Cercetări Marine	I.R.C.M.	Nr.10	225 - 242	1977

EFFECT OF PHENOL AND β-INDOLIL ACETIC ACID ON SOME UNICELLULAR ALGAE

Mihnea Pia Elena and Voinescu Iulia

Romanian Marine Research Institute - Constanța

ABSTRACT:

The work presents the result of some tests on the action of phenol and β-indolil acetic acid on a few unicellular algae, common in the Black Sea phytoplankton. The tested products change the membrane permeability for the trophic substratum, which causes modification in the rate of utilization of the medium and implicitely the variation of the generating time and of biomass of the studied algae.

In zones affected by domestic and industrial pollution, the increase in the quantity of mineral and organic substances entailed a significant increase in the phytoplanktonic biomass (10). Testing of the role of mineral substances showed that, in the majority of the observed algae, the obtained increments were lower than those produced by domestic waste waters (unpublished results). Consequently the organic componds in the waste water may be - among other things - that "factor" which is responsible for the massive growth of the unicellular algae (5; 11).

During its degradation, the organic matter liberates some compounds extremely variable in structure, most of which are slightly mineralized. But, there are some intermediary degradation

steps of the organic matter, marked by a particular resistance in time, such as phenol, scatol and indol.

There is a coincidence between the way of action of the "factors" existent in domestic waste waters on unicellular algae, and the quasi-permanent existence of indol, for example, in the mediums where anoxic decomposition of proteins occurs. This coincidence suggests that phenol and indol may be considered as some of the stimulating factors of the phytoplankton multiplication.

The way of action of these products is similar to that of some hormones (2; 11) and it is known about their effect that they modify the cellular division rate and - only in very few of the cases - the cells size in the sense of its growth (4).

The present study was aimed at: (1) pointing out the action of phenol and β - indolil acetic acid in concentrations comparable to those which may appear in the natural environment, and (2) making evident the way in which these products act.

MATERIAL AND METHODS

The algae making the object of this study were Cyclotella caspia Grun., Chaetoceros simplex var. calcitrans Pauls., Platymonas impellucida McLaughlan & Parke and Chlamydomonas sp. This selection had in view the following: Cyclotella caspia is a diatom most common to the phytoplanktonic populations, in both clean and polluted waters. The diatom Chaetoceros simplex var. calcitrans thrived in the coastal zone, reaching notable values only in the last ten years, when some modification occured as a direct consequence of the rising degree of sea.water pollution. This phenomenon entailed equally the development of some chlorophyceae, which determined the isolation of two green algae for the experiment: Platymonas and Chlamydomonas sp.

According to the references, when the cultures are young, the reactivity toward, the growth factors is stronger (3). In order to obtain evident responses from the aglae, we took into account this particular, inoculating our experimental variants with cells in the log phase.

Before the inoculation, the algae are minutely washed with fresh medium (MS) and separated by centrifugation.

The used medium has a simple structure, ensuring vigo-

rous growth of most of the algae, no matter the systematic group they belong to[x/].

The inoculum ensured an identical concentration to all the tested variants.

The culturing vessels are exposed at a luminous intensity of 2,000 lx, in a regime, L/D = 8/16 hours, with continuous air bubbling at the temperature of 19-20°C.

The concentrations of the two tested products were of o.1; o.5; 2; 6; 1o and 2o/g %o, taking a control vessel (only the MS medium) for each alga.

The number of cells/ml is estimated hemacytometrically and the dosing of $P\text{-}PO_4$ and $N\text{-}NO_3$ from the culturing medium was performed after GENOVESSE AND MAGAZZU (7).

The obtained results are related in percents to the control which are an expression of how much the response is larger or smaller as compared to the one obtained in the control solution.

The results which were obtaind during 48 hours' inoculation are analysed, as the tests with domestic waste waters indicated a very active log phase during the first three of four days of culture (Mihnea and Voinescu, in this volume). On the other hand, in the natural environment, the phenomena of intense multiplication also occur within a very short time interval.

While analysing the results, we considered the values surpassing the control by more than 1% as significant, owing to the large qantities in which the studied anions occured in the culturing medium.

RESULTS

1. Cyclotella caspia

a. Phenol effect. All phenol additions imply some increase in $P\text{-}PO_4$ absorption rate, exceeding the total quantity of absorbed phosphorus in the control solution by more than 2%. A good absorption is obtained by aditions of o,5 to 1o/g %o, when the phosphorus percent that was acumulated in the cells exceeds

x/ The MS medium /g%o: $N\text{-}NO_3$ 14,000; $P\text{-}PO_4$ 2,000; $Si\text{-}SiO_3$ 2,3oo; S 9oo; citric acid 6,000; Fe - traces.

the control variant percent by 6 to 10% and the maximum accumulation value occurs at 2 to 6 µg‰ of phenol (Fig. 1).

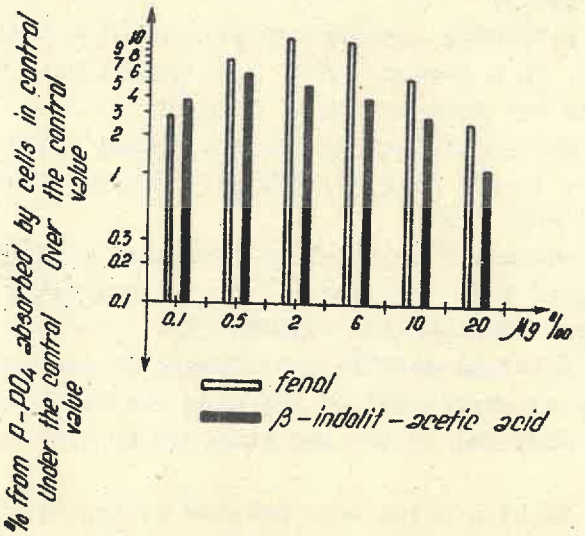

Fig. 1 - $P\text{-}PO_4$ absorption by Cyclotella cells in the presence of phenol and β - indolil acetic acid, as expressed in percents of the absorption determined in the control solution.

As concerns $N\text{-}NO_3$, a weak stimulation of absorption is obtaind at 0.1-0.5‰ concentrations. Above these values, a decrease in the absorptive power is found. The diminution of $N\text{-}NO_3$ utilisation reaches 0.3 to 1% of the value that was obtained in the control (Fig.2). Referring to the dependence of the absorption decrease on the phenol concentration, an inverse correlation was observed: increase in phenol addition implies decrease in the inhibitory process, so that at values of 20 ug ‰ phenol becomes again a weak stimulator of the $N\text{-}NO_3$ absorption process, i.e. the accumulation of this nutrient in the cells exceds the control by about 2%.

The cellular biomasses exceeded the one obtaind in the control solution, with a specification that this gain in growth is most significant within the range 2-20 µg ‰ of phenol. The ma-

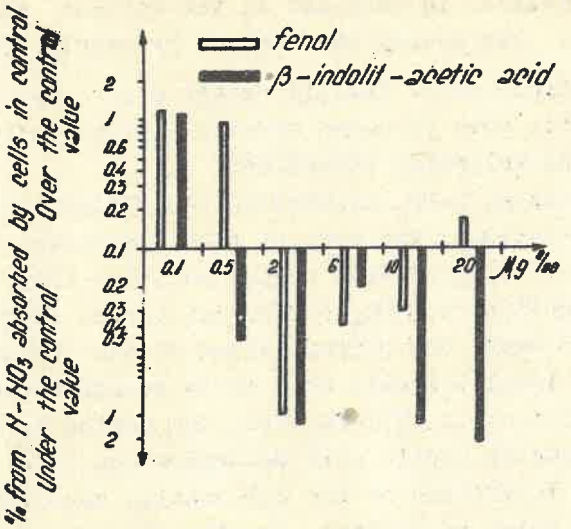

Fig.2 - $N\text{-}NO_3$ absorption by Cyclotella cells in the presence of phenol and β- indolil acetic acid, as expressed in percents of the absorption determined in the control solution.

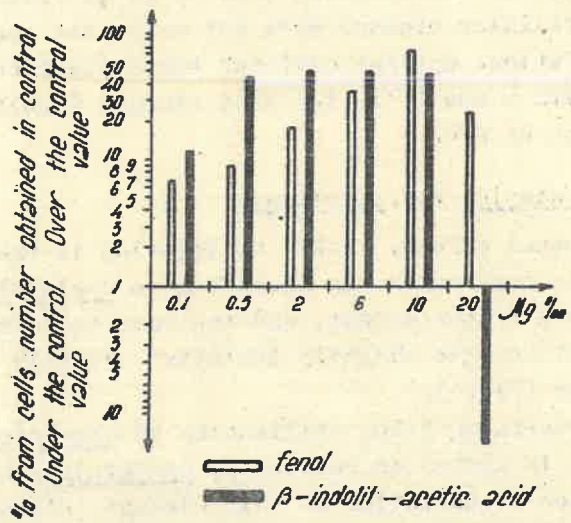

Fig.3 - Difference (%) between growths of the alga Cyclotella in the presence of phenol and of β- indolil acetic acid, as related to the value obtained in the control (MS), after 48 hours' culturing.

ximum biomass vallue is obtained in the variant with lo μg‰o of phenol (Fig.3), surpassing the control by nearly 7o%.

b. Effect of β- indolil acetic acid. The presence of β- indolil acetic acid produces effects similar to those given by phenol, with the following exceptions:

- although $P-PO_4$ absorption was stimulated for all the applied concentrations, the size of this absorption is slightly inferior to that obtained with phenol addition (Fig.1);

- significant $P-PO_4$ absorption occurs with additions between o.1 and 6 μg‰o, the maximum value of the process being obtained when β- indolil acetic acid is in concentrations of o.5μg‰o;

- utilisation of $N-NO_3$ from the medium is stimulated only when β- indolil acetic acid concentration is o.1‰; above this value, an inhibition of the utilization capacity of the discussed trophic anion is started, seeming that the inhibition depends on the value of insol concentration;

- multiplication of cells of Cyclotella is stimulated when addition of the considered substratum is done within o.1-lo μg‰o; the increase of the addition over lo ‰o is followed by a decrease in the cellular biomass with lo% below the one produced in the control solution. Optimum division takes place between o.5 and lo μg‰o, with a specification that biomass doubling occurs only by addition of 2‰o.

2. Chaetoceros simplex var. calcitrans

a. Phenol effect. $P-PO_4$ utilization in the phenol variants resembles that which was described in Cyclotella, except but the amplitude of the effect, and the fact that the addition of a quantity of 2o μg‰o slightly inhibits - in this case - its absorption power (Fig.4).

As concerning $N-NO_3$ utilization by Chaetoceros a clear-cut distinction is marked as related to Cyclotella: above 1‰o, phenol stimulates accumulation of this element (Fig.5).

Stimulation of cellular division is inversely proportional to phenol addition, being maintained above the control value. However the maximum tested concentration induced a slight decrease of multiplication, below the value that had been obtained in the control solution (Fig.6).

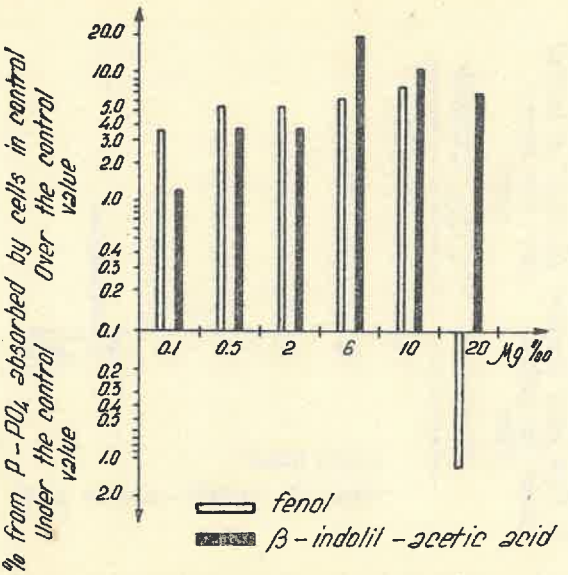

Fig.4 - P-PO_4 absorption by Chaetoceros cells in the presence of phenol and β- indolil acetic acid, as expressed in percents of the absorption determined in the control solution.

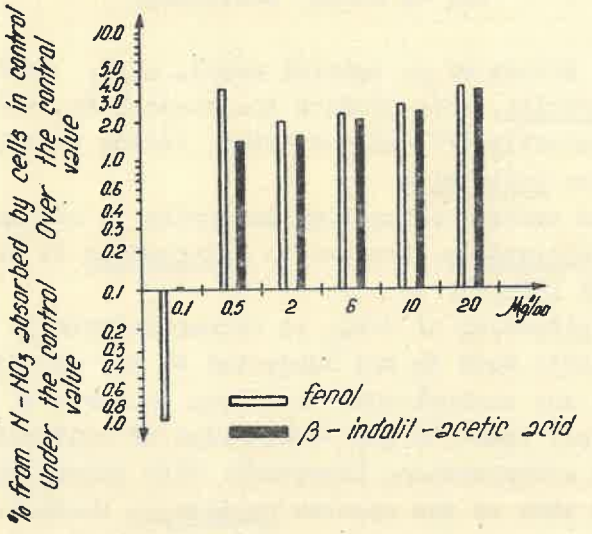

Fig.5 - N-NO_3 absorption by Chaetoceros cells in the presence of phenol β- indolil acetic acid, as expressed in percents of the absorption determined in the control solution.

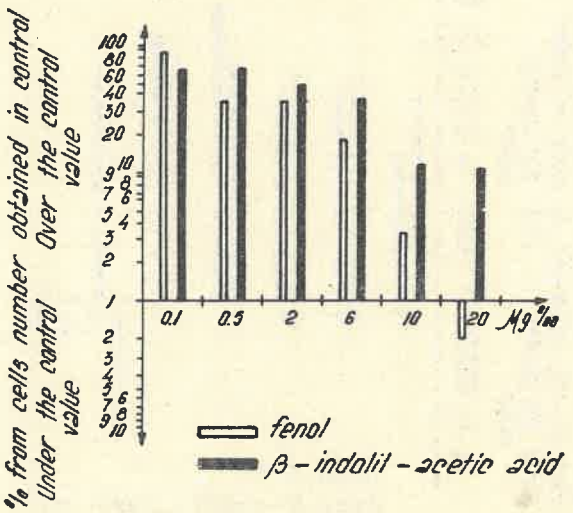

Fig.6 - Difference (%) between growths of the alga Chaetoceros in the presence of phenol and of β- indolil acetic acid, as related to the value obtained in the control (MS), after 48 hours' culturing.

b. Effect of β- indolil acetic acid. Just like in the species Cyclotella, this product increases P-PO_4 absorptive power at all the investigated concentration, taking values higher than in the species Cyclotella.

The maximum absorption intensity is obtained by addition o.5 μg‰ in Cyclotella, whereas in Chaetoceros it is produced by adding 6 μg‰ (Fig.4).

Utilisation of N-NO_3 at concentrations of o.1 μg‰ of β- indolil acetic acid is not subjected to any modification in comparison with the control concentration; increase of concentration in this product leads to the stimulation of utilization proportionally with the concentration increment. This physiologic behaviour differs from that of the species Cyclotella where it was shown that above o.5 μg‰, inhibition of N-NO_3 mobilization arose (Fig.5).

β- indolil acetic acid stimulates cellular division, the growth rate of the organism in question being inversely pro-

portional with the added quantity (Fig.6). Consequently, with some small differences, the two tested substances yield most similar efects.

3. Platymonas impellucida

a. Phenol effect. $P-PO_4$ absorption is stimulated by phenol concentrations ranging within o.1-1o μg‰. The process is optimized at values of o.1-6 μg‰ and reaches a maximum - over 1o% of the absorption in the control solution - when the addition is o.1 ‰. A phenol addition increment up to 2o‰ gives a limitation of absorption of which value is as much as 6% of the capacity of the Platymonas cells in the control variant (Fig.7).

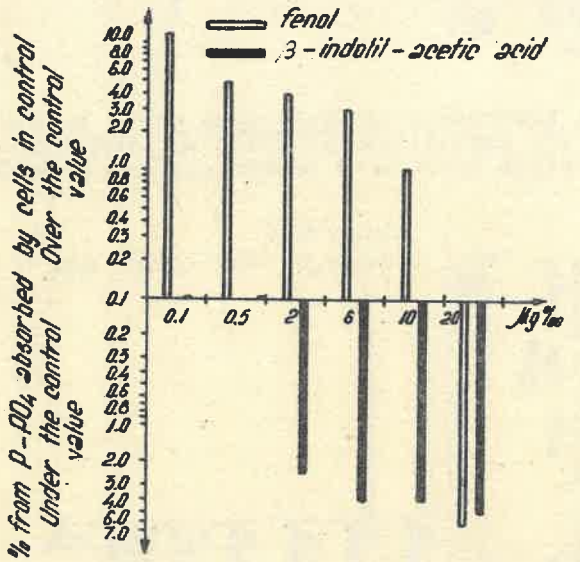

Fig.7 - $P-PO_4$ absorption by Platymonas cells in the presence of phenol and β - indolil acetic acid, as expressed in percents of the absorption determined in the control solution.

As regarding $N-NO_3$ utilization, it is disturbed by the presence of phenol, no matter the concentration in which it was tested.

However, the limitation of $N-NO_3$ utilization from the medium is of low amplitude, under 1% of that of the control for nearly all the variants. Exception is only made by 1‰ concentra-

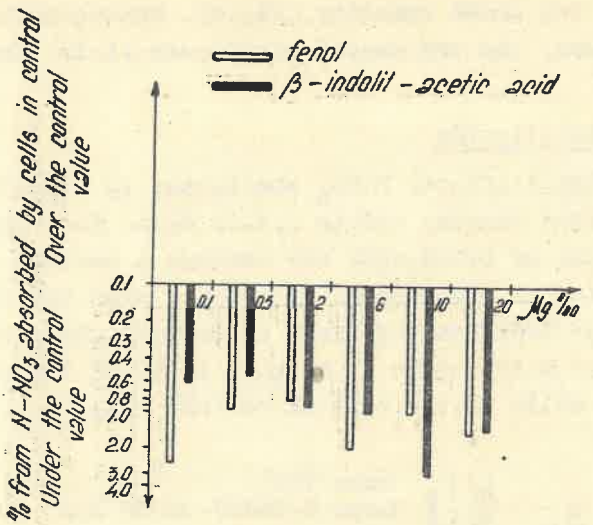

Fig.8 - $N\text{-}NO_3$ absorption by Platymonas cells in the presence of phenol and β- indolil acetic acid, as expressed in percents of the absorption determined in the control solution.

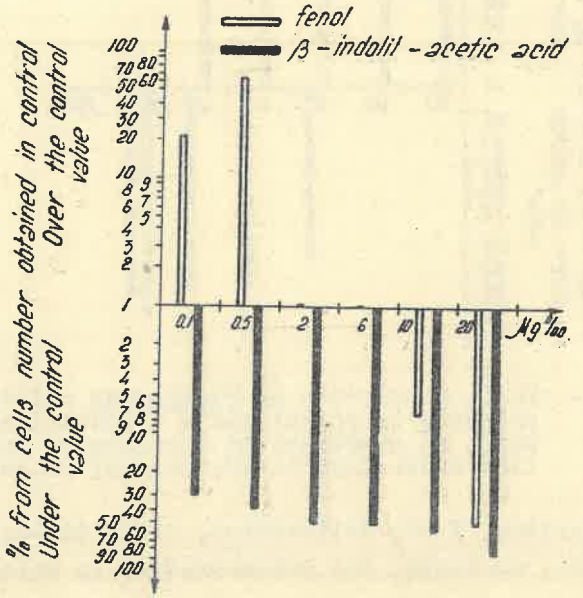

Fig.9 - Difference (%) between growths of the alga Platymonas in the presence of phenol and of β-indolil acetic acid, as related to the value obtained in the control (MS), after 48 hours' culturing.

tion,for which this slight inhibition becomes significant (Fig.8).

The multiplication process in the case of Platymonas is stimulated only at low phenol concentrations: o.1 and o.5 μg %o; values of 2 and 6 μg %o are neuter, while exceeding these concentrations implies inhibition of division, which results in algae biomass reduction by 7% and 5o% in comparison with the control, when the phenol is added in concentrations of 1o μg %o and 2o μg%o respectively (Fig.9).

b. Effect of β- indolil acetic acid. The experimentation of β- indolil acetic acid with Platymonas gave different effects from those of phenol.

$P-PO_4$ absorption is not influenced by concentrations ranging within o.1-o.5 μg %o; but the exceeding of these concentrations induces significant inhibition of the process of P utilization from the medium. A direct proportinality is also remarked between augmentation of β- indolil acetic acid and inhibation of $P-PO_4$ utilization.

$N-NO_3$ utilization in the presence of β- indolil acetic acid presents the same peculiarities as those mentioned for phenol, viz. absorption decrease is obtained, no matter the tested concentration (Fig.8).

As concerning cellular division, strong inhibition is noticed in the rate of this process as related to the one obtained in the control. The percents of division limitation are highly significant even beginning from concentrations of o.1 μg %o of β-indolil acetic acid (3o%) and are growing with the increase in the quantity of this product (Fig.9).

4. Chlamydomonas sp.

Of the four experimented algae, the strongest effect was obtained in Chlamydomonas sp.

The $P-PO_4$ quantity accumulated by absorption attains high percents as compared to that in the control for all tested variants (Fig.1o).

The greatest utilization value (more than 2o%) is obtained for concentration of 2 μg %o.

In contradistinction with the other species, not only the intensification of $P-PO_4$ utilization but also a slight stimu-

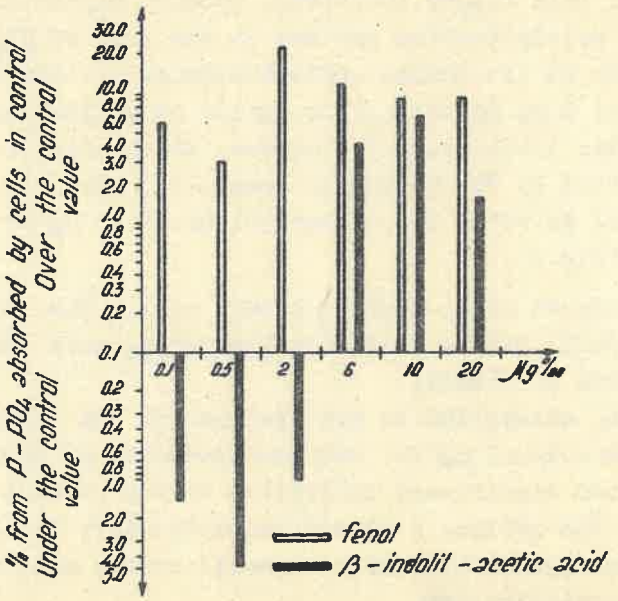

Fig.1o - P-PO_4 absorption by Chlamydomonas sp. cells in the presence of phenol and β- indolil acetic acid, as expressed in percents of absorption determined in the control solution.

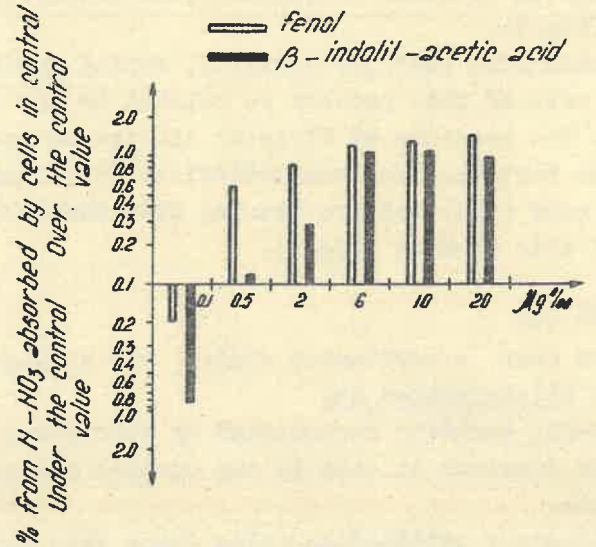

Fig.11 - N-NO_3 absorption by Chlamydomonas sp. cells in the presence of phenol and β- indolil acetic acid, as expressed in percents of the absorption determined in the control solution

lation of $N-NO_3$ utilization is observed (Fig.11).

β- indolil acetic acid concentration of o.1-2 μg %o slightly inhibit $P-PO_4$ absorption, whereas exceeding of these values increases the possibility of utilizing P from the medium. The effect obtained in case of $N-NO_3$ absorption is similar to that of phenol, with a specification that there were small differences only in the absorption amplitude (Fig.11).

As concerns the algae biomass too, the effect of the two tested products attains very high values. The cellular biomass increases by 2o to 242% as related to that obtained in the control solution with phenol and takes values of 21-73,7% in the presence of β- indolil acetic acid. We remark here a distinction between the effects of the two compounds: first, the phenol - induced cellular multiplication is about twice larger than that obtained by treating with β- indolil acetic acid. The phenol stimulates cellular division at all the stated concentrations, while the β- indolil acetic acid incites this process only at concentrations of o.1-6 μg %o; concentrations of 1o and 2o %o inhibit cellular division (Fig.12).

For analysing the average generating time (g), the following equations were used:

$$T.g = \frac{\log.2}{K_{1o}} \qquad (1)$$

where: T.g = generating time;
K_{1o} = constant of growth, h^{-1}

$$K_{1o} = \frac{\log.Nt/No}{t} \qquad (2)$$

where: Nt = number of cells at time of analysis;
No = initial number of cells;
t = number of hours passed when cell counting begins.

The average generating time is influenced in the sense of lengthening or shortening (Table 1) as the phenol or β- indolil acetic acid effect on cellular division was stimulatory or inhibitory. Shortening of generating time by 9 hours occured in Chlamydomonas sp. as corresponding to phenol addition in concentration of 1o μg %o. Generally, in Chlamydomonas are obtained the most significant reductions of the average generating time, while

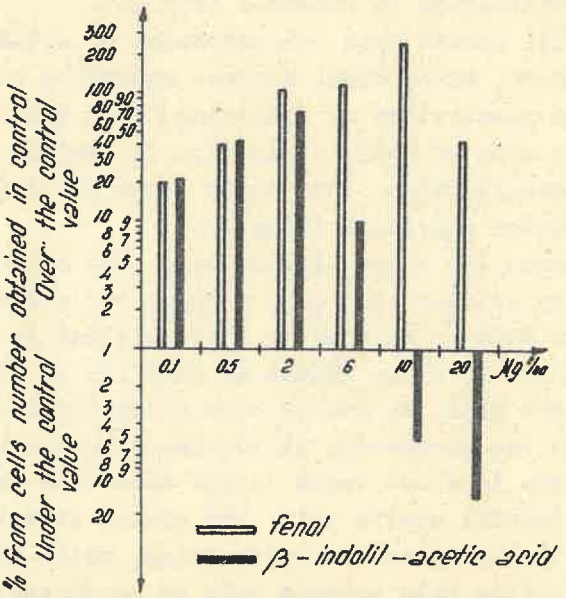

Fig. 12 - Difference (%) between growths of the alga Chlamydomonas sp. in the presence of phenol and of - indolil acetic acid, as related to the value obtained in the control (MS), after 48 hours' culturing.

in the other algae the reduction does not exceed 1-2 hours.

In Platymonas, the generating time is reduced by 1-2 hours in the presence of phenol (o.1 and o.5 µg %o) and it is lengthened both through addition of 1o and 2o µg%o of phenol and in all the β- indolil acetic acid variants. By addition 2o %o of β- indolil acetic acid, the generating time lengthens by 15 hours.

Supplementary samplings at short time intervals confirmed this modification of the average generating time, as the appearance of forms of sexual reproduction was not observed in any of the tested species, their multiplication occuring only as direct division.

Comparative analysis of the results outlines several characteristic aspects.

First, in three of the four algae, an increase, of $P-PO_4$ absorptive capacity is noticeable.

Table 1

Modification of generating time under the influence of phenol and β - indolil acetic acid

Variant	Tested species			
	Cyclotella c.	Chaetoceros s.v.c.	Platymonas i.	Chlamydomonas sp.
Phenol				
o.1	8	7	11	25
o.5	8	7	1o	18
2	8	7	12	15
6	7	7	12	15
1o	7	8	13	12
2o	8	8	17	18
Indol				
o.1	8	7	16	2o
o.5	7	7	16	18
2	7	7	16	16
6	7	7	17	2o
1o	7	8	16	21
2o	9	8	27	23
Control	8	8	12	21

It is known that there is a number of factors accounting for the excesive $P-PO_4$ utilization. The concentration gradient is one of them (6; 9). But the results are presented in relation to the values obtained in the control solutions with the same concentrations as in the variants. So, the concentration gradient cannot be implicated.

The pH (9) and the presence of organic substances can also explain an increase of phosphorus absorption.

But in our experiments the phenol and indol concentrations are too small and so, neither can this argument serve for founding the resulted effect.

The only possible way of interpretation is to consider the feature of a stimulating substance of both phenol and indol, besides the effect above mentioned.

FOGG (6) postulated the ability of the algae to synthe-

tize some enzyms irreversibly transfers P from polyphosphates to ATP whenever some processes requiring energetic overplus occur, as it is the case of protein or nucleic acid synthesis. As the polyphosphates are consumed, a new amount of P is uptaken from the medium. Consequently, as the metabolism is growing, an increase in P consumption can be expected.

Comparing the graphical representations of $P-PO_4$ consumption with those of cellular growths, one may find a confirmation of BENTLEY-MOWAT and REID's observations which assert that growth substances activate ADN of the cellular nucleus - an acid that controls the growth and raising of the cell.

In the algae Cyclotella, Chaetoceros and Chlamydomonas an increasing $P-PO_4$ absorption takes place, which is superposed on the activation cellular division and subsequently on the reduction of their generating time. Therefore it is certainly the matter of an influence of the tested substances at the level of the nucleic acid synthesis.

Platymonas has a reduced $P-PO_4$ absorption and a reduced cellular reproduction, so that both phenol and indol inhibit ADN synthesis during the experimental period.

It results that P is requested in large quantity for covering the energic necessities through ATP and ADN syntheses, the latter having as a consequence a rising of cellular division.

Another peculiarity that was noticed during these experiments consisted of the mode of $N-NO_3$ utilization. It must be specified that: (I) before starting the experiments, N and P starving circumstances were obviated and (II) the cultures utilized for the inoculation of the studied variants were in the log phase. Therefore, the increase or decrease in $N-NO_3$ consumption may indicate an enhance or a slowness in the processes of protein synthesis.

Concerning some algae, such as Chaetoceros and Chlamydomonas, the excessive $N-NO_3$ consumption proves the stimulation of the protein synthesis.

In Cyclotella $N-NO_3$ absorption shows an increase of protein synthesis only at very low phenol (o.1 and o.5 ‰) and β- indolil acetic acid (o.1‰) concentrations; exceeding these concentrations implies a decrease in $N-NO_3$ absorption and in correlation with this, in the protein synthesis rate.

Inhibition of $N\text{-}NO_3$ utilization for both studied products, at all concentration values, coincides in Platymonas with inhibition of division process and subsequently the protein synthesis is negatively affected, too.

CONCLUSIONS

1. Phenol and β - indolil acetic acid act in very small concentrations, determining either activation of metabolism, as in species Cyclotella, Chaetoceros and Chlamydomonas, or dominution of metabolism, as it was observed in Platymonas.

2. All the investigated processes prove that under the influence of phenol and β - indolil acetic acid a modification of cellular membrane permeability takes place, leading to increase or decrease of absorption capacity of the two trophic anions.

3. The excessive $P\text{-}PO_4$ absorption coinciding with cellular division in the algae Cyclotella, Chaetoceros and Chlamydomonas suggest the increase of ADN synthesis under the influence of tested products.

4. The alike increase of both $P\text{-}PO_4$ and $N\text{-}NO_3$ absorption in the algae Chaetoceros and Chlamydomonas proves that phenol and indol - in some definite concentrations - stimulate not only ADN synthesis but also protein synthesis processes.

5. Phenol and β - indolil acetic acid, in case when they are stimulatory, may have an effect on either ADN synthesis, or protein synthesis, or both processes.

6. Analysis of the multiplication process by calculation of the generating time too, proves once more the modifications caused by the two tested substances at the ADN level.

7. The variation of the number of cells occurs in all cases by modification of the generating time and not by appearance of sexual reproduction stage.

8. The effect of the two tested products differs as a function of species, being mostly an effect of stimulation of cellular division. In the natural environment, an increase of these products, resulted from the degradation of organic matter or from different industries, could cause chaotic multiplication in rather short time.

REFERENCES:

1. ANTIA N.J. and KALMAKOFF J., 1965 - Growth rates and cell yields from axenic mass culture of fourteen species of marine phytoplankters. Fish.Res.Bd.Canada - Manuscript Rep.Series (Oc. and Limnol): 1-23.

2. BENTLEY G.A., 1959 - Plant hormones in marine phytoplankton zooplankton and sea weeds: Reprints Int.Ocean.Congr.AAAS. 1: 910-1011.

3. BENTLEY-MOWAT J.A. and REID S.M., 1969 - Effect of gibberellins, kinetin and other factors on the growth of unicellular marine algae in culture. Bot.Mar. 12, 1-4: 185-193.

4. CONRAD H.M. and SALTMAN P., 1962 - Growth substances. In: Physiol. and Biochem. of algae (Ed.R.A.Lewin) Acad.Press N.Y., London: 663-669.

5. DUURSMA E.K., 1965 - The dissolved organic constituents of sea water. Chemical Ocean, London N.Y. Acad.Press., 1: 433-475.

6. FOGG G.E., 1973 - Phosphorus in primary aquatic plants. Water Res. Pergamon Press, 7: 77-91.

7. GENOVEZE S. and MAGAZU G., 1969 - Manuale d'analisi per le aque salmastre. Ed.Universit.Messina: 1-135.

8. JACOBI G., 1959 - Uber den Zusammenhang von Glykolsäure und lichtabhangiger Phosphorylerung. Planta, 53: 42-411.

9. KUHL A., 1962 - Inorganic phosphorus uptake and metabolism. In Physiol. and Biochem. of Algae (Ed.R.A.Lewin) Acad.Press N.Y. and London: 211-229.

10. MIHNEA P.E., 1978 - Effets produits par les eaux menagères sur les algues marines phytoplanctoniques. Revue Intern. d'Océan.Méd., 49: 89-98.

11. STIRN J., 1971 - Ecological consequences of marine pollution. Rev.Intern.d'Océan.Méd. 24: 13-46.