustacean gills apart from the respiration are also the primary site of osmoregulation. Such type of mucous secretion was quite prominent in the findings of Ghate and Mulherkar (1979) on *Macrobrachium kistenensis* and *Caridina sp.*; Murti and Shukla (1984) on *M. lamarrei*; Patil and Kaliwal (1986) on *M. hendersodayanum*; Nagabhushanam and Sarojini (1989) on *M. kistenensis, M. lamarrei* and *Scylla serrata* and Sharma and Shukla (1990) on *M. lamarrei* after exposure to various toxicants. The mucous secretion found in the present study may be an important factor to combat the toxic effects of metal solution. Plonka and Neff (1969) suggested that excessive mucous secreted by fish gills in response to toxic levels of heavy metals result in death by suffocation as a result of mucous coagulation and precipitation with metals on gill surface. Similar type of behavioral responses have also been reported in fishes such as, *Puntius conchonius* (Pant *et al.*, 1980) and *S. gairdneri* (Miller and Mackey, 1982) after exposure to copper and in the reviews of Atchison *et al.* (1987) and Weber and Spieler (1994).

Decreased swimming, chelepede scrapping and surfacing movements after 96hr exposure might be due to certain alterations in muscle fibers as indicated by Schultz and Kennedy (1977). Decrease in locomotion speed, chelepede scrapping and decreased response to gentle paddling may also be linked with their damaged tango, vibro and chemo receptors and loss of olfaction as also observed in fishes (Bardach *et al.*, 1965; Cancalon, 1980), in *Daphnia* (Stratton and Corke, 1981) and in prawns and shrimps (Ghate and Mulherkar 1979; Sarojini and Gyananath, 1983; Nagabhushanum and Sarojini, 1989; Sharma and Shukla, 1990). The blackening of undersurface of carapace, abdominal segments and on some of the appendages as observed in the present study has also been reported by Nimmo *et al*., (1977) in shrimp after exposure to cadmium and by Ghate and Mulherkar (1979) in Alyid prawn *Caridina* after exposure to copper sulphate.

The metal ions have a toxic effect on epidermal tissues of gills and general body surface. Damage to this tissue may result in a route of entry for chitinoclastic bacteria which intern result in a “shell disease”. Chitinoclastic bacteria are a normal part of the bacterial flora of the shrimp (Hood and Mayer, 1974) and hence are opportunistic pathogen of shrimp. Nimmo *et al*. (1977) concluded that the cell death in the gill tissue leads to gill blackening in crustaceans. The blackening could be probably of a metallic sulphide.

Although behavior has been shown to be a sensitive indicator of chemically induced stress in aquatic organisms particularly for crustaceans as also revealed in the present study but there are no standardized adequate data for behavioral responses that should be established for earliest detection of toxicity of various chemicals.

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