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EFFECT OF SOME NUTRIENTS AND THEIR COMBINATIONS ON THE GROWTH OF NITZSCHIA PALEA (KUTZ) W.SM.

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ABSTRACT

Mathematical regression models are given, which describie the dependence of *Nitzschia palea* growth on the available concentration of nitrogen, phosphorus, iron and silicon. Data of these experiments show that this dependence is a very complicated process. Culture growth is mainly affected by the concentration level of nitrogen, silicon and their intereffect. Nitrogen in its turn stimulates algal growth through its positive intereffect with phosphorus. At the same time silicon effect is inhibited by its intereffect with iron. On the other hand, the latter stimulates algal growth, specially at its early stages through its intereffect with phosphorus.

INTRODUCTION

Most studies on nutrients effects on algal growth relate to experiments where single element was applied. However in aquatic ecosystem, several nutrients are present together in different concentrations and chemical forms. The aim of this work is to obtain approximate mathematical picture of the potential impact of multielement concentration (nitrogen, phosphorus, iron and silicon) on the growth of Nitzschia pelea

Theoretical consideration and experimental design:

As the factors affecting the algal growth (Y) in the present experiments are quantitative variables, the forward can be taken as a function of the levels of these variables.

$$y = f \quad (N, P, Fe \text{ and } Si).$$

Where N, P, Fe and Si represents the variables, nitrogen, phosphorus, iron and silicon respectively. The mathematical solution of this function can be approximated in the following equation.

$$Y = b_o + b_N X_N + b_P X_P + b_{Fe} X_{Fe} + b_{Si} X_{Si} + b_{NP} X_N X_P + b_{NFe} X_N X_{Fe} + b_{NSi} X_N X_{Si} + b_{PFe} X_P X_{Fe} + b_{PSi} X_P X_{Si} + b_{FeSi} X_{Fe} X_{Si} + b_{NPFe} X_N X_P X_{Fe} + b_{NPSi} X_N X_P X_{Si} + b_{NFeSi} X_N X_{Fe} X_{Si} + b_{PFeSi} X_P X_{Fe} X_{Si} + b_{NPFeSi} X_N X_P X_{Fe} X_{Si}$$

Where

b_N, b_P, b_{Fe}; b_{Si}	are the regression coefficients of the linear effects of the different variables.
b_{NP}, b_{NFe}, b_{NSi}, b_{PFe} b_{PSi}, b_{FeSi}, b_{NPFe}, b_{NPSi} b_{NFeSi}, b_{PFeSi}, b_{NPFeSi}	are those of the interaction effects between the different variables.

These regression coefficients are considered as unit measurment of factor effect on culture growth.

In case of N. palea, the complementary effects of the four variables, were evaluated using 2^4 factorial experiment design given in (Table 1) Cochran and Cox (1957). In this design the concentration level (-1) for every factor represents a point lying within the limiting growth region on its growth curve, while the higher level (+1) represents a point within the stationary region (compiled from the previous experiments). It was decided that the scale of neutral variable change i,e the change of element concentration from lower level (-1) to the higher one (+1) through the intermidiate (o) level should be logarithmic, as the logarithmic transformation permits a more accurate pridiction of the polynomal describing the dependance of algal growth on the levels of the studied elements. The change from real element concentrations in mg/l to their coding levels -1, o, +1 in the experiments are calculated as follows.

$$X_N = \frac{\text{Log (N)}}{0.6021}$$

$$X_P = \frac{\text{Log (P)} + 0.5229}{0.4771}$$

$$X_{Fe} = \frac{\text{Log (Fe)} + 0.5969}{0.4031}$$

Table (1)

Central composite rotatable design for 4 variables.
Cochran and Cox (1957)
X = matrix of x variables.

No.$_u$	X_N	X_P	N_{Fe}	X_{Si}
1	-1	-1	-1	-1
2	1	-1	-1	-1
3	-1	1	-1	-1
4	1	1	-1	-1
5	-1	-1	1	-1
6	1	-1	1	-1
7	-1	1	1	-1
8	1	1	1	-1
9	-1	-1	-1	1
10	1	-1	-1	1
11	-1	1	-1	1
12	1	1	-1	1
13	-1	-1	1	1
14	1	-1	1	1
15	-1	1	1	1
16	1	1	1	1
17	0	0	0	0
18	0	0	0	0

$$X_{Si} = \frac{\text{Log (Si)} - 1.4314}{0.4771}$$

The real element concentrations in mg/l equivalent to the coding levels are given in Table II.

MATERIAL AND METHODS

The alga **Nitzschia palea** was cultured in clear round flasks with flat bottom of 100 ml capacity, the culture media used in the different experiments were prepared according to the matrix given in table (I) using the real element concentrations given in table (2). Three replecates of 30 ml of the different nutrient media were prepared for each experiment. After autoclaving, the pH was adjusted to 7.2 ± 0.1. The cultures were inoculated under sterile

Table (2)

Real element concentrations equivalent to coding levels (mg/l).

Coding level	(X_N)	(X_P)	(X_{Fe})	(X_{Si})
-1	0.25	0.1	0.1	9
0	1.00	0.3	0.253	27
+1	4.00	0.9	0.64	81

conditions with actively growing cells of N. palea, adjusting its initial concentration to 20 x 10^3 cell/ml. The cultures were grown under continious illumenation of 4 K Lux at constant temperature of 25 ± 1 °C for 10 days. Subsamples of 1 ml were taken every two days for estimation of algal growth. This was done optically using Carlzeiss colourimeter at wave length 420 um. Readings of the optical densities were converted to cell numbers/ml by using previously caliberated standard curves,

RESULTS AND DISCUSSION

The data in Table (3) represent the experimental mean growth values in 10^3 cell/ml recorded for the successive days of growth for the different experiments given in the matrix X of Table I. The regression coefficients computed from these data according to Yates (1937) are given in Table (4). The significance of these coefficients were tested using the 1 normal graphic method used by Danial (1959) at 10 percent level of significance.

Regression model equation describing the dependence of culture growth on the different levels of N, P, Fe and Si in the culture media are given in equations (1-5) for the successive days of growth expressed in (10^2 cell/ml). These models contain only the significant regression coefficients: The calculated algal growth values (Y^*) as obtained from the model equations are given in Table (3). The fit of these regression models was tested using F test, which indicated that these models are significant at 10 % level.

Table (3)

Experimental (Y) and calculated (Y^*) growth values in 10^3 cell ml^{-1} for the different days of growth.

$No._u$	Day 2		Day 4		Day 6		Day 8		Day 10	
	Y	Y^*	Y	Y^*	Y	Y^*	Y	Y^*	Y	Y^*
1	32.8	27.1	18.7	16.9	48.3	33.6	24.5	21.3	43.3	48.9
2	20.8	27.9	15.3	20.5	45.8	65.5	39.8	48.5	44.5	49.5
3	24.1	27.9	20.0	18.3	65.3	58.0	59.3	56.6	75.2	95.7
4	25.0	27.1	31.7	19.8	70.8	57.3	69.7	83.9	75.3	95.8
5	40.0	35.1	49.0	47.7	93.3	96.4	75.8	67.6	84.5	79.9
6	47.3	51.2	44.5	38.8	82.8	85.6	87.5	94.8	110.7	91.5
7	43.3	51.2	26.6	31.4	74.3	90.5	88.8	102.9	93.2	71.6
8	49.2	35.1	112.0	124.5	207.8	198.9	160.3	130.1	199.2	193.0
9	28.3	40.2	63.8	59.0	87.2	89.9	114.7	116.9	110.7	81.7
10	51.3	39.4	216.2	203.7	312.0	288.4	262.2	289.2	233.3	210.6
11	57.7	39.4	74.0	75.3	111.0	114.4	124.0	152.2	125.7	128.5
12	29.0	40.2	112.3	118.0	278.7	280.2	365.0	324.5	259.7	257.0
13	57.7	63.5	44.2	46.7	82.8	87.8	96.0	70.6	90.3	112.7
14	50.3	47.4	87.2	99.0	238.3	243.5	251.5	243.0	210.0	252.6
15	59.2	47.4	42.8	44.6	95.8	81.9	111.0	105.9	100.5	104.3
16	47.3	63.5	104.8	99.7	340.0	356.9	256.3	278.3	371.3	354.1
17	32.3	39.9	109.3	121.1	150.4	151.0	140.0	143.0	118.3	121.8
18	47.5	39.9	132.8	121.1	151.7	151.0	145.9	143.0	125.3	121.8

Table (4)

Regression coefficients for the different days of growth.

Regression coefficients	Time of culture growth in days. 2 nd.	4 th.	6 th.	8 th.	10 th.
b_0	41,474	66,443	139,656	136,656	139,208
b_N	-1,412*	24,057	57,385	49,885	48,792
b_P	319*	- 912*	15,823	17,656	23,292
b_{Fe}	7,818	-2,557*	16,240	4,260*	18,250
b_{Si}	6,151	26,724	53,573	60,927	48,479
b_{Np}	-2,786*	620*	10,469	8,635*	13,673
b_{NFe}	661*	- 818*	7,948	- 1,885*	16,542
b_{NSi}	-1,672*	12,901	41,635	36,281	32,104
b_{PFe}	78*	8,588	11,760	- 4,448*	10,292*
b_{PSi}	318*	- 8,755	- 2,677*	- 1,156*	3,313*
b_{FeSi}	-1,818*	-20;859	-16,240	-23,135	-12,896
b_{NPFe}	2,037*	12,995	18,615	- 2,427*	13,792
b_{NPSi}	4,214	-12,495	- 6,531*	- 1,781*	3,819*
b_{NFeSi}	-2,370*	- 9,891	- 7,052*	- 9,073*	188*
b_{PFeSi}	-1,151*	5,161*	3,760*	- 7,094*	5,979*
b_{NPFeSi}	3,839	3,630*	- 385*	-10,531*	3,687*

* Values are not significant.

$$Y^*_2 \cdot 10^2 = 415 + 78\ X_{Fe} + 62\ X_{Si} + 42\ X_{NpSi} + 38\ X_{NPFesi} \quad (1)$$

$$Y^*_4 \cdot 10^2 = 664 + 241\ X_N + 267\ X_{Si} + 86\ X_{PFe} + 129\ X_{NSi} - 88\ X_{PSi} - 209\ X_{FeSi} + 130\ X_{NPFe} - 125\ X_{NPSi} \quad (2)$$

$$Y^*_6 0\ 10^2 = 1397 + 574\ X_N + 158\ X_P + 162\ X_{Fe} + 536\ X_{Si} + 105\ X_{NP} + 79\ X_{NFe} + 118\ X_{PFe} + 416\ X_{NSi} - 162\ X_{FeSi} + 186\ X_{NPFe} \quad (3)$$

$$Y^*_8 \cdot 10^2 = 1367 + 499\ X_N + 177\ X_P + 609\ X_{Si} + 363\ X_{NSi} - 231\ X_{FeSi} \quad (4)$$

$$Y^*_{10} \cdot 10^2 = 1392 + 488\ X_N + 233\ X_P + 183\ X_{Fe} + 485\ X_{Si} + 137\ X_{NP} + 165\ X_{NFe} + 321\ X_{NSi} - 129\ X_{FeSi} + 138\ X_{NPFe} \quad (5)$$

From these models it is clear that w r the concentration range of the 4 elements studied the change from lower element concentration to a higher one eads to a significant increase in algal growth. This is indicated in the model equnations. (1-5) by the positive linear regression coefficients of nitrogen, phosphorus iron and silicon (b_N, b_P, b_{Fe} and b_{Si}). In some models some of these coefficients are missed as their values were non-significant, but the effect of these elements can be easily noticed through their intereffect with the other elements In general, beside the four positive linear effects, there are some intereffects which strongly affect culture growth All over the time of experiments cultures were affected by two unlike intereffects. The positive intereffect of nitrogen with silicon and the negative intereffect of iron with silicon. This means that although the effect of nitrogen on culture growth positively depends on the concentration level of silicon, the effect of the latter is weakened by increasing ferric level in culture media. From the chemical point of view, this may be due to the coating of insoluble silicates of iron formed after the absorbtion of iron or hydroxides of this element by silicon (Riley and Chester, 1971).

This complicated process for the sixth day regression model of growth (equation 3) is illustrated in figures 1 and 2. The frist one illustrates the dependence of culture growth on the concentration level of nitrogen and silicon at certain levels of iron and phosphorus. The high growth yield obtained in figure 1 (a) is mainly due to the positive interaction of phosphorus with iron. The negative interaction effect of silicon with iron in equation (3) is illustrated in figure 2 (a,b,c,d) From the first three figures it appears that the maximum culture growth can be obtained when iron lies at its lower level and silicon at its higher level. An exceptional case illustrated in figure 2 (d), where the culture growth at high level of both iron and silicon, slightly exceeds that when iron t its lower level (-1) and silicon lies at its high level (+1). This is attributed to the fact that the summation of the positive interaction effect of both nitrogen with phosphorus and nitrogen, phosphorus with iron (equation 3) exceeds the negative interaction of iron with silicon.

During the first six days of experiments, culture growth was largely affected by the positive intereffect of phosphorus with iron. This intereffect was previously noticed by Fedorov, et al (1970) and abdallah, (1986), but till now it is not well discussed. Beginning with sixth day of growth till the tenth day, the positive intereffect of nitrogen with phosphorus was more pronounced. The simultaneous nitrogen and phosphorus addition on culture growth have been discussed by several authors. Gatham and Rhee (1981) considered that algal growth is a function of external and internal content of nitrogen and phosphorus. Increasing nitrate concentration in culture media, stimulates both nitrogen and phosphorus uptake by algal cells establishing different amounts of cell nitrogen and phosphorus needed for cell division

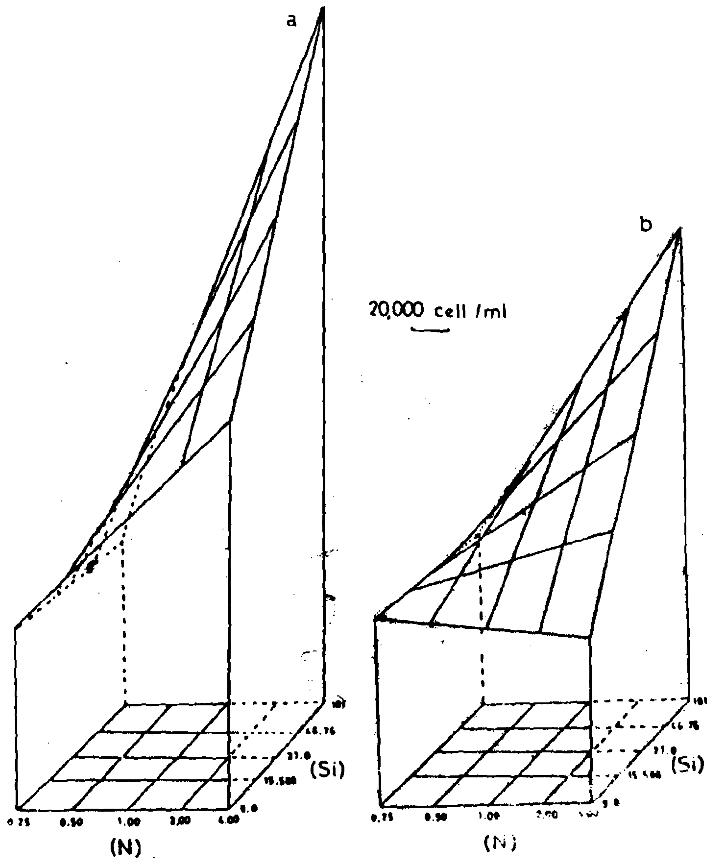

Fig. (1)

The relation between the culture growth of N. palea and the concentration of nitrogen and silicon at the sixth day.
(a) when P and Fe = (+1) = (0.9 mg P/l & 0.64 mg Fe/l)
(b) when P = (-1) and Fe = (+1) = (0.1 mg P/l & 0.64 mg Fe/l)

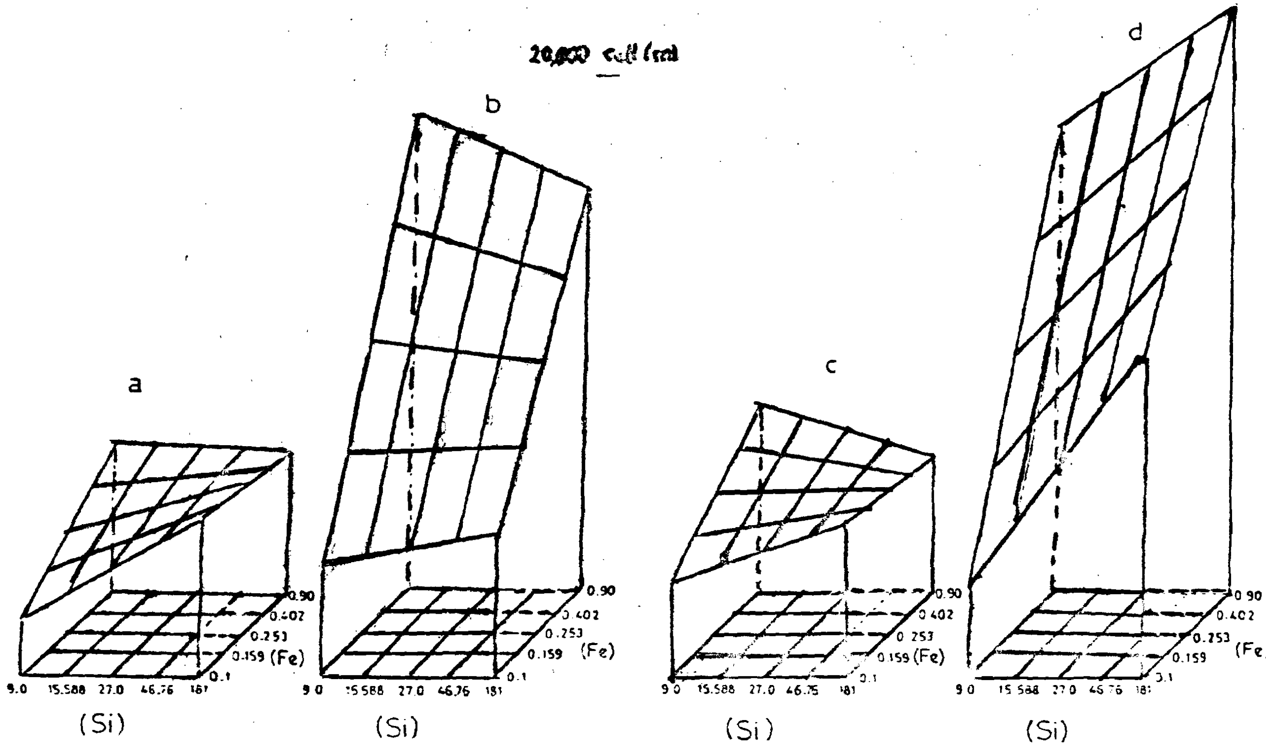

Fig. (2)

The relation between the culture growth of N. palea and the concentration of iron and silicon at the 6 th day.

(a) when N and P lie at (-1) = (0.25 mg N/l & 0.1 mg P/l).

(b) when N = (+1) = (4.0 mg/l) and P = (-1) = (0.1 mg/l).

(c) when N = (-1) = (0.25 mg/l) and P = (+1) = (0.9 mg/l).

(d) when N and P lie at (+1) = (4.0 mg N/l & 0.9 mg P/l).

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