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Accumulation and Transfer of Copper by *Oocystis pusilla*

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Copper (Cu) is an essential micronutrient that at higher concentrations is toxic to algae and other aquatic biota. Copper toxicity to algae depends upon the individual species, their physiological and environmental conditions, and the chemical forms of metals in the medium (Sunda and Guillard 1976). Similarly, uptake of copper in algae varies with metal concentrations (Hardstedt-Romeo and Gnassia-Barelli 1980), pH values (Les and Walker 1984) and growth conditions (Laube et al. 1980). Copper accumulated by phytoplankton can be transferred and cause toxicity to zooplankton (Wikfors and Ukeles 1982).

Meador (1988) showed that the green alga, *Oocystis pusilla*, was resistant to Cu contamination and significantly reduced the toxicity of Cu to other species in standardized aquatic microcosms (Taub 1989). Our experiments were conducted to better understand how *O. pusilla* reduced Cu toxicity. The specific objectives of this study were to determine the effect of Cu on the growth of *O. pusilla*, and to study accumulation by *O. pusilla* and transfer of Cu from *O. pusilla* to *Daphnia magna*.

MATERIALS AND METHODS

The *O. pusilla* and *D. magna* were obtained from Dr. F. Taub's laboratory, School of Fisheries, University of Washington. Unialgal stocks of *O. pusilla* were cultured in 1 liter glass bottles in low Si T82MV medium (Taub and Crow 1980) at 23 ± 1°C under a 12 hour light : 12 hour dark (12L:12D) photoperiod regime with mild aeration during incubation. Algae in the exponential growth phase were used for the experiments. *Daphnia* were cultured (12L:12D) in a 5 L plastic container in T82MV at 23 ± 1°C and fed *O. pusilla* every day. Newborn *Daphnia* were collected daily and neonates (≤24 hours old) were used for the experiments.

To measure Cu accumulation by *O. pusilla* batch cultures with an initial algal density of 5 x 10^4 cells mL^{-1} were established in 100 mL of sterile T82MV medium (pH=7.0) by inoculation from unialgal stock cultures and illuminated (12L:12D) at 23 ± 1 °C. At the beginning of the experiment (exponential growth) or during the stationary phase, triplicate cultures were spiked from a $CuSO_4$ stock solution to reach non-toxic concentrations of 50 or 200 ppb of copper. Cultures were shaken twice a day by hand and growth was monitored daily by counting cells under a compound microscope at 160x magnification. To determine Cu concentration, algae were collected on days 1, 3 and 7 after metal addition and filtered through a

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0.45 μm membrane filter (Millipore). The filter with algae was digested with 10 mL concentrated HNO_3 at 160°C for at least 4 hours and evaporated to dryness. After cooling the sample was dissolved in 0.1 N HNO_3 for analysis by atomic absorption spectrophotometry (AAS). Accumulation per algal cell was calculated as the total metal contents in algae divided by the total number of cells.

To estimate transfer and toxicity of Cu to *D. magna* , thirty neonates were used; twenty as treated animals and ten as controls, in each experiment. Individual *D. magna* were maintained in 20 mL T82MV medium in a 30-mL test tube at 23 ± 1°C (12L:12D). Every two days *Daphnia* were transferred to a fresh medium and fed 2 x 10^6 cells of *O. pusilla* which had been grown for more than one week in T82MV with 50 or 200 ppb added copper (no added metal for controls). Algae were centrifuged and the cell pellet was washed with distilled water (pH was adjusted to 9.0 with NaOH to prevent desorption of copper from cells) prior to being fed to *Daphnia.* Experiments lasted at least 20 days. *Daphnia* molts were recorded daily and collected for metal analysis. Newborn *Daphnia* and moribund eggs were counted and collected each day. *Daphnia* neonates from the first and third broods were collected for body length measurement; those from the second broods were collected for metal analysis. The length of newborn *Daphnia* was measured from the anterior end of the head to the end of the caudal spine. At the end of the experiment, individual *Daphnia* were collected for Cu analysis. At the same time, 50 neonates were kept in 500 mL of T82MV medium, fed metal-contaminated algae and transferred to fresh medium every two days. Ten of these *Daphnia* were collected on day 8, 15 and 20 for metal analysis. Adult *Daphnia*, neonates and molts were dried at 60°C for 24 hours, weighed on an electronic balance (Mettler H20T, d=0.01 mg) and digested with 10 mL concentrated HNO_3 at 160°C. The solution was evaporated to dryness, cooled to room temperature and dissolved in 0.1 N HNO_3 for AAS.

Copper was measured by AAS for samples prepared in a 0.1 N HNO_3 matrix. Analyses were conducted by acetylene flame or graphite furnace atomization with a Hitachi 180-70 Zeeman effect spectrophotometer. Absorption was measured as the signal peak at 324.8 nm. Graphite furnace atomization was performed on samples with concentrations less than 0.1 ppm copper. Subsamples of 10 μL were ashed and atomized with a five step program: 30 seconds at 600°C, atomizing, 7 seconds at 2,700°C, cleaning, 3 seconds at 2,800°C. Calibration was conducted at the beginning of each analytical session and verified periodically during each session. Standards were prepared by diluting a 1,000 ppm Reference Standard (VWR Scientific) in 0.1 N ultrex HNO_3. The recovery rate was 102% at 100 ppb Cu and detection limit was 4 ppb with graphite furnace atomization.

Analysis of variance (ANOVA) was used to compare the differences in metal accumulation in algae, growth of algae, time of first breeding, production of living newborn and moribund egg, and length of newborn *Daphnia.* When the null hypothesis of the ANOVA ($p<0.05$) was rejected, a Student-Newman-Kuels test was used for comparisons among treatments.

RESULTS AND DISCUSSION

Algal growth was depressed at high (>400 ppb) Cu concentrations but stimulated at lower concentrations (Fig. 1). Algal densities among control and treated algae were not statistically different but density at 200 ppb Cu was significantly higher than for higher Cu concentrations. All later experiments were conducted at Cu concentrations that were considered to be non-toxic to *O. pusilla.*

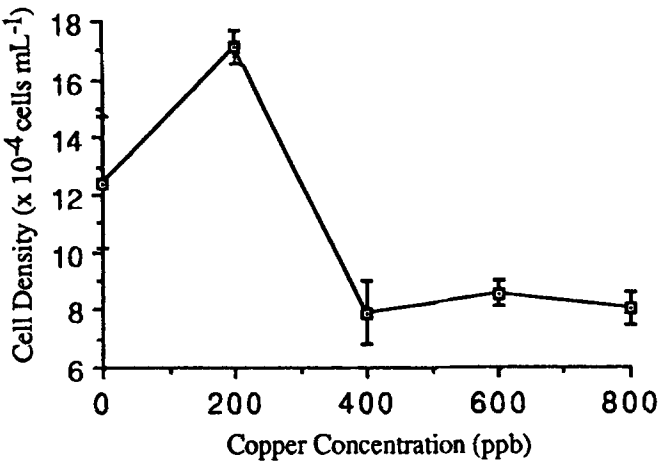

Figure 1. Density of *O. pusilla* in T82MV medium for different concentrations of copper. Density is plotted after 7 days of growth. Error bars are 1 standard error for triplicate cultures.

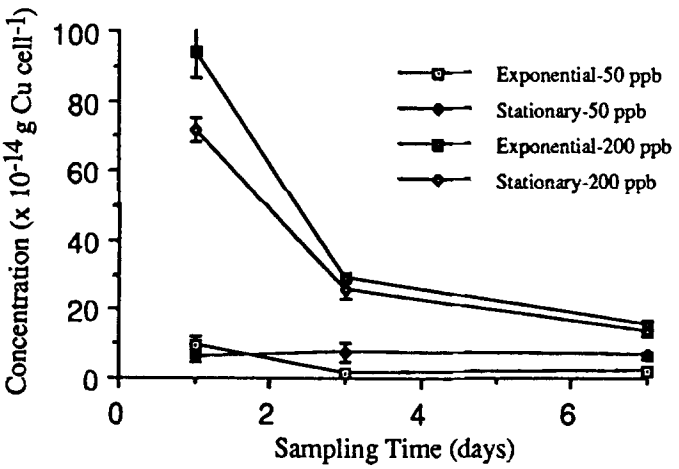

Figure 2. Concentration of copper in *O. pusilla* following addition of 50 and 200 ppb Cu. Error bars represent 1 standard error calculated for 3 replicates.

The uptake of Cu by *O. pusilla* varied with concentration, growth phase and exposure time (Fig. 2). At 50 ppb, the concentration of Cu in *O. pusilla* ranged from 1.06×10^{-14} g Cu cell^{-1} to 9.5×10^{-14} g Cu cell^{-1} during the exponential growth phase and between 5.9×10^{-14} g Cu cell^{-1} and 7.4×10^{-14} g Cu cell^{-1} during the stationary growth phase. The concentration of Cu in *O. pusilla* was significantly higher on day 1 than on day 3 or 7 during exponential growth. However, Cu concentration in *O. pusilla* did not vary significantly during the stationary growth phase. At 200 ppb, Cu concentration in *O. pusilla* decreased from 9.39×10^{-13} g Cu cell^{-1} on day 1 to 1.58×10^{-13} g Cu cell^{-1} during the exponential growth phase

and from 7.15 x 10^{-13} g Cu $cell^{-1}$ to 1.32 x 10^{-13} g Cu $cell^{-1}$ during the stationary phase. Accumulation/cell of Cu decreased over time in both the exponential and stationary growth phases. However, the number of algal cells increased during the experiment for both exponential and stationary phases. As a result the uptake by algae (% of total Cu) was considerably more stable than the concentration/cell (Chang 1991).

Copper-contaminated *O. pusilla* transferred Cu to *Daphnia* (Table 1). After 20 days, the concentration of Cu in *Daphnia* was 43 μg Cu g^{-1} in the control, 67 μg Cu g^{-1} when fed algae contaminated with 50 ppb Cu, and 535 μg Cu g^{-1} for algae from the 200 ppb Cu culture. The concentration of Cu in neonates increased with increasing Cu concentrations from 88 μg Cu g^{-1} in *Daphnia* fed control algae to 773 μg Cu g^{-1} for those fed algae contaminated with 200 ppb Cu. However, the concentration of Cu in molts was the highest (250 μg Cu g^{-1}) for those fed algae contaminated with 50 ppb copper and lowest (141 μg Cu g^{-1}) for 200 ppb algae. The Cu concentration in *Daphnia* increased with culture time for control *Daphnia*, decreased with time for *Daphnia* fed algae from the 50 ppb culture and remained relatively constant for *Daphnia* fed algae from 200 ppb Cu.

Table 1. Concentration of copper (μg g^{-1}) in *Daphnia*, neonates and molts.

O. pusilla	8-day *Daphnia*	15-day *Daphnia*	20-day *Daphnia*	Neonates	Molts
Control	23	29	43	88	205
50 ppb Cu	135	110	67	214	250
200 ppb Cu	578	531	535	773	141

Production of newborn *Daphnia* was significantly greater for controls than for treatment groups and the production of moribund eggs was significantly lower (Table 2). Time of first breeding was significantly different among control and treatments but did not follow a consistent pattern with increased Cu concentration. Body length of newborn *Daphnia* was not statistically different among control and treatments.

Table 2. The production of neonates and moribund eggs within 15 days, and the length (mm) of neonates and time(day) of the first breeding.

O. pusilla	Neonates	% of moribund eggs	Body length (1st breeding)	Body length (3rd breeding)	Time of first breeding
Control	45±12	8.2±0.7	1.04±0.11	1.12±0.05	7.00±0.48
50 ppb Cu	33±10*	28.3±5.5*	1.08±0.10	1.13±0.07	6.70±0.48*
200 ppb Cu	22±11*	59.2±7.2*	1.07±0.08	1.12±0.11	7.28±0.48*

* Significantly different from the corresponding control value, $p < 0.05$

Copper inhibited the growth of *O. pusilla* at concentrations above 400 ppb but stimulated growth at less than 200 ppb. Similar results have been observed previously for blue green algae (Laube et al. 1980; Wurtsbaugh and Horne 1982), freshwater green algae (Manahan and Smith 1973) and marine phytoplankton assemblages (Wood 1983). Stimulated growth at low concentrations occurs because Cu is an essential micronutrient for these algae.

Uptake of metals in algae increases with increasing metal concentration in solution (Hardstedt-Romeo and Gnassia-Barelli 1980; Laube et al. 1980). However, the concentration per cell depends upon algal cell density (Gipps and Coller 1980; Laube et al. 1980). In our experiments the cellular concentration of Cu declines as population density increases while the percentage of the total Cu bound to algae remains much more constant.

Transfer of Cu from algae to *Daphnia* represents one of two (food and water) principal sources of metals. Accumulation of metals is dependent upon the rate of uptake and excretion of metals in the organisms and may reflect the physiological condition of the organism more than the environmental conditions (Winner 1984). In our experiments, the Cu concentration in *Daphnia* that were fed algae contaminated with 50 ppb Cu decreased with exposure time while Cu in *Daphnia* that were fed algae from 200 ppb Cu cultures was relatively constant. This suggests that *Daphnia* may have a regulatory mechanism for copper and other essential metals as has been observed previously in decapod crustaceans (Bryan 1976). High concentrations of copper in neonates and especially in molts suggests that this may provide a mechanism for controlling Cu concentration in the adult *Daphnia* (Spacie and Hamelink 1985).

Although there were no apparent effects on adult *Daphnia*, the reduced production of live neonates indicates toxicity from Cu-contaminated algae. Biesinger and Christensen (1972) found that a Cu concentration of 35 ppb in Lake Superior caused a 50% decrease in *Daphnia* reproduction. However, Dave (1982) reported that a low concentration of copper stimulated reproduction in *D. magna* but reduced survival. The differences in neonate production between control and treatments might be determined by the condition of the female at the time of egg production or the direct effect of chemicals on reproduction (Winner 1984). Flickinger et al. (1982) reported that copper concentrations above 20 ppb caused a significant delay in the maturation of *Daphnia*. Time of first breeding was significantly different for copper treatments than controls, but was not consistent with increased Cu concentration.

The sensitivity of a response is very important for an indicator of toxicity. Flickinger et al. (1982) reported that negative phototaxis, body length of neonates, and filtration rate were more sensitive indicators than reproduction and survival. However, Ingersoll and Winner (1982) reported that body length of newborn *Daphnia* was not affected consistently by copper. In our experiments, body length of newborn and the total value of neonates and moribund eggs were not significantly different between control and copper treatments. The number of neonates, however, was less in the copper treatments than in control; therefore, the number of living newborn is a more sensitive indicator than body length of newborn or time of maturation.

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