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NUTRITIONAL REQUIREMENTS OF THE RED TIDE DINOFLAGELLATE PRORECENTRUM TRIESTINUM SCHILLER

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ABSTRACT

In order to examine the nutritional requirements of the Red Tide dinoflagellate ***Prorocentrum triestinium*** *Schiller, an axenic culture, was established.*

The combination of nitrate and phosphate enhanced the growth yield remarkably, comparing with their individual addition as well as vitamin B_{12} and thiamine. The soil extract significantly stimulated the growth, more than all treatments tested. Trace metals were also crucial elements. Their essential role triggering outbreaks of ***P. triestinum*** *must deserve attention.*

The experiment supports the results of the recent field ecological studies carried out in the eutrophicated coastal waters of Alexandria (Egypt).

INTRODUCTION

Prorocentrum triestinum is a well known Red Tide species in temperate coastal waters, its occurrence is closely related to land drainage (Iizuka, 1985). The species represents a significant member of the multi-species Red Tide blooms in the Eastern Harbour of Alexandria during summer and autumn (Labib, 1994), contributing a population size in 71.1 x 10^6 cell l^{-1} during April, 1993 (Labib 1995).

Since it is impractical to follow the growth rate of a given phytoplankton population under a natural condition of complicated processes (Takahashi, 1980), moreover, the knowledge gained from the ecological studies are not sufficient to explain factors developing the outbreaks of ***P. triestinum*** in eutrophicated coastal waters, the present culture experiment was established to examine its nutritional requirements.

MATERIALS AND METHODS

Prorocentrum triestinum was isolated from a semi-closed, highly eutrophicated marine basin (Aburatsubo Bay, Japan). Axenic culture strain was obtained using the sterile micropipette washing method. A culture medium was prepared by filtering one liter of seawater (36.5 ‰), through a glass fiber filter (Whatman GF/C), then enriched, individually with the chemicals or the soil extract at concentrations shown in Table 1.

Table (1) : Chemical and soil extract of enriched culture experiments

Filtered seawater 1000 ml			
$NaNO_3$	0.5 mg	Soil extract	10 ml
K_2HPO_4	0.1 mg	EDTA-Fe	1.3 µg
Vitamin B_{12}	1 µg	EDTA-Mn	1.6 µg
Thiamine	20 µg	EDTA-Zn	0.1 µg
		pH[1)]	7.8

1) adjusted by addition of HCL

The filtered seawater (served as a control) and enriched filtered seawater were sterilized by autoclaving for 15 min at 121°C abd 15 psi in 1-L teflon bottle. After being cooled for at least 24 h, 10 ml of both the control and the enriched seawater were dispensed into 25 x 150 mm clean sterilized culture tubes, and inoculated with living cells to a concentration of 100-150 cell ml^{-1}. Incubation was done at 22° ± 1°C, with illumination provided by cool white fluorescent light of 0.025 Ly min^{-1} under 12:12h light-dark cycle. The experiment lasted for 17 days. The soil extract was prepared by filtering a boiled with water

bottom sediment sample through a glass fiber filter (Whatman GF/C), then autoclaved. Both filtered seawater and the soil extract were stored in deep freezer for subsequent use. The initial nutrient concentrations in filtered seawater were 0.86, 1.3 and 0.44 μ mol l^{-1} for nitrate plus nitrite, ammonia and phosphate, respectively, whereas their levels in the soil extract were respectively, 5.4, 32.1 and 6.8 μ mol l^{-1}. The nutrient analysis was done according to the method of Strickland and Parsons (1972). Cells were preserved with glutaraldehyde (1%). Their numbers were evaluated very day and the growth curves were calculated using the average growth values of triplicated cultures. Diel increasing growth rate of cells (μ_2) of ***P. triestinum*** was estimated from cell counts, in the exponential phase, using the following equation:

$$\mu_2 = (\text{Ln } C_1 - \text{Ln } C_0) / (T_1 - T_0) \text{ Ln } 2,$$

where C_0 and C_1 are numbers at time T_0 and T_1, respectively

RESULTS AND DISCUSSION

The results are shown in Figs. 1& 2. The control profile have been omitted for reasons of clearity in Fig. 2.

The diel increasing rate of ***P. triestinum*** under control condition was limited (μ_2 = 0.14 d^{-1}, terminal density of 330 cell ml^{-1}).

Although there is a general assumption that nitrogen and phosphorous play a primary role in determining the growth of phytoplankton in the eutrophicated coastal waters of the Mediterranean Sea (Nixon & Pilson 1983), the present experiment showed that the growth of ***P. triestinum*** under the individual addition of nitrate (μ_2 = 0.24 d^{-1}, 680 cell ml^{-1}) and phosphate (nearly a similar value) was extremely poor. These results are in agreement with the recent field observations carried out in the eutrophicated coastal waters of Alexandria (Labib 1992, 1994), and it was suggested the importance of other promoting substances (s) rather than nitrate developing the outbreak of ***P. triestinum*** and other Red Tide flagellates. A culture experiment (Mingazzini ***et al.***, 1992) pointed out nitrogen as a second factor and its was also reported the addition of phosphate to have a limited influence on the growth of ***Prorocentrum minimum*** (Graneli ***et al.***, 1985).

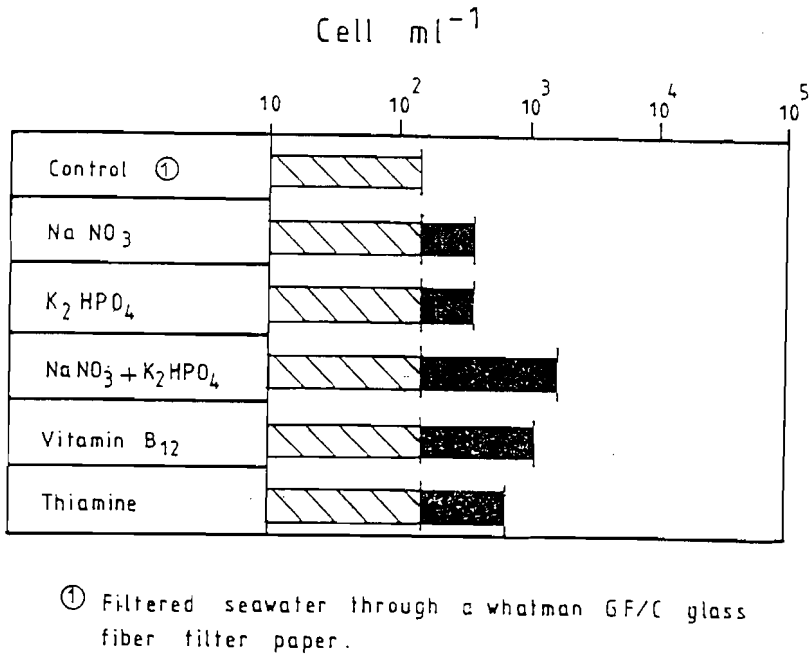

Figure (1) : Effect of macro-nutrients and vitamins on the growth yield of ***Prorocentrum triestinum*** after 17 days incubation. Growth yield exceeding the control is shown by black bars.

The growth of ***P. triestinum*** was remarkably enhanced (about 2 fold, μ_2 = 0.46 d^{-1}, 3450 cell ml^{-1}), by the addition of both nitrate and phosphate, confirming the essential role of combination of factors. According to Tomas (1978), there are instances in which the optimum for one factor is affected by another. The interaction of nitrate and phosphate was proved by Selli ***et al.*** (1992), that nitrate reductase, a key enzyme of nitrogen by phytoplankton was activated, to a certain extant by addition of phosphate.

The growth was stimulated by the addition of both vitamin B_{12} (μ_2 = 0.32 d^{-1}, 1100 cell ml^{-1}) and thiamine (μ_2 = 0.26 d^{-1}, 800 cell ml^{-1}). Vitamin B_{12} is essential for the growth of ***Prorocentrum micans*** (Hasting & Thomas, 1977) and other flagellates (Watanabe ***et al.***, 1982), yet phytoplankton species are greatly different in their nutritional life history and requirements (Kilham & Hecky, 1988). According to Ohwada & Taga (1969), vitamin B_{12} is an abundant vitamin in the sediment of highly eutrophicated waters.

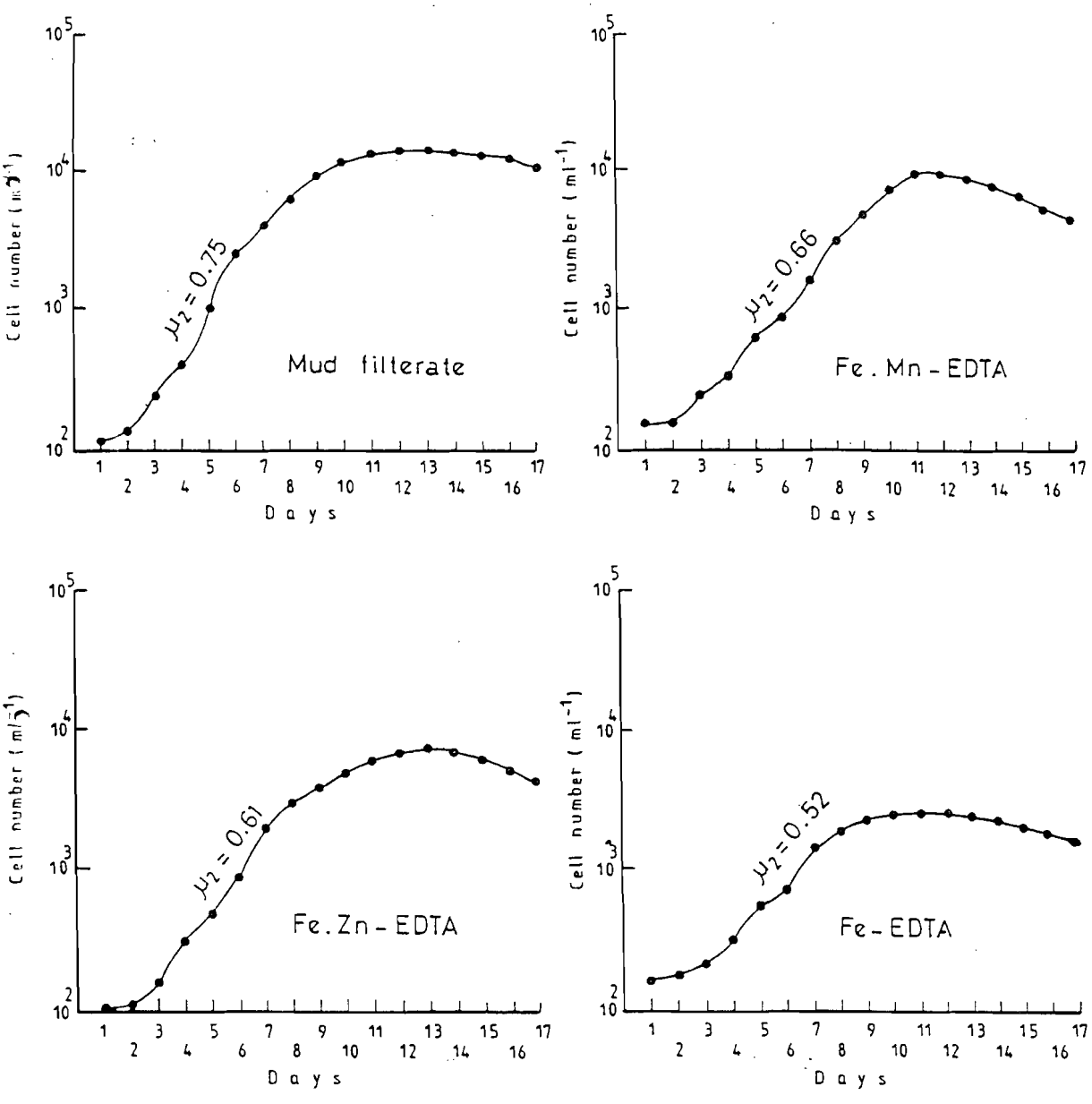

Figure (2) : Diel increasing rate of cells (u2) of ***Prorocentrum triestinum*** incubated under different trace elements.

The specific stimulation, more than of all treatments tested, occurred by the addition of the soil extract (μ_2 = 0.75 d^{-1}, 23 x 10^3 cell ml^{-1}), in agreement with the results of Iwasaki (1979) and Takahashi & Fukazawa (1982) for other Red Tide species.

Trace metals also exhibited significant stimulating effect, with 0.66 d^{-1}, 12 x 10^3 cell ml^{-1} and 0.61 d^{-1}, 8.75 x 10^3 cell ml^{-1}, for the addition of Fe-Mn EDTA and Fe-Zn EDTA). On the other hand, the effect of Fe-EDTA was relatively less than others (μ_2 = 0.52 d^{-1}, 4.5 x 10^3 cell ml^{-1}). Yamochi (1984) pointed out the growth yield nitrate-phosphate combination. Yamochi (1984) pointed out the growth yield of ***Prorocentrum micans*** to be enhanced by addition of Fe-EDTA. The essential effect of trace elements to algal growth is documented (O'Kelley, 1974).

The recent studies of Red Tide outbreaks in the Easter Harbour of Alexandria (Labib 1992, 1994) revealed an increase in ammonia towards the bottom and an opposite vertical profile for dissolved oxygen. These environmental variations suggest suddenly increased decomposition or release of sediment substances into the water column including metals as Fe and Mn (Hoshika ***et al.***, 1978), vitamin B_{12} (Ohwada & Taga 1969) and organic chelators which could significantly reduce possible heavy metals stress on Red Tide flagellates (Anderson & Morel 1979). Humic materials in the soil extract may also influence the growth (Graneli ***et al.***, 1985).

The present experimental results may have implications on Red Tide growth in eutrophicated coastal waters and suggest some relationship between the growth stimulation of ***P. triestinum*** and release of substance (s) from bottom sediments under poor oxygen and high ammonium levels. The stimalation of the mud filterate on ***P. triestinum*** strongly supported this possibility. The results confirm the essential role of combination of factors, rather than their individual effect, in agreement with the ecological studies in Alexandria waters.

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