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Effects of Copper on Respiration, Reproduction, and Some Biochemical Parameters of Water Flea *Daphnia magna* Straus

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In general, copper is being discharged into the environment from mining operations, metal processing facilities, chemical industries, thermal power plants, fossil fuel burnings and several other similar sources. Because of widespread uses, copper ions ultimately reach to the aquatic ecosystem and creates water pollution problem, being highly toxic to most of the freshwater organisms and tend to accumulate in them (Moore and Ramamoorthy, 1984; Khangarot and Ray, 1987; Khangarot *et al.,* 1987). Water flea *D. magna*, an important zooplankton, has been studied earlier (Biensinger and Christensen, 1972; Maki, 1979; Adema, 1978) and on the basis of its sensitivity to environmental pollutants it has been recognized as a general representative of freshwater animals (Baudouin and Scoppa, 1974; Buikema *et al.*, 1980). The advantage of using cladocerans with particular reference to *D. magna* as test species include their short life-cycle, easy to culture and maintains, small size, easy to perform bioassays with little space, etc, etc. These characteristics make *D. magna* an ideal candidate for aquatic toxicity studies. Numerous studies have been reported on the effects of environmental pollutants on survival, growth, and reproduction of cladoceran species (Bellavere and Gorbi, 1981; Reading and Buikema, 1980). Biesinger and Christensen (1972) reported that sub lethal copper concentrations caused a decreased in animal weight, total protein and glutamate oxaloacetate transfrase (GOT) activity in *D. magna* in 21 days exposure.

The purpose of this study is to determine the acute toxicity of copper on survival, respiration rate, animal weight, total protein, and metabolism as measured by glutamate oxaloacetate transfrase (GOT), glutamate pyruvate transferase (GPT), acid and alkaline phosphatases activities. The chronic tests were run for 21 days to determine the effect of Cu on the survival, adult total length and number of neonate production.

MATERIALS AND METHODS

Water flea *D. magna* were collected from a natural pond situated in Gheru Campus of Industrial Toxicology Research Centre, Lucknow. A stock culture was maintained in the laboratory. Daphnids were fed once a day, 6 days a week, with a fish food and dried yeast homogenate. Test organisms were fed during acclimatization, culture and chronic toxicity periods but were starved during acute

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toxicity studies. Daphnid food was prepared by dissolving the Shalimar Fish Food in distilled water. Ten percent stock solution was prepared and 0.1 g of dried yeast was added to it. During the chronic toxicity test, culture and acclimatization period, 0.5 mL of stock solution was added to the medium. For acute toxicity studies, test procedure as described earlier elsewhere is followed (Khangarot and Ray, 1987). For chronic tests ten daphnids (24 hr old) were placed in 100 mL water. Test containers were covered with a pane of glass to minimize evaporation. Survival of initial animals was noted after 21 days. Test solutions were renewed after every 7 days. Reproduction was measured by counting neonates produced each week and were discarded.

For oxygen consumption studies, glass bottles of approximately 60 mL capacity were used as respirometer. Ten daphnids were placed in each glass bottle containing test solution at room temperature ($15\pm2^{o}C$). Bottles were sealed by stoppers and carefully checked for air bubbles. Five sets of replicate for each experiment were made. After the end of 24 hr, amount of dissolved oxygen in each bottle was determined by Winkler's method (APHA *et al.,* 1995). Oxygen consumed was determined by subtracting the corrected oxygen content of the experiment from blank values. Oxygen consumption was converted to μL/mg dry weight/hour.

Biochemical studies were carried out using 0.2 to 0.3 g group of water fleas initially in 500 mL glass containers. For protein and enzyme activity analysis, 100 mg wet weight group of water flea (w/w) was homogenized in ice cold 0.2M phosphate buffer using chilled potter-Elvhjem homogenizer up to a total volume of 2.5 mL for 15-30 seconds. After centrifugation for 10 minutes (at 2500 rpm) and the supernatant was withdrawn for protein and enzymes activity. Alkaline phosphatase (Orthophosphoric monoester phosphodehydrolase, EC.3.1.3.1.) and acid phosphatase (Orthophosphoric monoester phosdehydrolase, EC.3.1.3.2.) were determined by using disodium phenyl phosphate as reagent (Wotten, 1964). Glutamate oxaloacetate transferase (GOT, L-aspirate 2-oxaloglutrate aminotransferase, EC.2.6.1.1.) and glumate pyruvate transferase (GPT, L-alanine aminotransferase, EC.2.6.1.2.) were determined according to method of Reitman and Frankel (1957). Total protein content in the homogenate was estimated in TCA precipitate by the method of Lowry et al. (1951) using bovine serum albumin as standard.

The 24 and 48 hrs EC_{50} values and their 95% confidence limits were calculated by moving average angle method (Harris, 1959). The test described by Fisher (1959) was employed to calculate the statistical significance between control and experimental values. P values less than 0.05 were considered to be significant.

RESULTS AND DISCUSSION

The important mean and range of physicochemical cartelistic of test water were as follows: pH 7.6 (7.5-7.7); temperature 13 (12-15) ^{o}C; dissolved oxygen 5.6 (5.2-6.2) mg/L; total alkalinity 400 (390-415) mg/L as $CaCO_3$; total hardness 240 (235-260) mg/L as $CaCO_3$; and total solids 224 (210-280) mg/L.

Table 1. The effect of 48 hr of exposure of copper on the rate of oxygen consumption of *Daphnia magna**

Cu conc. (mg/L)	Oxygen consumption (μL /mg dry wt./hr)	% Difference from control
Control	6.282± 0.153	
0.01	6.648± 0.051	+9.27
0.032	6.420± 0.068	+2.20
0.056	4.521± 0.070**	-32.95
0.10	4.625± 0.089**	-26.38
0.18	5.093± 0.051	-18.93

± values represent the mean ± SE of 5 of replicate values

* 5 replicates per test

** Significantly different from control at P<0.001

Table 2. The effect of 48 hr of exposure of copper on the dry weight of *Daphnia magna**

Cu conc. (mg/L)	Mean dry weight μg +SE (per animal)	% Difference from control
Control	388±6.77	
0.01	358±8.24	-7.73
0.032	260±6.33**	-32.99
0.056	272±4.82**	-29.90
0.10	240±9.25**	-38.14
0.18	210±7.86**	-45.88

± values represent the mean ± SE of 5 of replicate values

* 5 replicates per test

** Significantly different from control at P<0.0012

The EC_{50} values (i.e. 50% survival of daphnid) and their 95% confidence limits of copper show a progressive decline between 24 and 48 hr of exposure, suggesting an increase in toxicity with the increase of exposure period. The 24 and 48 hrs EC_{50} values and their 95% confidence limits were 0.536 (0.466-0.643) and 0.093 (0.081-0.112) mg/L of Cu, respectively. At higher test concentrations of Cu (0.1mg and 0.18mg/L), daphnids showed a signs of hyperactivity and increased locomotive activity during the early hour of exposure followed by a decrease of swimming activity prior to death. At lower test concentrations, animals showed normal movements for a longer period of exposure. In most of the cases, before immobilization, daphnids remain on the surface of the water and then settle down at the bottom and showed uncoordinated body movement and finally become immobilized. The 24 and 48 hrs EC_{50} values and 95% CL were in the same range as earlier reported for *D. magna* by other investigators (Biesinger and Christensen, 1972; Winner, 1976; Khangarot and Ray, 1987). In soft water Cu is more toxic than in hard water (Baudouin and Scoppa, 1974; Howarth and Sprague, 1978). Acute toxicity tests are helpful in establishing the water quality criteria for diverse uses of water. The effect of acute Cu exposure on rate of oxygen consumption per hour is shown in Table 1. In control tests, rate of oxygen consumption was 6.282 μL /mg dry wt/hr; whereas at 0.1 mg/L of Cu,

Table 3. Activities of enzymes and protein content in *D. magna* following copper exposure for 48 hr

Parameter	Control animal	0.1 mg/L of Cu (% change)	0.05 mg/L of Cu (% change)
Glutamate oxaloacetate transferase[a]	10.62±0.82	48.52±7.81** (+356.9)[c]	61.80±1.53[d]** (+481.9)
Glutamate pyruvate transferaase[a]	27.13±1.80	11.98±1.25** (-55.84)	24.62±2.60 (-9.25)
Acid phosphatase [b]	34.45±3.10	15.77±1.20** (-55.22)	25.79+3.10* (-25.14)
Alkaline phosphatase[b]	35.85±2.62	29.02±2.93* (-19.05)	40.12±3.91 (+11.91)
Protein (mg/g)	22.30±0.98	18.02±1.61* (-17.94)	20.10 ±1.88 (-9.87)

[a]μM Pyruvate formed/min/mg protein
[b]nM phenol librated/min/mg protein
[c]percent change, + sign denotes percent increase and - sign percent decrease
[d]± values represent the mean ± SE of 5-6 of replicate values
Significantly different from the respective control value, *P<0.05; **P<0.001

Table 4. Effect of chronic toxicity of copper for 21 days on the survival, production of neonates and total length of *Daphnia magna**

Cu conc. (mg/L)	Survival of adult (%)	Number of neonates produced per adult	Adult total length (mm)
Control	93.3	59.8±1.9**	3.62±0.020
0.01	87.7	64.4±2.4**	3.58±0.024
0.032	70	51.2±1.6**	3.40±0.016**
0.056	53.3	30±2	3.10±0.022**
0.10	0	0	-
0.18	0	0	-

±Values represent the mean ± SE of 5 replicates
*5 replicates per test
** Significantly different from control at P<0.05; **P<0.01

it was 4.625 μL/dry wt/hr. Similar decrease trends were also noted with other Cu concentrations. The results of rate of oxygen consumption indicate a significant decrease in respiratory rate following acute Cu exposure. Variability in daphnid oxygen consumption has been reported for environmental pollutants. Reading and Buikema (1980) observed a significant variability in oxygen consumption and filtering rate following selenium exposure in *D. pulex*. On the basis of present and other studies it appeared that the rate of oxygen consumption is a good indicator of pollutant stress. Date for changes in body weight, protein content, GOT and GPT, alkaline and acid phosphatases activities at 0.05 mg/L of Cu after 48hr of exposure are given in Tables 2-3. The pollutant exposure causes a significant

decrease in body weight and protein contents during 48 hr of exposure. The GOT and alkaline phosphatase enzyme activities were stimulated at 0.05 mg/L of Cu concentrations. However, the activities of GPT and acid phosphatase were inhibited by short-term Cu exposure. Results of 21day of Cu exposure are summarized in Table 4. At 0.056 and 0.032 mg/L of Cu the survival values are significantly lower than the control experiments. In chronic toxicity studies, reproductive impairment was found to be more sensitive measure than survival during the short-term exposure. Reproductive impairment can be defined as reduction in average cumulative number of young produced per adult. No significant difference was noted in neonate production at 0.01 mg/L of Cu and control experiments. Furthermore, reproductive impairment, percent survival, and body weight of test animals are significantly ($P<0.05$) decreased as compared to control tests. Concentrations below than 0.01mg/L of Cu were not found to increase the mortality of daphnids during 21day of exposure.

In the present study it has been shown that Cu exposure caused a significant decrease in GPT activity in dose dependent manner. Similarly, significant changes in alkaline and acid phosphatases activities at 0.05 mg/L of Cu were noted. Biesinger and Christensen (1972) observed a decrease in GOT activity, protein content and animal weight after a 21 day of Cu exposure in *D. magna.* Copper is known to increase the fragility of lysosomal membrane and thus release hydrolyzing enzymes, which causes cell damage (Todd and Thompson, 1965). However, there is no clear-cut explanation of mode of action of Cu on transferases and phosphatases in aquatic organisms. The chronic toxicity values of the present studies for Cu are higher than those of earlier 21days study of Biesinger and Christensen (1972). The differences between water hardness in these two studies are the main reason for wide differences in results. As water hardness increases, Cu toxicity decreases and vice-versa. To clarify the mechanism of toxic action of Cu at the cellular and molecular levels of aquatic test species with special reference to *D. magna,* further concurrent studies on the use biochemical markers should be encouraged. The biochemical markers have the utility in predicting the chronic toxicity effects using the short-term bioassays. The acute and chronic toxicity studies of *D. magna* suggests that relatively, short life cycle, durations of the tests, ease in handling and maintain, little space, less expensive and higher reproductive rate make the daphnid test system as an attractive alternative test model for the screening of environmental pollutants. In fish, similar tests would take between 14 days for acute and one and half year for chronic study to complete the tests depending on the purpose of study (Maki, 1979). The *D. magna* might fill the need for a valid desirable test organism for toxicant screening to handle the backlog of thousands of untested environmental chemicals for which the classical long-term testing using fish and mammalian test models can not be considered at present.

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