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Effect of pH and Time on the Acute Toxicity of Copper Sulfate to the Ciliate Protozoan *Tetrahymena thermophila*

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Ciliate protozoans have been shown to be extremely sensitive to the effects of heavy metals such as cadmium and copper (Ruthven and Cairns 1973) and, thus, have been used extensively as eukaryotic models to study the effects of pollution on aquatic environments (Carter and Cameron 1973; Slabbert and Morgan 1982). Advantages of using such cellular systems include rapid cell-cycles (2.5 hr) and their ability to be grown in axenic culture at high densities (Nanny 1986). The ciliates used in previous acute toxicity studies have all been anucleate species. *T. thermophila* is a micro-nucleated ciliate having a genetic organization that in some ways mirrors the complex diversification of specialized cell types found in metazoa (Nanney 1986).

Several heavy metals are known to interact with nuclear components eliciting a toxic cellular response (Goyer 1986). Cadmium has been shown to bind to DNA as well as other critical cellular proteins disrupting normal cell function leading to mutagenesis or cell death (Goyer 1986). The Fenton-catalyst, copper, can produce oxidative stress caused by the production of reactive oxygen metabolites, leading to DNA damage (Chubatsu and Meneghini 1993). Consequently, the purpose of this study was to examine the effects of two heavy metals, cadmium and copper, on cell viability and to assess the role of the extracellular factors, time and pH, in the acute toxicity of copper in a micro-nucleated organism.

MATERIALS AND METHODS

Cultures of *T. thermophila* were grown axenically in a medium comprosed of 10 g proteose peptone No. E (Difco), 0.5 g dehydrated yeast extract, 2.5g dextrose and 0.5 g sodium chloride in 1 l of deionized water (pH 6.9). Cultures were incubated at 30°C in capped 250-ml flasks containing 50 ml of medium in a rotary shaker for 3 days and grown to a density of 1-1.5 $x10^6$ cells per ml. Cell density was measured using a hemocytometer. Cultures of three days were in maximum growth phase and very few of the cells were reproducing.

Cells were pelleted by centrifugation (2500 x g for 10 min) washed and re suspended in reconstituted deionized water (hardness 211 ppm $CaCO_3$; alkalinity 200 ppm) with various pH values (6-8). Assays were carried out in petri dishes containing 30 ml volumes of reconstituted water using 2.0 x 10^5 cells per ml at room temperature (27°C). Organisms were exposed to 10 μM (as the salt)

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concentrations of cadmium chloride and copper sulfate for 24 hr. In subsequent studies, cells were exposed for 24-96 h at various concentrations of copper sulfate, as the salt (0-10 ppm). Each concentration was performed in triplicate. Lethality was measured by lack of organism movement and structural abnormalities of the cellular membrane. Lethality counts of each sample were performed in duplicate. Statistical evaluations (ANOVA and Tukey Comparison of Means)were performed using Toxstat 3.4^{R}(West Inc., Cheyenne, WY).

RESULTS AND DISCUSSION

Significant differences were observed between the toxicity of cadmium chloride (100 % lethality) and copper sulfate (98.5 $\pm$ 4) to *T. thermophila.* In previous studies performed on another species of *Tetrahymena (pyriformis),* the 96-hr LC50 for cadmium sulfate was 1.67 ppm and 6.67 ppm for zinc sulfate in water containing 400 ppm $CaCO_3$ (Carter and Cameron 1973). Ultrastructural damage was observed in *T. pyriformis* exposed to approximately 2 ppm cadmium sulfate for 20 h with an 76-84% loss of viability (Dunlop and Chapman 1981). However, data regarding hardness or alkalinity were not present. Since a 24 h exposure to 2 ppm cadmium chloride (10 μM) in water containing 200 ppm $CaCO_3$ led to 100% mortality in the present study, *T. thermophila* appears to be more sensitive to cadmium toxicity than *T. pyriformis.* In a third *Tetrahymena* species (*vorex*), a decline of 62% viability was observed after a steady state exposure of only 40 ppb cadmium (Lawrence et al. 1989). In seven other ciliates, 24 hr LC50s to cadmium ranged from 0.2 to 2.6 ppm (Madoni et. al. 1992). Consequently, *T. thermophila* appears to be similar to other ciliates in its sensitivity to cadmium.

Although sensitive to cadmium chloride, *T. thermophila* was resistant to copper sulfate toxicity when compared to other ciliate species. *T. pyriformis* was shown to "tolerate" copper concentrations of only 0.32 ppm (Ruthven and Cairns 1973). When comparing 24 hr LC50 values of multiple heavy metals between seven ciliate species, copper was 10-100 times more toxic than cadmium in every species (Madoni et al. 1992). Although alkalinity, hardness and pH endpoints were not reported, the 24 hr LC50s for copper shown in the present study were 100-300 times greater than the values in the seven ciliate species. Thus, *T. thermophila* appears to be unique in its resistance to copper toxicity.

Copper toxicity to aquatic organisms can be affected by intracellular and extracellular factors (Sprague 1985). Extracellular factors include exposure time, water hardness, alkalinity, and pH while intracellular factors might include proteins such as metallothionein. In the present study, the effect of exposure time and pH was examined.

With the exception of copper sulfate concentrations greater than 3 ppm at pH 7 (Fig. 2) and 7.5 ppm at pH 8 (Fig. 3), toxicity did not appear to be influenced by time of exposure. The most significant reduction in LC50 over time occurred in the pH 7 group where an approximately 30% decrease was observed from 24 to 96 hr. Although the 96 hr LC50 for cadmium sulfate has been determined in ciliates (Carter and Cameron 1973), exposure times for copper toxicity have never exceeded 24 hr. Thus, 24 hr exposure times may over-estimate the acute toxicity of some compounds, especially metals.

As pH was changed from 6 to 8, lethality of copper generally decreased. However, concentrations above 3 ppm at pH 7 appeared to exert more toxicity

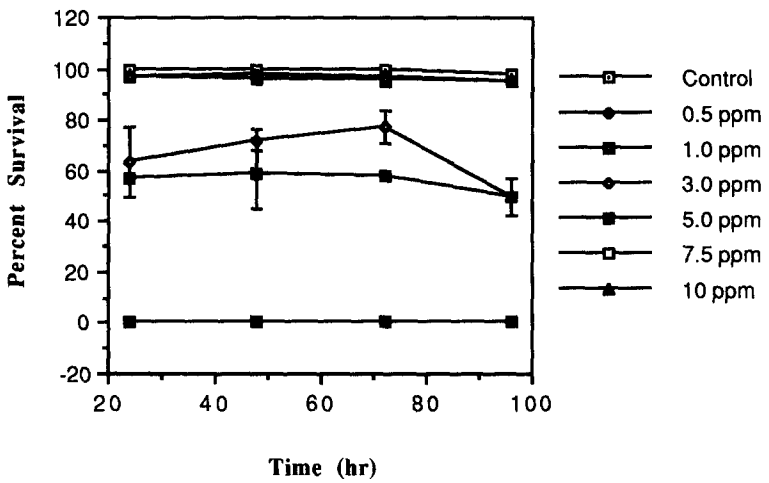

Figure 1. Effect of copper sulfate on *T. thermophila* at pH 6.

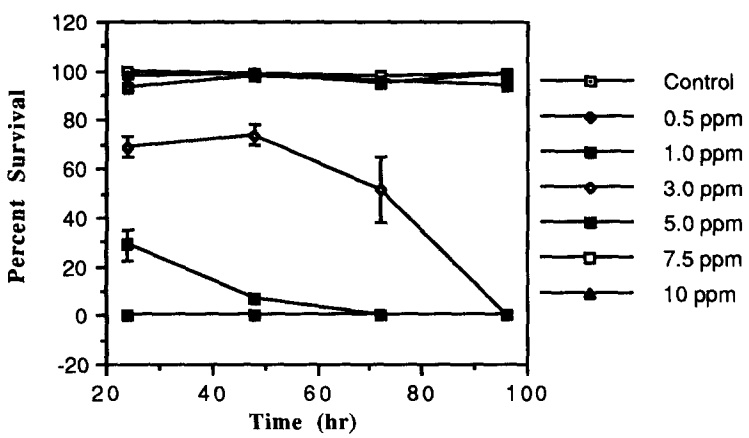

Figure 2. Effect of copper sulfate on *T. thermophila* at pH 7.

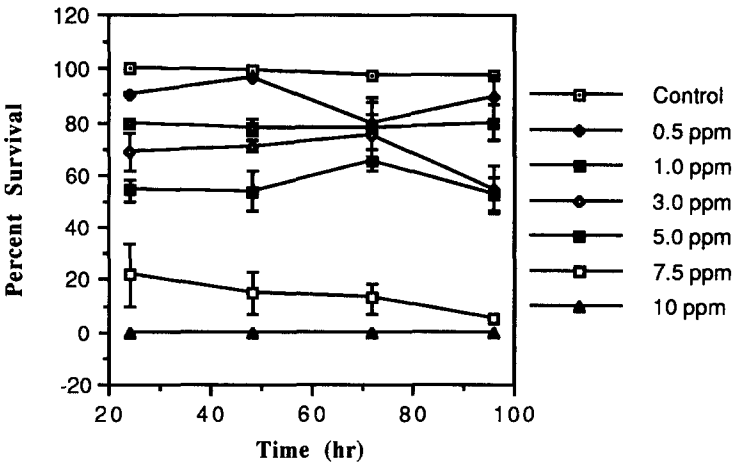

Figure 3. Effect of copper sulfate on *T. thermophila* at pH 8.

than the same concentrations at either pH 6 or 8. Percent survival between pH 7 and 8 at concentrations of greater than 3 ppm were significantly different ($p \leq 0.05$) at 48, 72 and 96 hr. Comparing survival between pH 6 and 7, only concentrations above 5 ppm were significantly different at each time-point with the exception of the 96 hr 3 ppm concentration which was significantly more lethal at pH 7 than 6. Survival was only observed at pH 8 with concentrations of 7.5 ppm. The decrease in copper toxicity with increasing pH is consistent with other studies examining the toxicity of copper in aquatic organisms (Sprague 1985). At lower pH (i.e., pH 5), a large proportion of copper is present as Cu^{2+}, the more toxic copper ion, but at higher pH there is a variety of hydroxides and carbonates (Stiff 1971). *T. thermophila* may be unique by having an affinity for one of these forms of copper at pH 7 that is more toxic than the other forms. Or perhaps at pH 7, this form of copper is more readily taken up across the cell membrane. Moreover, since the optimum pH for *T. thermophila* culture is 7.4, metal uptake pathways would be optimal at pH 7. (Nanney 1986).

In summary, *T. thermophila* is resistant to copper sulfate toxicity. The greatest toxicity of copper sulfate was observed at a pH range where optimal metal uptake would occur. Since mortality was still significantly lower in copper-treated cells even at 96 hr than that observed for a 24 h cadmium exposure, an intracellular mechanism appears to be involved in this resistance. Future studies will focus on various intracellular mechanisms of metal protection such as the production of metallothionein.

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