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Acute Toxicity of the Fungicide Copper Oxychloride to the Freshwater Prawn *Macrobrachium rosenbergii* De Man

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The Freshwater prawn *Macrobrachium rosenbergii* is cultured throughout the tropical and subtropical zones in all of the world, especially in some countries in Asia and South America. As with any other type of aquaculture, the quality of the water is a critical factor related to the success of this activity, while the runoff of pesticides in agriculture is one of the main sources of pollution in aquaculture ponds.

Copper oxychloride is a fungicide used extensively on crops throughout the world, particularly in Brazil. The toxicity of this pesticide to aquatic organisms has not so far been investigated. Although there are some studies on copper toxicity focusing on freshwater prawns (Ghate and Mulherkar 1979; Murti and Sukla 1984; Liao and Guo, 1990; Natarajan et al. 1992), none have been carried out with copper oxychloride. The objective of this study was to use the copper oxychloride in order to determine the acute toxicity of copper to the freshwater prawn *Macrobrachium rosenbergii*, according to static and renewal methods of testing, as well as to determine the bioaccumulation of copper in the organisms submitted to those tests.

MATERIAL AND METHODS

Postlarvae of *Macrobrachium rosenbergii* were produced under rigorously controlled conditions in the hatchery of the Fisheries Institute – SP – Brazil. After 15 days from metamorphosis prawns were transferred to the bioassay laboratory, and acclimatised for 48 hours in a glass aquaria of 100 litres capacity. During this period the postlarvae were fed on 35% protein commercial pellets. At the end of the acclimatization period, mortality was less than 2%.

Reconstituted standard water was used for acclimatizing prawns and conducting tests. It was prepared according to the standard methods (APHA et al. 1989), which state the composition of soft waters by adding the required salts ($NaHCO_3$, $CaSO_4.2H_2O$, $MgSO_4$ and KCl) to deionized fresh water. Temperature was controlled by air conditioning and adjusted to 28°C, and the photoperiod was 12 hr L:D cycle.

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The fungicide used in this study was the copper oxychloride (Cuprogarb 500™ - wettable powder with 99% purity), purchased from Oxiquímica Ind. e Com. Ltda. Stock solutions were prepared by dissolving a calculated quantity of active ingredient (500 g of copper/Kg) in distilled water. A series of five concentrations (toxic range determined by preliminary tests: 0.05, 0.10, 0.20, 0.30 and 0.40 mg L^{-1}) was prepared by adding a calculated volume from the stock solution into test containers, considering the equivalent on Cu^{++}. One container was kept as the unexposed control group. Test containers were constituted of retangular glass aquaria of 16 litres capacity filled with 5 litres of test solution. Each aquaria was covered with a plastic film to prevent volatilization. Aeration was provided by air pumps and individual stone diffusers. Twenty postlarvae (mean weight of 0.022 $\pm$ 0.008 g) were randomly distributed into each test container (4 postlarvae L^{-1}, n =20). No feed was supplied during the experiments.

The bioassays were carried out according to two different methods: static and renewal tests (APHA et al., 1989). In the static test there were no solution replacements during the 96 hours of exposure. In the renewal one test solutions were replaced by fresh ones of the same respective concentrations after every 24 hours until 96 hours of testing. A parallel aquaria battery was kept with the fresh solution and the animals were carefully transferred from the occupied aquaria (with the 24 hr used solution) to the empty aquaria (with the fresh solution in the same initial nominal concentration). Aquaria batteries were replicated four times in the static method and three times in the renewal one.

Dissolved oxygen (mg L^{-1}), temperature (^0C), pH and electric conductivity ($\mu S\ cm^{-1}$) were recorded individually in each test container at exposure times of 24, 48, 72 and 96 hours. Hardness and alkalinity (mg $CaCO_3\ L^{-1}$), and total ammonia (mg L^{-1}) were determined by standard methods (APHA, 1989) only after the ending of the experiments.

Mortalities were recorded at 24, 48, 72 and 96 hours of exposure and dead organisms were removed regularly from the test solutions. The criterion for death was taken to be the total lack of movement, observed when prawns did not respond to gentle touching with a glass rod. The obtained data were analysed through the softwares Multi-method LC_{50} and Gwbasic 3.10 according to the four statistical methods for estimating Median Lethal Concentrations (LC_{50}): Binomial (Stephan, 1977), Moving Average Interpolation (Bennett, 1952; Stephan, 1977), Probit (Finney, 1971) and the Trimmed Spearman Karber (Hamilton et al., 1977), and the results were compared by one way ANOVA. The median values of LC_{50} obtained in static and renewal tests were analysed by student *t*-test. Safe concentration levels were estimated by the quotient of LC_{50}(96hr) / 100, according to the same methodology used by Natarajan et al. (1992).

After toxicity testing all prawns was grouped by concentration and reserved for analysing copper bioaccumulation. The whole body of the prawns was used in the measures of copper bioaccumulation, since their small size did not allow individual analysis for the different parts of the body. Samples were dried, and digested in a 2,5 mL mixture of (2:1) nitric acid (64%) and perchloric acid (96%), according to the adapted method related by Sarruge and Haag (1974). All samples were analysed on a GBC 932 AAS. The Standard was Merck aqueous copper metal (99,99%). The lower limit of detection was 0.01 μg/g dry weight. As the respiratory pigment in prawns is a copper-containing (haemocyanin), the natural concentration of copper (estimated from the control group) was subtracted from the trial groups for calculating only the accumulated copper contents.

RESULTS AND DISCUSSION

The acute toxicities of copper are presented in the tables 1 and 2, according to the four methods used for estimating LC_{50}'s, and there were no statistical differences among them. It indicates that any statistical method can be used with the same accuracy. Therefore, mortality data can be transformed by the four methods (if they all were available), and the reported LC_{50} must be the lower one. It will provide a large security margin. The reported LC_{50}'s ranged from 0.22-0.25 mg L^{-1} at 24 hr of exposure to 0.06 mg L^{-1} at 96 hr of exposure in the static bioassay. Similarly, LC_{50}'s in the renewal bioassay ranged from 0.22-0.26 mg L^{-1} at 24 hr of exposure to 0.05 mg L^{-1} at 96 hr of exposure.

Comparisons between LC_{50}'s from static and renewal tests indicated that there were no statistically significant differences during all time exposures (Table 3). It demonstrates that there were no alteration of the fungicide active ingredient during the static exposure. It can be attributed to the high stability of the metal Cu^{++} in the water.

The physico-chemical parameters analyzed during the bioassays showed no differences among the range of 5 concentrations, neither between concentration ranges and control groups, therefore it was possible to calculate the respective averages (Table 4). Similarly, there were no significant variations between static and renewal test methods for any parameter, except for the total ammonia concentration, which was higher in the static test (Figure 1). Toxic exposures must have stimulated the excretion mechanism in the prawns. This figure also shows that lower ammonia concentrations were detected at higher copper concentrations. It can be attributed to the high toxic action of these concentrations, which caused higher mortality. Therefore, aquaria with lower concentrations of copper had more ammonia detection, since there were more surviving prawns in constant metabolism of excretion during all of the time exposure (96 hours). Anyway, total ammonia concentrations were higher in the static bioassay, increasing above the 1.0 mg L^{-1} reported by Sandifer et al. (1983) as the critical level to postlarvae of *M. rosenbergii*. Therefore, the

renewal test seems to be the best method for carrying out bioassays with no toxic interferences of ammonia.

Table 1. Comparative values of copper LC_{50} (mg L^{-1}) to *M. rosenbergii* estimated under four methods of data transformation for static acute toxicity test with copper oxychloride.

Exposure Time (hr)	Binomial	Moving Average	Probit	Spearman Karber	ANOVA S	ANOVA F
24	0.25 (0.12-0.40)*	0.24 (0.18-0.35)	0.22 (0.15-0.29)	0.23 (0.18-0.29)	0.04	0.50^{ns}
48	0.14 (0.05-0.25)	0.11 (0.07-0.14)	0.10 (0.07-0.13)	0.12 (0.08-0.17)	0.03	0.98^{ns}
72	0.09 (-)**	0.08 (0.04-0.11)	0.08 (0.05-0.10)	0.08 (0.04-0.17)	0.09	0.57^{ns}
96	0.06 (-)	0.06 (0.00-0.07)	0.06 (0.04-0.08)	0.06 (0.03-0.13)	0.01	0.33^{ns}

* = 95% of confidence limit
** = non calculated values
s = standard deviation
ns = non significant

Table 2. Comparative values of copper LC_{50} (mg L^{-1}) to *M. rosenbergii* estimated under four methods of data transformation for renewal acute toxicity test with copper oxychloride.

Exposure Time (hr)	Binomial	Moving Average	Probit	Spearman Karber	ANOVA s	ANOVA F
24	0.26 (0.05-0.40)*	0.22 (0.15-0.42)	0.23 (0.14-0.41)	0.22 (0.16-0.31)	0.07	0.19^{ns}
48	0.11 (-)**	0.11 (0.07-0.17)	0.10 (0.04-0.13)	0.11 (0.06-0.20)	0.07	2.28^{ns}
72	0.09 (-)	0.08 (0.02-0.11)	0.07 (0.03-0.16)	0.09 (0.04-0.14)	0.08	0.45^{ns}
96	0.05 (-)	0.05 (-)	0.05 (0.03-0.07)	0.05 (-)	**	**

* = 95% of confidence limit
** = non calculated values
s = standard deviation
ns = non significant

Table 3 - Comparative values of copper LC_{50} (mg L^{-1}) to *M. rosenbergii* for static and renewal acute toxicity tests with copper oxychloride

Exposure time (hr)	LC_{50} Static	LC_{50} Renewal	Student *t*-test	
			t	d.f.
24	0.24	0.23	-0.033^{ns}	26
48	0.12	0.11	0.775^{ns}	25
72	0.08	0.08	-0.780^{ns}	26
96	0.06	0.05	1.953^{ns}	17

ns = non significant
d.f.= degrees of freedom

Table 4 – Physico-chemical parameters of water used in bioassays

Parameter	Static	Renewal
Temperatura (°C)	27.42 ± 0.35	27.11 ± 0.40
pH	7.40 ± 0.18	7.53 ± 0.17
Electric conductivity (μS cm^{-1})	173.42 ± 3.86	174.36 ± 2.95
Dissolved oxygen (mg L^{-1})	6.93 ± 0.20	7.18 ± 0.15
Hardness (mg $CaCO_3$ L^{-1})	46.55 ± 1.34	48.07 ± 2.50
Alkalinity (mg $CaCO_3$ L^{-1})	32.60 ± 2.22	23.26 ± 5.45

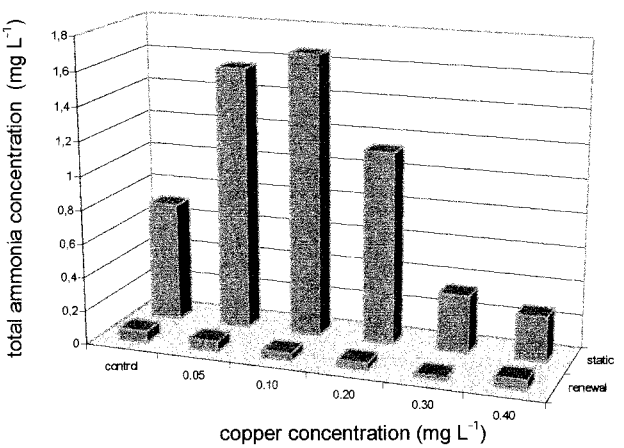

Figure 1 – Total ammonia detected during bioassays with copper oxychloride

Different levels of copper sensitivity were determined for some macrocrustaceans (Table 5). The variety of forms of Cu^{++} used on these studies may explain in part some of these differences. The LC_{50}'s determined by Natarajan et al (1992) to *M. rosenbergii* differ from those obtained in the present study, especially in 24 and 48 hours of exposure. These differences can be related to the higher water alkalinity (200 ± 20 mg $CaCO_3$ L^{-1}) used by Natarajan et al. (1992). According to Spear and Pierce (1979), freshwater invertebrates can express different sensitivities to copper, according to the water alkalinity and hardness. On the other hand, LC_{50}'s determined by Ghate and Mulherkar (1979) to *M. kistnensis* and by Liao and Guo (1990) to *M. rosenbergii* are very similar to those obtained in the present study. Liao and Guo (1990) found that in shrimps (*Penaeus* sp) copper toxicity was three orders of magnitude less than in freshwater prawns (*macrobrachium* sp). Conversely, Ahnasullah and Ying (1995) and Bambang et al. (1995) reported copper toxicities to marine shrimps, which were more similar to those measured in *M. rosenbergii*.

Table 5 – Acute toxicity of copper to some macrocrustaceans

Specie	Exposure time (hours)	LC_{50} (mg L^{-1})	Form of Cu^{++} used*	Reference
Caridina sp	48	0.281	1	Ghate and Mulherkar (1979)
Echinogammarus tibaldii	96	0.59	2	Pantani et al. (1997)
Gammarus italicus	96	0.17	2	Pantani et al. (1997)
Macrobrachium kistnensis	48	0.279	1	Ghate and Mulherkar (1979)
Macrobrachium lamarrei	96	0.247	1	Murti and Shukla (1984)
Macrobrachium rosenbergii	**24**	**0.23 – 0.24**	**3**	**Present study**
Macrobrachium rosenbergii	**48**	**0.11 – 0.12**	**3**	**Present study**
Macrobrachium rosenbergii	**72**	**0.08**	**3**	**Present study**
Macrobrachium rosenbergii	**96**	**0.05 – 0.06**	**3**	**Present study**
Macrobrachium rosenbergii	24	0.39	1	Liao and Guo (1990)
Macrobrachium rosenbergii	24	1.22	1	Natarajan et al. (1992)
Macrobrachium rosenbergii	48	0.035	1	Natarajan et al. (1992)
Macrobrachium rosenbergii	96	0.012	1	Natarajan et al. (1992)
Metapenaeus ensis	24	465	1	Liao and Guo (1990)
Paratya australiensis	96	0.034	1	Daly et al. (1990)
Penaeus japonicus	24	427	1	Liao & Guo (1990)
Penaues japonicus	96	1.45	2	Bambang et al. (1995)
Penaeus merguiensis	96	0.38	4	Ahsanullah & Ying (1995)
Penaeus monodon	24	436	1	Liao and Guo (1990)
Penaeus penicillatus	24	319	1	Liao and Guo (1990)
Penaeus semisulcarus	24	231	1	Liao and Guo (1990)

(*) = 1-copper sulphate; 2-copper chloride; 3-copper oxychloride; 4-mentioned as dissolved copper

Sublethal limit for copper exposure was recorded as 0.0005 mg L^{-1} in this study, whereas the brasilian environmental legislation (CONAMA, 1986) reported 0.02 mg L^{-1} as the safe level of copper for rearing aquatic organisms. Although this reccomendation does not suit *M. rosenbergii* requirements, it sems to be acceptable to some species of fishes, since the same safe level of 0.02 mg L^{-1} was equally determined by Yang and Chen

(1996) for rearing the eel *Anguilla japonica*. Furthermore, Nussey et al. (1996) have also detected copper levels of 0.055 and 0.085 mg L^{-1} as non toxic for the natural populations of tilapia (*Oreochromis mossambicus*) from the Olifants River – South Africa.

Analysis of copper accumulation revealed that this pattern was inversely proportional to the range of acute concentrations (Figure 2). Certainly, the fast death of the animals under higher fungicide concentrations should have interrupted copper accumulation, while surviving animals must have accumulated more copper in lower concentrations due to the longer exposure time. Despite this, Borgmann et al. (1993) have determined that the amphipod *Hyalella azteca* can accumulate copper only under acute exposures. Therefore, the pattern of copper bioaccumulation in crustaceans is not well defined yet. More studies should be carried out in acute and chronic exposures in order to clarify this aspect.

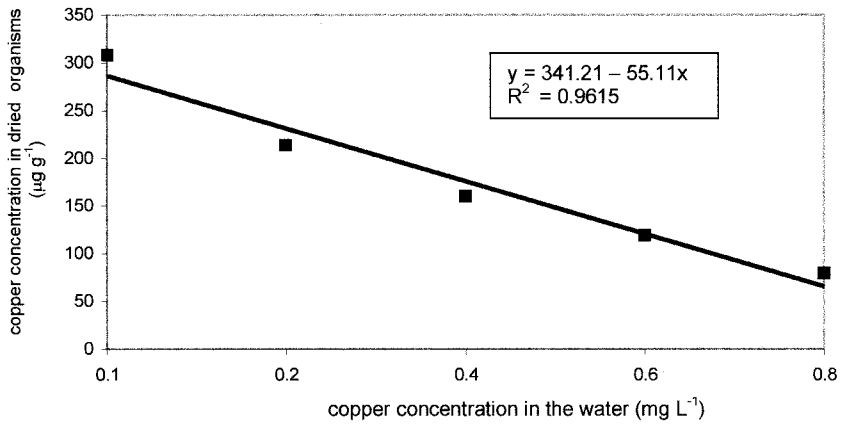

Figure 2 – Copper bioaccumulation in *M. rosenbergii* during 96h of copper oxychloride exposure

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