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UTILIZATION OF THE PRINCIPAL TROPHIC ANIONS BY THE MARINE UNICELLULAR ALGAE AS A FUNCTION OF THE SPECIFIC ENVIRONMENTAL CONDITIONS

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ABSTRACT:

The results of the experiments on the utilization of trophic anions N ($-NO_3$,$-NO_2$) and P ($-PO_4$) by the unicellular algae *Cyclotella caspia* GRUN. and *Chaetoceros simplex* var. *calcitrans* PAULS. are presented in this paper. The dependence of the influx-efflux phenomena on the specific temperature of the synthesis process of the two organisms, as well as on the total salt gradient value in the medium is thought. The ability of the algae to take part in the processes of the nitrogen state modification is discussed, together with the form of nitrogen storage in the cells.

GENERALITIES

This study followed several directions, among which:

1. establishing of the modality of utilization of $N\text{-}NO_3^-$, $N\text{-}NO_2^-$ and $P\text{-}PO_4^-$ as a function of their concentration in the medium;
2. establishing of the possible interrelations between these nutrition factors;
3. observation on temperature influence on the trophic substrate utilization process;
4. deduction of ecological implications of nitrogen and phosphorus concentration increase in the natural environment.

It was experimentally proved that N and P utilization by the algae depends on a multitude of physical and chemical agents (3, 4, 6, 9, 13, etc.).

The mechanism of the utilization of these anions when concentrations are very low (between lo and loo µg/l) is less known, and so are the possible interrelations between the utilization rate of a substrate with such low levels and agents as temperature or total concentration gradient.

It is known that the nitrogen cycle in the upper water layer is determined by the activity of the bacteria of the genera *Nitrosomonas*, *Nitrosocystis* and *Nitrobacter*, requiring certain conditions of temperature and organic as well as inorganic chemical compounds concentration (1). At least for a sequence of this cycle, the algae have an important role, too.

VACCARO and RYTHER (15) observed that, by culturing the algae *Phaeodactylum tricornutum* BOHLIN. and *Chaetoceros* sp. in media containing $N\text{-}NO_3^-$ as the only source of nitrogen, a $N\text{-}NO_2^-$ accumulation occured. An active production of $N\text{-}NO_2^-$ from $N\text{-}NO_3^-$ takes place in the natural environment in the upper layer of the eutrophic zone.

It is a well known fact that $N\text{-}NO_2^-$ is an intermediary on the way of $N\text{-}NO_3^-$ toward cellular metabolism. WADA and HATORI (18) mentioned the important role the phytoplankton may have in transforming NO_3^- into NO_2^-. After reduction to nitrite, another photosynthetically reduction of the latter to NH_4^+ takes place (5). Nitrogen, under this form, is drown into the metabolic cycle.

Under certain circumstances, nitrogen may be storaged in the cells and reutilized when it lacks in the medium (11).

As regards $P\text{-}PO_4^-$, it was proved that the algae may utilize it excessively, storaging most of it as orthophosphate (4, 6). The reserves are also utilized when P disappears from the culture medium.

MATERIAL AND METHODS

The planktonic diatoms *Cyclotella caspia* and *Chaetoceros simplex* var. *calcitrans* were chosen taking into consideration the following: *Cyclotella* is one of the common representatives of the Black Sea phytoplankton, which is known well enough from the ecologic and physiologic point of view. *Chaetoceros simplex* var. *calcitrans* is one of the species the frequency of which has grown in the natural communities according to the growth of N and P content of the littoral waters, from several tens of µg/l to several hundreds of µg/l - as average values (7).

Utilization of nitrogen under the form of NO_3^- and NO_2^- was tested, as well as utilization of phosphorus under the form of PO_4^-.

In choosing the concentrations (Table 1), the very low concentration levels of these anions prior to the marine eutrophication phenomenon were considered (2, 16, 17), as well as the numerous occurances when the massive development of an alga (algae) causes the N and P decay, and in these conditions, still there are possibilities for other algae to develop. The control MS*) medium has a much higher concentration, representing the comparison term in the consideration of the trophic substrate utilization processes.

Table 1

$N\text{-}NO_3^-$, $N\text{-}NO_2^-$ and $P\text{-}PO_4^-$ concentrations (µg/l) in the tested variants of the medium

Nb. of combination	Experimented combinations					
	(1) $N\text{-}NO_3^-$ variable		(2) $N\text{-}NO_2^-$ variable		(3) $P\text{-}PO_4^-$ variable	
	$N\text{-}NO_3^-$	$P\text{-}PO_4^-$	$N\text{-}NO_2^-$	$P\text{-}PO_4^-$	$P\text{-}PO_4^-$	$N\text{-}NO_3^-$
1	10	75	10	75	10	83
2	50	75	30	75	30	83
3	70	75	50	75	50	83
4	83	75	70	75	75	83

Two lots were prepared from all the experimented variants; one was exposed to 6-8°C and the other, to 19-20°C. All variants had duplicates.

The culture medium was prepared with artificial**) sea

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*) $N\text{-}NO_3^-$: 14 x 10^3 µg/l; $P\text{-}PO_4^-$: 5.4 x 10^3 µg/l

**) After PORA and OROS (8), calculated for the Black Sea salinity water to which the nutrient salts enclosed in the Table 1 were added.

The algae Cyclotella and Chaetoceros in the log phase were separated by centrifugation from the medium in which they grew and were thoroughly washed with artificial sea water. The resuspension in the experimental media ensured an initial density of 4oo cells/ml.

During the observation period, the algal suspentions were continuously air- bubbled, being exposed to an illumination/darkness regime of 16/8 hours (2 x 10^3lx).

The division rate was controlled every day and so were the N-NO_3^-, N-NO_2^- and P-PO_4^- levels in the culture medium.

Cell counting was performed hemacymetricly, chemical dosage following standard methods: MULLIN and RILEY for nitrates, BENDSCHNEIDER and ROBINSON for nitrites, and MURPHY and RILEY for phosphorus (12).

RESULTS AND DISCUSSION

1. Cellular division

Cyclotella caspia

Testing the three trophic anions N-NO_3^-, NO_2^- and P-PO_4^- proved the existence of a proportional relation between their concentration and cellular division, regardless of the thermic regime to which the alga was exposed (Figs. 1-6).

Although with the two nitrogen forms the maximum cellular division value is reached after 48 hours, both lag phase aspect (Figs. 5, 6), and the maximum density reached show that Cyclotella makes better use of N-NO_3^-. This holds for both thermic domains experimented.

The exposure to the two temperatures still caused differentiated growth in Cyclotella, no matter the contents of the nutritive solution. At the temperature of 6-8°C, the growth expressed both the low division rate and the maintaining of the development ability for a long period in any of the studied variants. In the conditions of 19-20°C, the growth curves shown that after a very active division, the growth of the alga is limited by certain factors. The explanation appears to be logical: at temperatures of 19-20°C, the nutritive substrate absorption rate is higher from the very initial 24 hours of culture (Table 2).

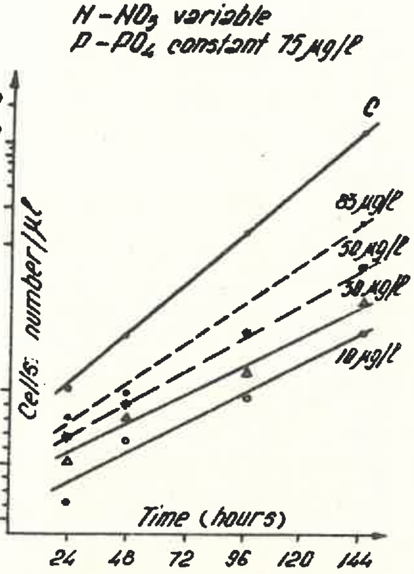

Fig. 1

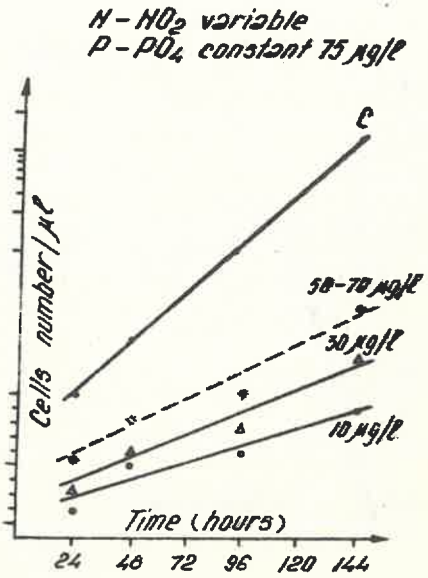

Fig. 2

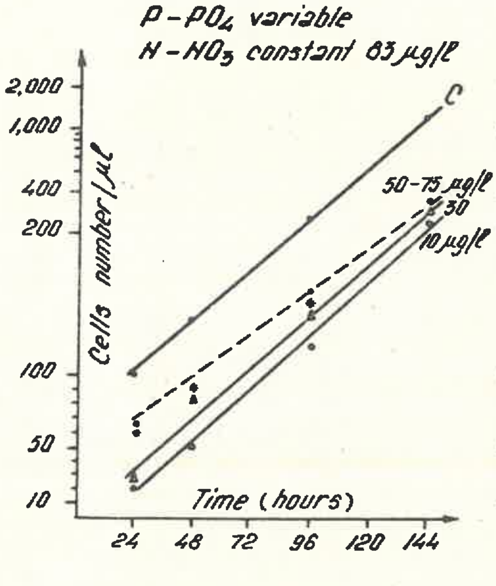

Fig. 3

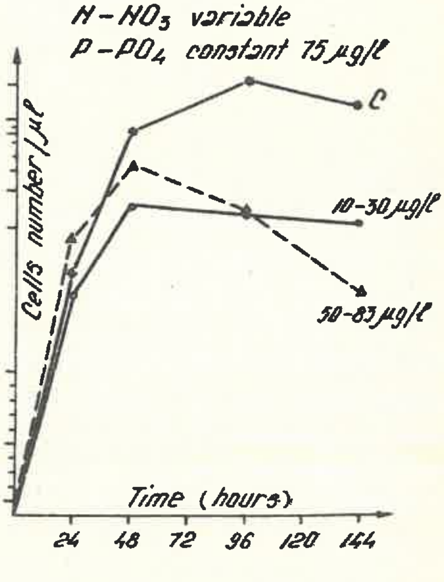

Fig. 4

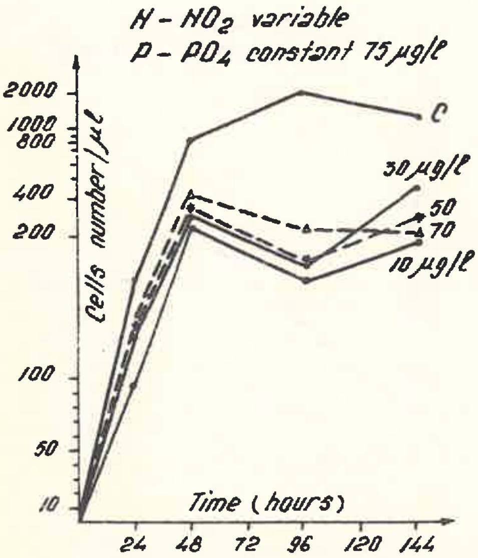

Fig. 5

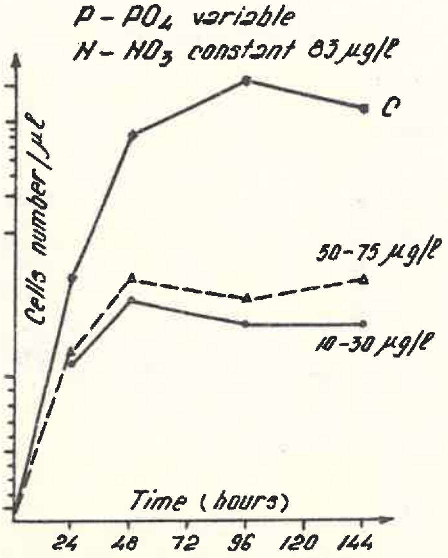

Fig. 6

Figs. 1-6 : The development of Cyclotella in different concentrations of N-NO_3^-, N-NO_2^- and P-PO_4^- as µg/l (Figs. 1-3: 6-8°C and Figs. 4-6: 19-20°C)

Having been absorbed, N and P are integrated in the metabolic and energetic cycles, which gives possibility of rapid cellular syntheses and consequently, of shortening the generating time. At 6-8°C temperatures,absorption takes place much slowlier (Table 2), neoformation of live organic substance is slow and generating time is dilated. Then again, even after a nearly complete absorption of the nutritive substrate, the low cellular metabolism causes very low division rate, which reflects the lengthy consumption of the intracellularly accumulated N and P.This explains the maintaining of the division availability for a longer time at 6-8°C.

Table 2

The dynamics of N utilization ($N-NO_3^-$, NO_2^-) by *Cyclotella* in different conditions of temperature (the days to which the most significant data correspond, are given)*

Experimented variant (µg/l)	The determined quantity of N (µg/l), thermic conditions and the day of observation							
	Temperature: 6-8°C				Temperature: 19-20°C			
	Initial	I	IV	VI	Initial	I	IV	VI
$N-NO_3^-$ variable								
10	10	9.38	1.05	0.59	10	0.53	0.65	0.71
50	50	6.20	1.08	0.79	50	3.74	0.59	0.68
70	70	8.84	1.16	0.79	70	0.93	0.59	0.62
83	83	7.71	1.13	0.74	83	4.74	0.82	0.51
$N-NO_2^-$ variable								
10	10	8.12	2.78	0.28	10	4.09	0.93	1.59
30	30	15.15	10.17	0.17	30	10.20	0.54	0.85
50	50	21.19	11.42	0.08	50	10.07	0.51	0.59
70	70	28.26	16.45	0.08	70	12.65	0.62	0.54
$P-PO_4^-$ variable								
10	83	42.02	11.59	0.79	83	3.37	0.68	0.62
30	83	23.79	1.08	0.56	83	13.92	0.68	0.63
50	83	34.94	12.98	0.98	83	9.30	0.88	1.05
75	83	30.09	19.31	1.42	83	9.01	0.68	1.35
Control: MS medium	14,000	64.13	87.92	7.36	14,000	53.95	161.68	251.10

* All determinations were performed on the nutritive solutions

Chaetoceros simplex var. *calcitrans*

The proportionality between the concentration of the three trophic anions and cellular division was manifest in this alga, too in both thermic regimes experimented (Figs. 7-12). The two nitrogen sources give different effects according to the temperature: $N-NO_3^-$ is better utilized at 6-8°C while $N-NO_2^-$ at 19-20°C.

When the temperature was 6-8°C, the growth curves had a hyperbolic aspect, indicating the cessation of the division process as a consequence of a very active log phase which begins from the moment of the cell suspending in the culture medium (Figs. 7-9).

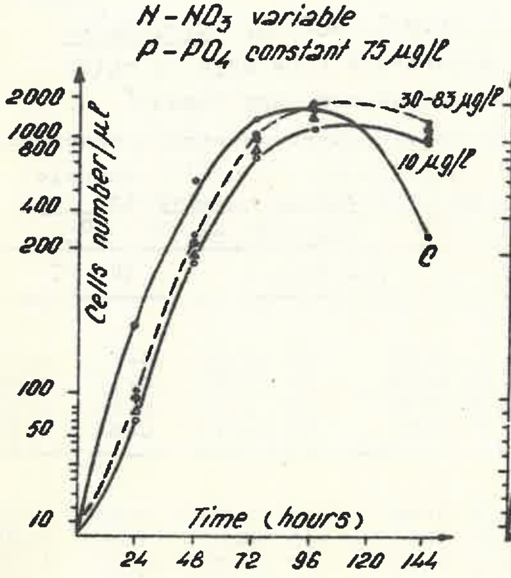

Fig. 7

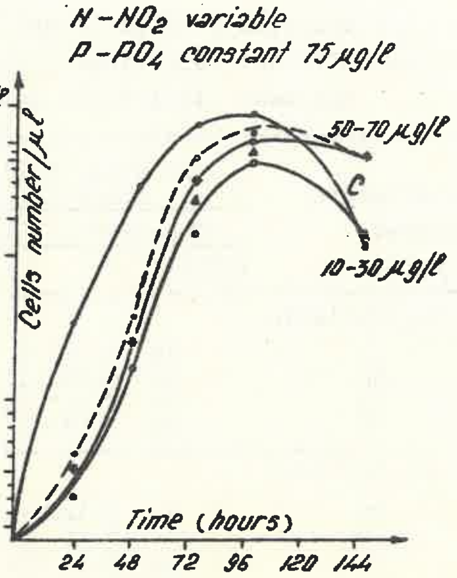

Fig. 8

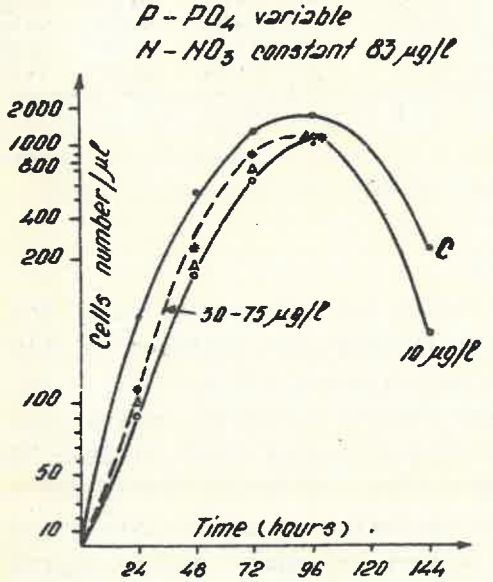

Fig. 9

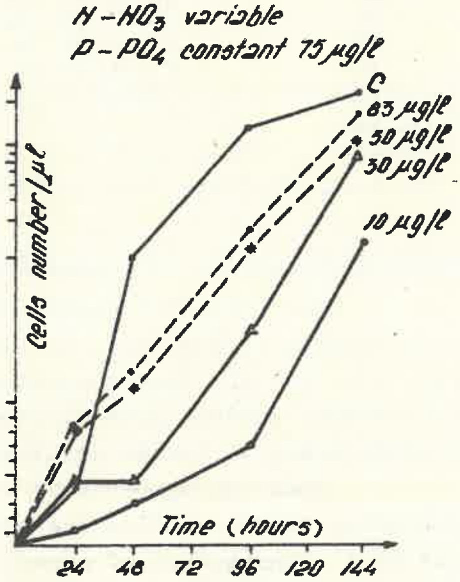

Fig. 10

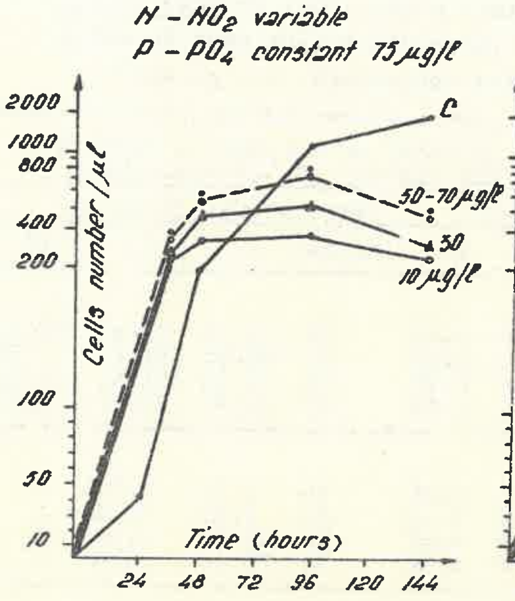

Fig. 11

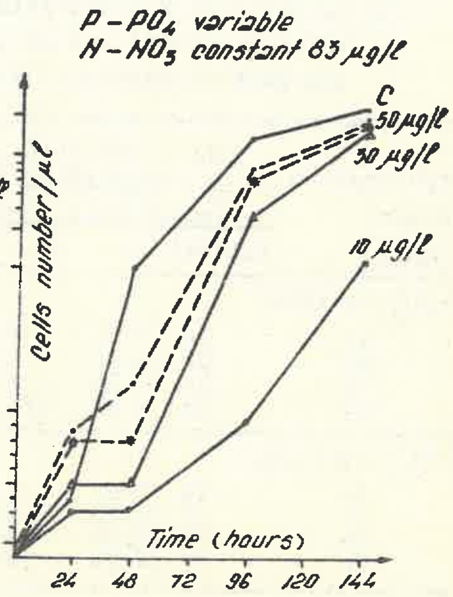

Fig. 12

Figs. 7 - 12: The development of <u>Chaetoceros</u> in different concentrations of $N\text{-}NO_3^-$, $N\text{-}NO_2^-$ and $P\text{-}PO_4^-$ as µg/l (Figs. 7-9: 6-8°C and Figs. 10-12: 19-20°C)

In the variants with $N\text{-}NO_3^-$ and $P\text{-}PO_4^-$, the 19-20°C temperature causes decrease in the division rate; the development tends to a linear aspect (Figs. 10-12) with lag phases of 24 to 48 hours. In the same thermic regime a clearly higher division rate as compared with the control was obtained in the presence of $N\text{-}NO_2^-$, even during the initial 24 hours; the lag phase characteristic of the all other variants exposed to this temperature was absent.

The behaviour differences of the algae in the two regimes of temperature are also evident in the trophic substrate utilization rate (Tables 2,3).

Table 3

The dynamics of N utilization ($N-NO_3^-$, NO_2^-) by <u>Chaetoceros</u> in different conditions of temperature (the days to which the most significant data correspond, are given)*

Experimented variant (µg/l)	The determined quantity of N (µg/l), thermic conditions and the day of observation							
	Temperature: 6-8°C				Temperature: 19-20°C			
	Initial	I	II	IV	Initial	I	II	IV
$N-NO_3^-$ variable								
10	10	8.65	0	0.76	10	79.29	79.97	82.18
50	50	4.33	0	0.82	50	59.20	81.82	0.56
70	70	14.50	0	0.76	70	82.18	81.42	10.98
83	83	4.57	1.88	0.88	83	81.88	79.57	9.31
$N-NO_2^-$ variable								
10	10	5.09	0	0.76	10	2.35	1.13	0
30	30	11.10	0	3.53	30	7.38	1.11	0
50	50	18.47	0	0.82	50	14.68	0.99	0
70	70	24.24	0	0.82	70	25.10	0.85	0
$P-PO_4^-$ variable								
10	83	64.56	6.60	1.30	83	82.90	86.46	70.37
30	83	25.63	1.17	1.05	83	82.90	86.80	0.85
50	83	24.28	2.89	0.99	83	71.57	71.57	0.48
75	83	24.99	3.53	1.02	83	79.20	67.42	0.57
Control: MS medium	14,000	72.91	92.24	87.28	14,000	146.11	165.97	195.23

* All determinations were performed on the nutritive solutions

2. Nutritive substrate utilization

Using the following formula, the influx and efflux rate of N and P anions was established in all the combinations of the experimented media in the two thermic regimes.

$$\Delta = \lg \frac{C_t / C(t - t_1)}{t - (t - t_1)}$$

where:

Δ = absorbed or excreted quantity of the considered trophic anion;

C_t = quantity of the considered anion in the medium at time t;

$t - (t - 1)$ = time in days or hours between two determinations.

The results showed that N and P utilization is strictly dependent on the concentration of these anions in the medium and their influx and efflux may be related to large time intervals.

The succession of the efflux - influx processes is determined by the anion concentration: as a rule for the tested variants, an efflux takes place first,after which the absorbtion of trophic elements begins, while in the control, where the concentration of the tested anions is much higher, an intense influx is produced, followed by a slighter efflux.

The particularities of this oscillating utilization are specific for each of the investigated algae and depend not only on the trophic anion concentration, but also on the temperature.

During the determinations on the culture medium, an alternation was found between the two forms of N, no matter the source of this anion.

Cyclotella caspia

Nitrate utilization

In all the experimented variants, the nitrate begins to fade away from the medium during the initial culturing hours (Figs. 13-16). The nitrate reduction to nitrite and the absorption of the latter has a much more active dynamics at 19-20°C. The oscillation periods of $N\text{-}NO_2^-$ generation and influx are much shorter at these temperature values as compared to the 6 - 8°C thermic regime.

$N\text{-}NO_3^-$ utilization seems to be independent on $P\text{-}PO_4^-$ concentration.

Nitrite utilization

When the N source was the nitrite, an extrusion of N, which was measured just from the initial 24 hours as both NO_2^- and NO_3^-, was noticed. The nitrate accumulation in the culture medium increases in direct proportion to the initial $N\text{-}NO_2^-$ concentration in the medium (Figs. 17,18). The nitrate disappears from the culture medium after 48 hours, except the variant in which the initial nitrite was in concentration of lo µg/l,and the exposure

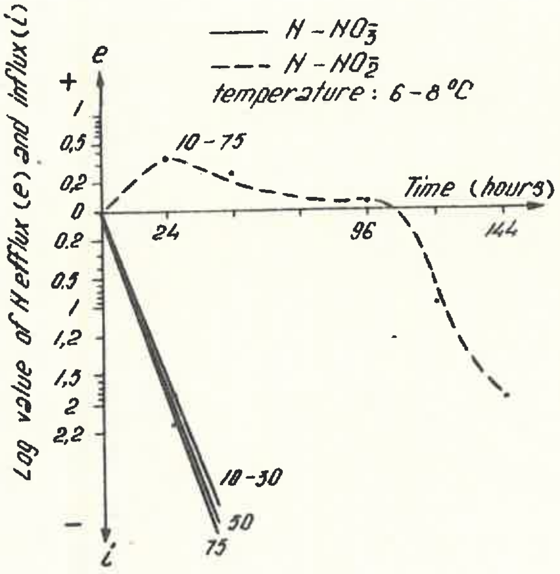
N - NO_3^-
N - NO_2^-
temperature : 6 - 8 °C
Log value of N efflux (e) and influx (i)
e
+
i
−
1
0,5
0,2
0
0.2
0.5
1
1,2
1,5
2
2
2,2
10 - 75
Time (hours)
24
96
144
10 - 30
50
75

Fig. 13

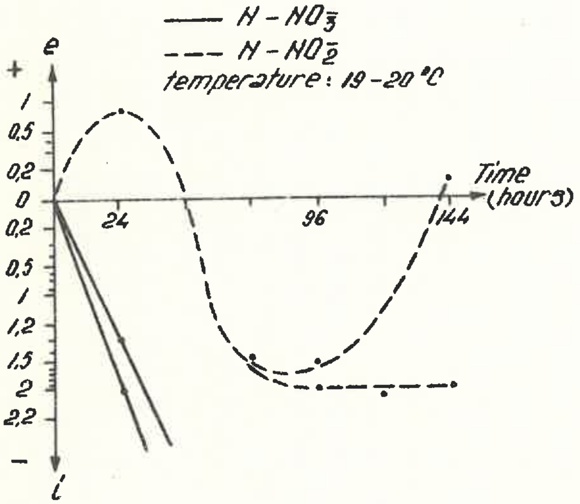
N - NO_3^-
N - NO_2^-
temperature : 19 - 20 °C
e
+
i
−
1
0,5
0,2
0
0,2
0,5
1
1,2
1,5
2
2,2
Time (hours)
24
96
144

Fig. 14

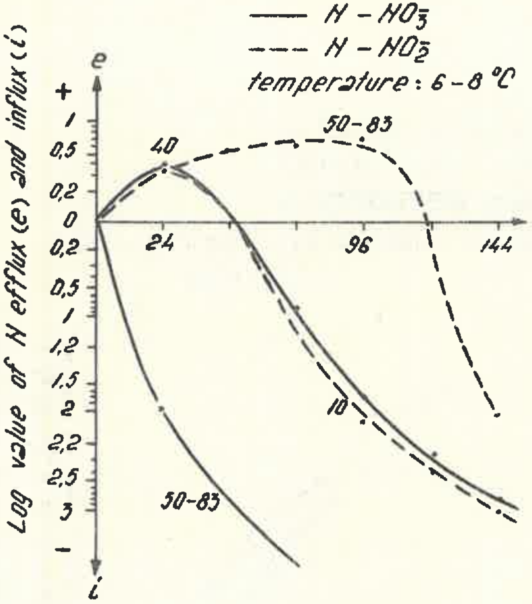

Fig. 15

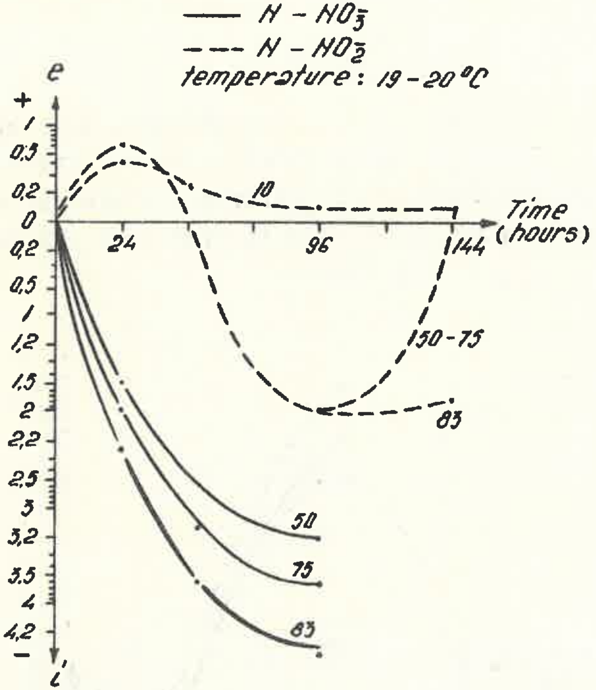

Fig. 16

Figs. 13-16: The dynamics of nitrogen and the ratio N-NO$_3^-$ / N-NO$_2^-$ influenced by *Cyclotella* growth, in different conditions of temperature and trophic anions concentration (Figs. 13,14: N-NO$_3^-$ constant 83 µg/l, P-PO$_4^-$ variable: lo-75 µg/l; Figs. 15,16: P-PO$_4^-$ constant 75 µg/l, N-NO$_3$ variable: lo-83 µg/l)

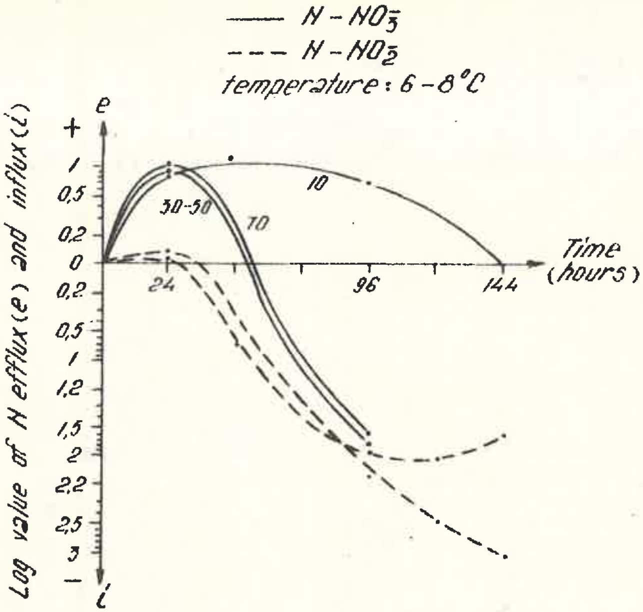

Fig. 17

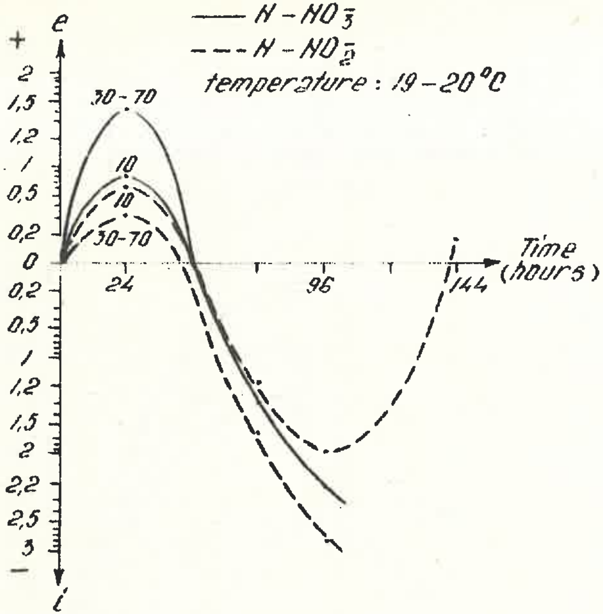

Fig. 18

Figs. 17, 18 - The dynamics of nitrogen and the ratio N-NO_3^- / N-NO_2^- influenced by *Cyclotella* growth in different conditions of temperature and trophic anions concentration (for both cases P-PO_4^- constant, 75 µg/l; N-NO_2^- variable : lo - 7o µg/l) temperature was 6-8°C. Here, the nitrate is preserved for a longer period, the influx being evident after 96 culturing hours. The influx rate is low in this case.

Phosphate utilisation

The efflux amplitude is in inverse ratio with P concentration in the medium, and the efflux period is much shorter at temperatures of 19-20°C (Figs. 19-24).

The influx rate increases according to the increase of phosphate concentration in the culture medium, reaching the highest values by exposing to 19-20°C.

No matter the temperature, the presence of $N\text{-}NO_2^-$ in the medium implies more rapid $P\text{-}PO_4^-$ absorption (Figs. 23,24).

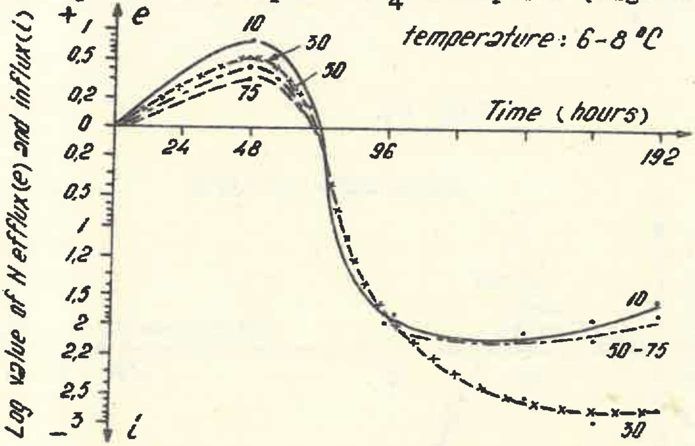

Fig. 19

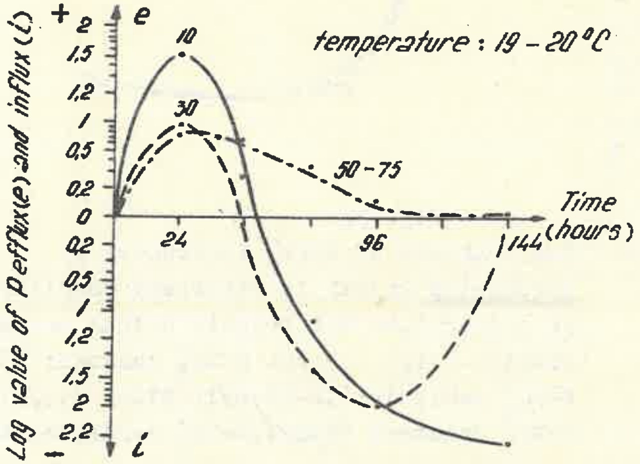

Fig. 20

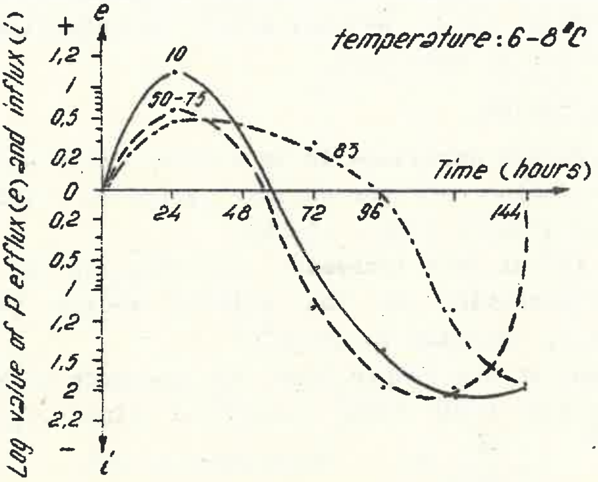

Fig. 21

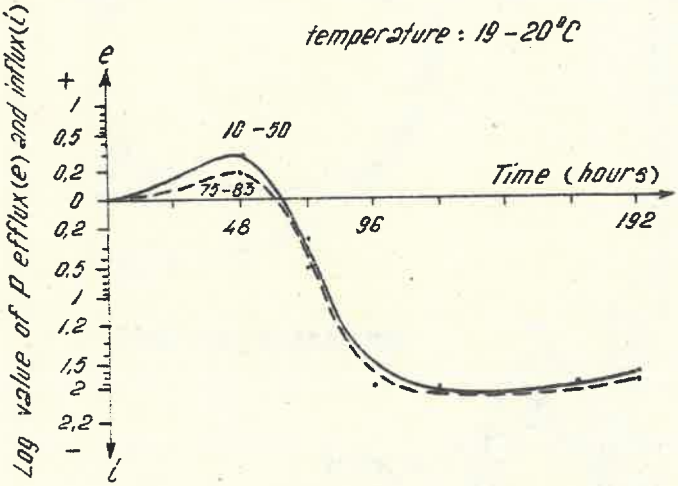

Fig. 22

Figs. 19-22 - The dynamics of P-PO_4^- influenced by Cyclotella growth in different conditions of temperature and trophic anions concentration (Figs. 19,2o: N-NO_3^- constant 83 µg/l, P-PO_4^- variable: lo-75 µg/l; Figs. 21,22: P-PO_4^- constant 75 µg/l, N-NO_3^- variable: 10-83 µg/l)

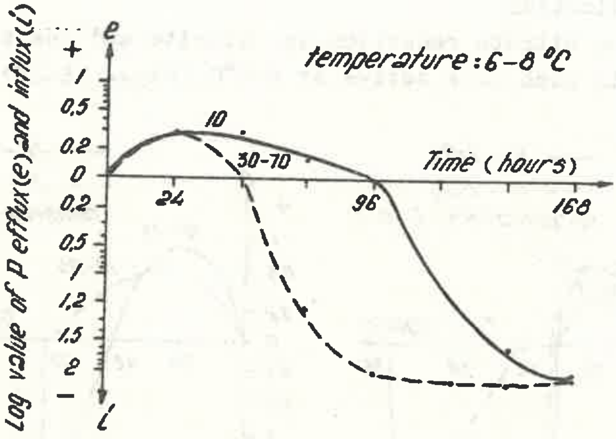

Fig. 23

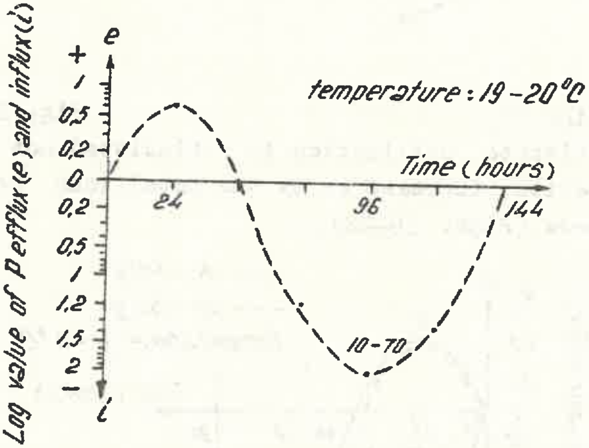

Fig. 24

Figs. 23, 24 - The dynamics of $P-PO_4^-$ influenced by *Cyclotella* growth in different conditions of temperature and trophic anions concentration ($P-PO_4^-$ constant 75 µg /l; $N-NO_2^-$ variable: lo - 7o µg/l)

Chaetoceros simplex var. calcitrans

Nitrate utilization

The nitrate reduction to nitrite and the absorption of the latter is much more active at 6-8°C (Figs. 25-28).

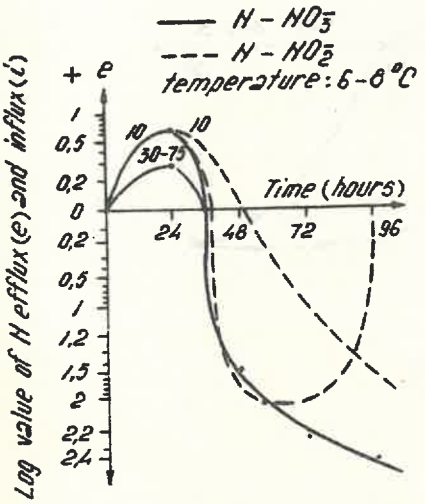

Fig. 25

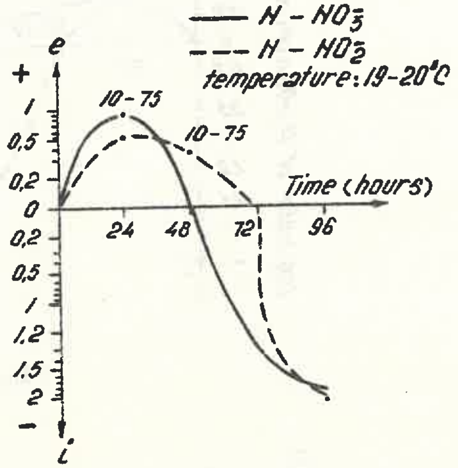

Fig. 26

The nitrate utilization is stimulated not so much by P-PO_4^- concentration increase as by the total ions concentration gradient increase (Figs. 25-28).

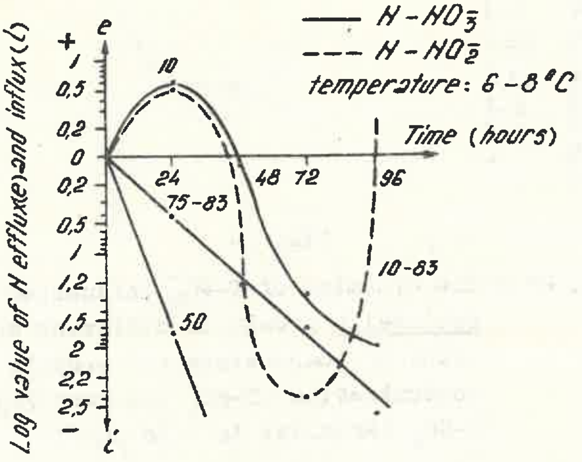

Fig. 27

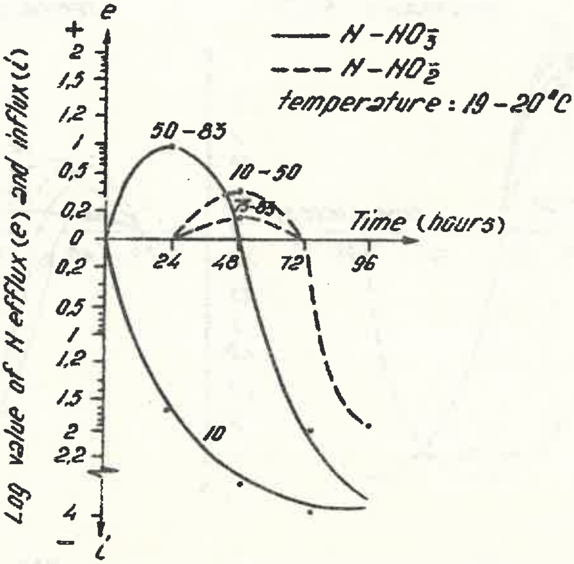

Fig. 28

Figs. 25-28 - The dynamics of nitrogen and the ratio $N\text{-}NO_3^-$ / $N\text{-}NO_2^-$ influenced by Chaetoceros growth, in different conditions of temperature and trophic anions concentration (Figs. 27,28: $N\text{-}NO_3^-$ constant 83 µg/l; $P\text{-}PO_4^-$ variable: 10-75 µg/l)

Nitrite utilization

In the 6-8°C thermic regime, the nitrite absorption as well as the nitrate accumulation in the medium is an direct proportion to the initial concentration of the nitrogen source (Fig.29). Exposure to 19-20°C produces a delay in the membrane transport processes; the nitrogen excretion, as well as the modifications of the two oxidized forms, begin only after 48 hours and show a much lower development rate (Fig.3o).

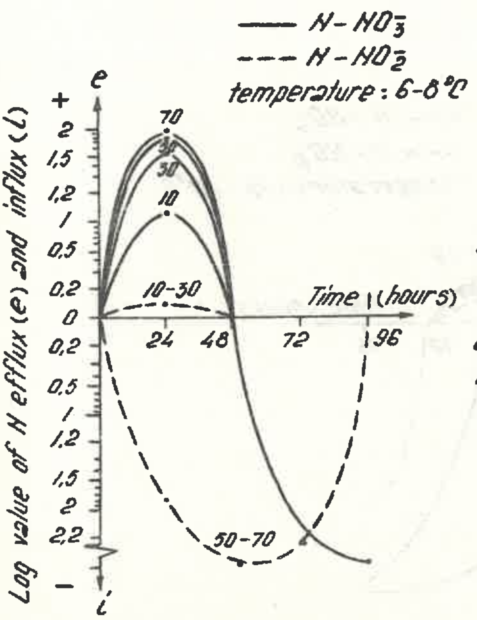

Fig. 29

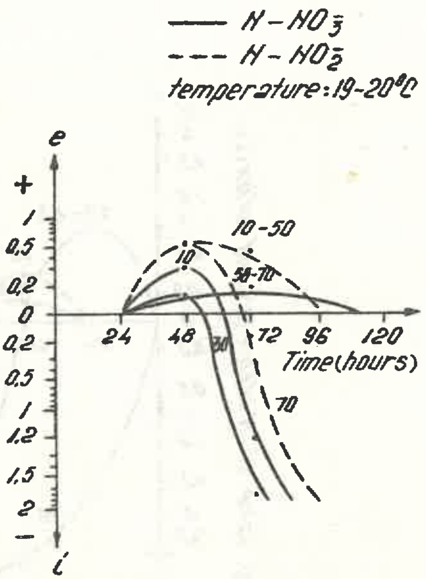

Fig. 3o

Figs. 29,3o - The dynamics of nitrite and the ratio N-NO$_3^-$ /N-NO$_2^-$ influenced by Chaetoceros growth in different conditions of temperature and trophic anions concentration (P-PO$_4^-$ constant 75 μg/l; N-NO$_2^-$ variable: lo-7o μg/l)

Phosphate utilization

The efflux value is in inverse proportion to the initial P-PO$_4$ concentration in the medium, while the influx is in direct proportion to the concentration increase of this anion in the medium (Figs. 31,32). The influx rate is optimized at 6-8°C.

* * *

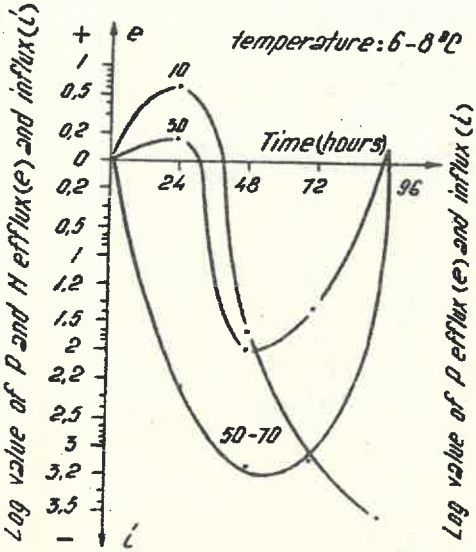

Fig. 31

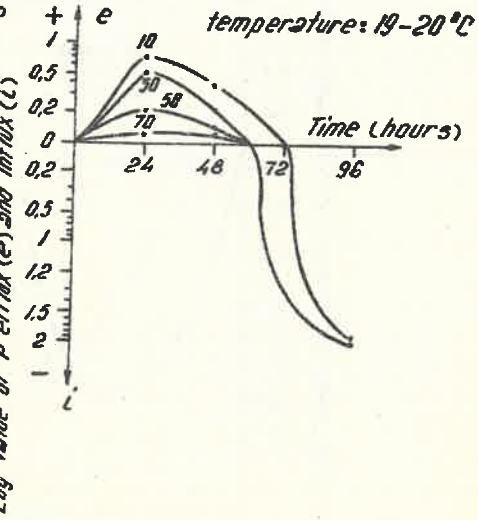

Fig. 32

Figs. 31, 32 - The dynamics of P-PO_4^- influenced by Chaetoceros growth, in different conditions of temperature and trophic anions concentration (N-NO_3^- constant 83 µg/l; P-PO_4^- variable: 10-70 µg/l)

The cultivation in the control solution where the concentration was much higher than that in the so far discussed variants (N-NO_3^- : 14×10^3 µg/l; P-PO_4^- : 5.4×10^3 µg/l), showed some differences regarding the particularities of the substrate utilization in both algae. An intense influx occurs, followed by a slight efflux after the saturation for both trophic anions (Figs. 33, 34 and 35,36). The modification of the ratio between efflux and influx is explaned only by the increase of the concentration gradient.

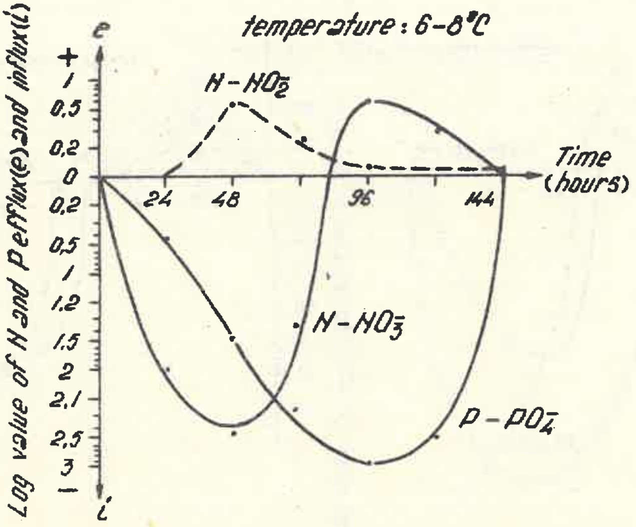

Fig. 33

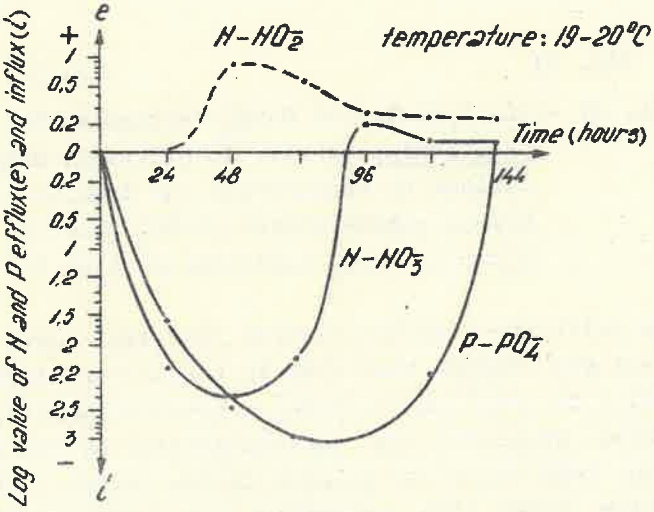

Fig. 34

Figs. 33,34 - The dynamics of nitrogen, the ratio N-NO$_3^-$/N-NO$_2^-$ and the P-PO$_4^-$ dynamics influenced by Cyclotella growth in different conditions of temperature, in control nutritive solution (N-NO$_3^-$: 14 x 10^3 μg/l; P-PO$_4^-$: 5.4 x 10^3 μg/l)

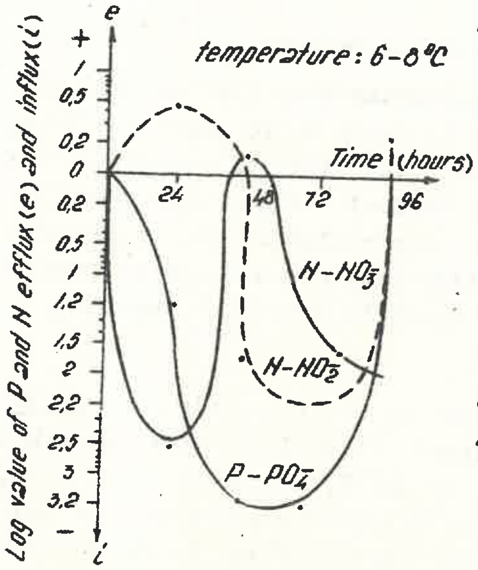

Fig. 35

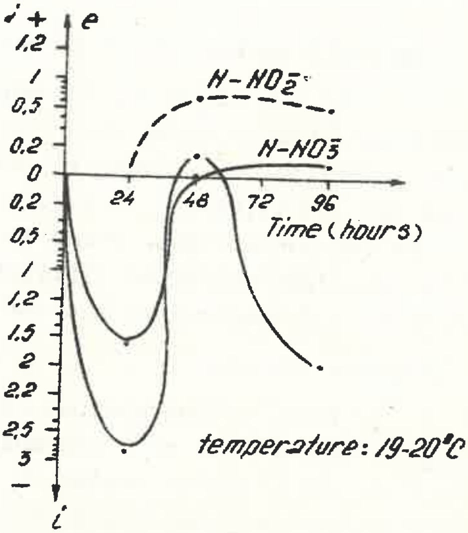

Fig. 36

Figs. 35,36 - The dynamics of nitrogen, the ratio $N-NO_3^-$/$N-NO_2^-$ and $P-PO_4^-$ dynamics influenced by *Chaetoceros* growth in different conditions of temperature, in control nutritive solution ($N-NO_3^-$: 14 x 10^3 µg/l; $P-PO_4^-$: 5.4 x 10^3 µg/l)

PRIBIL and MARVAN (9, 10) obtained activation of nitrogen consumption in *Scenedesmus quadricauda* (TURP.)BREB. in direct ratio to its concentration, when 1,000-3,000 mg N/l were added to the growing solution. In the same alga, $P-PO_4^-$ utilization is in direct ratio to the phosphorus concentration in the medium within the range of 3 - 300 mg P/l.

In the algae *Cyclotella* and *Chaetoceros*, a higher absorption rate is obtained at much lower N concentrations; but the P concentration to which such a high rate corresponds is confined to the limits cited by the authors above.

Another interesting peculiarity is in the fact that,for the concentration levels of N and P which were reached in the control, the utilization of these anions seems not to depend any more in a significant measure on the temperature value.

3. Interconditioning of the investigated factors

In the course of the experiments described so far (concentration limits: $N\text{-}NO_3^-$ 10-83 µg/l; $N\text{-}NO_2^-$ 10-70 µg/l and $P\text{-}PO_4^-$ 10-75 µg/l) it was observed that the division process of the algae was conditioned both by the chemical state of the trophic factors and temperature. There was also obviously the dependence of the nutrient substrate utilization rate on the concentration gradient, the temperature and the specific metabolic patterns.

a. Cellular division

$P\text{-}PO_4^-$ concentration seems to be limitative in *Cyclotella*, regardless of the temperature (Figs. 1-6). When there is a constant $N\text{-}NO_3^-$ concentration of 83 µg/l, a variation of $P\text{-}PO_4^-$ concentration between 10 and 75 µg/l induces stimulation of cellular division in proportion to the concentration increase. When $P\text{-}PO_4^-$ holds constantly 75 µg/l, high division rates are obtained even in variants 10-30 µg/l $N\text{-}NO_3^-$, $N\text{-}NO_2^-$.

At the same values of P or N concentration, the 19-20°C temperature favours a higher division rate as compared to that obtained at 6-8°C.

The growth of *Chaetoceros* in thermic regime 19-20°C seems to depend on both P and N concentration increase, while at 6-8°C this dependence is lost and the obtained division values are very close together and very close to the control values, no matter the study variant (Figs. 7-12). It may be assumed that under 19-20°C thermic regime the metabolic activity in *Chaetoceros* develops with a high energetic and plastic consumption; the increase in concentration of both trophic ions compensates the unicellular consumption,making cellular division be possible. The growth of the alga in the control solution, where N and P concentration satisfy the metabolic requirements, shows attenuation of temperature - dependence.

Under 6-8°C thermic regime, the efficiency of nutrient substrate utilization is higher; a good economy is observed in the utilization of the extracellular nutrient substrate and the intracellular storage as well.

b. Nutrient substrate utilization

The optimum thermic regime for mobilizing the studied trophic ions is different for the two of the algae: 19-20°C for Cyclotella and 6-8°C for Chaetoceros. Temperature is a determining factor for N and P utilization when these anions are at small concentrations. The higher the concentration of the essential nutritional anions, the much less the significance of this factor.

$N-NO_3^-$, $N-NO_2^-$ and $P-PO_4^-$ utilization in both algae depends on the increase in both N and P concentration gradient.

$P-PO_4^-$ efflux amplitude is in inverse ratio to its concentration in the medium, while the influx amplitude is in direct ratio to its concentration in the nutrient solution.

With Cyclotella, the presence of $N-NO_2^-$ in the medium induces more rapid absorption of $P-PO_4^-$.

c. $N-NO_3^-$ and $N-NO_2^-$ balance

Utilization of both nitrogen forms showed that accumulation of both $N-NO_2^-$, when $N-NO_3^-$ is the nitrogen source, and $N-NO_3^-$, when $N-NO_2^-$ is the only nitrogen source, is possible in the culture medium.

$N-NO_2^-$ accumulation in the culture medium of the algae which were grown on $N-NO_3^-$ is well known, as the algae reduce the nitrate to be able to integrate it into the metabolic cycle (11, 15, 18).

The $N-NO_3^-$ occurance in the culture medium when $N-NO_2^-$ was the only N source has a single explanation. As N concentration is critical in the medium, an extrusion of the intracellular N storage occurs. Up to now it was positively known that unicellular algae accumulate N as $N-NO_2^-$. Only one mention (11) was made on the possibility for seaweeds of the genera Porphyra to accumulate considerable quantities of $N-NO_3^-$. In our study Cyclotella and Chaetoceros proved also the unicellular algae to have this ability. It is the intracellular storage that are reutilized in case of deficit in the medium.

The problem of N and P ion efflux when their concentrations in the medium are from 10 to 100 µg/l constitutes one of the multiple features of the membrane transport phenomenon. The following reasoning may be accepted in the present instance: the mechanism of the nutritive anions integration along different metabolic path ways is not triggered before the moment when a balance occurs between the intracellular and extracellular trophic ion concentration. The need for ionic balance, which is necessary for the multiplication of algae, is quoted in the litterature. TOKUDA (14) found that the start of the cellular division in the alga Nitzschia closterium (EHR.) W.SM. is connected with the synthesis and excretion of glycolic acid into the medium. Division process is triggered only when an equilibrium occurs between extra- and intracellular concentrations of this compound. The author eliminates the lag phase by addition of glycolic acid to the nutritive solution.

4. Ecological implications

The algae Cyclotella and Chaetoceros can be found in natural communities all the year round. But massive developments occur only during certain seasons: in summer for Cyclotella and in early spring and late autumn, for Chaetoceros. Temperatures of 15-21°C are characteristic of the summer season, while spring and autumn are characterized by less than 10°C.

The nutrient level*) oscillates within the following limits: 65 - 205 μg/l P-PO_4^- and 64 - 143 μg/l N(NO_3^-, NO_2^-) in summer, 57 - 339 μg/l P-PO_4^- and 92 - 334 μg/l N(NO_3^-, NO_2^-) in autumn and 27 - 1,179 μg/l P-PO_4^- and 65 - 455 μg/l N(NO_3^-,NO_2^-) in spring. Thus, even when the concentration level of trophic anions is falling, if there is optimum temperature for their utilization, a massive development of the species Cyclotella is possible.

As our experiments have proved, Chaetoceros can grow when N and P have low concentrations, too; but, in the natural environment, a decrease in nutritive substances is followed by a temperature increase which is unfavourable to the processes of trophic substrate utilization, keeping a low division rate in the alga. In 1975, an excessive growth of the nutrients was recorded

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*) The data correspond to 1977, after the determinations made by Hydrology Laboratory of the Romanian Marine Research Institute

and as an effect of this, a massive development of *Chaetoceros* in summer, too. This confirms our observation: very high P and N concentrations diminish the significance of temperature.

These observations can be one of the explanations of *Cyclotella caspia* and *Chaetoceros simplex* v.*calcitrans* biological cycle. Twenty years ago, when the N and P mean level was up to 100 µg/l their biological cycle was tight correlated with temperature. When the N and P concentrations have increased the temperature dependence have diminished, beeing possible the *Cyclotella* and *Chaetoceros* presence, in the nearshore phytoplanktonic communities, all through the year.

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