

TOXIC EFFECTS OF COPPER AND LEAD ON A TROPICAL MARINE BLUE GREEN PHYTOPLANKTER, *OSCILLATORIA THIEBAUTII*

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Abstract

Toxic effects of copper and lead on *Oscillatoria thiebautii* (Gomont et Gomont) Geitler were studied. Copper was more toxic than lead and even lethal. The extent of inhibition by both metals was uniform throughout different stages of growth of the species with linear inverse relationship between the two parameters. Effect of copper and lead was synergistic.

Introduction

Oscillatoria thiebautii (Gomont et Gomont) Geitler, a eurythermal blue green phytoplankter occurs mainly in tropical seas (Desikachary, 1959; Sournia, 1968). It has the ability to fix elemental nitrogen (Carpenter & McCarthy, 1975). It occurs commonly in coastal waters of Karachi, a large industrial city, with vast amounts of pollutants discharged into the sea. Since metals coexist and interact with each other in nature (Break *et al.*, 1976), the individual and combined effect of copper and lead on *O. thiebautii* was, therefore, studied.

Materials and Methods

All cultures were batch cultures and were maintained in a medium modified after von Stosch & Drebes (1964) containing NaNO₃, 45.5 mg; Na₂HPO₄ . 12 H₂O, 10.75 mg; MnCl₂ . 4 H₂O, 0.0198 mg; FeSO₄ . 7 H₂O, 0.278 mg; Na₂SiO₃, 15.0 mg; and Vit. B₁₂, 0.001 mg in 1 litre of sea water. Chelating substances were not used in the experimental cultures. Stock cultures in addition contained 3.72 mg Na₂EDTA. 2H₂O and 1 ml soil extract prepared with a Soxhlet apparatus (Kornmann, 1972).

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All glasswares, sea water and media were sterilized in an autoclave at 121°C and $15\text{ lb}^{-2}\text{ inch}$. A 2 day old culture was used as inoculum. Twenty ml of stock culture was inoculated in 1 litre Erlenmeyer flasks and kept at $28^{\circ} \pm 2^{\circ}\text{C}$ with a 10 hr. dark; 14 hr. light cycle and illuminated from both sides of the flasks using two fluorescent day light tubes giving 6000 lux. In another set copper and lead dissolved in sterilized distilled water were added to the experimental cultures. The culture flasks were shaken vigorously for few minutes twice daily so that the filaments may not stick to sides and bottom. At 24 hr. interval an aliquot of 50 ml of the culture was pipetted and chlorophyll 'a' content of *O. thiebautii* estimated using SCOR/Unesco method (Anonymous, 1966).

Results and Discussion

O. thiebautii was very sensitive to copper, since the inhibition in growth was noted as early as the first day of incubation (Figs. 1 & 2). At low concentrations of copper chlorophyll 'a' values increased with time at a rate slower than the control. At high concentrations ($10 - 20\ \mu\text{g Cu l}^{-1}$) a lag phase in growth was observed as chlorophyll values even lower than the inoculum values were noted but this remained only for

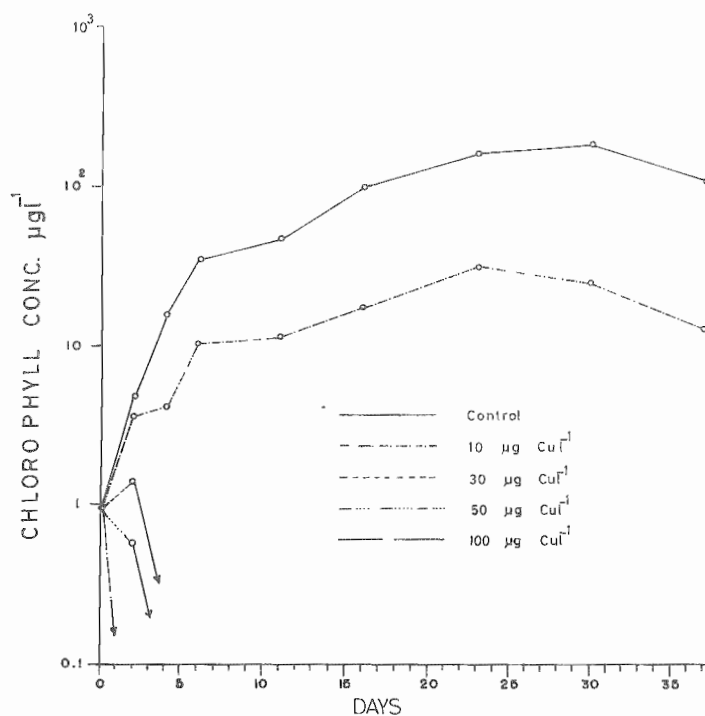


Fig. 1. Effect of copper on chlorophyll 'a' concentrations at an inoculum size of $0.96\ \mu\text{g chl. 'a' l}^{-1}$ (arrows indicating zero values).

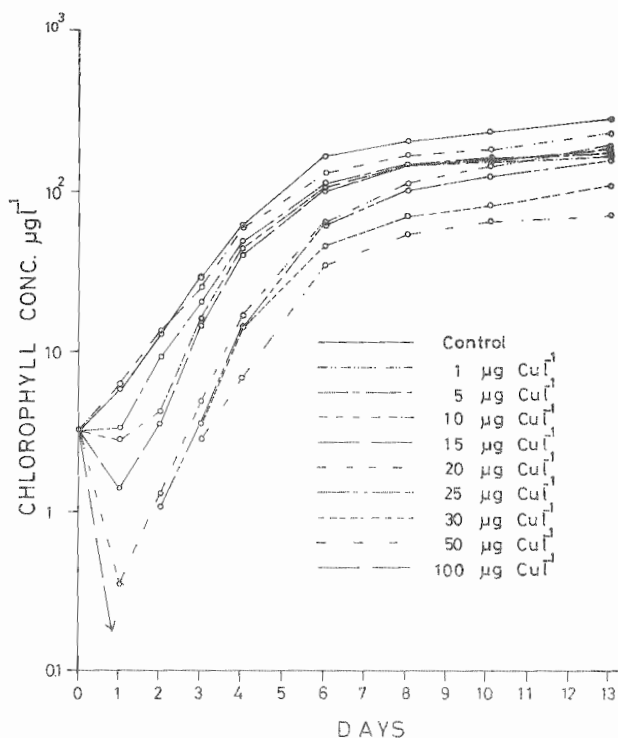


Fig. 2. Effect of copper on chlorophyll 'a' concentrations at an inoculum size of $3.17 \mu\text{g chl. 'a' l}^{-1}$ (arrows indicating zero values).

a day (Fig. 2). It seems that the cells took one day to adapt to higher concentrations of the metal most probably by excreting dissolved organic matter to bind it (Steemann Nielsen & Wium-Andersen, 1971). At higher concentration (above $20 \mu\text{g Cu l}^{-1}$) however, the species could not adapt. Such similar inhibitory effect of copper on other algae has been reported (Davey *et al.*, 1973; Saifullah, 1978).

Copper inhibited carbon assimilation in *O. thiebautii* and low values affected the species since cupric ion activity instead of copper concentrations were used (Rueter *et al.*, 1979). Growth of *Skeletonema costatum* was inhibited at $50 \mu\text{g Cu l}^{-1}$ and above (Mandelli, 1969; Berland *et al.*, 1977; Braek *et al.*, 1976). Such high inhibitory concentrations may be accounted for the complex media containing chelating substances.

Lead inhibited the growth of *O. thiebautii* at all concentrations ranging from 50 to $2000 \mu\text{g Pb l}^{-1}$ and it was not lethal (Fig. 3). Like copper, the cells entered a lag phase on the first day of incubation at high concentrations. Inhibitory effect of lead on other groups of algae is known (Dayton & Lewin, 1975; Saifullah, 1976; Berland, *et al.*, 1977).

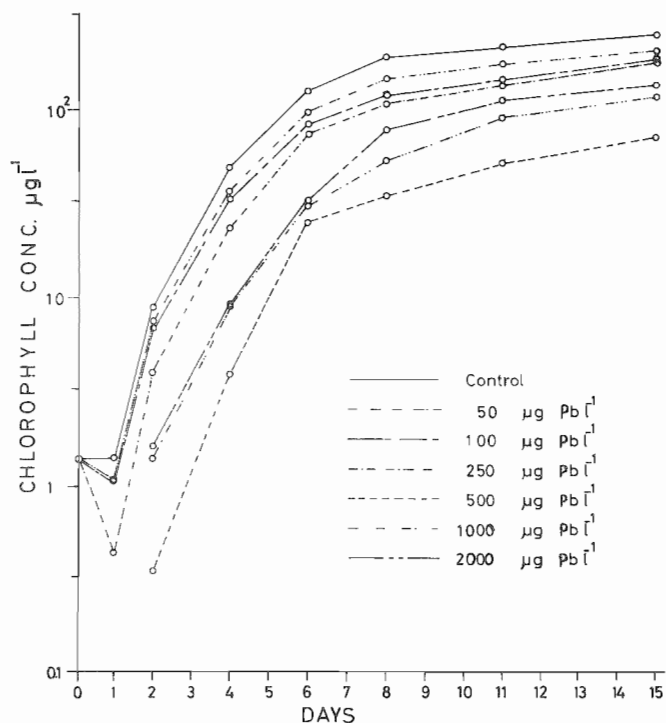


Fig. 3. Effect of lead on chlorophyll 'a' concentrations at an inoculum size of $1.39 \mu\text{g chl. 'a' l}^{-1}$

The results indicate that chlorophyll 'a' values progressively decrease with an increase in concentrations of both metals. Chlorophyll 'a' values on the sixth day of incubation were 66, 38, 28 and 22% of the control, respectively, at 5, 20, 30 and $50 \mu\text{g Cu l}^{-1}$ (Fig. 2). Similarly, the values were 71, 59, 57, 47 and 38% of the control, respectively, at 50, 100, 250, 1000 and $2000 \mu\text{g Pb l}^{-1}$. When the chlorophyll 'a' values at different concentrations of copper (Fig. 4) and lead (Fig. 5) observed on a certain day of the experiment during the exponential phase were plotted against each other and fitted to a least square regression equation, they were linearly inversely related with each other. The relationship was significant, and may be generalized as follows:

- i) $\log \text{chlorophyll 'a' conc.} = 1.253 - 0.0147 \text{ Cu conc.}$
- ii) $\log \text{chlorophyll 'a' conc.} = 1.195 - 0.0002 \text{ Pb conc.}$

It is interesting to note that maximum inhibition of *O. theibautii* was noted at $500 \mu\text{g Pb l}^{-1}$ and such anomalous observations have been found in *Ochromonas malhamensis* (Malanchuk & Gruendling, 1973) and *Prorocentrum micans* (Saifullah, 1976).

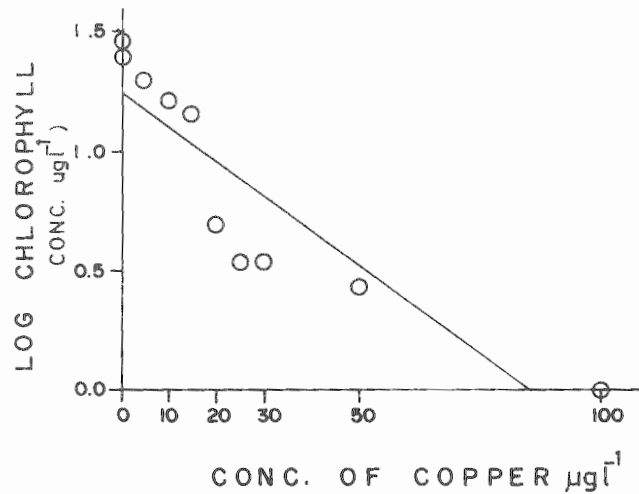


Fig. 4. Relationship between chlorophyll 'a' and copper concentrations during third day of exponential phase ($\log Y = 1.253 - 0.0147 X$; $P < 0.001$).

Comparing effects of copper and lead, the rate of inhibition in growth at a given concentration of metals remained almost the same throughout different phases of growth (Figs. 1-3). In this respect blue green algae may be distinguished from other groups of algae, where the inhibitory effect of heavy metals considerably increase with the age of culture (Hessler, 1974; Saifullah, 1976, 1978; Berland, *et al.*, 1977). Algae are known to accumulate the metals with time (Riley & Roth, 1971) and become physiologically weak with age (Fogg, 1966). In the present study, however, the inhibitory effects of heavy metals were not pronounced during stationary phase than during exponential phase. Whether this is true with other blue green forms needs investigations.

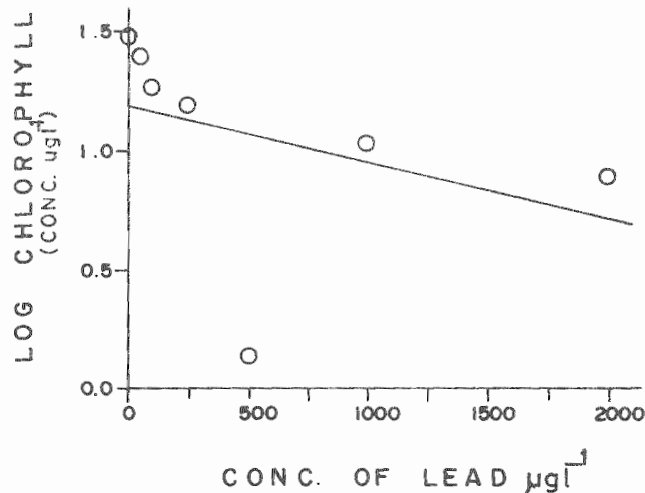


Fig. 5. Relationship between chlorophyll 'a' and lead concentrations during second day of exponential phase, ($\log Y = 1.195 - 0.00024 X$; $P < 0.5$).

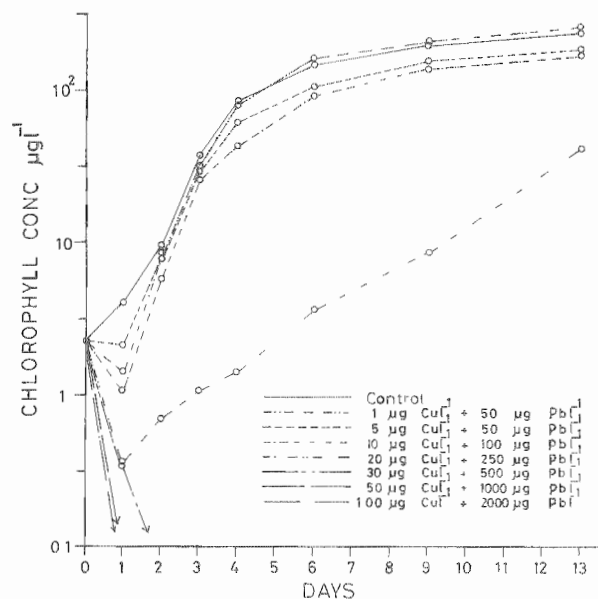


Fig. 6. Combined effect of copper and lead on chlorophyll 'a' concentrations (arrows indicating zero values).

Size of inoculum is known to modify the inhibitory effects of heavy metals. Steemann Nielsen & Kamp-Nielsen (1970) and Saifullah (1978) have reported large size inocula to reduce the effect of copper on algae resulting in an increased excretion of organic matter by the cells which binds the heavy metals (Davey *et al.*, 1973; Bentley-Mowat & Reid, 1977). In the present study also the effect of both the metals was retarded by large size inoculum since *O. thiebautii* died at $30 \mu\text{g Cu l}^{-1}$ when smaller inocula were used (Fig. 1) as compared to large size inoculum (Fig. 2) when $100 \mu\text{g Cu l}^{-1}$ showed this effect. Similarly, chlorophyll 'a' values did not decline upto $250 \mu\text{g Pb l}^{-1}$ on the first day of incubation with larger inoculum but showed a reduction at low concentrations of lead with smaller inocula (Fig. 3).

Combinations of copper ($20 \mu\text{g l}^{-1}$) and lead ($250 \mu\text{g l}^{-1}$) showed greater inhibition in growth of *O. thiebautii* (Fig. 6) than used separately (Figs. 2 & 3). On the sixth day of incubation, chlorophyll 'a' values were 2% when Cu and Pb were used together as compared to 37% in Cu alone related to the control. Similarly, the species died within two days of incubation at 30 and $50 \mu\text{g Cu l}^{-1}$ in combination with lead, whereas no lethal effects were recorded at these concentrations in copper alone (Fig. 2). These observations would suggest a synergistic effect of Cu and Pb. Of the four species of marine phytoplankton, Braek *et al.*, (1976) found a synergistic effect of Cu and Zn on three species only. Lead affects the metabolism indirectly by causing phosphate defi-

ciency in the medium (Monahan, 1973; Dayton & Lewin, 1975), arrests cell division and uptake of essential elements as it deposits on the cell membrane (Hessler, 1974; Schulz-Baldes & Lewin, 1976). On the other hand copper is known to inhibit plant metabolism in unicellular algae (Greenfield, 1942; McBrien & Hassal, 1967). The present studies also confirm that the inhibitory effect of copper increases in the presence of lead.

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