THE EFFECTS OF METAL MIXTURE IN THE CULTURE MEDIUM ON THE GROWTH OF PHAEODACTYLUM TRICORNUTUM UNDER NUTRIENT LIMITATION

KÜLTÜR ORTAMINDAKİ METAL KARIŞIMININ BESİN ELEMENTİ SINIRLAYICI KOŞULLARDA PHAEODACTYLUM RICORNUTUM'UN ÜREMESİNE ETKİLERİ

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Abstract

A mixture of copper, zinc, cobalt, manganese and iron was added to the medium in which cultures of *Phaeodactylum tricornutum* (Bohlin) were grown under nitrate and phosphate limitation. Growth was determined by counting cells, measuring *in vivo* fluorescence and monitoring ^{14}C uptake. It was found that maximum cell concentrations were reached at concentrations over 5 mg N l^{-1} and 0.5 mg P l^{-1} in the media whether or not there was enrichment with metals,. The fluorescence intensities, growth rates, ^{14}C uptake rates of the cells and chlorophyll-a: cell ratio in metal enriched media were higher than those in unenriched media.

Introduction

It is difficult to determine what limits primary production in natural waters. Light, temperature, nutrients and various trace elements all play roles in the

regulation of primary production i.e. there is no single factor limiting algal growth. Generally the two major nutrients nitrogen and phosphorus limit primary production. However, not only nutrients but the trace metals are also responsible for the development of eutrophication. The idea that trace elements may also limit algal production in marine environments is not new (Harwey, 1947 ; Ryther and Guillard, 1959) and recent experimental evidence suggests that Fe, for example, limits primary production in the open ocean (Martin and Fitzwater, 1988). Indeed Di Tullio *et al.*, (1993) produced data supporting Martin and Fitzwater's hypothesis; i.e. Fe is vital in controlling diatom growth in the equatorial Pacific. In recent decades, the injection into the aquatic environment of many trace metals from terrestrial and atmospheric sources has increased (Förstner and Wittmann, 1981) and generally coastal waters are always relatively rich in such trace metals as molybdenium, copper, cobalt, manganese, zinc and iron which have all been shown to be essential for algae (Bryan, 1976).

However, the metals are found in very low concentrations in open sea water and required in very small amounts by algae and the tolerance of algae to trace metals is very limited. Indeed, for this reason, algal bioassays are used extensively to assess realistic water quality criteria for trace metals in natural waters (Maestrini *et al.* 1984). Although a large number of studies on the effects of single metals on the growth of algae have been reported (Barlett *et al.* 1974; Christensen and Scherfig, 1979), only limited knowledge exists about the combined effects of metals on algae. In general, since many metals are present in the natural environment, it is perhaps more realistic to determine their combined effects rather than to investigate their influence individually.

In the present study we determined the combined effects of the mixture of some metals on the growth of phytoplankton under nitrate and phosphate limiting conditions.

Materials and Methods

A pure culture of marine diatom *Phaeodactylum tricornutum*, widely used in bioassays, was chosen as the test algae. Inocula were taken from the cultures preincubated under the same experimental conditions. The culture was inoculated into nitrate and phosphate free media twice so that the algae used up their inner reserves before bioassay experiments were performed. The algae were added to the media to give concentrations of 10^4 cells ml^{-1}.

The constituents of f medium (for nitrate, phosphate and silicate) and f/2 medium (for metals except iron) (Guillard and Ryther, 1962) were slightly modified (Table 1) and changed according to the aim of the bioassay. The culture medium was prepared as 5 separate stock solutions (1000 fold concentrated of the original medium) to prevent precipitation. Nitrate concentrations between 0 and 24.7 mg N l^{-1} and phosphate concentrations between 0 and 2 mg P l^{-1} were used to achieve nitrate and phosphate limitations respectively. A metal mixture of Cu, Zn, Co,

Mn, Fe was added in order to obtain enriched medium. Sea water collected from the surface waters of the Marmara Sea with a salinity of 20 ‰, consisting of Black Sea water modified by vertical mixing with underlying Mediterranean water, was used as dilution water after filtration through 0.45 mm and sterilization in an autoclave at 120°C for 20 min.

All bioassay experiments were carried out in 1000 ml erlenmayer flasks. The flasks containing 500 ml working solutions and cells were placed on a rotary

Table 1. Modified f Medium Composition

Solution Nr	Compound	Concentration (mg l^{-1})
1	$NaNO_3$	150
2	$NaH_2PO_4.H_2O$	10
3	$Na_2SiO_3.5H_2O$	25.7
4	$CuSO_4.5H_2O$	0.009
	$ZnSO_4.7H_2O$	0.022
	$CoCl_2.6H_2O$	0.010
	$MnCl_2.4H_2O$	0.180
5	$FeCl_3.6H_2O$	1.818

shaker and illuminated by cool-white fluorescence lamps placed horizontally over the shaker. The light intensity at the surface of culture media was 3500-4000 lux. Temperature was maintained at 20±1°C in an isolated air-conditioned room.

Cells were counted directly under the microscope in a haemacytometer. Chlorophyll-a was estimated from *in vivo* fluorescence measurements using a Shimadzu Model RF-540 fluorescence spectrophotometer. The ^{14}C uptake rate was measured by the method described by Damgaard and Nyholm (1980). The radioactivities were counted with a Packard 1550 Tri-Carb liquid scintillation counter. Nutrient analyses were performed by a Technicon Autoanalyzer following standard procedures (Standard Methods for the Examination of Water and Wastewater, 1985) modified for a continuous analyzing system (Technicon Industrial Method a, 1977; b, 1977).

Two replicates were performed for each set of experiments and the culture media containing no NO_3-N and PO_4-P were considered as control samples.

Cell counts, fluorescence intensities, ^{14}C uptake rates and NO_3-N, PO_4-P concentrations were measured every 24 hours for the first 5 days and also 3-4 times to the end of the bioassay.

Results and Discussion

Figures 1 and 2 show fluorescence intensity, cell concentration and the rate of ^{14}C uptake with respect to the duration of culturing under nitrate limitation. Figures 3 and 4 show the change of the same parameters under phosphate limitation. It is evident from the figures that fluorescence intensities and cell numbers show similar trends and maximum cell concentrations were reached with concentrations of 5 mg N l^{-1} and 0.5 mg P l^{-1} whether or not the media were enriched with metals. ^{14}C uptake is consistent with the other two growth curves (Fluorescence intensity and cell concentration versus days) i.e. the ^{14}C uptake was a maximum on the day on which the population entered its stationary phase.

By comparing figures 1 and 2 or 3 and 4, it is seen that the enrichment with metals prolonged the lag phase to become from 0-2 days (non enriched) to 2.5-3 days (enriched). Enrichment with metals increased the chlorophyll content of each cell during the lag phase. The cells living in metal enriched medium always possessed higher concentrations of chlorophyll but these concentrations diminished as the cultures grew. All the metal enriched cultures grew more rapidly. Maximum rates of assimilation of ^{14}C which were reached after lag phase were in order of magnitude greater in metal enriched media. It was also observed an increase in the rate of assimilation at higher concentration of nitrate and phosphate. For comparison of bioassays, the exponential growth rate determined using the cell numbers and the equation, $m=\ln(X_2/X_1)/(t_2-t_1)$, is taken as representative of each culture. The effect was also confirmed by calculations of growth rates. One finds the growth rates to lie between 1.20-1.40 day^{-1} and between 0.70-0.75 day^{-1} in metal enriched and nonenriched media respectively i.e. metal enrichment approximately doubled the rate of growth.

Since cells in metal enriched media grew more rapidly it should not be surprising that they exhausted the supply of nitrate and phosphate more readily. Figures 5 a and b show the development of *Phaeodactylum* cultures as described by their *in vivo* fluorescence as a function of the concentration of nutrient remaining in the media. It is clear that, in general, the lag phase is terminated by the exhaustion of nutrient. The growth may persist for several days after all phosphate in the medium has been exhausted. During this period the cells appeared to have been utilising previously assimilated phosphate for their growth.
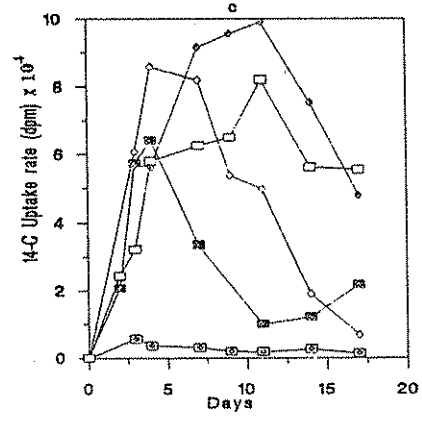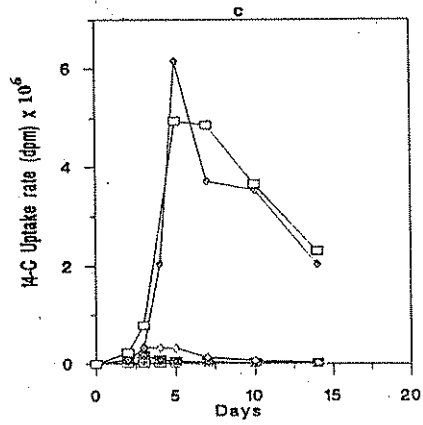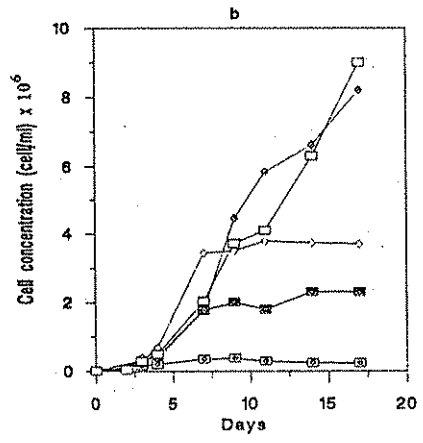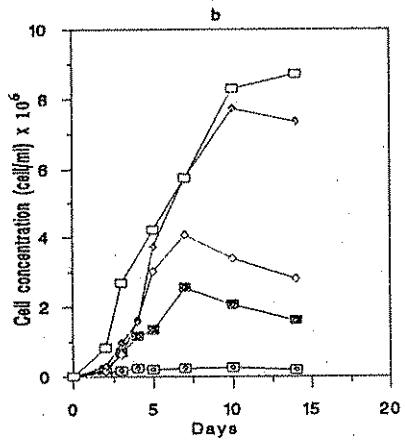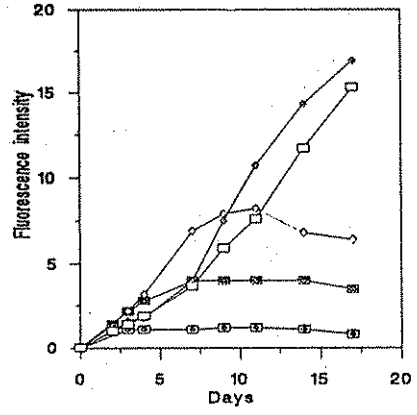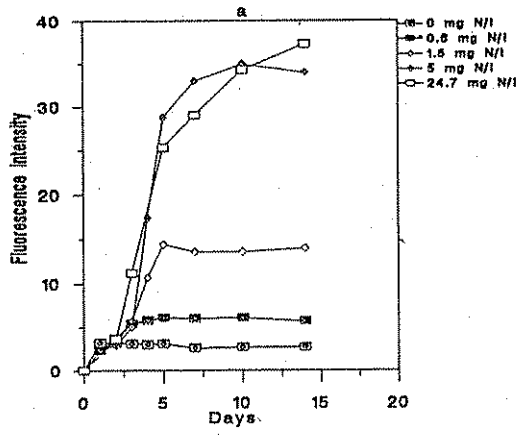
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Figure 1. The results of metal enriched bioassays under nitrate limitation.

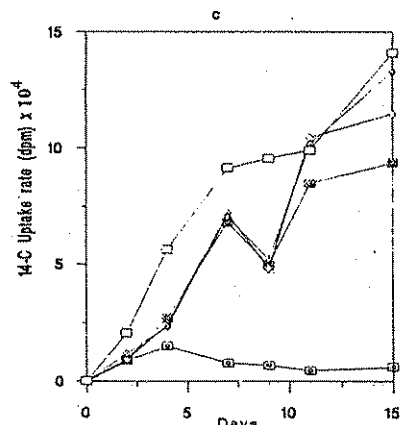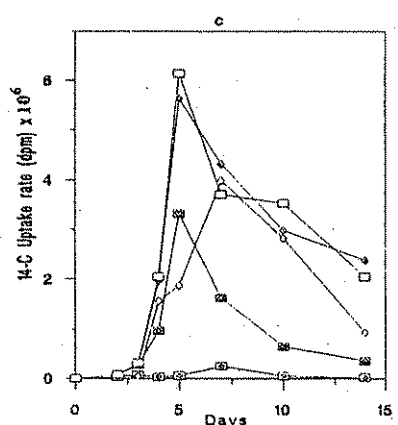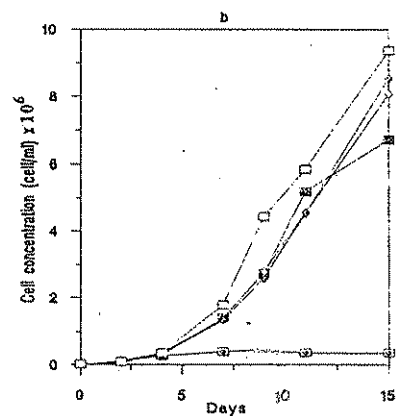Figure 2. The results of metal nonenriched bioassays under nitrate limitation.
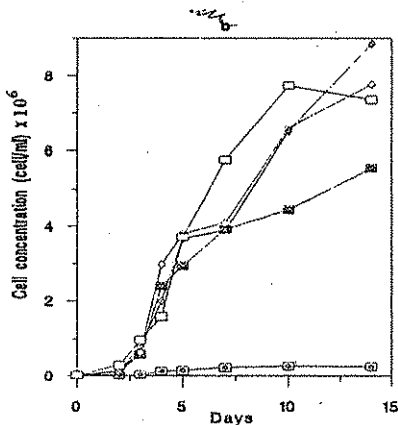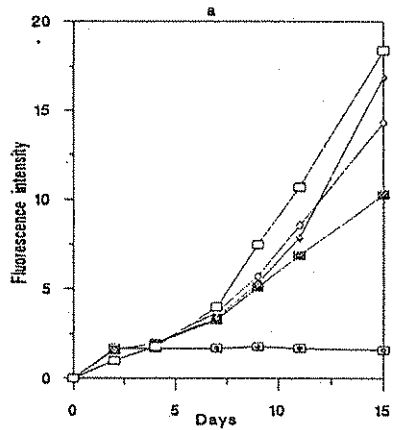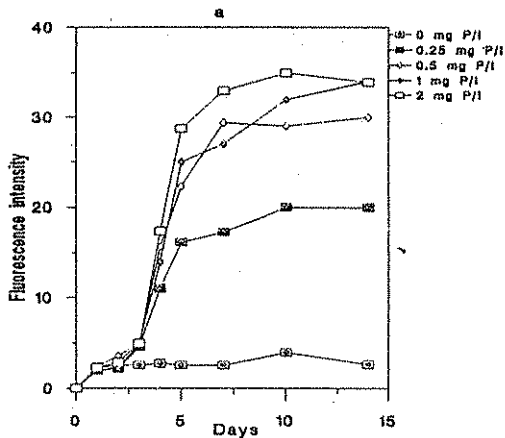
Figure 3. The results of metal enriched bioassays under phosphate limitation.

Figure 4. The results of metal nonenriched bioassays under phosphate limitation.
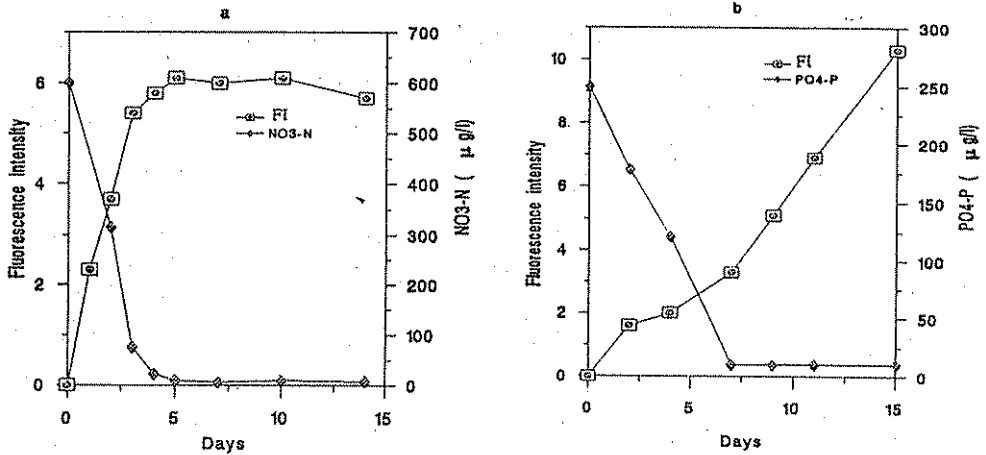
Figure 5. The relationship between fluorescence intensity and nutriente uptake.

Thus, ഛe maximum populations which the media could support were little changed by metal enrichment-and were, therefore, presumably determined by the total amount of nitrate or phosphate which was supplied consistent with the whole of this discussion, the major effect of the extra metal was to expedite growth.

Conclusions

1. Although concentrations of 0-24.7 mg N l^{-1} nitrate and of 0-2 mg Pl^{-1} phosphate were used, maximum cell concentrations were achieved with 5 mg N l^{-1} and 0.5 mg P l^{-1} in both metal enriched and nonenriched media.
2. Maximum growth rates were calculated to lie between 1.20 -1.30 day^{-1} and approximately 0.75 day^{-1} for nitrate limited bioassays and between 1.30-1.40 day^{-1} and approximately 0.70 day^{-1} for phosphate limited bioassays in metal enriched and onenriched media respectively.
3. ^{14}C uptake rates were greatly increased when the media were enriched with metals.

4. The maximum cell concentrations reached were the same for both enriched and nonenriched media, the chlorophyll fluorescence intensities were increased by metal enrichment. For this reason the ratios of chlorophyl-a:cell were higher in metal enriched media than in nonenriched media.

5. Both nitrate and phosphate were exhausted faster from the medium when it was enriched with metals.

6. Growth persisted after the exhaustion of phosphate from the medium suggesting that the cells had previously stored phosphate (but not nitrate).

7. Enriching the media with metals prolonged the lag phase of the cultures and the concentrations of chlorophyll in each cell increased. Subsequently, rates of growth approximately doubled and in consequence both nitrate and phosphate were exhausted more rapidly. Thus, in a sense, the additional metals catalysed the growth of the phytoplankton. However, the total biomass (number of cells) produced was rather independent of the enrichment by metals being determined presumably by the availability of the major nutrients.

Acknowledgement

Özet

Nitrat ve fosfat sınırlayıcı koşullarda besi ortamına bakır, çinko, kobalt, mangan ve demir karışımı eklenerek *Phaeodactylum tricornutum*'un üremesi incelenmiştir. Üreme hücre sayımı, süspansiyonda floresans şiddeti ölçümü ve ^{14}C kullanım hızının takibi ile izlenmiştir. Ortamın metallerle zenginleştirilmesi maksimum hücre konsantrasyonlarını etkilememiş ve bu hücre sayılarına 5 mg N l^{-1} ve 0.5 mg P l^{-1} konsantrayonları ile erişildiği gözlenmiştir. Floresans şiddeti değerleri, üreme hızları, ^{14}C kullanım hızları ve klorofil-a:hücre oranları ise metaller ile zenginleştirilmiş ortamda, zenginleştirilmemiş ortama oranla daha yüksek bulunmuştur.

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