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Static Non-Renewal Acute Toxicity of Copper (II) Sulfate Pentahydrate on Adult Shrimp *Litopenaeus vannamei* in São Vicente, Cabo Verde

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Abstract

This paper presents the acute toxicity effects of Copper (II) sulfate pentahydrate on adult *Litopenaeus vannamei*. The experimental treatments were 20 mg/L, 60 mg/L, and 80 mg/L $CuSO_4 \cdot 5H_2O$, while mortalities after 96 hours were 20%, 100%, and 66.7%, respectively. Accordingly, the 96-h LC_{50} (Lethal Median Concentration) of copper to the adult *L. vannamei* was estimated to be 41.69 mg/L. Physicochemical parameters such as pH, dissolved oxygen, and temperature were monitored every 24 hours. A positive relationship was observed between copper concentration and mortality, indicating that increased copper sulfate concentrations led to higher mortality rates. These findings suggest that precise control of copper sulfate concentrations is crucial in aquaculture to prevent toxicity effects and ensure successful shrimp farming. These findings contribute to safer aquaculture by establishing the threshold levels of toxicity that minimize environmental contamination and ensure ecosystem health.

Keywords

Shrimp, *Litopenaeus vannamei*, Copper Acute Toxicity, LC_{50} Determination, Aquaculture, Aquatic

1. Introduction

White Leg Prawns (*Litopenaeus vannamei*) which are widely known as "vannamei," are native to the warm marine waters of the Eastern Atlantic and Pacific [1]. Copper sulfate is commonly applied to shrimp ponds to eliminate filamentous

algae [2] while it's very effective in reducing the abundance of phytoplankton, including Microcystis and other blue-green algae [3]. The application rate of copper sulfate varies from 0.025 to 2 mg/L and is directly related to total alkalinity [4]. The report from field experiments showed that shrimp farmers often apply excess amounts of copper sulfate [5] [6] in pond management which is of primary concern. Past studies [7]-[11] have extensively reviewed the toxicity of copper in teleosts with a copper concentration ranging from 0.5 to as much as 2.5 mg/L, which have been reported as 96-h LC_{50} median lethal concentration values. This paper, therefore, provides information on the effects of a change in concentration of Copper (II) sulfate pentahydrate on adult *L. vannamei* and the toxicity endpoint (median lethal dose; LC_{50}).

2. Materials and Methods

Adult *L. vannamei* used in this study were obtained from the shrimp farm at Calhau, São Vicente, Cabo Verde. They were transported in the same seawater used for the bioassays to the experimental aquarium and acclimated for at least 24 hours before the experiment. Shrimps were maintained in well-oxygenated seawater under a fixed temperature (28.08 ± 0.54˚C), dissolved oxygen level (5.4 ± 0.8 mg/L), and pH (7.3 - 8.12) during acclimation to minimize stress. No copper sulfate was added during this period.

Six treatment groups of T_1, T_2, T_3, T_4, and T_5, with the exception of T_0 (control test), were prepared with Copper (II) sulfate pentahydrate solution of 5 mg/L, 20 mg/L, 40 mg/L, 60 mg/L, and 80 mg/L respectively. The control group (T_0) of seawater was such that $CuSO_4{\cdot}5H_2O$ was not added to establish a baseline for comparison with treatment groups. The same handling procedures and water quality conditions were maintained across all groups, ensuring uniformity in experimental conditions. In order to ensure accurate copper sulfate ($CuSO_4{\cdot}5H_2O$) concentrations in each treatment group, we prepared a 100 mg/L stock solution by dissolving 0.01 g $CuSO_4{\cdot}5H_2O$ in 500 mL deionized water. The desired final concentrations of each treatment were then achieved via the dilution equation (Equation (1)).

$$(M_1 V_1 = M_2 V_2) \tag{1}$$

where:

M_1 = Initial concentration of stock solution (100 mg/L)

V_1 = Volume taken from stock solution

M_2 = Desired concentration in treatment

V_2 = Final volume of solution (500 mL)

Volumes of the stock solution (**Table 1**) were taken with a calibrated precision pipette and transferred to individual flasks. These were diluted with deionized water to the final volume of 500 mL, mixing well to ensure homogeneity. The solutions thus prepared were then added immediately to the respective treatment aquaria, each containing 4.5 L of seawater, and agitated to ensure even distribution.

Table 1. $CuSO_4 \cdot 5H_2O$ concentration of treatment groups.

Treatment Group	M_1 (mg/L)	V_1 (ml)	M_2 (mg/L)	V_2 (ml)
T_0 (Control)	0	500	0	0
T_1	5	500	100	25
T_2	20	500	100	100
T_3	40	500	100	200
T_4	60	500	100	300
T_5	80	500	100	400

10 adult *L. vannamei* were selected and shared per aquarium containing 4.5 L of seawater in 6 treatment groups of T_0, T_1, T_2, T_3, T_4, and T_5 with an average weight of 9.75 ± 2.28 g, 10.96 ± 1.20 g, 11.59 ± 1.40 g, 9.69 ± 2.06 g, 12.24 ± 2.01 g and 9.99 ± 1.46 g respectively and an average length of 11.88 ± 0.69 cm, 12.05 ± 0.37 cm, 12.15 ± 0.56 cm, 11.51 ± 0.83 cm, 12.43 ± 0.63 cm and 11.6 ± 0.74 cm respectively (see **Supplementary Table S3**). Oxygen was introduced to each aquarium using an automated pump. Physiochemical parameters such as pH, Dissolved oxygen, and temperature were measured at intervals of 24 hrs, 48 hrs, 72 hrs, and 96 hrs. The mortality rate was also recorded at these time intervals, as shown in Appendix 1.

Short-term median lethal concentration LC_{50} toxicity tests were carried out following the method described by Buikema *et al.* [12] and APHA-AWWA-WPCF [13]. The adult shrimps were taken from the acclimation tank and transferred to each solution. Bioassay experiments to establish tolerance limits were conducted in 4.5 L plastic tanks containing 0.5 L of test solution. Each tank contained 10 shrimps, and the water was aerated continuously by an air stone. There were sextuplicates for each test solution with 60 adults (10 per replicate for each test solution). During the experiments, the shrimps were fed on two selected species of marine microalgae (*Thalassiosira weissflogii* and *Chaetoceros muelleri*) contained in the seawater from the shrimp farm. Water temperature was maintained at 28.08 ± 0.54 C, dissolved oxygen was 5.4 ± 0.8 mg/L, and the pH ranged from 7.3 to 8.12. Observations were usually made at 24-h intervals up to 96 h. Death was assumed when shrimps were immobile and showed no response when touched with a glass rod. The concentration-response of test organisms was determined for LC_{50} of copper with a Microsoft Excel at 95% confidence limits [14], which consists of transforming the mortality data in probit mortality. With this method, the estimated probit line and logarithm concentration curve were determined.

3. Results and Discussions

Results from only three treatment groups were captured in the analysis due to experimentation errors resulting from the loss of oxygen to the aquaria on day

one. The number of live shrimps was adjusted accordingly (see **Supplementary** Table S1) with a focus on three treatment groups (T_2, T_4, and T_5), which resulted in the presentation shown in Table 2 below.

Table 2. Parameters from treatment groups.

Date	Time	Treatment Group	Conc mg/L	Total	Alive	Dead	Mortality (%)	pH	Temp (C)	DO (mg/L)
		T_2	20	5	5	0	0	7.5	27.5	5.85
10/1/2021	24 hr	T_4	60	5	5	0	0	7.3	27.5	5.23
		T_5	80	6	6	0	0	7.3	27.5	5.4
		T_2	20	5	5	0	0	8.1	27.6	6.09
10/2/2021	48 hr	T_4	60	5	5	0	0	7.8	27.5	4.7
		T_5	80	6	5	1	16.7	7.8	27.8	5.11
		T_2	20	5	5	0	0	8.12	27.8	6.04
10/3/2021	72 hr	T_4	60	5	4	1	20	7.78	27.9	4.3
		T_5	80	5	3	2	40	7.75	27.8	3.9
		T_2	20	5	4	1	20	7.9	28.6	5.81
10/4/2021	96 hr	T_4	60	2	0	2	100	7.6	28.9	5.7
		T_5	80	3	1	2	66.7	7.8	28.8	5.75

Results obtained from Table 2 showed that no shrimp died after 24 hours of exposure to 20 mg/L, 60 mg/L, and 80 mg/L of $CuSO_4{\cdot}5H_2O$ during day one. In 80 mg/L of $CuSO_4{\cdot}5H_2O$ after 48hr, 16.7% mortality was recorded, while 20% and 40% mortality were noted in 60 mg/L and 80 mg/L of $CuSO_4{\cdot}5H_2O$, respectively, after 72 hours. There were 20%, 100%, and 66.7% mortality recorded for 20 mg/L, 60 mg/L, and 80 mg/L of $CuSO_4{\cdot}5H_2O$, respectively, after 96 hr exposure time.

The result from LC_{50} computation (see **Supplementary** Table S2) using Table 3 showed that at over 41.69 mg/L $CuSO_4{\cdot}5H_2O$, the resultant effect would be the mortality of over 50% of the adult shrimp. Figure 1 shows that the mortality of shrimps increases with the concentration of Copper (II) sulfate pentahydrate, showing a positive correlation and response of shrimp to higher concentrations of toxicity in their seawater.

Table 3. Probit analysis and LC_{50} computation.

Conc (mg/L)	log10 (concentration)	% Mortality	probit
20	1.301029996	20	4.16
60	1.77815125	60	5.25
80	1.903089987	83	5.95

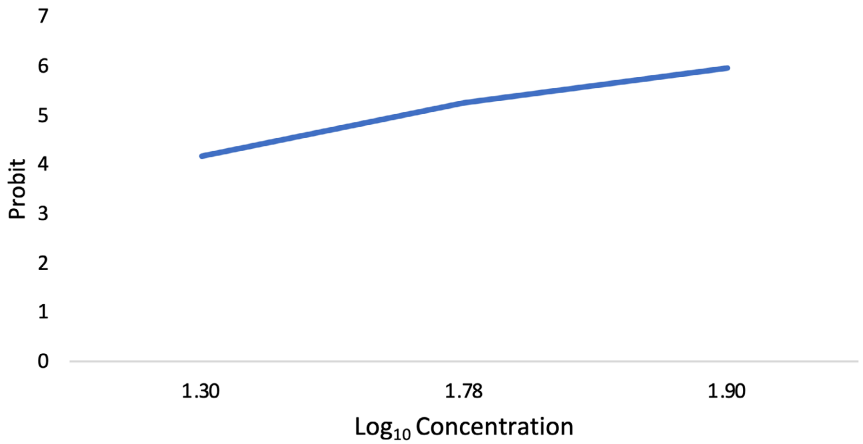

Figure 1. Dose-response curve of *L. vannamei* to $CuSO_4 \cdot 5H_2O$.

4. Conclusions

Copper exposure greatly affects the survival rate of shrimp, particularly in their early developmental stages, namely the nauplii and zoeae, which are marked by high sensitivity [15]. As development progresses, tolerance to copper is increased, with juveniles being the most tolerant. In this study, the 96-h LC_{50} of *L. vannamei* adults was determined to be 41.69 mg/L, which aligns with reports that LC_{50} values are lower in earlier development stages. For example, *Penaeus japonicus* nauplii have a 48-h LC_{50} of 1 μg/L, while juveniles have a 96-h LC_{50} of 2050 μg/L [15].

Molting in crustaceans increases sensitivity to copper because of increased water and ion uptake, and copper toxicity can synergistically increase molting stress and thus increase mortality. Furthermore, exposure also disrupts osmoregulation, reducing both hypo- and hyper-osmoregulatory capacity in a dose- and time-dependent manner [15]. Chronic copper exposure is toxic to growth, with higher levels causing reduced weight gain and some growth rates. Still, there have been reports that copper at lower levels (0.3 mg/L) can increase final weight, body length, survival, and yield, but no differences were statistically significant [16] [17].

While our study primarily focused on acute lethality, other studies highlight that long-term exposure to high copper concentration could result in structural damage to the hepatopancreas, which is a common effect, with higher copper concentrations causing lumen enlargement, cell rupture, and tissue necrosis [16] [17], others include immune suppression, and gut microbiota shifts [17] [18]. The findings of our study demonstrate that there is an affirmative correlation between mortality rates and copper levels, as other researchers have concluded [15]. Additional research indicates that reduced copper levels exert physiological effects even after extended periods of exposure [17].

Moreover, excessive use of copper in aquaculture, especially as an algaecide, may cause environmental pollution and can indirectly result in shrimp health

problems [3] [17]. Kubitza [19] suggests uncontrolled use of copper sulfate in shrimp ponds can lead to adverse effects, including copper-resistant cyanobacteria, increased toxicity, reduced growth of shrimp, and environmental problems, with consequences for shrimp health, productivity, and survival. The results necessitate the monitoring and control of copper concentrations continuously, not just to avert acute toxic impacts but also to reduce chronic and sublethal impacts that can impair the health and productivity of shrimp.

Although the initial experiment had lags in oxygen supply, which resulted in the death of some shrimps, nonetheless, conclusive results were obtained from the live shrimps that finished the experiment. The data is indicative of the precise control and management of copper sulfate concentrations in an aquaculture environment. This study gives a basis for safer shrimp farming and sustainable aquaculture by determining lethal concentrations of the chemical compound so that hazards associated with environmental contamination could be limited and ecosystems kept healthier. Further, constant monitoring and control should be ensured in order to minimize those errors which could result from external factors besides the toxicity effect being considered in the experiment. The findings highlight the importance of continued monitoring and regulation of copper levels, not only to prevent acute toxic impacts but also to minimize chronic and sublethal impacts that threaten the health and productivity of shrimp. In addition, ensuring sustainable copper management in aquaculture aligns with broader efforts to enhance self-sufficiency and sustainability [20], where seafood production must balance efficiency with environmental stewardship.

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Author Contributions

T.O., N.K. conceived and planned the study. T.O. processed the data, performed the analysis, designed the figures, and drafted the manuscript. T.O., N.K. contributed to the interpretation of the results. N.K. provided critical feedback and helped shape the research, analysis, and manuscript. All authors discussed the results and commented on the manuscript.

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Conflicts of Interest

The authors declare no known conflict of interest that could have biased the work results in this study.

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Appendix

Supplementary Tables

Table S1. Summary of variables measured for treatment groups.

Date	Time	Treatment Group	Conc mg/l	Total	Alive	Dead	pH	Temp (C)	DO (mg/L)
10/1/2021	24 hr	T_0	0	9	0	9	-	-	-
		T_1	5	8	0	8	-	-	-
		T_2	20	9	5	4	7.5	27.5	5.85
		T_3	40	11	0	11	-	-	-
		T_4	60	11	5	6	7.3	27.5	5.23
		T_5	80	9	6	3	7.3	27.5	5.4
10/2/2021	48 hr	T_2	20	5	5	0	8.1	27.6	6.09
		T_4	60	5	5	0	7.8	27.5	4.7
		T_5	80	6	5	1	7.8	27.8	5.11
10/3/2021	72 hr	T_2	20	5	5	0	8.12	27.8	6.04
		T_4	60	5	4	1	7.78	27.9	4.3
		T_5	80	5	3	2	7.75	27.8	3.9
10/4/2021	96 hr	T_2	20	5	4	1	7.9	28.6	5.81
		T_4	60	2	0	2	7.6	28.9	5.7
		T_5	80	3	1	2	7.8	28.8	5.75
							7.85	28.07777778	5.266666667
							0.166883193	0.535671956	0.801592166
						Average ± SD	7.85 ± 0.17	28.08 ± 0.54	5.4 ± 0.8

Table S2. Summary of LC_{50} computation.

Summary Output			
		Intercept	0.499598056
		X Variable 1	2.782105828
Regression Statistics			
Multiple R	0.979953852	y = ax + b	
R Square	0.960309551	y = 2.78x + (0.50)	
Adjusted R Square	0.920619102	5 = 2.78x + 0.50	
Standard Error	0.254150027	5 − 0.50 = 2.78x	
Observations	3	x = (5 − 0.50)/2.78	
		x = 1.62	

Continued

ANOVA							
	df	*SS*	*MS*	*F*	*Significance F*	LC50 = antilog x LC50 = antilog 1.62	41.68693835
Regression	1	1.562807764	1.562807764	24.19497842	0.127684672	LC50 = 41.69	
Residual	1	0.064592236	0.064592236				
Total	2	1.6274					

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.499598056	0.950719364	0.525494772	0.691981207	−11.58043683	12.57963294	−11.58043683	12.57963294
X Variable 1	2.782105828	0.565602117	4.918839134	0.127684672	−4.404550471	9.968762127	−4.404550471	9.968762127

Table S3. Length and weight measurement.

concentration (ppm)	number of adult shrimps	length(cm)	weight(g)	Average (length)	Average (weight)
0	1	10.5	6.27	11.88 ± 0.69	9.75 ± 2.28
	2	11.5	10.75		
	3	11.8	10.62		
	4	12.5	12.06		
	5	12.3	6.72		
	6	12.8	12.35		
	7	12.6	12.13		
	8	11.5	7.8		
	9	11.8	10.4		
	10	11.5	8.4		
5	1	11.8	11.2	12.05 ± 0.37	10.96 ± 1.20
	2	13	13.1		
	3	12.2	11.2		
	4	11.9	9.3		
	5	12	12.4		
	6	12	10.3		
	7	12.1	11.8		
	8	12	10		
	9	11.8	9.9		
	10	11.7	10.4		
20	1	11.2	9.6	12.15 ± 0.56	11.591 ± 1.404
	2	12.1	11.01		

Continued

20	3	12.7	13		
	4	12.6	13		
	5	13	14		
	6	12.5	11.4		
	7	11.5	10		
	8	11.8	12		
	9	11.9	10.7		
	10	12.2	11.2		
40	1	12.5	12.4	11.51 ± 0.833	9.69 ± 2.062
	2	11.1	8.9		
	3	12.1	11.6		
	4	12	10.4		
	5	10.5	7.3		
	6	10	5.7		
	7	11.6	10.3		
	8	12.4	11.2		
	9	11	8.6		
	10	11.9	10.5		
60	1	12.3	12.7	12.43 ± 0.63	12.24 ± 2.01
	2	13.2	14.5		
	3	12.5	11.5		
	4	13.2	15.3		
	5	12.3	12.1		
	6	12.8	13.2		
	7	12.5	11.1		
	8	12	11.4		
	9	12.5	12.6		
	10	11	8		
80	1	11.5	9.6	11.61 ± 0.74	9.99 ± 1.46
	2	12	10.2		
	3	12.5	12.3		
	4	11.8	10		
	5	11.5	9.3		
	6	11.6	10		
	7	12.5	11.8		
	8	11.9	10.8		
	9	10.2	7.3		
	10	10.6	8.6		