



RESEARCH ARTICLE

Effect of sub-acute exposure of copper sulphate on oxygen consumption and scaphognathite oscillations of fresh water prawn *Macrobrachium lamarrei* (Crustacea- Decapoda)

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Abstract

Heavy metal pollution is the out come of population explosion, unplanned industrialization and various natural processes like soil erosion, weathering of rocks and volcanic eruption across the globe. Heavy metals after entering into environment ultimately finds their way into aquatic ecosystem and affects aquatic flora and fauna as well as human beings. Copper, though an essential micronutrient, when present in excess causes various deleterious effects on aquatic flora and fauna by altering their behaviour, physiology and histoarchitecture. Smaller fresh water prawns are important members of aquatic food chain and having economical as well as medicinal value. Noninvasive parameters like behavioural patterns, scaphognathite oscillations, haemocyte profile, heart beat rate and oxygen consumption rate etc. can be used to assess environmental impact without sacrificing the animal.

Present study deals with the effect of copper sulphate (0.075 mg/L, 25% of 96 h LC 50) on oxygen consumption rate and scaphognathite oscillations of fresh water prawn, *Macrobrachium lamarrei* (Crustacea-Decapoda) for 10, 20 and 30 day exposure. oxygen consumption was found continuous decreasing upto 30 days ($F = 35.35, p \leq 0.01$) than controls whereas scaphognathite oscillations were decreased after 10 days, thereafter further increased after 20 days and finally tend to decrease after 30 days ($F = 0.74, p \leq 0.05$) than controls. Mechanism of copper toxicity in reference to respiration; role of both noninvasive parameters as biomarkers and role of *M. lamarrei* as bioindicator in relation to heavy metal copper has also been discussed.

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Introduction

Fresh water prawns (Crustaceans), besides being important members of aquatic food chain, having high nutritional, medicinal and economical value for human beings are greatly affected by pollutants (Shukla and Sharma, 1993, Verma et al., 2010). Increased industrialization, urbanization during last decade have provoked some serious concern for the environment. Heavy metal contamination in river is one of the major quality issues in many fast growing cities, because maintenance of water quality and sanitation infrastructure did not increase along with population and urbanization growth (Akoto et al., 2008; Reza and Singh, 2010 and Ahmed et al., 2010). Some of the heavy metals are essential to living organisms and they are commonly found in natural waters but high concentration and accumulation of them may become so toxic.

Copper is "grey listed metal" (Mason, 1996) and occurs naturally in plants and animals as essential micronutrient to perform various physiological and bio-chemical processes, yet the hyper concentration (even a little bit more than needed) causes serious threat to life. Copper concentration far exceeds the background of copper level of water

(0.5 µg /l). Copper sulphate is used in fresh water aquaculture to control external parasites, bacterial diseases herbicide and also as fungicide (Reddy et.al.,2006). 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. Thus excessive amount of copper accumulates in water bodies and cause toxicity to aquatic fauna and flora and ultimately to man. Copper salts are widely used in agriculture as fungicide, algacide and nutritional supplement in fertilizers. Due to toxic effects of dissolved copper, shrimp hatcheries routinely use water that treated with ethylenediamine tetra acetic acid to chelate free copper. In decapode crustaceans, three types of circulating hemocytes are recognized: hyaline, semigranular and large granular cells (Tsing et.al.,1989). They are involved in cellular immune responses that include phagocytosis and constitute the primary method of eliminating micro-organisms or foreign particles (Bayne et.al.,1990). In addition to phagocytosis, hemocytes are involved in coagulation and in the production of melanin via the prophenoloxidase system (Johansson and Soderhall,1989 , Soderhall and Johansson,1996). Several physico-chemical parameters and environmental contaminants have been reported to affect the immune response in crustaceans and there have been reviewed by (Moullac and Haffner,2000).

Keeping in view, the ecological, nutritive, academic and overall economic value of the fresh water prawns, present study was carried out to evaluate the effects of copper sulphate on scaphognathite oscillations and oxygen consumption of fresh water prawns *Macrobrachium lamarrei* , potential animal for fresh water aquaculture.

Materials and Methods

Fresh water prawn, *Macrobrachium lamarrei* were collected from river Gomti, Lucknow (U.P.), India with the help of fisherman and brought the laboratory (N -26° 49' 55'' E- 80° 55' 58'') in large plastic containers. The animal were maintained in glass aquaria of 20 L capacity in dechlorinated water having physico-chemical characteristic as follows-pH 7.80 ± 0.18 mg/L, temperature 28°C, dissolved oxygen 10.7 ± 0.80 mg/L, hardness- 292 ± 2.3 mg/L, alkalinity 78 ± 1.6 mg/L (APHA,1998). Stock solution of CuSO₄, AR grade, molecular weight 249.68 gm/mole, manufacture by quickems fine chemicals pvt.Ltd., New Delhi, 110060 India was prepared by dissolving weighed amount of salt in double distilled water. Two drops of glacial acetic acid was added to prevent the precipitation.

Adult inter-moult staged *M.lamarrei* (Length-4.83 ± 0.54, weight 1.108 ± 0.23 gm) were being utilized in experiments after 5-7 days laboratory acclimation. Acute exposure was carried out on predetermined 25% of 30 days LC-50 value of copper sulphate (0.075 mg/L). One aquarium with dilute water only served as control for each set. Food supply and change of medium in test aquarium was given on each alternate day and throughout experiment. Continuous air supply was provided with the help of air pumps and diffusers to both control as well as test aquaria. Experiments were replicated thrice and carried out according to guidelines of APHA (1998). General behaviour conditions, heart beat and oxygen consumption was observed after 10 days, 20 days and 30 days. Scaphognathite oscillations were recorded under stereoscopic dissecting binocular microscope with the help of stop watch from both control and experimental animals. The respiratory rate was determined by hourly monitoring of dissolved oxygen contents according to Winkler's method (APHA,1998). The oxygen consumption rate was calculated as oxygen consumed mg/ L/gm. Body weight/days. Experiment was replicated thrice and data were subjected to statistical analysis for student " t " test and ANOVA using MINITAB software on PC.

Results & Discussion

Response of sub-acute toxicity of copper sulphate on , behavioural alterations , oxygen consumption and scaphognathite oscillations in fresh water prawn, *M. lamarrei* have been summarized in Table-1 and 2 , Figure 1 & 2. Acute toxicity is caused by a relatively large dose of chemical, the onset of symptom is sudden and the intensity of effects rises rapidly and may result death (Shuhaimi-Othman, M. et.al.,2011), therefore acute toxicity tests are most widely used methods for determining the toxic range of heavy metal. Long term exposure was carried out on 96 h LC-50 values (0.075 mg/L ; 25% of 96 h LC-50), experimental animals resided at bottom of aquaria became less active to gentle prodding and light stimuli. Mucous secretion was observed at carapace , gills and abdominal region. Exposed animals consumed less food due to inability to detect the food. Blackening in gills, carapace and abdomen was noticed in 50% animals after 30 days exposure. Acute toxicity induced behavioural alterations are quite comparable to the observation on other crustaceans (Sharma and Shukla,1990 ; Yang et.al.,2007, Murti and Shukla,1984). Fresh water prawns appear to be more sensitive to copper than most other species of crustaceans that have been studied. Na et.al. (2008) studied the toxic effect of copper on the giant fresh water prawn *Macrobrachium rosenbergii*, they recorded that, exposure to elevated copper levels might damage the ultrastructure of gills and might further weaken their normal physical activities. The structural alterations observed in the gills in the present study were similar to those of *M.lamarrei*, *M. dayanum* exposed to copper (Lodi et.al.,2006), *M.rosenbergii* (Kaoud and Ahmed, 2013), profused secretion of mucous on whole body parts and more pronounced in gill region. In long

term exposure experimental prawns showed continuous decline trend in oxygen consumption rate through out the experiment from 10 days to 30 days exposure than control animals. The differences between means of control and experimental prawn were highly significant at 10 days ($t=36.23$, $p \leq 0.01$) and 30 days ($t=48.76$, $p \leq 0.01$) exposure. The overall changes were significant in control ($F= 28.66$, $p \leq 0.01$) as well as in experimental animals ($F=35.35$, $p \leq 0.01$).

Experimental prawns showed continuous decline trend in oxygen consumption (0.355 ± 0.0072 , 0.256 ± 0.0036 , 0.228 ± 0.0018 mg/ L/gm. Body weight/h) throughout the experiment (10,20 and 30 days exposure) than control ones. The differences between means of control and experimental animals were highly significant at 10 days ($t=15.99$, $p \leq 0.01$), 20 days ($t=36.23$, $p \leq 0.01$) and 30 days ($t= 48.76$, $p \leq 0.01$) as well as in experimental animals ($F=28.66$ $p \leq 0.01$). Continuous decrease in oxygen consumption as observed in present study has also been reported in various crustaceans (Murti and Shukla, 1984, Reddy and Venugopal, 1993; Osunde et.al., 2003; Yang et.al., 2007; Verma, D.R., 2010). Change in oxygen consumption rate are a good index to measure altered metabolic activity in organism exposed to various toxic metals in surrounding medium. Similar effect has also been reported in other crustaceans (Moullac and Haffner, 2000, Lombardi et.al., 2000). Frias-Eepericueta (2008) studied the effect of three concentrations of copper (3.512, 1.756 and 0.877 mg/l) on juvenile *Litopenaeus vannamei* and found that there were severe time and dose dependent structural damages such as necrosis, loss of regular structure and infiltration of haemocytes in the gill tissues, as well as atrophy, necrosis and irregular tubular structure in the hepatopancreas, thus affecting the exchange of gases and the fusion of lamellae causes a decrease in the total respiratory area of the gills, resulting in a decreased oxygen uptake capacity of fish gills (Nowak, 1992).

Scaphognathite oscillations of experimental animals (169.2 ± 2.16) were found decreased than the controls (175.4 ± 1.74) ones ($t= 2.23$, $p \leq 0.05$) after 10 days of exposure. A further increase in heart beat was noticed in experimental animals (171.3 ± 1.87), which was below controls (177.2 ± 1.27) after 20 days exposure. Increase of scaphognathite oscillations may be due to avoidance reaction followed by hypoxia due to metal induced irritation and coagulation of mucous on gill surface (Cheng and Chen, 2001). A further decrease in experimental animal animals (165.7 ± 2.68) was noticed after 30 days of exposure than the controls (176.9 ± 1.84) ($t= 3.44$, $p \leq 0.01$). The overall changes in heart beat of experimental animals were significant ($F= 0.74$ and $p \geq 0.05$). The further decrease in heart beat in later stage of experiment may be due to altered carbohydrate metabolism (Radhakrishnaiah and Bussapa, 1986) or due to alterations in muscle fibres operating scaphognathite oscillations (Schultz and Kennedy, 1977). The decrease in scaphognathite oscillation may be result of some neurological impairment. Almost similar alterations in ventilator structure have been reported in other crustaceans (Patil and Kaliwal, 1986, Sharma and Shukla, 1990, Cheng and Chen, 2001).

The present study reveals that knowledge of the toxicity of copper will be helpful to water quality management in fish farms with specially to prawn culture. oxygen consumption rate and scaphognathite oscillations can be used as monitoring tools to assess the status of aquatic body in reference of heavy metal pollution, which is global concern now days.

Table-1: Effect of Copper sulphate on oxygen consumption in *Macrobrachium lamarrei*.

Duration of exposure (Days)	Rate of oxygen consumption (mg/ L)/ gm body weight/h	
	Control	Exposed
10 days	0.484 \pm 0.0038	0.355 \pm 0.0072
20 days	0.445 \pm 0.0037	0.256 \pm 0.0036
30 days	0.432 \pm 0.0038	0.228 \pm 0.0018

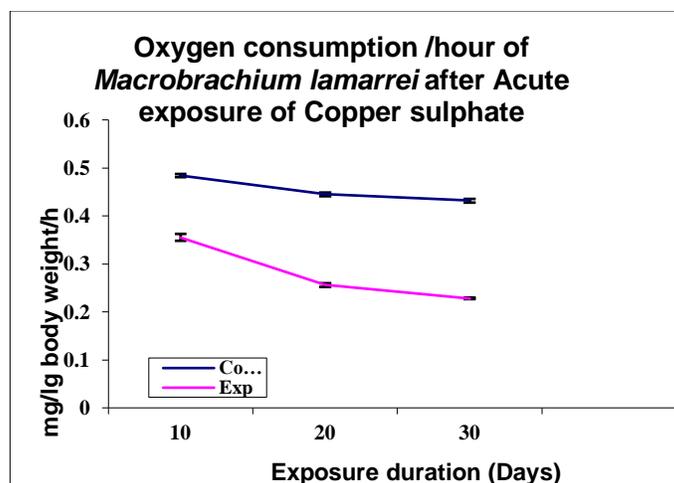
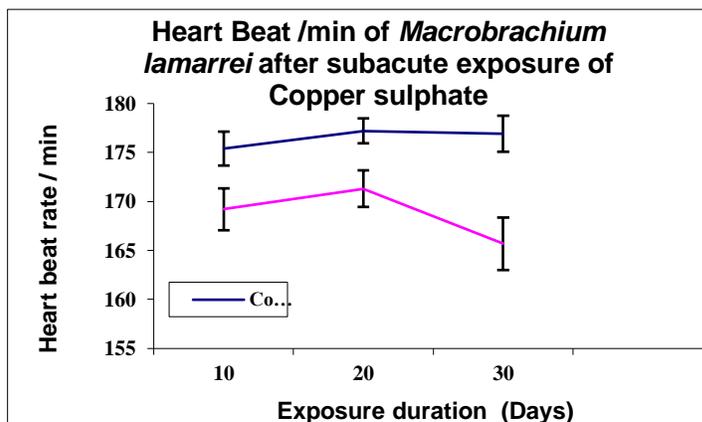


Table-2: Effect of Copper sulphate on scaphognathite oscillations in *Macrobrachium lamarrei*.

Duration of exposure (Days)	scaphognathite oscillations / min (Mean \pm S.E.)	
	Control	Exposed
10 days	175.4 \pm 1.74	169.2 \pm 2.16
20 days	177.2 \pm 1.27	171.3 \pm 1.87
30 days	176.9 \pm 1.84	165.7 \pm 2.68

Values are mean \pm S.E.



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