

Effect of Some Metal Ions on the Photosynthesis of Microplankton & Nannoplankton

A. RAJENDRAN, SUMITRA-VIJAYARAGHAVAN & M. V. M. WAFAR
National Institute of Oceanography, Dona Paula 403004, Goa

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In larger algae (microplankton) and smaller algae (nannoplankton) photosynthesis got either stimulated or inhibited at higher concentrations of manganese, cobalt, copper, iron, zinc and molybdenum. The response in both the groups of phytoplankton varied with different metals. Copper stimulated photosynthesis of both fractions up to 5 µg/litre conc. Zinc was less toxic to nannoplankton than to microplankton. Iron showed inhibition of photosynthesis from 4.5 µg/litre conc. onwards. A marked rate of photosynthetic increase was observed with molybdenum. Some of the ecological and ecotoxicological aspects of heavy metals on marine photosynthesis are discussed.

MANGANESE, iron, copper and zinc are of special interest because they are not only essential micronutrients but also toxic pollutants when their concentrations exceed a certain level. This dual role implies that both upper and lower limits of their acceptable concentrations exist in marine and estuarine ecosystems. These heavy metals find their way into estuaries as pollutants along with industrial and domestic wastes and also as a result of mining activities and erosion. Their concentration in sea water may not seem to be high enough to be toxic at times but their toxic effect as pollutants will be magnified when they are concentrated in various trophic levels. Though the primary producers form a very significant basic trophic level in the ecological system, only few reports¹⁻³ are available on the effects of heavy metals on planktonic algae. Riley and Roth⁴ have also shown that the amount of an element taken up is related to its concentration in the medium.

Toxicity studies in a culture medium with an axenic unicellular culture will present some practical difficulties and the results obtained may not essentially reflect the natural environment. For example, toxicity of arsenate and selenate is markedly reduced in the presence of excess of phosphate or sulphate respectively⁵. Toxicity can also be altered in the presence of organic matter which may chelate specific metals. The number of cells present in the culture can also alter the toxic effect on the cells. On the other hand, experiments in the natural environment also have some disadvantages such as the superimposition of the effects of naturally occurring environmental variables over those of toxicants. In spite of this, *in situ* experiments would provide a more realistic picture than the laboratory culture experiments. In the present investigation, such an attempt has been made to find the effects of 6 biologically important micronutrients (Cu, Zn, Mn, Mo, Fe and Co) at different

concentrations, on the photosynthetic rate of microplankton and nannoplankton fractions in a tropical estuary.

Materials and Methods

Experiments were carried out in the Zuari estuary Goa (Dona Paula Bay). Microplankton and nannoplankton fractions were separated by passing the samples through a 22 µ mesh bolting silk. Stock solutions of different concentrations of metals were prepared with sterile artificial sea water and the required concentration of each metal was prepared by adding the stock solution (mostly 1-5 ml) to the light and dark bottles. For each concentration of metal, 2 light and 1 dark bottles, along with controls, were inoculated with 1 ml of 4 µ Ci of NaH¹⁴CO₃ supplied by BARC, Bombay. *In situ* incubations were done immediately after the collection for 3 hr from 8 to 11 a.m. Photosynthetic rates of both micro- and nannoplankton were measured at different concentrations of metals as ¹⁴C uptake. The filters were counted for radioactivity in a Geiger-Muller counter.

Results

Carbon assimilation was measured as mg C/m³/hr but in order to generalize and compare the effects of all the metals on the photosynthesis of both microplankton (larger) and nannoplankton (smaller), the values were converted to per cent carbon assimilation, taking the rate of microplankton (control in which there is no addition of metal ion) as 100% (Fig. 1).

Copper — Copper enhanced microplankton and nannoplankton productivity only up to a concentration of 8 and 4 µg/litre respectively and above this level photosynthetic rate decreased enormously, even up to 52% in the case of nannoplankton fraction, and 73% in the case of microplankton fraction. Inhibition due to copper was much more than with

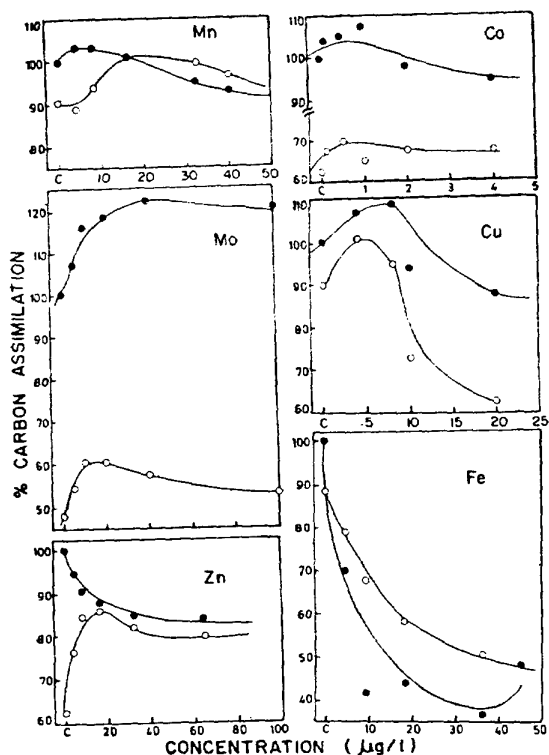


Fig. 1—Variations in the rate of carbon assimilation by micro-(●) and nannoplankton (○) at different concentrations of metals

other metals. Copper has been shown by several workers⁶⁻⁹ to be toxic in its ionic form even at small concentrations to several species of phytoplankton. However, Steemann Nielsen and Wium-Andersen¹⁰ found that in spite of the decrease in photosynthetic rate at higher concentrations, a concentration of 3 µg/litre of copper stimulated the excretion of complex organic extracellular products which diminished the concentration of active ions in the solution and, consequently, their toxicity.

Zinc—Photosynthetic rate of nannoplankton fraction increased as the concentration of zinc increased up to 16 µg/litre, but the microplankton production decreased gradually at all higher concentrations. It appears that moderately high concentration of zinc did not affect the growth of nannoplankton.

Iron—Both the fractions of phytoplankton showed only a decrease in photosynthetic rate from a concentration of 4.5-45 µg/litre (Fig. 1). Most of the iron present in sea water is in the form of micro colloidal ferric hydroxide and the dissolved organics maintain this form of iron in a filter passing state. In these experiments, though iron was added as an inorganic salt, some portion might have been flocculated in sea water which had a higher pH. But, any enhancement in the photosynthetic rate, however, was not found, perhaps due to very high concentration of iron. It is interesting to note that at higher concentrations

above 4.5 µg/litre, photosynthetic carbon assimilation of nannoplankton fraction was greater than that of microplankton.

Molybdenum—There was a marked increase in photosynthetic rate in the case of molybdenum up to a concentration of 20 µg/litre and at further higher concentrations, this was not significantly reduced. Molybdenum is known to play an important role through dual functions in influencing nitrate reductase enzyme systems and also in nitrogen fixation capacity¹¹. Sournia and Citeau¹¹ also observed a probable relation between the level of molybdenum and photosynthetic assimilation of carbon in nearshore waters of Nosy-Bé (Madagascar). Among other metals, only molybdenum increased photosynthetic rate of microplankton up to a concentration of 40 µg/litre and the carbon assimilation of nannoplankton decreased beyond 20 µg/litre (Fig. 1).

Manganese and cobalt—Manganese and cobalt, though increased the rate of carbon assimilation at low levels (8 µg/litre with Mn and 1 µg/litre with Co), these did not appear to be very toxic to both micro- and nannoplankton (Fig. 1). Many of the aquatic algae require vitamin B₁₂ which contains cobalt. Hohn-Harsen *et al.*¹² demonstrated that a blue-green alga needed cobalt, for it did not utilize an exogenous source of vitamin B₁₂.

Discussion

In all the conditions, nannoplankton productivity varied from 50 to 90% of the total productivity and the response to increased concentrations of metals had a different effect on the rate of photosynthesis of nannoplankton as compared to microplankton. In some situations nannoplankton productivity was greater than that of microplankton. This may be due to the fact that the uptake of nutrients and trace metals during photosynthesis of nannoplankton might be more efficient than in larger algae or due to some variations in the physiological response between these 2 fractions of phytoplankton to varied conditions of environmental parameters. Malone¹³ pointed out that the growth rate of nannoplankton, as indicated by the productivity index, was higher than that of microplankton by 88%.

Present results indicated that the response of both fractions of algae to different concentrations of the metals is highly varied. Photosynthetic carbon assimilation was greatly affected in nannoplankton when they were exposed to higher concentrations of copper than the other metals. Berland *et al.*¹⁴ studied the sublethal effects of mercury, cadmium and copper on the diatom *Skeletonema costatum* grown in batches and in bacteria-free culture (division rate, maximum yield growth, mean cell volume, particulate C and N, and ¹⁴C uptake were used as toxic impairment criteria). It was observed that the division rate which was the most sensitive feature was affected first. However, they also observed that the algal response varied according to the metal. Mercury produced an acute decrease in division rate, followed by temporary recovery of growth capacity within the first 48 hr after the metal addition. Cadmium, on the other hand, increased the division rate,

followed by an obvious decrease. Copper reduced the division rate slowly or quickly depending on the metal concentration. They also observed that the cell synthetic capacity was less affected than the division rate, especially with mercury. Berland *et al.*¹⁵ in an earlier study on the inhibitory role of certain heavy metals on a number of marine phytoplankton species observed different results with different species. They identified some of the species as more sensitive and some very resistant. They also noticed varied response in the same species to different levels of toxicity, especially in the case of cadmium and copper. Mandelli⁷ found that the growth of several species of dinoflagellates and diatoms in batch cultures under continuous illumination was inhibited at 20°C by concentrations of copper between 0.055 and 0.265 mg/litre. *Coccolithus elebens* failed to grow at 0.03 mg/litre at 40°C where as *Dumaliella tertiolecta* survived 0.6 mg/litre at 30°C. He also observed that *Skeletonema costatum* in cultures with maintenance of constant number of cells and copper ions, the growth was inhibited at 0.05 µg/ml.

Present results show that except iron, all other metals stimulated photosynthesis up to an optimum concentration. Because of the high concentration of iron added, photosynthesis was inhibited from 4.5 µg/litre onwards. It is well known that the photosynthetic rate of diatoms increased due to the addition of chelated iron to the medium^{16,17}. Goldman¹⁸ pointed out that chelation with EDTA slightly lowered toxic level of a metal and that nitriolacetic acid (NTA) also acted in a similar way in lowering the toxic level and stimulating the photosynthesis. But it was also observed in some cases that 100 ppb chelated iron with phosphate and nitrate, the levels of which were significantly above the initial environmental levels, inhibited the photosynthetic rate¹⁸. The same case was observed in the present study also where the levels of iron added were well above the normal concentration and also they were not in chelated condition.

Bowen⁵ pointed out that metal-organic compounds may be either much more toxic than metal ions (e.g. ethyl mercuric chloride and mercuric ion) or much less so (e.g. cupric ion and copper salicylaldehyde). Organo-mercuric compounds reduced the photosynthesis of phytoplankton at a concentration of 0.1 ppb which was much lower than that found in potable waters¹. However, Young and Barber¹⁹ found that in spite of high amounts of potentially toxic organic matter and heavy metals, inhibition of phytoplankton growth was not consistently demonstrated by their studies on the effect of sewage sludge and dredge-spoil dumping, that were loaded with organic materials and heavy metals on the growth of natural populations of phytoplankton.

Phytoplankton concentrate Mn, Fe, Cu and Zn from sea water to high levels²⁰. It is said that much of the uptake is probably due to passive adsorption rather than through active processes. Bachman²¹ found that fresh water phytoplankton concentrated ⁶⁵Zn from solution as well or better when dead than alive. Davies²² found some marine

diatoms to accumulate iron by adsorption on their surfaces as ferric hydroxide. Umezumi and Saiki²³ observed that while ³²P was accumulated by living organisms, ⁶⁵Zn was adsorbed more rapidly by dead organisms. In spite of these observations, it is well known that marine organisms, especially autotrophic algae obtain all their nutrients directly from sea water and are able to take up elements against a concentration gradient. Among cations (including metallic elements such as iron which may exist as colloids in the sea), the order of affinity for living cells is: tetravalent and trivalent elements > divalent transition metals > divalent group IIA metals > univalent group I metals⁵. Among the tetravalent and trivalent subgroup, iron has got high affinity for plankton and brown algae. Among the divalent transition metals, Bowen⁵ gives the following affinity rate for plankton: Zn > Pb > Cu > Mn > Co > Ni > Cd.

Concentration of various heavy metals by marine algae may be controlled by various environmental factors, especially salinity, temperature and light. Hiyama²⁴ carried out experiments to determine the concentration factors of several radionuclides and found that in the case of iron taken up by algae, the concentration factor was affected by light. Rate of uptake of a metal by algae may depend on the metal, the species, its age and temperature of the water.

The important mechanism of toxic action is considered to be poisoning the enzyme system. Copper, being more electronegative metal, acts in this way. Some metals are acting as antimetabolites. Others form stable precipitates or chelates with essential metabolites. For example, iron forms a stable chelate with ATP and thus becomes a toxic element. Most of the metals like cadmium, copper, mercury and lead combine with the cell membrane affecting its permeability.

Whenever a laboratory experiment is set up to find the effect of a pollutant on the algal growth and photosynthesis, certain environmental factors like grazing, bio-degradation, temperature and nutrients should be taken into account. The *in situ* photosynthesis experiment alleviates this problem to some extent. Berland *et al.*¹⁴ also felt that ¹⁴C assimilation method gave much coherent results, especially with regard to biomass increase as a function of time. The study of the speed of cell division has not been extended to *in situ* studies of natural populations and, in this case, measurements of photosynthesis by ¹⁴C appear to constitute a rapid and relatively sensible way to detect and measure the toxicity. It may be worth while to study the exposure time of a pollutant to the phytoplankton community for detectable changes in their metabolic activities.

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