EFFECTS OF VARYING CONCENTRATIONS OF COPPER SULFATE ON THE POPULATION DENSITY OF CHLORELLA SP., NITZSCHIA SP. AND OSCILLATORIA SP.

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The effects of varying concentrations of copper sulfate ($CuSO_4$) on the population density of the algae *Chlorella* sp., *Nitzschia* sp. and *Oscillatoria* sp. were studied. $CuSO_4$ was applied to the algal cultures grown in the laboratory at ordinary room conditions. The growth rates of *Chlorella* sp. and *Nitzschia* sp. were greatly reduced at concentrations 0.1 ppm. *Oscillatoria* sp. was more sensitive to $CuSO_4$, showing a significant reduction of growth at 0.05 ppm. Good logarithmic growth for all three species was observed in the controls.

Algae are beneficial to man and other organisms as food sources and gas exchangers (Jackson, 1964). However, some of their effects may be disturbing to man, such as the putrefaction of masses of algae in recreational lakes and in reservoirs for water supply (Prescott, 1959). An indirect effect is their ability to kill other aquatic organisms.

The freshwater plankton *Chlorella* sp. and *Oscillatoria* sp. tolerate any type of environment except intense heat and extreme chemical toxicity (Lackey, 1964; Carr and Whitton, 1973). Prescott (1959) has demonstrated that "bloom"-producing species, those which cause disturbances, are abundant in waters which are enriched with phosphates, nitrates and bicarbonates. *Chlorella* sp., a free-floating type of alga, grows well even in a carboy of distilled water on a laboratory shelf and may cause greenish coloration of the water (Lackey, 1964).

On the other hand, *Oscillatoria* sp., a clinging type, forms dense populations and produces a dark crimson coloration near the surface of the water (Carr and Whitton, 1973). Scharff (1958) has reported that other clinging types of algae appear as black spots or as a brownish or greenish mossy layer. Growth of this alga in pools or reservoirs results in an unsightly greenish or brownish appearance, accompanied by turbidity of the water.

The occurrence of certain diatom species in the water is associated with pollution, and the abundance of *Nitzschia* sp. is specifically related to the amount of water nitrogen (Patrick, 1964). Further research is needed regarding the toxic effects of *Nitzschia* on other aquatic organisms.

A polluted environment with dense algal blooms may affect other aquatic life maintained in a particular ecosystem. It is known that fish have been killed when certain algae bloomed (Lackey, 1964). Algal growths have often fostered bacterial development, imparting a very disagreeable odor to the water (Scharff, 1958; Carr and Whitton, 1973).

Controlling algal growth partly solves the above-mentioned problems. Since algae have long been known for their extreme sensitivity to excess Cu, copper salts are frequently used as algicides. Moore and Kellerman of the United States Department of Agriculture were the first to introduce the application of $CuSO_4$ over the surface of a reservoir in 1904. In later years, $CuSO_4$ and other inorganic substances were used to remove algae from artificial pools, industrial cooling waters, an open-air swimming bath, irrigation water, salmon spawning channels, etc. (Scharff, 1958; Round, 1965; Yeo and Dechoretz, 1976).

Studies have shown Cu to be toxic to algal cells when added in excess amounts, leading to suppression of growth or death of the cells (Greenfield, 1942; Den Dooren de Jong, 1965; Soeder et al., 1967; Whitton, 1970; Levitt, 1972; Steward, 1974). According to Greenfield (1942), photosynthesis in Chlorella is inhibited by Cu concentrations higher than 0.1 um. From the data of Fitzgerald and Faust (1963), it is evident that $CuSO_4.5H_2O$ is toxic to Chlorella at a concentration of 2 mg/l or more after seven days of contact time; 0.05 mg/l (0.05 ppm) was toxic to Microcystis aeruginosa and Gloeotrichia echinulata; 0.1 mg/l was toxic to Anabaena circinalis and 0.4 mg/l, to the diatoms.

Determining the toxic level of $CuSO_4$ to destroy each type of alga is especially important, since Cu is also required as a micronutrient (Stewart, 1974). Deficiency symptoms appear when Cu is absent or when the concentration in the medium is lower than the growth requirements. It has been shown that Cu is important in photosynthesis in Chlorella ellipsoidea for plastocyanin (Katoh, 1960), a component of the photosynthetic system essential for the electron transport mechanism of pigment system I (Bishop, 1964).

This study, therefore, aims to investigate the effects of varying concentrations of $CuSO_4$ on the population density of Chlorella sp., Nitzschia sp. and Oscillatoria sp., and determine the levels of the chemical that will suppress algal growth. The results of this study may be applied in homes where aquaria are maintained for decorative purposes.

MATERIALS AND METHODS

Collection and analysis of water samples.

Algal samples were taken from a pond with an area of 21.5 m x 8 m and a depth of 0.875 m, fronting the Administration

Building of the University of the Philippines at Los Baños, College, Laguna. The depth of the water was 65 cm.

Samples were collected using a conical, fine-mesh nylon plankton net. Organisms collected with this net were 93% phytoplankton.

The pond water was stirred before dipping the net through it. The temperature of the water was taken and recorded; the pH was also measured immediately after collection using a Chem-mate pH meter model no. 72 to ensure that the same conditions would be maintained in the laboratory.

Preparation of media.

Pond water samples of about 10.5 liters were filtered using coarse filter paper, Whatman No. 1, to remove sediments. Filtered pond water and distilled water were mixed at a 1:1 ratio, and pH was adjusted to 7.9, optimum for algal growth. From the above mixture, aliquots of 7.5 ml were transferred to cotton-plugged 18 x 45 ml test tubes.

For mass culturing of algae, both organic and inorganic media were used. The organic medium was soil water extract (SWE) (25 g sieved garden soil + 100 ml tap water). The inorganic media used were modified Chu ##10 (MC 10) (as described by Gerloff et al., 1950) and Tris Buffered Inorganic Medium (TIBM) (as described by Bellis, 1968). All media were sterilized in an All-American pressure cooker #941.5 for 15 minutes.

Culture experiments.

Samples were inoculated into two tubes each of SWE, MC 10 and TIBM in an effort to grow all algal species present in the pond. Test tubes with the culture were shaken daily to provide oxygen to the cells and to maintain the homogeneity of the suspension. Weekly sub-culturing was conducted for one month to obtain unialgal cultures of Chlorella sp., Nitzschia sp. and Oscillatoria sp.

When pure cultures were obtained, a final transfer was made to test tubes with a 1:1 ratio of filtered pond water and distilled water. The cells were allowed to grow for 48 hours, then copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$) was applied at varying concentrations to the cultures. Five ml of the chemical was added to each tube.

All cultures were maintained under ordinary room condition (25-27 ^oC), under approximately 155 foot candles of daylight fluorescent light, so as to obtain results that would be applicable to small aquaria in Philippine homes.

Quantitative determination.

Quantitative determination of the algal population was by microscope counting with the use of a haemacytometer, as described by Martinez et al. (1975).

The first count was after a 24-hr incubation of the cells in filtered pond water and distilled water; the second, after a 48-hr incubation. First and second countings represent algal population not affected by $CuSO_4$. Successive counts were taken for four consecutive days after $CuSO_4$ application.

RESULTS

The three species differed in their degree of sensitivity to $CuSO_4$. Treatment with 0.01 ppm of $CuSO_4$ resulted in an increased number of cells per cu mm per day in the three species; however, a slight reduction in growth rate was observed, compared to the control (Figs. 1, 2 and 3). Cell division was still evident in Nitzschia sp. and Chlorella sp. at 0.05 ppm, but the rate was again lower than the control's (Figs. 1 and 2). There was 76.1% reduction of growth rate in Nitzschia and 76.7% reduction in Chlorella.

Reduction of growth rate of both Nitzschia and Chlorella was significantly different from the control at 0.1 ppm of $CuSO_4$. A slight increase in the number of cells was recorded on the sixth day of observation in Nitzschia at 0.1 ppm, but the count was only about 38% of the control, indicating a reduction in cell division.

On the other hand, there was no change in the cell count in Chlorella on the fifth and sixth days of observation, indicating absence of cell division. Since the rates of growth of both Nitzschia and Chlorella were greatly affected by $CuSO_4$ at the same minimum level (i.e. at 0.01 ppm), both organisms have the same sensitivity to the chemical. Complete suppression of growth was observed with succeeding higher concentrations (i.e. 0.01 ppm).

The blue-green alga, Oscillatoria sp., appeared to be the most sensitive among the three species studied. A sharp decrease in growth rate occurred at a concentration of 0.05 ppm $CuSO_4$, with 48.8% reduction. Such a reduction of growth rate is significantly different from the control (Fig. 3).

DISCUSSION

The selectivity of the toxic action of Cu for different algal species is probably a function of processes within the algal cells (Fitzgerald and Faust, 1963). It has been demonstrated by Steemann-Nielsen and Wium-Andersen (1971) that the growth of Nitzschia is less influenced by low concentrations of Cu. When Cu is present, the diatom excretes organic matter which may bind to the chemical, and thus make the medium suitable for growth by removing the toxic effect.

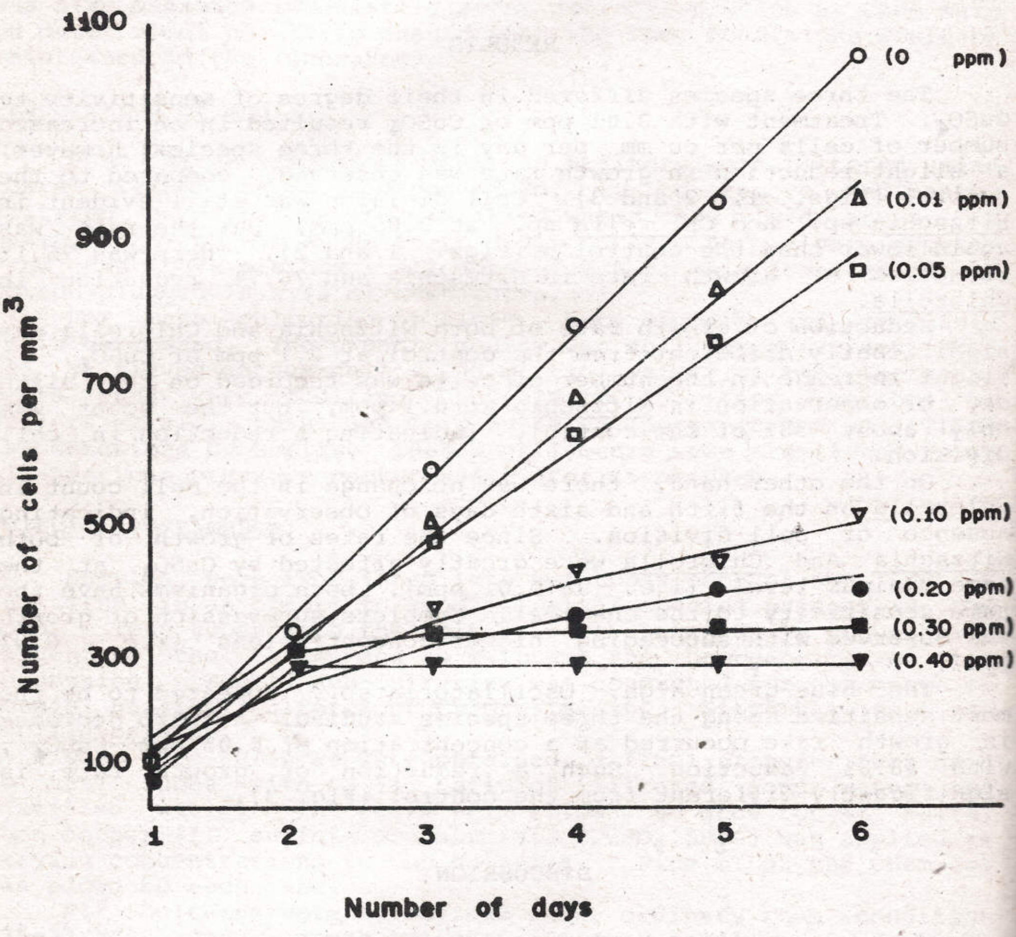

Fig. 1. Effect of varying concentrations of $CuSO_4$ on the population density of Nitzschia sp.

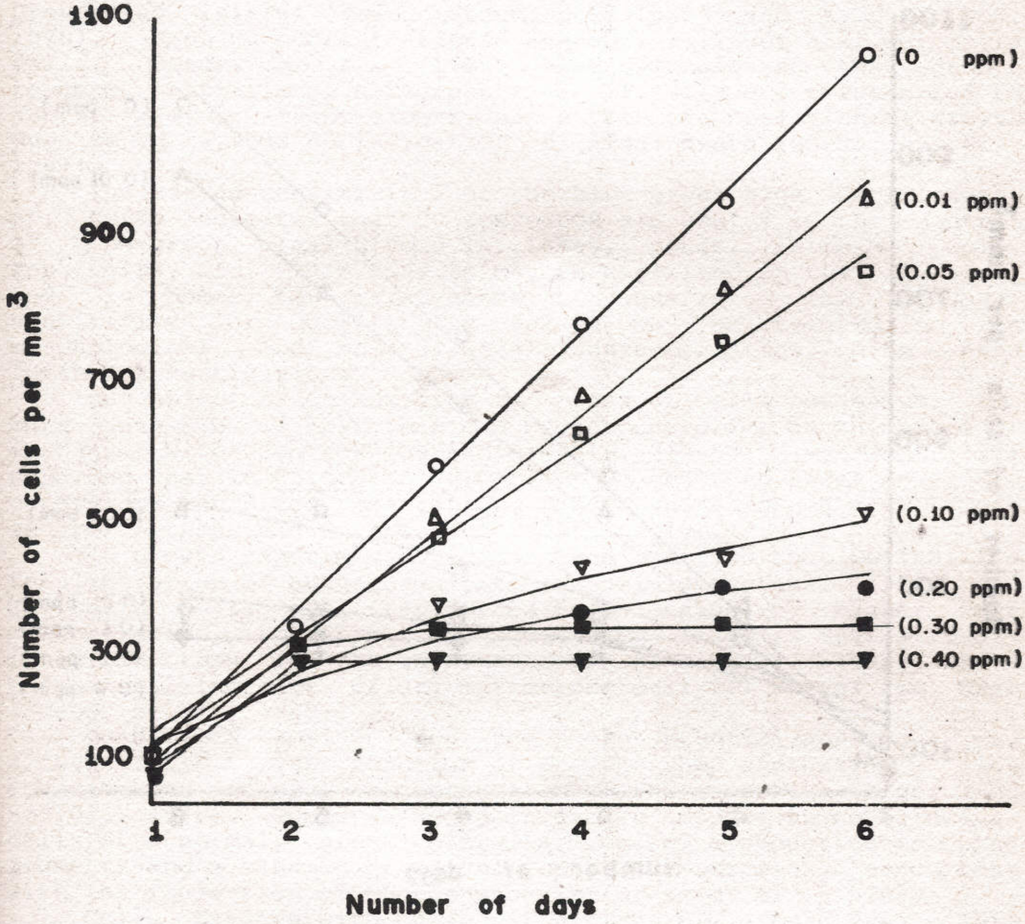

Fig. 2. Effect of varying concentration of $CuSO_4$ on the population density of Chlorella sp.

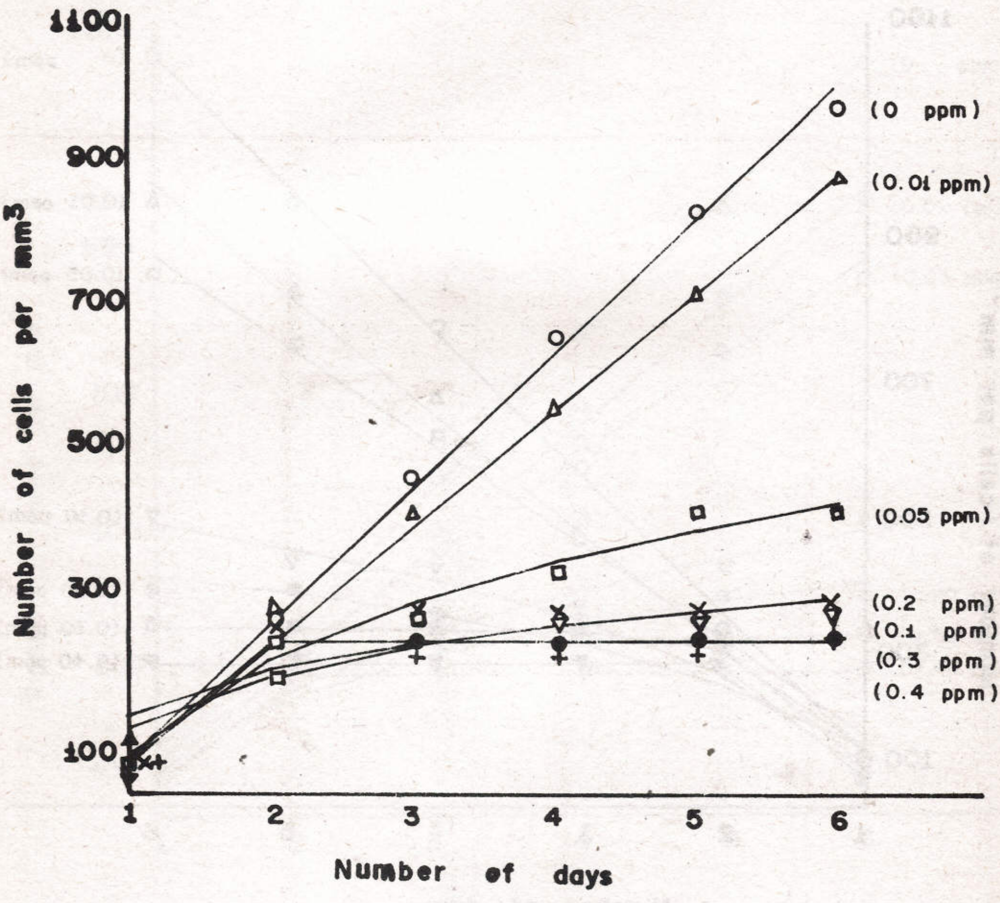

Fig. 3. Effect of varying concentrations of $CuSO_4$ on the population density of Oscillatoria sp.

Addition of low concentrations of Cu to the medium also has little effect on the growth of Chlorella. It has been shown by Steemann-Nielsen and Kamp-Nielsen (1970) that Chlorella reestablishes a normal growth rate with minute amounts of Cu added. Cu is, again, bound by the alga such that no lasting poisonous effect is observed. However, no regeneration of growth rate is seen in the cultures from a medium with Cu concentrations preventing initial growth (Steemann-Nielsen and Kamp-Nielsen, 1970). Such Cu concentrations, however, will not kill Chlorella cells. Soeder et al. (1967) have demonstrated that growth resumes, initially at a reduced rate, if cells are transferred to another medium without excess Cu. A complete cessation of growth due to Cu is thus no indication of algal death (Soeder et al., 1967).

The marked sensitivity of the blue-green alga Oscillatoria sp. to Cu indicates that Cu compounds are useful in the control of blue-green algal blooms in lakes, ponds, swimming pools, reservoirs, etc. This finding may be exploited in the laboratory and in homes for maintenance of aquaria. The unusual sensitivity of Oscillatoria to Cu may be related to its morphological and physiological characteristics, which need further investigation.

One effect of Cu toxicity is loss of cell potassium, but more potassium is lost than can be accounted for on the basis of exchange with Cu (McBrien and Hassall, 1965). McBrien (1967) reported that in Chlorella vulgaris, Cu absorbed under anaerobic conditions inhibited respiration, photosynthesis and growth more severely than did Cu absorbed aerobically.

In growth experiments, it has been observed that Cu inhibits the liberation of autospores (Steemann-Nielsen et al., 1969). If Chlorella cells are transferred to a medium of higher salt concentration, the formation of daughter cells is more strongly inhibited than is the synthesis of biomass, resulting in a transitory increase of dry matter per cell for one or two days (Soeder et al., 1967).

Herbicides acting upon the photosynthetic apparatus may either inhibit its function or prevent the synthesis of its constituents and cause a destruction of the apparatus (Boger and Schlue, 1976). Copper, added in excess amounts to non-growing cells of a normal, green Chlorella, caused a reduction in total pigments and a blue shift of chlorophyll absorption, concurrent with the inhibition of photosynthesis (Gross et al., 1970).

Cells treated with Cu appear bluish- or grey-green. This color change in poisoned cells may indicate different sub-cellular distributions, constituting another important cellular change (Hassall, 1963). It is suggested that chlorophyll is metastable with reference to Mg and the green Cu pheophytin is very stable (Hill, 1963). Perhaps, a replacement of the ionically bound Mg by a Cu atom took place, but an oxidation of chlorophyll or some other reaction cannot be excluded (Gross et al., 1970).

The action of Cu takes place primarily on the plasmalemma, which, together with the cell walls and slime envelopes, is able to bind Cu to some extent, as in the case of Chlorella (Steemann-Nielsen et al., 1969). Steemann-Nielsen et al. (1969) further reported that if both the cell walls and slime envelopes are composed of pectic acid, 12 carbon atoms at least are necessary for binding one Cu ion.

Gross et al. (1970) suggested that the mechanism of action of Cu may be influenced by the structure of the cell, with the rupture of membrane barriers as the first step in the damage sequence, or the primary toxic effect of Cu. Therefore, membrane integrity may be altered by the action of Cu. In Nitzschia, Steemann-Nielsen (1971) has shown that organic matter is lost by the cells as Cu penetrates them. This implies that Cu loosens the cell membranes.

Other evidence to show that the permeability of the cells is increased or that there is alteration in the retentive properties of the cell due to the presence of Cu is shown by McBrien (1967), in that even when only small amounts of Cu are taken up by the cells, substantial amounts of cellular K+ are released. Normally, this membrane retains K+ within the cells.

The demonstration that cells poisoned with Cu contain little soluble P suggests either that P metabolism of growing cells is severely disturbed when the ratio of sorbed Cu to nucleotide P rises above about 0.2 or, alternatively, that the Cu renders the cell permeable to solutes (Hassall, 1963).

Related to the rupture of membrane barriers (Gross et al., 1970) is the ability of the salts of heavy metals to inactivate enzymes (Myrback, 1965) and precipitate proteins (Brian, 1964, as quoted by Sutton et al., 1970; McBrien, 1967).

A major metabolic process also affected by Cu is respiration. Cell respiration is enhanced by shaking the culture. In this particular study, normal daily shaking of the tubes was stopped on the day that $CuSO_4$ was applied to the cells. Since Cu is highly toxic, it greatly reduces respiration, especially when applied to cells under anaerobic conditions or when cells are not shaken (Hassall, 1962, 1963). Respiration of mature autospore mother cells is inhibited in a more concentrated medium (Stewart, 1974). Furthermore, excess Cu in the medium delays cell division, causing a reduction of growth rate (Soeder et al., 1967). This accounts for the reduction in the number of autospores or daughter cells with higher concentrations of $CuSO_4$.

SUMMARY AND CONCLUSION

Increasing concentrations of $CuSO_4$ caused a reduction in the growth rates of Nitzschia sp., Chlorella sp. and Oscillatoria sp. Each species had a certain degree of sensitivity to toxic doses of the chemical. The toxic dose of $CuSO_4$ for Nitzschia sp. and

Chlorella sp. was 0.1 ppm with r value of 0.99 and 0.97, respectively. For Oscillatoria sp., the toxic dose was at 0.05 ppm with r = 0.97.

Given the combined toxic effects of Cu on algal cells, it is safe to say that $CuSO_4$ is a good algicide to suppress algal growth in home aquaria. $CuSO_4$ is not poisonous to fish in the concentrations ordinarily used in water treatment, Moore and Kellerman (as cited in a brochure of the Mountain Copper Company, Ltd.) have studied the toxic effect of $CuSO_4$ on various species of fish and have set the limiting safe dosage for goldfish at 0.50 ppm.

RECOMMENDATIONS

When using $CuSO_4$ as an algicide, the toxic dose for the specific algal species to be eliminated should be strictly observed. Lower concentrations than the toxic dose enhance growth and reproduction of the algae. On the other hand, higher concentrations than the toxic dose might have some toxic effect on the fish maintained in the aquaria.

It should also be noted that 24 hours after $CuSO_4$ application, the water in the aquaria must be changed. Treated water may contain considerable dead organic matter resulting from the action of $CuSO_4$, and this organic matter may clog the gills of the fish (The Mountain Copper Co., Ltd.). It is also suggested that $CuSO_4$ should be distributed evenly in the water. Uneven distribution of $CuSO_4$ may result in toxicity to fish in areas with greater concentrations of the chemical.

ACKNOWLEDGEMENTS

I wish to thank Dr. Macrina T. Zafaralla of the Institute of Biological Sciences, University of the Philippines at Los Baños for allowing me to conduct this study at the Phycology Laboratory. I also wish to express my sincere thanks to Dr. Angel C. Alcala and Ms. Louella Dolar of Silliman University for their constructive criticism and suggestions.

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