Implementation of Anti-Fouling Tests with Copper Sulphate Pentahydrate Using the Coral *Flavia gravida* (Cnidara, Scleractinia)

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The use of antifouling compounds in paints on ships and oil platforms has caused environmental damage to marine species. Thus, the demand for environmentally friendly compounds and technologies has increased. The objective of this article was to perform toxicological experiments with the antifouling biocide copper sulfate pentahydrate to identify concentrations that have the least impact on the native coral *Favia gravida*. The acute test showed mortality at concentrations of 0.02 and 0.025 mg/mL, although reactions in mobility, cilia movement, and initial development were observed at concentrations above 0.01 mg/mL. The chronic test showed continued development only in the control and at a concentration of 0.005 mg/mL. The high mortality rate in the control group indicates the need for further testing. This is the first toxicological test with an antifouling agent in *F. gravida*.

Keywords: Ecotoxicology. Coral Reefs. Acute Essay. Chronic Essay.

Antifouling compounds were highly commercialized in the 1970s due to increasing demand in boat paints [1]. The importance of using antifouling compounds is linked to marine biofouling, a term used to define the action of species that establish themselves on natural and artificial surfaces, such as ships, smaller vessels, and oil platforms [2]. However, since then, several ecological consequences have been documented worldwide, including the impact on marine organisms and ecosystems [1].

Scleractinian corals are one of the organisms that can be affected by antifouling compounds. They are animals belonging to the phylum Cnidaria and are the leading builders of coral reefs [3].

These organisms secrete calcium carbonate, forming the structure of the reefs, and shelter unicellular, photosynthetic algae called zooxanthellae that live in symbiosis within the coral tissue, providing them with food [8].

However, studies on the effect of antifouling compounds on these organisms in Brazil are in their incipient stages.

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A common substance used in antifouling paints is copper sulfate. This substance is a salt formed by sulfuric acid and copper, as in Equation 1:

$$Cu^{2+} + SO_4^{2-} \leftrightarrow CuSO_4 \quad (1)$$

It has a molecular formula ($CuSO_4$) and high solubility in water [4]. Copper sulfate is one of the most widespread pollutants in the marine environment. Among its applications are its use as a fungicide, bactericide, algaecide, and fertilizer [4]. It is already used in antifouling paints.However, despite its high solubility in water, several factors, including alkalinity, hardness, and pH, influence the action of copper in water and impact marine organisms [5]. Therefore, understanding the reactions of organisms to different concentrations of copper sulfate is of great importance.

The objective of this work is to test whether the antifouling compound copper sulfate pentahydrate ($CuSO_4.5H_2O$) affects the initial development of the coral *Favia gravida*. To achieve this, acute and chronic toxicological tests were conducted at various concentrations. *Favia gravida* is a zooxanthellate coral that is highly resistant to variations in temperature, salinity, and water turbidity [6]. It forms massive, spherical, and encrusting colonies [6]. In Brazil, *F. gravida* occurs from Maranhão to Espírito Santo, being

easily found on adjacent reefs and rocky shores, and is often exposed to contamination in urbanized regions [6,8].

Materials and Methods

Coral Collection

Favia gravida colonies were collected in June 2024 on Boa Viagem beach, Salvador, Bahia. They were transported to the laboratory in thermal boxes containing seawater, aeration, and light. The larvae were released 2 days after collection and sampled with a Pasteur pipette for the experiment.

Experimental Design

To carry out the ecotoxicological tests, a control solution with seawater (without copper sulfate pentahydrate) and five treatments with different concentrations of the copper sulfate solution were used: 0.005, 0.010, 0.015, 0.020, and 0.025 mg/mL. Each treatment and control had three replications. Each one of them contained five larvae of *F. gravida*. The response variables observed were larval mobility, larval cilia mobility, larval development, settlement, and mortality.

Acute and Chronic Tests

The acute test consisted of observing the response variables every four hours over 24 hours. The chronic test consisted of analyzing the same response variables for seven days, with observations made once a day, always around 3 pm. Larvae were observed with a stereomicroscope.

Experiment Preparation

The replicas consisted of Petri dishes with saline water (35 ppm) at a range of 23-25 ºC. These were previously washed with an extra 4% and decontaminated in a muffle at 400°C. Each Petri dish had a volume of 50 mL. Initially, a stock solution of the contaminant was prepared and subsequently diluted in the seawater in the Petri dishes. The stock solution contained 15 mg of copper sulfate pentahydrate ($CuSO_4.5H_2O$) in 100 mL of seawater. Dilution calculations for the different concentrations were made according to the following Equation 2:

$$C_1 \cdot V_1 = C_2 \cdot V_2 \quad (2)$$

Where:
C1 = The concentration of the control solution;
V1 = The volume we want to find;
C2 = The concentrations delimited above;
V2 = The volume of the Petri dishes.

Results

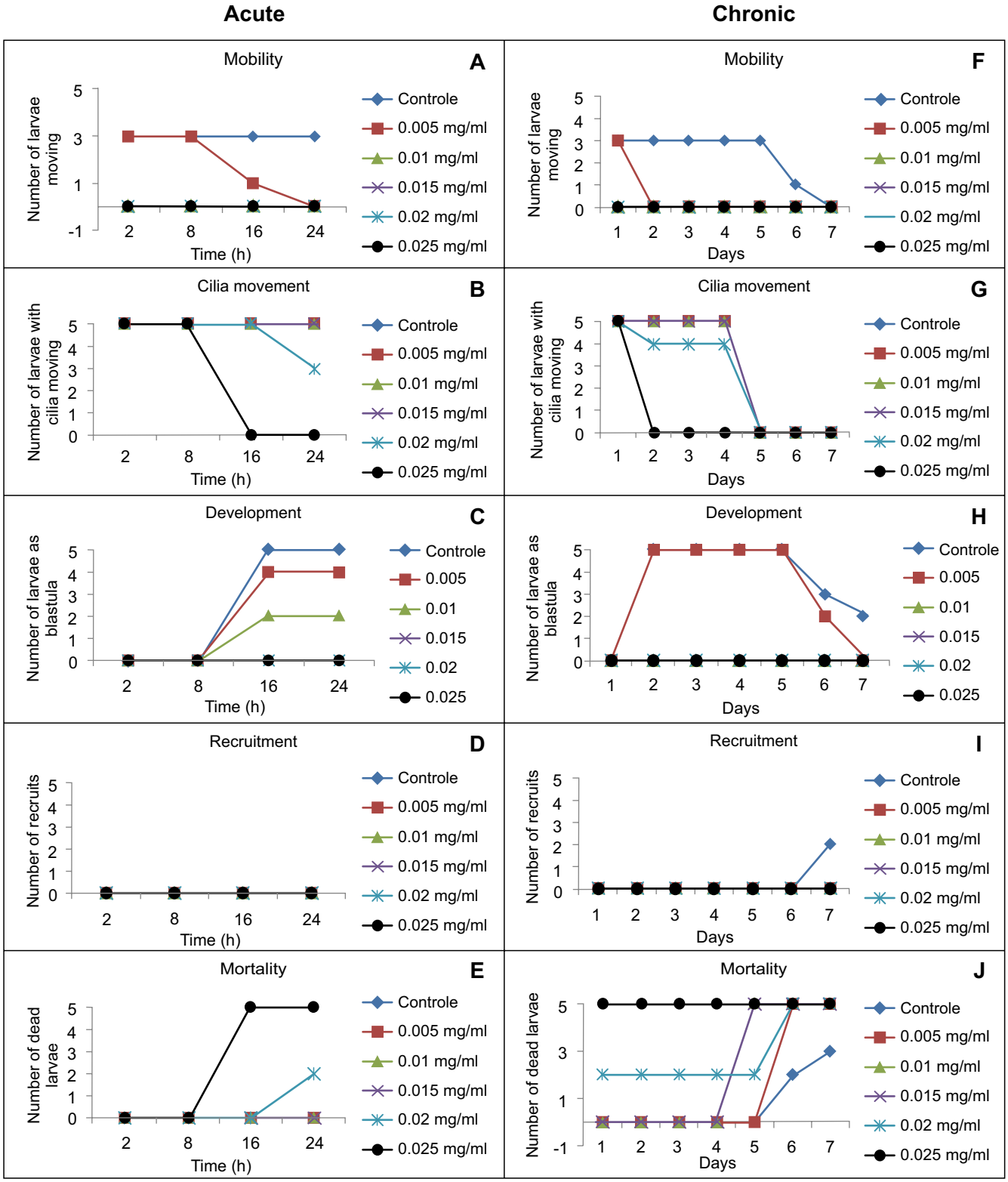
Acute Essay – 24 Hours

The acute test showed that during the first 24 hours, only the control and the concentration of 0.005 mg/mL showed movement of the larvae (Figure 1A). The beating of the larvae's cilia occurred during the first hours of almost all treatments. At the end of 24 hours, an average of two larvae from the 0.02 mg/mL treatment stopped the cilia movement, and all larvae of the 0.025 mg/mL treatment stopped the cilia movement. (Figure 1B). The initial development of the polyp occurred only in the control and at a concentration of 0.005 mg/mL (Figures 1C and 2C). No larval settlement was observed during this period (Figure 1D). Mortality was observed in all larvae treated with 0.025 mg/mL 16 hours after the start of the experiment. (Figure 1E).

Chronic Essay – 7 Days

The chronic test mainly aimed to evaluate larval development. Larvae mobility was observed in the control and 0.005 mg/mL treatment until the fifth day of the experiment. The other concentrations did not show larval displacement from the first day (Figure 1F). Cilia movement was observed in the control and at concentrations of up to 0.02

Figure 1. Response of the larvae of *Favia gravida* to different concentrations of copper sulfate pentahydrate.



A, B, C, D, E: Acute experiment; F, G, H, I, J, K: Chronic experiment.

mg/mL until the fourth day (Figure 1G). Larvae development began at the end of the first day in control and concentrations of 0.005 and 0.01 mg/mL. However, it continued to develop only in the control and 0.005 mg/mL treatment until the fifth day (Figure 1H). Recruitment occurred only in two larvae from one petri dish of the control (Figure 1I). At the end of the experiment, all larvae were dead except for the two that settled on the control (Figure 1J).

Discussion

This work presents the first toxicological test of copper sulfate pentahydrate on larvae of the coral *Favia gravida*. The results of the acute test (24 h) showed that 100% of the larvae died at a concentration of 0.025 mg/mL after one day of exposure, and that 40% of the larvae died at a concentration of 0.02 mg/mL. Mobility and blastula development only occur in the control and at a concentration of 0.005 mg/mL. The movement of the cilia is maintained at other concentrations. Regarding the results of the chronic test, the pattern remained similar. Movement and development occur in the control, and at a concentration of 0.005 mg/mL, cilia movement remains constant at other concentrations until the third and fourth day, after which recruitment occurs only in the control. We observed that the concentration of 0.025 mg/mL is highly toxic to the larvae of this species, and that only at 0.005 mg/mL does larval development occur, although they have not yet reached the recruitment stage.

Copper sulfate has already been used in toxicological tests with other organisms (Table 1). Comparing the results, it is possible to observe that the effects observed in *F. gravida* occurred at low concentrations, often lower than those reported in other studies. Chen and Lin (2000) observed mortality of the *Penaus monodon* at concentrations from 0.003 mg/mL at 15% salinity and 0.011 mg/mL at 25% salinity. Li and colleagues (2005), working with *Macrobrachium rosenbergii*, observed mortality at a concentration of 0.00042 mg/mL. Viana and Rocha (2005) showed that the diatomacea *Aulacoseira granulata* is less sensitive to copper sulfate than to atrazine, although an effect was observed at a concentration of 0.0001 mg/mL. Le Jeune and colleagues (2006) observed

Table 1. Comparison of our results with results from other articles that tested copper sulfate pentahydrate in other organisms.

Reference	Organism	Concentrations Tested (mg/mL)	Effect Concentrations (mg/mL)
Chen and Lin (2000)	Shrimp, *Penaeus monodon*	0.000, 0.001, 0.003, 0.006, 0.009, 0.012, 0.015 (15% salinity) 0.000, 0.008, 0.011, 0.014, 0.017, 0.020, 0.023 (25%salinity)	0.003, 0.006, 0.009, 0.012, 0.015 (15%salinity) 0.011, 0.014, 0.017, 0.020, 0.023 (25%salinity)
Li and colleagues (2005)	Shrimp *Macrobrachium rosenbergii*	0.00000, 0.00032, 0.00042, 0.00056, 0.00075, 0.001, 0.00135, 0.0018	0.00042, 0.00056, 0.00075, 0.001, 0.00135, 0.0018
Viana and Rocha (2005)	Diatom *Aulacoseira granulata*	0.0001, 0.00032, 0.001, 0.0032, 0.01	0.0001, 0.00032, 0.001, 0.0032, 0.01
Le Jeune and colleagues (2006)	Phytoplanktonic communities	0.00008, 0.00016	0.00008, 0.00016
Our study	*Favia gravida*	0.005, 0.010, 0.015, 0.020, 0.025	0.010, 0.015, 0.020, 0.025

changes in the phytoplankton communities with the following concentrations: 0.00008 and 0.00016 mg/mL.

Therefore, it is evident that very low concentrations can already have an impact on organisms.

Finally, the low recruitment proportion, especially in the control (with only two recruits in one petri dish), may indicate that the conditions offered in the experiment were not ideal for the larvae. The slick substrate of the petri dish may not be suitable. Some articles suggest using rougher substrates [7]. Perhaps the temperature, which had an average of 23ºC, also influenced the process. Finally, the presence of fungal contamination is a possibility, as we observed a white mucous membrane in each larva after death.

Conclusion

This is the first study to test copper sulfate pentahydrate in *Favia gravida* larvae. In a 24-hour acute assay, this substance caused mortality at concentrations of 0.02 and 0.025 mg/mL. However, reactions in the behavior of the larvae (mobility, cilia movement, and development) were observed at concentrations as low as 0.001 mg/mL. The chronic test showed continued development only in the control and at a concentration of 0.005 mg/mL, and recruitment only in the control from the fifth day onwards. The high mortality rate observed after seven days of the experiment may be related to the type of substrate and temperature used, and this relationship needs to be evaluated in future experiments.

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