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EFFECT OF COPPER SULPHATE ON THE RESPIRATORY TISSUES OF DRAGON FLY NYMPHS (ANISOPTERA) AT VARIABLE TEMPERATURE

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ABSTRACT
Assessment of toxicity on aquatic insects has gained significance for their suitability in bioassay studies. They also act as indicator species for many toxicants including heavy metals. In the present investigation dragonfly nymphs were selected as odonate larvae are important organisms in aquatic ecosystems. The present study tries to establish a correlation between the copper uptake and associated histological alterations and the subsequent changes in the respiratory tissues at different temperature ranges. It was found that accumulation of copper increased with duration of exposure till it reached a threshold limit beyond which the dragon fly nymphs failed to survive. The absorption of copper inside the body of the nymph also led to the deformities in the tracheal tissue as revealed by scanning electron micrograph investigation.

INTRODUCTION

The physiology of aquatic insects is governed by several factors including their respiratory strategies, life cycle, distribution and tolerance to pollutants. Aquatic insects constitute only 3-5% of total insect species but are highly diverse taxonomic groups (Daly *et al*., 1998). They form an integral part of both terrestrial and aquatic food chains. Moreover, they are often used to determine water quality based on number and type of species present. Laboratory bioassays are performed in order to determine toxicological effects of pollutants on aquatic insects. These can be in the form of toxicants, domestic and industrial wastes, which find their way into the aquatic water bodies or as surface runoffs in the form of toxic heavy metals. Such bioassays are commonly used to ascertain effects on non-target aquatic insect species and thus have a direct relationship to environmental protection Hilsenhoff, 1988; Plafkin *et al*., 1989. Although aquatic insects are mostly used to assess water quality, their respiratory strategies have received little attention (Cooper, 1994). Research works have demonstrated the importance of biological barriers in relation to the mechanism of contaminant uptake and fluxes in aquatic insects (Saouter *et al.,* 1991). Elevated temperatures pose major problems to aquatic organisms (Buchwalter *et al.,* 2003). However, few physiological studies with insects assess the role that respiratory system morphology plays in determining temperature responses in different aquatic insect species and their correlation with heavy metal uptake. The uptake of heavy metal pollutants in aquatic insects is the result of many variables such as the concentration of the pollutant in the water, the physico-chemical form of the pollutant, the membrane permeability of the organism, the type and quantity of food and its degree of contamination, the physiological state of the organism and the characteristics of the physical environment. The temperature range and respiratory strategies influence the organism as well as the pollutant.

Copper is an essential element involved in numerous physiological processes, hence it is important element for all organisms. However, Copper can be extremely toxic, depending on its concentration in water. The toxicity of Copper depends on a variety of factors including chemical and physical characteristics of water and the biology of the species (Fernandez and Mazon, 2003). The present paper deals with effect of copper sulphate on the respiratory tissues of Dragon fly nymphs under variable temperature regimes.

MATERIALS AND METHODS

Collection and maintenance of test insects

Live dragonfly nymphs were collected from local ponds of Ranchi. They were taken to the laboratory in polythene bags from their respective locations. The insects were acclimatized to the laboratory condition prior to experimentation.

Water quality assessment

The control and test waters were analysed for the determination of pH, conductance, DO and TDS by autoanalyser (model EI- 161E).

Exposure period

Separate glass jars were maintained for control and Cu treatment groups. Insects were exposed to copper salt ($CuSO_4$) for 72 and 96 hr at different temperature ranges (10°C, 15°C, 20°C and 25°C).

Calculation of LC_{50} at 72 and 96 hr

LC_{50} of Cu in test insect was determined by Probit analyses (Finney, 1971) by using the software: 'Biostat 2008' at different temperature ranges.

Copper accumulation studies

For dose selection 1/4th of the LC_{50} value at 72 hr and 96 hr and at different temperature range were administered. At the end of stipulated time insects were pooled together weighing approximately 1.00g wet weight for both control and treated groups. The samples were then digested with 0.1 N nitric acid in water bath and analyzed for the assessment of Cu uptake by ICP (Perkin Elmer, USA, DVD 2100) at RDCIS Analytical Laboratory, Ranchi.

SEM of the respiratory tissues

SEM studies were performed to assess the histological changes due to Cu exposure. Live odonate nymphs were cold anaesthetized and then the complete portion of rectal gill chamber was dissected out. The tissues were fixed in 2.5% Gluteraldehyde in 0.1 M sodium cacodylate buffer for four hrs and dehydrated in graded alcohol and dried by critical point dry (CPD) method (Hitachi, Model HCP-2). Finally the tissues were gold coated (Eiko Engineering, Model-IB-2) and examined under scanning electron microscope (Hitachi, Model- S 530).

Statistical analysis

Multi data analysis was performed using Stagraphic Centurion software for analysis of ANOVA to find out the significant difference in the uptake rate of Cu between 72 hr and 96 hr treatment groups. To determine which means are significantly different from which others, Multiple Range Tests was performed, represented graphically and range of spread of individual data was shown by plotting Box and Whisker Plots.

RESULTS

LC_{50} determination

LC_{50} value of Cu was calculated at 10°C, 15°C, 20°C and 25°C. The LC_{50} value gradually decreased from 72 to 96 hr along with temperature (Fig. 1) and it agrees with the finding of Sprague (1969) that LC_{50} value is dependent upon test species and experimental factors. 10 replicates for each dose were taken and a control was taken simultaneously. Mortality was recorded for each replicate. The calculated Probit value was 0.60±0.06 at 10°C which decreased to 0.41±0.05 at 25°C at 72 hr. While after 96 hr exposures the calculated values declined from 0.55±0.05 at 10°C to 0.40±0.05 at 25°C.

Observation of activity

With increase in temperature, duration and toxicant nymphs showed rapid trips to the surface. The rate of jet propulsion markedly increased, which gradually reduced with duration of exposure.

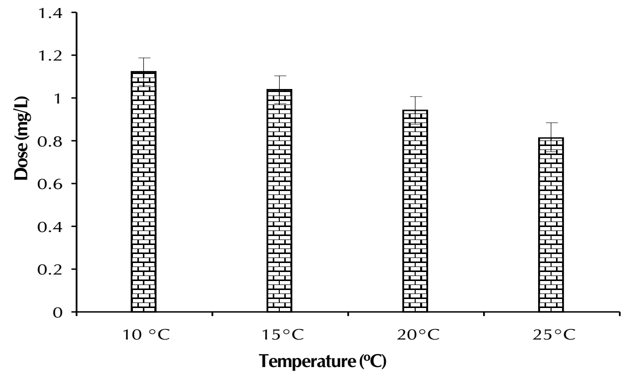

Figure 1a: 72 hr LC_{50} value of Cu(mg/L) in Dragonfly nymph at different temperature

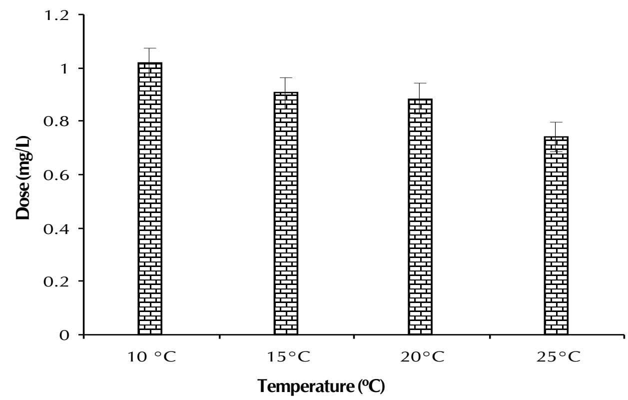

Figure 1b: 96 hr LC_{50} value of Cu(mg/L) in Dragonfly nymph at different temperature

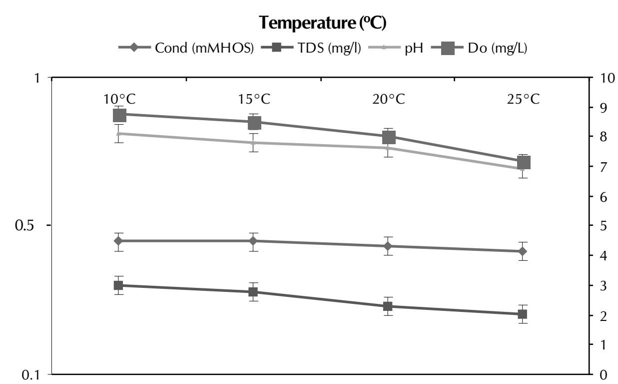

Figure 2: Water parametres of aquaria water at control condition

Water Quality Parameters

The control and test waters were analyzed to assess the physical parameters and also to ascertain whether the values are conducive for the test insects. It was observed that there were no significant changes with alteration in temperature. The pH varied from 8.1±0.08 at 10°C to 6.9±0.07 and 6.1±0.1 to 5.2±0.06 at 25°C, conductance from 0.26±0.003 to 0.28±0.003 and 1.14±0.003 to 1.18±0.002 μ mho, DO from 8.77±0.04 to 7.2 ± 0.06 mg/L and 8.62±0.03 to 6.54±0.04 mg/L while TDS (mg/L) in control water varied between 0.16± 0.003 at 10°C to 0.2± 0.004 at 25°C and

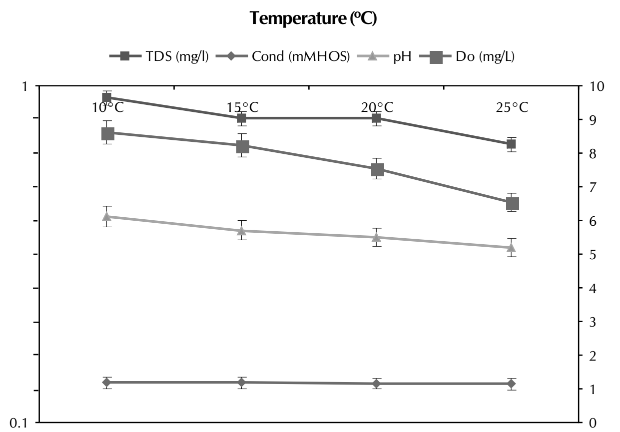

Figure 3: Water parameters of aquaria water when treated with Cu

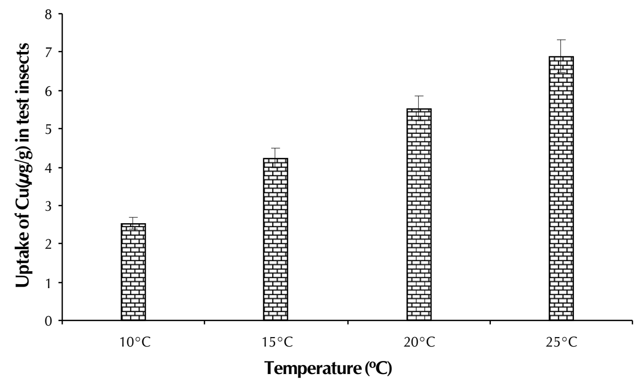

Figure 4b: Cu uptake at 96 hr in Dragonfly nymph

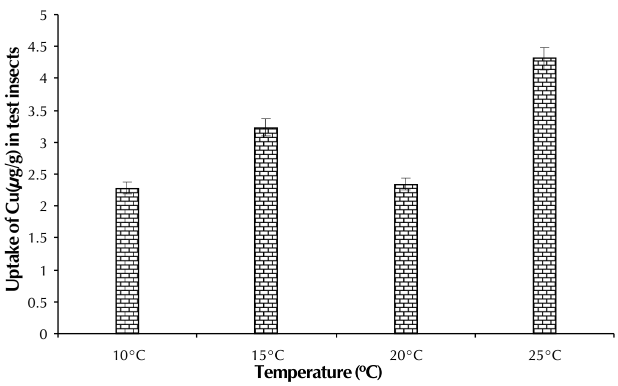

Figure 4a: Cu uptake at 72 hr in Dragonfly nymph

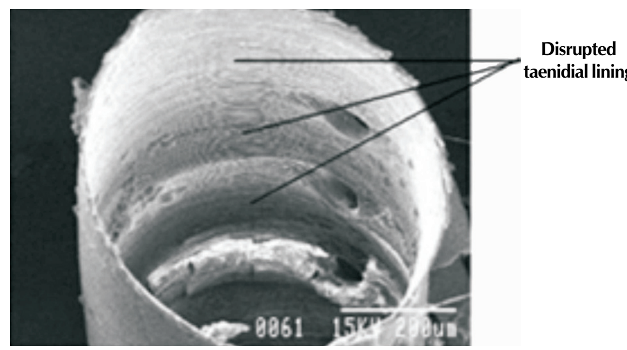

Figure 7: Section of trachea treated with Cu after 96 hr

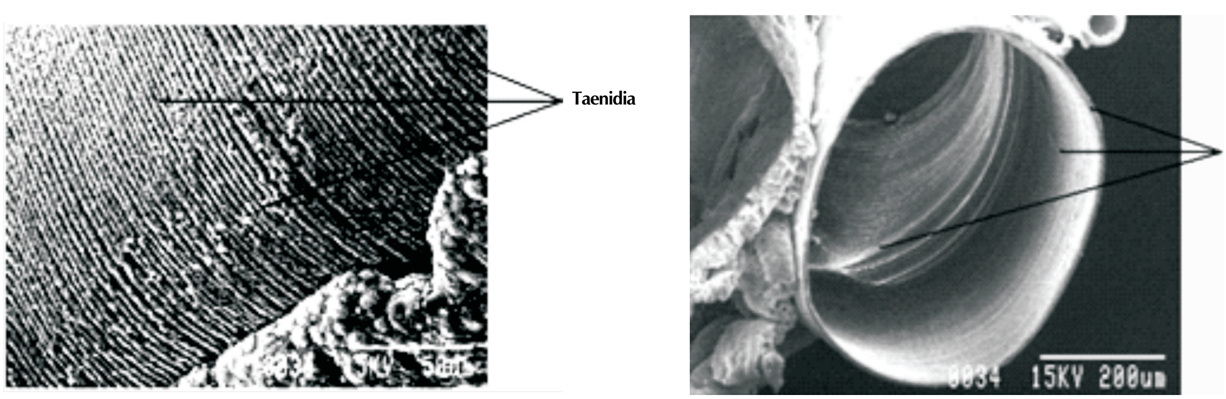

Figure 5 and 6: Trachea of a control dragonfly nymph showing smooth contour and taenidia

0.67±0.03 at 10°C to 0.92±0.02 25°C as shown in Fig. 2 and 3.

Measure of Cu in tissues

Cu uptake rates were compared between 72 and 96 hr time intervals at different temperature ranges and the result showed that there was a gradual increase in the uptake value, more so at 96 hr. While it increased from 2.282±0.03 μg/g at 10°C to 4.314±0.01 μg/g 25°C at 72 hr exposures, the value showed a significant rise at 96 hrs exposure, which varied from 2.524±0.004μg/g to 6.889±0.003μg/g. The uptake of Cu increased proportionally with the increasing period of exposure and temperature as shown in Fig. 4.

SEM Investigation

Scanning Electron micrographic investigation of Control (Fig. 5 and 6) and treated tissues (Fig. 7, reveal extensive tissue damage of the respiratory surfaces indicating impairment of respiratory functions. The trachea occurs to have a smooth lining of taenidium at control condition (Fig. 5 and 6).

After treatment with copper it was found that the smooth lining of tracheal surface gets disrupted as well as the epithelium layer gets damaged (Fig. 7).

The Box and Whisker Plot of the uptake data indicated in the Fig. 4 plots a summary of the distribution of Cu uptake data at 72 and 96 hr. It clearly reveals how data is spread out and

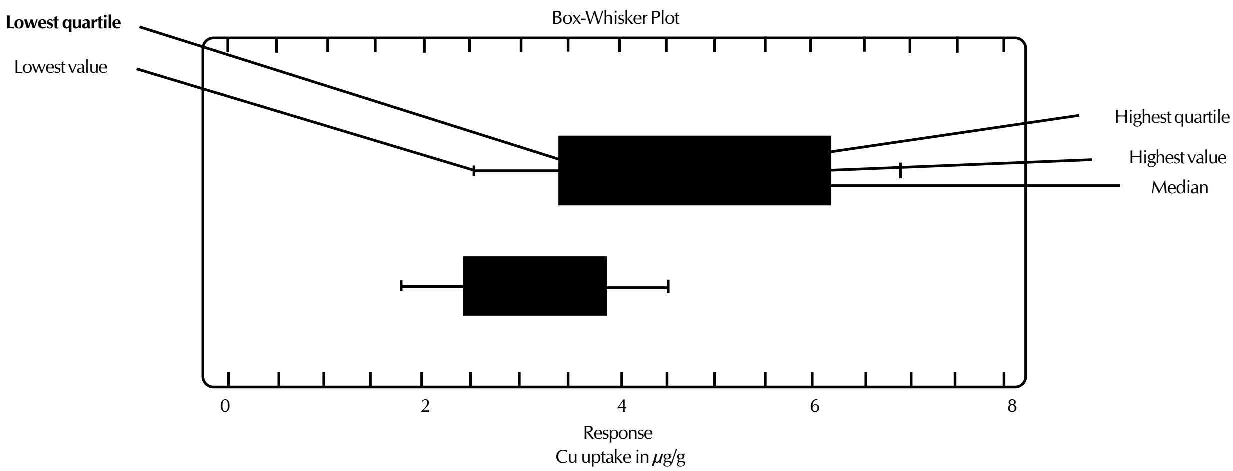

Figure 8:Box and Whisker plot to show difference in range of Cu uptake (µg/g) at 72 and 96 hr

how much variation there is. While the 72 hr data is negatively skewed the 96 hr is positively skewed (indicate by shift of the median), indicating that the data range has more variation in 96 hr, substantiating the ANOVA result of a significant difference both at 72 h ($F = 19.75$, $p < 0.01$) and 92 hr ($F = 9.32$, $p < 0.010$)

DISCUSSION

Odonata larvae can be found in most aquatic habitats, from fresh to brackish water, fast-flowing, and in both permanent and temporary water bodies. Dragonfly larvae can move rapidly by contracting their rectal chamber and shooting along with jet propulsion. The presence of rectal gills in the anal chamber of dragonfly nymphs is a noteworthy adaptation. These may be the sites for uptake of metal ions. It was observed that nymphs showed frequent and vigorous movements when exposed to Cu and the possible reason might be due to damage of the trachea that might have energetic costs, which would increase oxygen demand (Timothy *et al.,* 1999).

It is possible that larvae exposed to Cu were not meeting their oxygen demand due to disruption in their respiratory surface that limited the uptake and oxygen diffusion from the water. Thus the present observation might be a compensatory response. This might be explained by the fact that low oxygen concentrations (oxygen deficiency) are combined with a slightly lower pH and thus with a different copper speciation, resulting in a higher bio-availability of copper and consequently, in a higher copper uptake (Neuhoff, 1983).

Cu gets accumulated in the tissue of the dragonfly nymphs mainly through the gills as they are more permeable than the more sclerotized cuticle (Wigglesworth, 1979; Merritt and Cummins, 1996). The uptake of the heavy metal is influenced by temperature changes (Merritt and Cummins, 1996; Qaiser *et al.,* 2007; Denton and Burdon, 2004). Literature surveyed indicated that one of the possible routes of copper uptake is through Calcium channel, while there is partial inhibition of sodium and potassium pumps (Rogers *et al.*, 2005), resulting in homeostasis disturbances of ionic contents affecting the overall metabolism leading to stress condition.

Cu was found to increase with increasing temperature. Gill surfaces are damaged by several environmental stressors including heavy metals (Simpson, 1980). It was observed that with gradual increase in temperature there was subsequent enhancement of Cu uptake, which might have been aided by the presence of extensive rectal gill epithelial surface in dragonfly nymphs. The metal accumulations would tend to deform the gill structures thereby damaging the respiratory tissues of the nymphs (Simpson, 1980; Hare, 1992). The progressive and irreversible accumulation of these metals in various body tissues ultimately results into metal related disorders in the long run, thereby even endangering the aquatic biota and other organisms. Research with fish suggests that elevated temperatures may result in morphological changes in gill epithelia (Jacob *et al.,* 1981) and osmoregulatory stress (Fry, 1967; Garside *et al.,* 1972) confirming the present findings, substantiated by statistical evaluations.

To authenticate that there is actually cellular damage caused by Cu, Scanning Electron Micrographic investigation was performed which clearly reveals how much damage it can cause to the aquatic organisms. Uptake of Cu resulted into extensive tissue necrosis of the gills. Extended biochemical and physiological alterations induced by copper on the gill cells cause morphological changes in the gill structure. Copper can also be absorbed from integument, from absorbed water, from intestine and other exposed tissues. Grosell and Wood (2002). Cerqueira and Fernandes (2002) also got Identical observations in case of *Prochilodus lineatus*. Cu accumulation in the gills leads to several deformities, characterized by lifting up of the outer layer of lamellar epithelium and hyperplasia that occasionally resulted in fusion of adjacent lamellae (Martinez *et al.,* 2004).

It can be concluded that heavy metals in aquatic environment cause serious ecological disbalance, whose effects on the aquatic biota is magnified with rise in temperature and the respiratory activities and health of the aquatic insects is also affected. The bioaccumulation of Cu and other toxic heavy metals indirectly affect the entire food chain. In larger quantities, Copper can be toxic and have deleterious effects, especially to the lower trophic levels of food chains. Because of their high surface-volume ratios and readily permeable membranes, aquatic organisms are very sensitive to copper (Kosalwat and

Knight, 1987) Copper has a greater affinity than most metals for organisms and organic matter. A significant portion of Copper is complexed with organic matter in both freshwater and seawater.

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