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Acute and chronic toxicity of copper on aquatic insect *Chironomus ramosus* from Assam, India

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Abstract

Acute toxicity of copper (Cu) on *Chironomus ramosus* was determined by exposing third-instar larvae to graded concentrations of copper sulphate ($CuSO_4 . 5H_2O$). Median lethal concentrations (LC_{50}) of Cu as $CuSO_4$ at 24, 48, 72 and 96 hr were determined as 3280, 1073.33, 780, and 183 $\mu g\ l^{-1}$, respectively. For determining the effects of chronic toxicity, small first-instar larvae were individually exposed to sublethal concentrations of copper sulphate (1.0-18.0 $\mu g\ l^{-1}$) for a period of 21 days. Discoloration and thinning of body were detected at 1 $\mu g\ l^{-1}$ and ventilation movements, pupation and adult emergence were significantly affected at 1.8 $\mu g\ l^{-1}$. At 10 $\mu g\ l^{-1}$ $CuSO_4$ concentration, growth and tube-building activities of the larva were significantly different from the control.

Key words

Acute and Chronic toxicity, Median lethal concentrations, *Chironomus ramosus*, Copper sulphate

Introduction

Copper (Cu) is an essential trace element that beyond certain threshold levels can be extremely toxic and readily available to aquatic organisms. Elevated levels of Cu in water is long known to adversely affect survival, growth, reproduction, feeding and even cause morphological deformity (Hodson *et al*., 1979). Members of the Dipteran family Chironomidae are known to find wide use in single species acute and chronic toxicity testing (Rosenberg, 1992) and this group of insects, along with odonates and trichopterans, have been recommended for biomonitoring of metal pollution in freshwater systems (Zhou *et al*., 2008). The cupric ion was found to be more toxic to *Chironomus plumosus* larvae than the cuprous ion as well as several other metals like manganese, nickel, vanadium and molybdenum (Fargasová, 1998). Among lindane, Cu, dichloroacetic acid (DCA) and atrazine, Cu was next to lindane in toxicity to *C. riparius* larvae (Taylor *et al*., 1991). Available literature suggests that the toxicity of Cu can vary considerably among larvae of different species of the genus *Chironomus*. The 96 hr LC_{50} for the third instar larvae of *C. tentans* was found to be 1446 $\mu g\ l^{-1}$ for Cu (Nebeker *et al*., 1984) and 17.2, 18.55 and 18.21 mg l^{-1} for copper chloride, sulphate and nitrate, respectively (Warrin *et al*., 2009). For the fourth instar larvae of *C. decorus,* the 48 hr LC_{50} of anhydrous $CuSO_4$ was found to be 739 $\mu g\ l^{-1}$ (Kosalwat and Knight, 1987a). The 72 hour LC_{50} of Cu for the larva of a tropical chironomid *C. crassiforceps* was 0.64 mg l^{-1} in water with pH 6.0 (Peck *et al*., 2002). In contrast, the 96 hr LC_{50} of Cu in another chironomid *Tanytarsus dissimilis* was 16.3 $\mu g\ l^{-1}$, Cu being less toxic than Cd but more toxic than Zn and Pb in this species (Anderson *et al*., 1980). In *Chironomus thummi* larvae, Zn showed the highest toxicity followed by Pb and Cu (Bat and Akbulut, 2001). *C. riparius* was more sensitive to Cu than *Hyalella azteca*, *Heptagenia* spp. and *Tubifex tubifex* (Danielle *et al*., 2003).

In terms of chronic, sublethal toxicity, Kosalwat and Knight (1987b) found larval growth to be the best endpoint of chronic Cu toxicity with larval deformity as a "quick tool" to detect Cu pollution, while time taken for adult emergence was not found to be an effective endpoint. However, Cd exposure resulted in reduced emergence rate in *C. riparius*, and the reduction was proportional to both concentration and duration of exposure (McCahon and Pascoe, 1991). Copper along with other heavy metals such as Hg, Cd, Pb and Zn was shown to induce deformities in chironomid larvae (Janssens de Bisthoven *et al*., 1998; Bhattacharyay *et al.,* 2005). The self-protective mechanisms employed by the larva include construction of tubes, which in *C. luridus* protect them from toxins such as copper sulphate, besides serving other functions in feeding, respiration, and as an anti-predation shelter (Halpern *et al*., 2002).

The state of Assam in the northeastern region of India is a part of the Indo-Burma 'biodiversity hotspot' (Myers *et al*., 2000) and harbours various freshwater ecosystems such as large rivers, hill streams, floodplain lakes, swamps and marshes, ox-bows and the like. Metal contamination of these ecosystems could consequently be of great ecological and conservational concern. Besides information on the nature and extent of metal bioaccumulation by aquatic taxa (Gupta, 1995, 1996, 1998), it is also necessary to establish comprehensive regional toxicological databases (Buikema *et al*., 1982) for xenobiotic substances such as pesticides and heavy metals, ideally using indigenous species, for setting effective standards to control and regulate the entry of toxicants into the freshwater systems of this region. The present study therefore attempts to investigate the acute toxicity of $CoSO_4.5H_2O$ on the third instar larvae of *C. ramosus*, as well as the effects on sublethal endpoints such as growth, emergence, pupation, ventilation movement and tube construction.

Materials and Methods

Third instar larvae (6-7mm) of *C. ramosus* were collected from a small stream in Haflong, North Cachar hills district, Assam, and acclimatized for 1 week in laboratory conditions and provided clumps of *Spirogyra* filaments as food. The larvae were starved during the experimental period and exposed to graded concentrations of copper sulphate ($CuSO_4$. $5H_2O$, from Qualigens) at: 100, 180, 320, 560, 1000, 1800, 3200 and 5600 µg l^{-1} by making appropriate dilutions from a stock solution of 10000 µg l^{-1}. The concentrations of Cu are nominally expressed as that of $CuSO_4$.

For determining LC_{50} values, 10 larvae were placed in 100 ml of test solution of each concentration in PVC containers of 200 ml capacity. The larvae were starved during the 4 day exposure. Mortality criterion was failure to respond to light probing with the tip of a blunt needle, and was recorded at 24, 48, 72 and 96 hr. Dead organisms were removed and test solutions renewed every 24 hr. Each concentration was tested in 10 replicates. Acute toxicity of $CuSO_4$ was determined by estimating median lethal concentrations (LC_{50}) with the help of log-probit analysis (Finney, 1971; Buikema *et al*., 1982) using SPSS 10 software for Windows.

For chronic toxicity studies small first instar larvae (1-2 mm) were exposed individually to 1.0, 1.8, 3.2, 5.6, 10 and 18 µg l^{-1} $CuSO_4$. The length of each larva was measured under a stereoscopic binocular microscope fitted with an ocular micrometer and it was placed individually in 100 ml of test solution in a PVC container. Twenty larvae were exposed to each concentration of the toxicant. One set of control comprising 20 larvae was also similarly maintained in toxicant free test solution during the experiment. Solutions were removed and mortality checked every 24 hr and dead larvae, if any, were removed and measured. Growth was recorded at 7 day intervals and finally expressed as mm d^{-1}. Each larva was provided approximately equal amount of *Spirogyra* filaments as food. The larvae also constructed tubes out of the algal filaments. Percentage of larvae undergoing pupation and emergence, able to construct tubes and produce ventilation movements were noted. Other changes such as discoloration, thinning and longitudinal contraction of body could only be visually assessed. The duration of the test was 21 days.

Statistical analysis comprised one-way analysis of variance (ANOVA) for estimating the significance of difference among treatments and control and Tukey test for multiple comparisons, using SPSS 10 for Windows.

Results and Discussion

Mean 24, 48, 72 and 96 hr LC_{50} values for Cu as $CuSO_4.5H_2O$ in the larvae of *Chironomus ramosus* are presented in Table 1. The 24 hr LC 50 for $CuSO_4$ was 3280 µg l^{-1} which sharply declined to 1073.33, 780 and 183 µg l^{-1} at 48, 72 and 96 hr, respectively. The 48 hr LC_{50} for anhydrous $CuSO_4$ was 739 µg l^{-1} in *C. decorus* fourth instar larvae (Kosalwat and Knight, 1987a); and the 96 hr LC_{50} in *C. riparius* and *Tanytarsus dissimilis* 43 and 16.3 µg l^{-1}, respectively (Danielle *et al*., 2003; Anderson *et al*., 1980). These values are considerably lower than the corresponding

Table - 1: LC_{50} values of $CuSO_4.5\ H_2O$, for *Chironomus ramosus* 3rd instar larva from Assam

Hours of exposure	LC_{50} (µg l^{-1})	95 % CL
24	3280	3124-3436
48	1073	929-1217
72	780	735-825
96	183	177-189

CL = Confidence limit, LC_{50} = Median lethal concentrations

Table - 2: Toxicity of $CuSO_4$. $5\ H_2O$, on different sublethal end parameters on *Chironomus ramosus* 1st instar larva from Assam

Treatments	End parameters				
(µg l^{-1})	Growth (mm d^{-1})	Tube formation (%)	Ventilation movement (%)	Pupation (%)	Emergence (%)
Control	0.18 ± 0.08	70 ± 16.6	100 ± 0	60 ± 12.8	60 ± 16.6
1.0	0.14 ± 0.06	70 ± 16.6	100 ± 0	50 ± 6.1	49 ± 10.8
1.8	0.13 ± 0.06	70 ± 17.0	80* ± 11.2	45* ± 9.4	40* ± 9.4
3.2	0.11 ± 0.07	65 ± 17.3	70* ± 9.4	30* ± 7.9	20* ± 6.1
5.6	0.10 ± 0.07	45 ± 16.6	50* ± 6.1	25* ± 3.5	20* ± 6.1
10	0.07 ± 0.03*	40* ± 16.2	40* ± 5	0	0
18	0.07 ± 0.04*	30* ± 14.6	30* ± 3.5	0	0

Values are mean of ten replicates ± S.D, * Indicates significant differences with control at $p \leq 0.05$

values in *C. ramosus*, indicating higher vulnerability to Cu in the former two species. On the other hand, *C. tentans* with 96 hr LC$_{50}$ of 1446 μg l^{-1} for Cu (Nebeker *et al*., 1984) and 18.55 mg l^{-1} for CuSO$_4$ (Warrin *et al.*, 2009) appears to be more tolerant of Cu toxicity. The 72 hr LC$_{50}$ of 780 μg l^{-1} in *C. ramosus* is comparable to that of 640 μg l^{-1} reported in a tropical chironomid *C. crassiforceps* in water with pH 6 (Peck *et al*., 2002). The average pH and dissolved oxygen content of test water were 6.8 and 6.6 mg l^{-1}, respectively. Thus, the available evidence suggests that the spectrum of Cu sensitivity in Chironomidae is fairly wide having both highly sensitive and tolerant species with *C. ramosus* occupying a somewhat intermediate position. Variations also exist among different chironomid species in their relative vulnerability to heavy metals: Cu was found to be at least 100 times more toxic than Cd, Al and Zn in *C. plumosus* (Fargasova, 2001). Conversely, Anderson *et al.* (1980) found Cu to be less toxic than Cd, but more toxic than Zn and Pb in *Tanytarsus dissimilis*. Thus, there appear to be considerable intergeneric, if not interspecific, differences in response to metal toxicity in chironomids. Among other groups, larvae of the odonates *Pachydiplax longipennis* and *Erythemis simplicicollis* were found to be more vulnerable to Cu than Cd or Pb, mortality from the former recorded at concentrations above150 μg l^{-1} (Tollett *et al*., 2009). This compares well with the 96 hr LC$_{50}$ of 183 μg l^{-1} found in the present study for *C. ramosus*.

Effects of exposure to sub lethal concentrations of Cu on growth, mortality, morphology, movement, tube formation, pupation and emergence of *C. ramosus* are shown in Table 2. The average growth in larval length per day was 0.18 mm in control, which was significantly higher than that in larvae exposed to 10 and 18 μg l^{-1} CuSO$_4$. Kosalwat and Knight (1987b) found larval growth to be the best endpoint of chronic Cu toxicity, while time taken for adult emergence was not found to be an effective endpoint. In contrast, pupation and emergence were found to be one of the most sensitive indicators of sublethal Cu toxicity in *C. ramosus*, along with ventilation movements of the larvae, both of which were significantly reduced at 1.8 μg l^{-1}. Paumen *et al*. (2008) also found emergence to be a powerful endpoint for detecting life cycle effects of polycyclic aromatic compounds (PACs) in *C. riparius*. Tubes constructed by the larvae of *C. luridus* were found to protect them from toxicants such as CuSO$_4$ (Halpern *et al*., 2002). In the present study, tube building in *C. ramosus* was significantly reduced at 10 μg l^{-1}. At this concentration growth also showed significant reduction. It is probable that as the tube building capacity is increasingly impaired, the larvae become more vulnerable to Cu toxicity that suppresses growth and finally cause mortality. *C. ramosus* larvae were observed to undergo thinning and discoloration from red to white starting at 1.0 μg l^{-1}. Although these two effects could only be visually assessed and not quantified in the present investigation, they have the potential to be used as early indicators of Cu toxicity that could also be easily monitored visually. While examining the effects of chloramines and Cu on the larvae of *C. luridus*, Halpern *et al*. (2002) observed larval color change from red to white when exposed to high concentrations of chloramines, but not Cu. Higher mortality from Cu observed in many aquatic insect species might be due to the ability of aquatic insects like dragonfly larvae to more readily bioaccumulate Cu (Tollett *et al*., 2009). Thus, it appears that although Cu is an essential trace element for most animals including insects, it could be potentially highly toxic beyond certain threshold tolerance limits, which for *C. ramosus* is about 1.8 μg l^{-1} CuSO$_4$ after a 21 day exposure. This is about 1/100th of the 96-h LC$_{50}$ value, thereby indicating its high long-term toxicity. Copper sulphate is still commonly used in the study area as well as in the other parts of India and many developing countries to control algal blooms in water bodies including fish ponds. It is also used as a fungicide in agricultural fields and tea gardens of the study area wherefrom it could be transported into the freshwater ecosystems via surface run off. Therefore, the high toxicity of CuSO$_4$ to non-target organisms like aquatic insects needs to be taken into careful consideration before applying it indiscriminately in freshwater systems.

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