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Toxicity of Copper Sulfate to Juvenile Grass Prawn, *Penaeus monodon**

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ABSTRACT

Copper sulfate has been generally used for many years to reduce algal density in ponds, and to prevent culture animals from being infected with diseases. The purpose of this study was to investigate the toxicity of copper sulfate at therapeutic level and at toxic level when exposed to juvenile *Penaeus monodon* for 24 hours. It showed that at toxic level of 40 ppm, copper sulfate caused gill damage and reduced the survival rate of *P. monodon*. Residual copper in the prawn was markedly eliminated during a short period of immersion into clean seawater. Prawn exposed to therapeutic level of 0.4 ppm of copper sulfate did not exhibit any side effects. It is suggested sublethal level of copper sulfate also did not cause any undesirable effect to the prawn which is transfered into clean seawater after exposure. The result of this study showed that at therapeutic levels, that is, at a level of up to 4 ppm and with exposures of up to 24 hours, copper sulfate can be safely applied to ponds with juvenile *P. monodon*.

INTRODUCTION

Copper sulfate is being commonly used in aquaculture to reduce algal density in ponds. Its effect is largely attributed to cupric ions. On the use of copper sulfate as algicide, it was shown that it acts mainly on the cell membrane by increasing the permeability of the cells. Cupric ions inhibit both respiration and photosynthesis, but cupric ions were found to restrict photosynthesis more than respiration (Boyd, 1982). On the other hand, copper is a heavy metal and thus, easily accumulated by organisms. Therefore, the toxicity of copper to organisms has been extensively studied and investigated (Steemann Nielsen and Wium-Andersen, 1970; Engel and Sunda, 1979; Chen and Slinn, 1980; Ahsanullah *et al.*, 1981; Chen, 1981; Chou *et al.*, 1985; Liao and Hsieh, 1988; Reardon and Harrell, 1990; Harland and Nganro,

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1990). Studies on cultured animals showed that copper first shows its effects at the gills, disrupting mainly the gill functions including ionic disturbances and acid-base changes. Toxic doses of copper were shown to greatly restrict respiratory gas-exchange, which ultimately lead to tissue hypoxia and eventual death. The toxicity of copper sulfate was also found to increase with a reduction in water hardness, salinity, temperature, pH value, alkalinity, and/or dissolved oxygen, and to decrease in the presence of chelating agents and organic materials such as humic acid, amino acids and suspended solds (Alabaster and Lloyd, 1982). Many studies have also been made regarding the toxicity of copper sulfate to *P. monodon* (Chen and Hsiech, 1978; Chen *et al.*, 1979; Chou *et al.*, 1985). Chronic toxicities to *P. monodon* continuously exposed in copper sulfate was reported by Liu in 1980. His study showed that the growth and survival of postlarval prawn decreased during long term copper exposure, and the toxic effects increased with copper concentration. He also found that oxygen consumption decreased with increasing copper concentration and copper toxicity increased with decreasing salinity. Moreover, experiments on the accumulation of copper were extensively studied also. These studies showed that copper accumulated more in the gill than in the shell and muscle. Furthermore, accumulation increased with decreasing salinity and increasing temperature. These studies, however, concentrated on long-term copper exposure. Thus, we focused our investigations on the toxicity of copper sulfate to juvenile *P. monodon* exposed for 24 hours and to determine a safely therapeutic dose.

MATERIALS AND METHODS

In a our previous study, we obtained 24-hour median lethal concentration (LC_{50}) value of copper sulfate to postlarval *P. monodon* (Liao and Guo, 1989). The value, 400 ppm, plus 1/10, 1/100, and 1/1000, were used to derive the concentrations, 40, 4, and 0.4 ppm, respectively, used in the present study. Juvenile *P. monodon* with average body weight of about 0.3 gram were selected from the rearing ponds and exposed to the test and control solutions in triplicate FRP tanks filled with 100 L seawater. The tanks were aerated by an air stone with blower throughout the experiment. During the experiment, the juvenile prawn were fed squid containing 3.01 μg of Cu/g body weight four times a day. The chemical characteristics of the seawater are as follows: salinity, 28-30 ppt; pH value, 7.7-7.8; and alkalinity, 127.4 mg/l $CaCO_3$. Water temperature ranged from 25.2 to 29.5°C. The prawn were exposed for 24 hours to the solutions. Tests were then made after the exposure, during which the tanks were cleaned and refilled with clean seawater. Cultural practices were continuously performed throughout the experiment. Growth tests were made every two weeks after the 24-hour exposure. Survival tests were conducted to count mortality at 24-hour exposure and every two weeks in culture period. Histological examination was made at 24-hour exposure and during the culture period by routinely fixing the prawn in Davidson's solution, dehydrating, embedding in paraffin, sectioning at 4 μ, staining with hematoxylin and eosin, and observing it using a light microscope. The copper content of the whole body was analyzed using an atomic absorption spectrophotometer (Hitachi, Model 170-30) based on AOAC (Association of Official Analytical Chemists) methods (1980). Samples took from prawn exposed for 24 hours and during the culture period.

RESULTS AND DISCUSSION

Survival and growth tests

The mortality rate of grass prawn after exposure is shown in Fig. 1. At a concentration of 40 ppm copper sulfate, about 30% mortality were observed after 24-hour exposure. There were no significant differences ($P>0.05$) in the rest of the test groups. Fig. 2 shows the growth of prawn treated with copper sulfate. There were no significant differences ($P>0.05$) in all treatments. Several studies also showed similar results. Lightner (1980) reported that low concentrations of copper (0.9-1.0 ppm) from Cutrine-Plus or copper salts may cause some discoloration and irritation on the gills of penaeid prawns following 24-hour exposure. No mortalities were observed from treatment levels of up to 4 mg Cu/L following 24 hours exposure. Johnson (1974) also tested copper sulfate toxicity on *P. duorarum*, *P. stylirostris* and *P. setiferus*, and found juvenile prawn of these species to be unaffected at treatment levels ranging from 0.5 to 20 ppm. These studies indicated that there was no undesirable effect on the survival and growth of juvenile

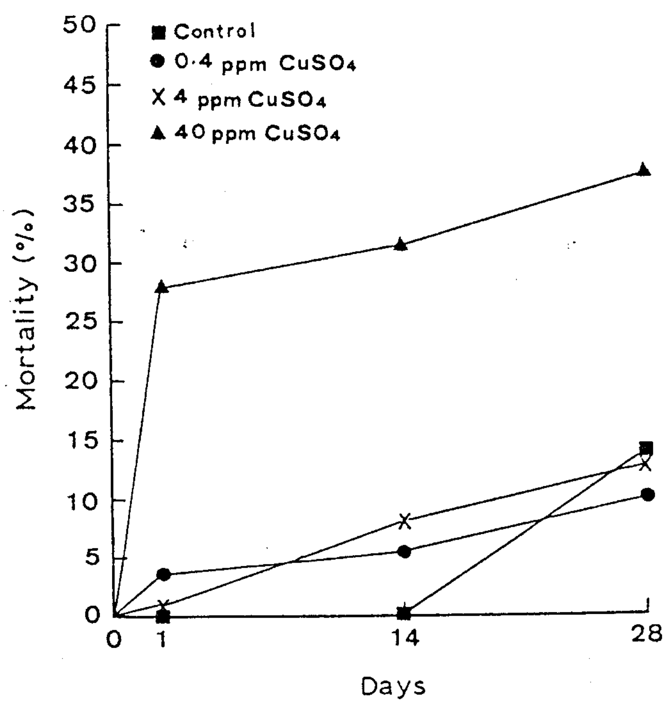

Fig. 1. Mortality rate of juvenile *Penaeus monodon* exposed for 24 hours to different concentrations of copper sulfate.

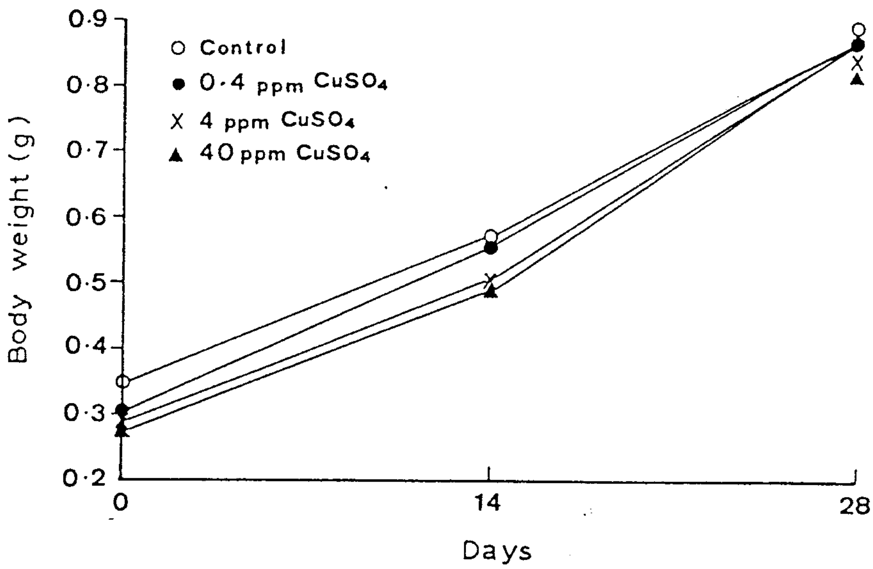

Fig. 2. The effects of different concentrations of copper sulfate on the growth juvenile *Penaeus monodon*.

P. monodon exposed to copper sulfate doses below 4 ppm for 24 hours.

Histological examination

Light microscopic studies showed that morphological changes in the gills were evident only at concentrations of 40 ppm copper sulfate and exposure of 24 hours.

Fig. 3. Gill lamellae from prawn exposed to 40 ppm copper sulfate. Epithelium, which has a balloonlike appearance, contains dense material (arrow). Base-lamellar region is reduced in thickness (arrow head).

These changes included an extracted appearance in gill lamellae; a balloon-like appearance which contained dense material in the efferent vessel of primary filament; and reduced thickness in the basi-lamellar region (Fig. 3). Jeremy (1969) also got a similar result at low levels of copper poisoning in the winter flounder (*pseudopleuronectes americanus*). His study showed that copper may be absorbed by the gill epithelium and act primarily on the cell enzymes resulting in the formation of lysosome, vacuoles, and vesicles. Boitel and Truchot (1989) were able to characterize copper intoxication in seawater-acclimated shore crabs (*Carcinus maenas*) in their study. Their study showed that at a toxic level, respiratory gas-exchanges were affected, most probably because of anatomical gill damage. Thus, it can be concluded that the mortality of prawn at concentrations of 40 ppm copper sulfate and exposure of 24 hours resulted from disorder of gill function and inhibition of respiration.

Residue and elimination tests

Fig. 4 shows the copper residue in the prawn after 24 hour-exposure and copper elimination thereafter. At concentrations of 40 ppm copper sulfate, prawn has higher copper residue at 24 hour-exposure, and showed a significant difference ($P<0.01$). The rest of test groups and control showed no significant difference ($P>0.05$). When the prawn treated with 40 ppm copper sulfate was placed in clean seawater, copper residue in the body was quickly eliminated. Copper residue content was almost similar to normal prawn after 6 days, although the residue content may have been eliminated markedly even before the tests were

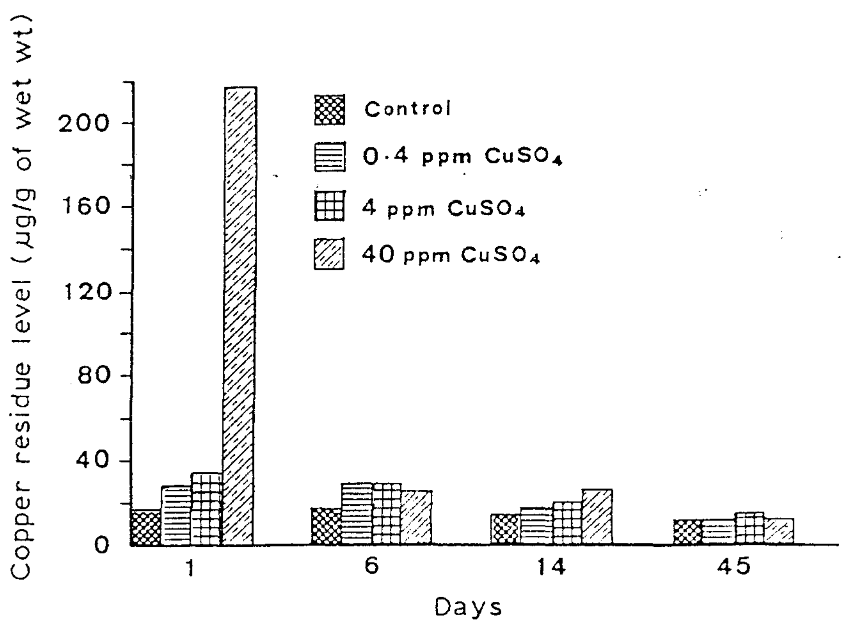

Fig. 4. Copper residue level in juvenile *Penaeus monodon* that were exposed for 24 hours to different concentrations of copper sulfate and then placed in clean seawater.

made. Our study confirmed the results of Liao and Hsieh (1986) on the toxicities of copper to *P. japonicus.*

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硫 酸 銅 對 草 蝦 之 毒 性 研 究

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摘 要

硫酸銅用來去除池中過多之藻類及防治水產動物細菌性疾病已有數十年的歷史。本研究主要目的乃在探討硫酸銅對草蝦的毒性，擬了解治療及中毒劑量在藥浴草蝦 24 小時所能產生之慢性毒反應。結果顯示，硫酸銅在中毒劑量 40 ppm 時，對草蝦的鰓部會造成損害，亦會降低蝦的活存率；在蝦體中的殘留情形，則發現藥浴後之蝦體一移至新海水中，銅在短時間內會大量釋出。至於，以治療劑量 0.4～4 ppm 藥浴的草蝦，發現硫酸銅對蝦體不會造成不良的影響。因此，使用硫酸銅防治蝦病，其劑量在 4 ppm 以下藥浴時間低於 24 小時是很安全的。