Pak. J. Bot., 18(1): 137–145, 1986.

BIOASSAY STUDIES OF PHYTOPLANKTON OF COASTAL WATERS OF KARACHI IN RELATION TO HEAVY METAL POLLUTION: I. EFFECT OF COPPER AND LEAD ON *SKELETONEMA COSTATUM*

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Abstract

Toxic effects of copper and lead on a local diatom species *Skeletonema costatum* were studied using bioassay technique. Copper inhibited growth at a concentration of 10 μg Cu l^{-1} and killed the cells at and above 30 μg Cu l^{-1}. Lead inhibited growth, the lowest tolerance limit being 50 μg Pb l^{-1}. The effect of both metals increased with age of culture and a significant inverse relationship existed between their concentrations and growth of the species. The combined effect of copper and lead on the species was synergistic.

Introduction

According to an estimate there are about 11,000 industrial units of various kinds located in and around Karachi, a city of about 7 million people. The effluents from these industries and sewage are dumped without any prior treatment into the sea near Karachi Harbour through Lyari and Malir rivers (Beg *et al.*, 1975). Other likely source of heavy metal pollution are the numerous ships plying through Karachi Harbour and Port Qasim area. Such activities are partly responsible for heavy metal pollution in harbours (Young *et al.*, 1979).

In the present study the effect of copper and lead were tested against *Skeletonema costatum,* a common phytoplankter in the coastal waters near Karachi. The two metals are known to be common hazardous pollutants in coastal waters of all industrial cities (Ruino, 1972). Most complex media with soil extract and EDTA which are known to chelate heavy metals have been used (Mandelli, 1969; Erickson *et al.*, 1970; Morris & Russel, 1973; Break *et al.*, 1976; Berland *et al.*, 1977) and hence high values many times greater than natural concentrations reported to inhibit the growth of these organisms. In the present study culture media used was sterilised sea water enriched with only few salts and chelators and soil extracts were not used. In this study individual and combined effects of copper and lead was also tested.

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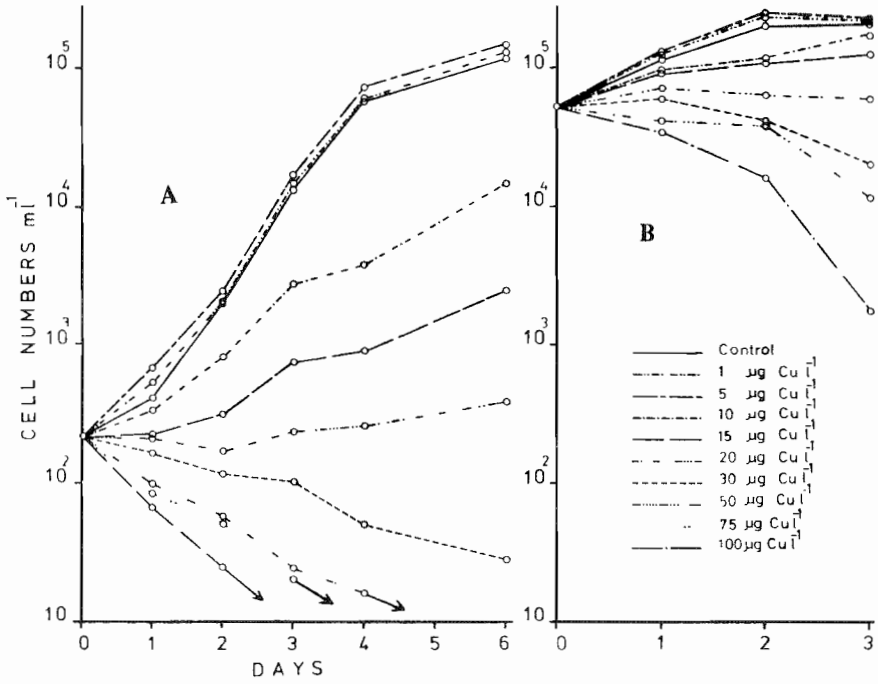

Fig. 1. Effect of copper on growth at an inoculum size of A) 220 cells ml^{-1} B) 51,400 cells ml^{-1} (arrows indicating zero values).

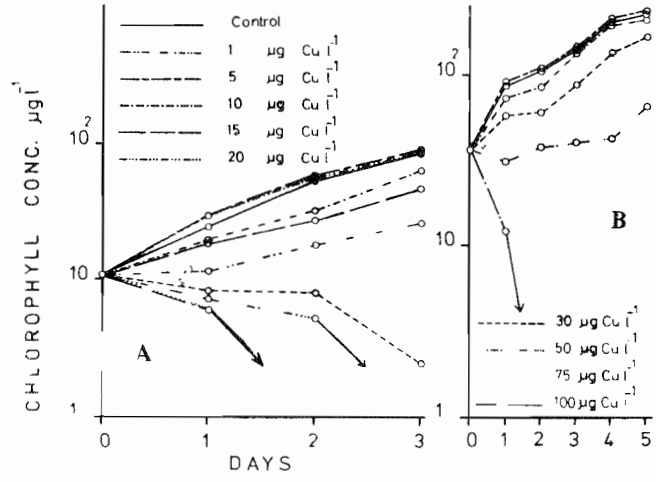

Fig. 2. Effect of copper on chlorophyll 'a' concentration at an inoculum size of A) 10.5 µg Chlorophyll 'a' l^{-1} B) 36.5 µg chlorophyll 'a' l^{-1} (arrows indicating zero values).

Materials and Methods

All being batch cultures were maintained in a medium modified after von Stosch & Drebes (1964). The medium contained:

Sea water	1.0 litre,
$NaNO_3$	45.5 mg.,
$Na_2HPO_4 . 12H_2O$	10.75 mg.,
$MnCl_2 . 4H_2O$	0.0198 mg.,
$FeSO_4 . 7H_2O$	0.278 mg.,
$Na_2 SiO_3$	15.0 mg. and
Vit. B_{12}	0.001 mg.

About six months aged sea water was used, as it is suitable for growth of organisms (Kayser, 1973).

The cultures of *S. costatum* were maintained in 500 ml Erlenmeyer flasks at 20°C, subjected to a daily cycle of 16 h light and 8 h darkness. All glasswares, seawater and media were wet sterilized at 15 psi for 15 min. Since sterilization precipitates heavy metals (Jones, 1967; Erickson *et al.*, 1970); it is therefore assumed that sterilized medium had no copper or lead in the ionic form except that added artificially in the experimental cultures. The cultures were not axenic but care was taken to minimise bacterial contamination. Copper and lead were dissolved in sterilized distilled water in such proportions that addition of 1 ml of solution gave the desired concentrations. The inoculum consisted of 1 ml of stock cultures at a time when the species was in the exponential phase of growth and that healthy cells were being picked up. The culture flasks were shaken vigorously for few minutes twice daily for homogenous distribution and aeration was not applied since it is known to precipitate dissolved substances (Riley *et al.*, 1965).

Daily observations were taken using an aliquot of 1 ml of the experimental culture for cell counting purpose during exponential phase and early growth period and on alternate days at later stages. Simultaneously 10 to 20 ml aliquot of the sample was taken for chlorphyll 'a' measurement (Anon, 1966), which may be used as an index of phytoplankton standing crop in healthy cultures (Krey, 1958).

Results and Discussions

In the present study as low as 10 μg Cu l^{-1} inhibited the growth of *S. costatum* (Figs. 1-2) in contrast to 50 μg Cu l^{-1} (Mandelli, 1969; Berland *et al.*, 1977 Break *et al.*, 1976). Jensen *et al.*, (1976) however reported a low value of 10 μg Cu l^{-1} inhi-

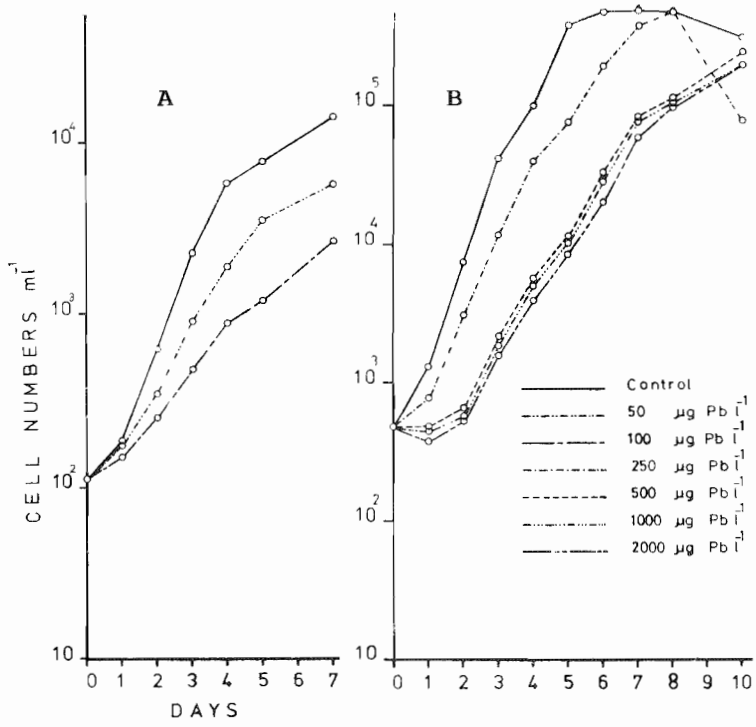

Fig. 3. Effect of lead on growth at an inoculum size of A) 110 cells ml^{-1} B) 475 cells ml^{-1}

biting growth of *S. costatum* using dialysis culture technique which is more close to natural environment. The chelating and quenching effect of EDTA on activity of copper is shown in Fig. 6. In the presence of EDTA and soil extract the growth of *S. costatum* increased at 10 µg Cu l^{-1} instead of being inhibited as observed in absence of EDTA (Fig. 1). Similarly a concentration of 30 µg Cu l^{-1} was lethal (Fig. 1) but in the presence of EDTA and soil extract the species survived at 50 µg Cu l^{-1} (Fig. 6). Similar results were obtained with lead. Comparing Figs. 3 & 6 lead inhibited more growth of the species in absence of EDTA and soil extract than in its presence. *S. costatum* showed good growth in the enriched seawater as in the presence of soil extract and EDTA (Fig. 6). Since the experiments were run for a few days only, absence of chelators did not affect growth. However the presence of chelators is necessary for sustaining long term growth in stock cultures.

Several workers have found copper toxic to marine phytoplankton including *S. costatum* (Mandelli, *et al.*, 1969; Break *et al.*, 1976; Berland *et al.*, 1976; Morel *et al.*, 1978; Gavis *et al.*, 1981). Studies with chlorophyll 'a' concentration gave almost the same

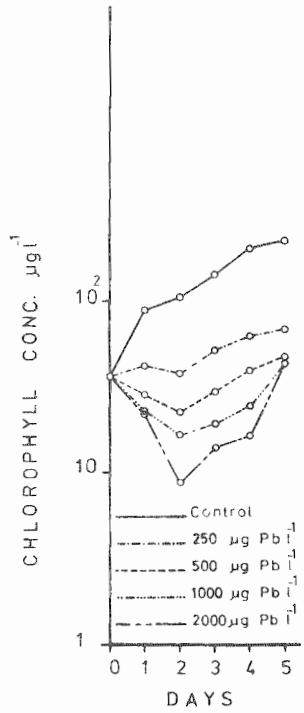

Fig. 4. Effect of lead on chlorophyll 'a' concentration at an inoculum size of 36.6 µg chlorophyll 'a' l^{-1}.

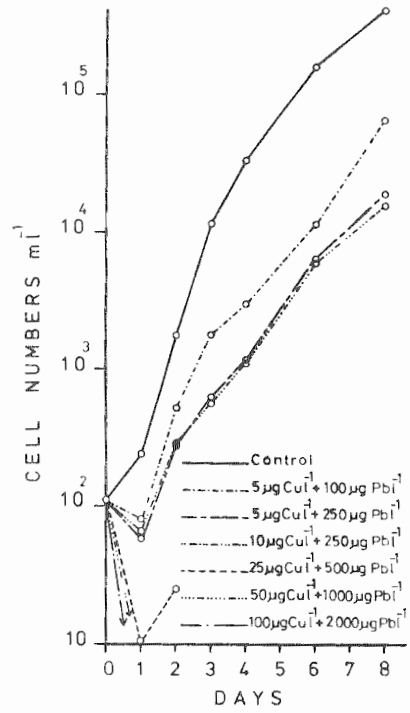

Fig. 5. Combined effect of copper and lead on growth at an inoculum size of 110 cells ml^{-1} (arrows indicating aero values).

results with copper (Fig. 2A & B) as with cell numbers (Fig. 1A & B) they progressively decreased with increase in concentration of the copper. A similarity between the two parameters would indicate that chlorophyll 'a' declined as a result of decrease in cell numbers. A linear direct relationship between chlorophyll 'a' concentration and cell numbers is represented to exist in a growing and healthy culture (Krey, 1958). The possibility of a direct effect of copper on chlorophyll 'a' can not be ruled out since Greenfield (1942) also found that copper inhibits chlorophyll synthesis.

Lead inhibited *S. costatum* at all concentrations ranging from 50-2500 µg Ph. l^{-1} but was not lethal (Figs. 3, 4). Dayton & Lewin (1957) and Saifullah (1976) have found similar results with other algae. Berland *et al.*, (1977) have reported the inhibition of the same species at 1000 µg Ph l^{-1}, which may be due to the complex media used. Hessler (1974) has also reported higher values of lead inhibiting growth. The effect of lead on chlorophyll 'a' concentration of *S. costatum* (Fig. 4) followed the same pattern as that of cell numbers. Saifullah (1976) also found similar results and Hampp & Lendzian (1974) reported that lead inhibits chlorophyll synthesis.

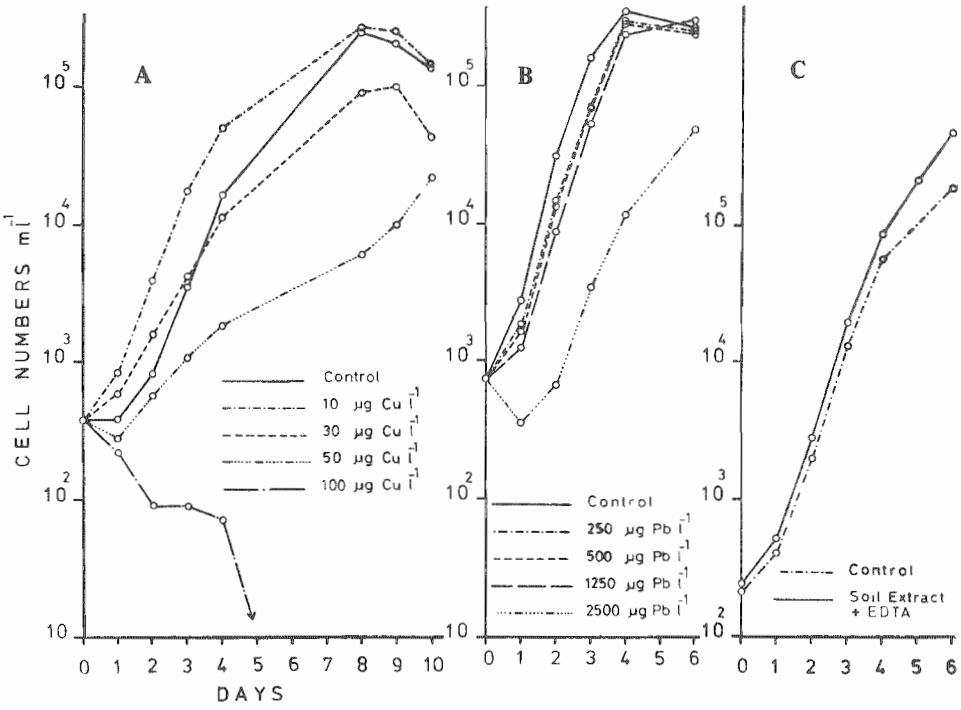

Fig. 6. Effect of A) copper on growth with EDTA and soil extract at an inoculum size of 380 cells ml^{-1} B) lead on growth with EDTA and soil extract at an inoculum size of 740 cells ml^{-1} C) EDTA and soil extract on growth (arrow indicating zero value).

The effect of both metals increased with age of the culture. On the second day of incubation with Cu the number of cells were 14.11, 8.59, 5.81, 2.83, 2.63 and 1.26% of the control respectively, but on the fourth day they were 6.62, 0.45, 0.09, 0.03, 0.02 and zero % of the control at 10, 20, 30, 50, 75 and 100 µg Cu l^{-1} respectively. With lead, the rate of inhibition was not so drastic, as also found by others (Hessler, 1974; Saifullah, 1976, 1978; Berland *et al.*, 1977). The possible explanation for increased inhibitory effect of metals with age of culture lied in the fact that algae progressively accumulate them with time (Riley & Roth, 1971) and also that the cells grow physiologically week with age (Fogg, 1966).

When the cell numbers at different concentrations of copper (Fig. 7A) and lead (Fig. 7B) observed on a certain day of experiment during the exponential phase of growth were plotted against each other and fitted to a least square regression equation, it was found that they were linearly inversely related with each other. With an increase in concentration of the metals, both cell numbers and chlorophyll 'a' concentration of the species declined.

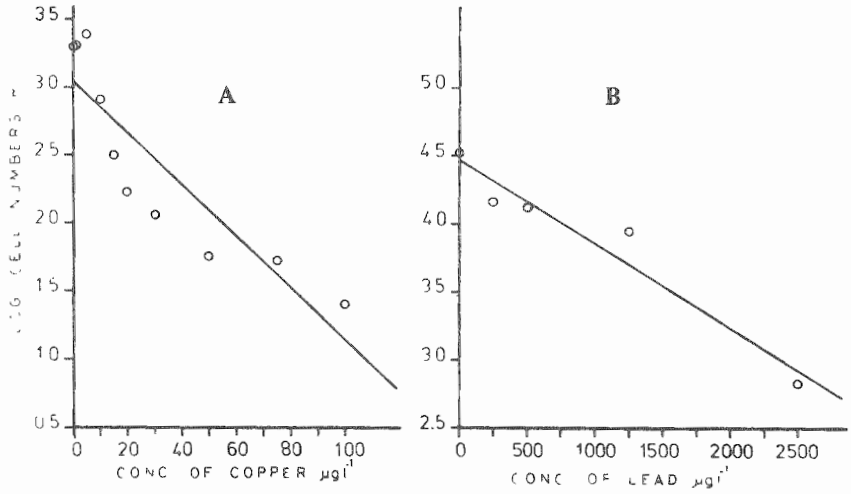

Fig. 7. Relationship between cell numbers and concentration of A) copper during second day of exponential phase (Log Y = 3.037 – 0.019 X; p 0.001), B) lead during second day of exponential phase (Log Y = 4.469 – 0.00062 X; p 0.01).

Steemannn-Nielse & Kamp-Neilsen (1970) and Saifullah (1978) have reported that large size inocula mask the effect of copper on algae due to increased excretion of organic matter by large number of cells which bind heavy metals (Davey *et al.*, 1973; Bentley – Mowat & Reid 1977). In the present study 30 and 50 μg Cu l^{-1} were lethal to *S. costatum* when the inoculum size was 11 μg chlorophyll 'a'. l^{-1} (Fig. 2A); but no such mortality was found at the same concentration of copper when the inoculum size was 36 μg chlorophyll 'a'. l^{-1} (Fig. 2B). Lead did not seem to have any significant effect on inoculum size.

Copper and lead proved to be synergistic in their inhibitory effect (Fig. 5). The combined inhibitory effect of both metals was more pronounced than when applied alone (Fig. 4) since 5 μg Cu l^{-1} inhibited growth of the species more in combined form than alone. Similarly 250 μg Pb l^{-1} used alone did not inhibit growth as compared to use of 100 μg Pb l^{-1} in combination with 5 μg Cu l^{-1}. Similar synergistic effect of copper and lead on *Oscillatoria thiebautii* has been observed by Khan & Saifullah (1984). The cumulative effect of copper and zinc is synergistic on three other species of phytoplankton (Braek *et al.*, 1976).

Acknowledgements

The first author acknowledges the facilities provided by the Centre of Excellence in Marine Biology, University of Karachi and a fellowship from University grants Commission during 1976-78.

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(Received for publication 10 September 1985)