I.C.W.B.-C.I.P.S.

MATHEMATICAL MODEL OF
POLLUTION IN THE NORTH SEA

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# THE INFLUENCE OF EXTRACTS OF NORTH SEA SEDIMENTS ON THE GROWTH CURVE OF A MARINE UNICELLULAR ALGA *Dunaliella viridis* TEODORESCO

by

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# The influence of extracts of North Sea sediments on the growth curve of a marine unicellular alga Dunaliella viridis TEODORESCO.

## 1. Introduction

Last year we have carried out a series of culturing experiments with marine unicellular flagellated algae (PERSOONE and UYTTERSPROT, 1972), in order to evaluate the quality of North Sea waters. The goal was to try to detect the possible detrimental effect of polluting (growth inhibiting or toxic) substances, or the presence of growth stimulating nutrients (due to f.ex. eutrophication).

Growth curves of Monochrysis lutheri DROOP and Dunaliella viridis TEODORESCO in seawaters from samplings at the 25 main points of the Mathematical Model, have been compared to controls grown in artificial seawater.

Two series have been run :

a. the former with addition of the usual culture medium to detect if there was any inhibition ( by pollutants ) on"well-fed" algae;
b. the latter in the unknown seawater without any extra nutrient enrichment, in order to find out how much the growth stimulating effect of the nutritive minerals of the seawater was depressed or totally countered by the toxic action of pollutants.

From the results it was quite clear that the area of the North Sea in front of the Scheldt estuary, as well as the one just offshore the belgian coast gave much lower, and in many cases, negative results as compared to the waters of the open sea.

Although these types of experiments proved most valuable to assess the influence of pollutants present in the seawater at the very moment of sampling, it is quite clear that the

results can only be related to that particular water sample. It is not at all sure that the pollution load of the water masses just floating or passing by the next day would have been of the same order of magnitude.

So in order to characterize the degree of pollution of any aquatic biotope (in which there is a steady movement of the water masses) it would be necessary to repeat the experiments in time.

This is one of the main reasons why for fresh water biotopes, scientists have since a fairly long time emphasized the importance of analyses of the benthos as being a much more indicative part of the ecosystem for the detection of the presence or the effects of pollution.

Chemicals indeed tend to be concentrated in the sediments and their thorough analyses, as well from the biological as from from the chemical view-point are usually much more relevant than the analyses of the water.
Not only are the detected levels much higher in the former but, as the pollutants are very often leaching out quite slowly, one can still detect them (as well as one can still detect their detrimental effects on the benthic fauna and flora) a long time after the concentration of the toxicants in the water have dropped to an undetectable level.

Bearing this in mind we thought that it would be interesting to compare the results of our previous algal culturing experiments made on water, to those made this time with extracts of sediments.

The result normally should be quite relevant. Indeed, since a long time, algologists make extensive use of either biphasic media composed of soil and water, or of soil extracts : the so-called "Erdschreiber media" as food media for culturing algae. This procedure is based on the assumption that all the necessary elements for a good algal growth are usually present in soils.

As marine sediments are normally richer in nutrients than the water layer above (due to the bacterial break-down of organic matter during the normal food chain cycle) sediment extracts should be normally growth promoting. The presence of toxicants on the other hand should be reflected by a decrease in growth.

There are, however, several facts of which we have to be aware. First of all the different physical structures of the sediments at various places would certainly result in a different "leaching out" as well of the nutrients as of the pollutants. Secondly, it is well-known that inorganic pollutants tend to adsorb on particulate organic matter, and according to FROELICH et al. (1971) : "the highest concentrations of organic carbon are associated with the finest grain size sediments, probably as a function of grain size available for adsorption".

The method of extraction which would have reflected at best the natural "leaching out" of chemicals from sediments, would have been the extraction of identical volumes of sediment representing exactly the same surface area.
This was, however, impossible to realize in practice, since the samples taken with the grabs were mostly highly disturbed when arriving on deck.

So we finally decided to start the extraction not from samples with the same weight, or volume, but from samples containing exactly the same quantity of organic matter as criterion for the "adsorption capacity" of pollutants.

## 2. Materials and Methods

From sediment samples taken with a "Van Veen grab" at points 1 through 25, subsamples (about 0,5 liter in volume) were taken from the upper sediment layer and immediately deep-frozen in polyethylene bottles.
They were brought to the laboratory and kept in a deep-freezer at -16°C until further treatment. After thawing, small aliquots were taken to determine dry weight, ash weight and organic matter (as the difference of the latter 2).

The sediment extraction was carried out as follows : For each sample the exact quantity of wet weight sample, corresponding with (i.e. containing) 10 gr. of organic matter was calculated, weighed carefully and transferred to a bottle containing 1 liter of artificial seawater (prepared according to the formula of DIETRICH and KALLE 1963). The bottles were then placed on a "roller drive" for 8 hours at 23°C. After settling of the material in suspension, the liquid was drained off and filtered through a Millipore filter (0.45 micron).

The culturing experiments with algae were carried out exactly as described in our previous reports (PERSOONE and UYTTERSPROT, 1972).

Sediment samples of 100 ml extract were inoculated with a certain volume of algal cells of a stock in the exponential growth phase to obtain a starting concentration of 100.000 cells per ml.

Two series were run : one with addition of the usual culture medium, i.e. medium of VLASBLOM (for composition see PERSOONE and UYTTERSPROT, op. cit.), the second one in sediment extract without any addition.

All the experiments were carried out in two parallels. The controls were run on artificial seawater, either or not enriched with VLASBLOM culture medium.

The number of cells in each culturing tube was determined daily by taking 1 ml of the suspension, diluting it with an artificial seawater formaline mixture and counting it with an electronic particle counter. From both replications, only the best growth curve was retained for interpretation, since it is indicative for the best possible algal growth in that particular type of extract.

To mathematically characterize algal growth, many scientists rely either on the "growth rate" which can, however, only be computed from the logaritmic growth phase, or they take the number of algae present a certain number of days after the start of the experiment into consideration.

In our opinion both methods fail to reflect exactly the growth curve.
Indeed, the growth rate in the exponential phase can be very fast, but the latter phase can be delayed for a certain period by action of a pollutant. This will not appear from the computed "r" value.
The second manner does not give any indication whether the obtained value is situated in the exponential phase, plateau phase, or the decreasing phase of the curve.

In other words to really interpret how the algae have reacted on the unknown medium, the only way is to follow the growth until the "plateau" is attained.
In practice, however, there are two main objections :

a. growth experiments cannot be prolonged indefinitely (i.e. for weeks);
b. in order to compare the results obtained with different samples it is necessary to convert them to a summarizing figure.

We have tried to solve these difficulties by limiting the experiments to 5 days and by reducing the growth curve data to 2 numbers :

a. the integral of the curve as the expression of the "dynamics" of the growth (the integrals were calculated with a computer by means of a "BODE-formula of six points").
b. the number of algae obtained at the end of the experiment (the so-called 5th day value).

The comparison of these 2 figures relating to "unknown samples" to the homologs of the controls, gives a fairly good idea of the influence of the unknown medium on the algal growth.

For practical reasons we compute the ratios of these figures and consider the procentual value. If the latter is larger than 100, it means a growth stimulation as compared to the control; when smaller than 100 there was either a factor limiting the development of the culture or there was a toxicant present.

We usually diagram the value above 100 as indicative for growth stimulation, the one below 100 for growth inhibition.

Figure 1 is a schematic representation of the 9 most probable combinations which can occur :

1. This particular case where the 2 calculated ratios are exactly 100 (thus same integral and same 5th day value of both the unknown sample and the control) should normally reveal an identical growth in the unknown and in the blank.
   Two alternatives can exist but are highly improbable to occur :
   a) faster growth in the unknown sample at the start and slacking off at the end;
   b) slower start but faster growth in the unknown at the end of the culturing period.
2. Growth has been faster in the unknown sample but the number of cells after 5 days is the same.
3. The integrals of the growth curves are the same but the 5th day values indicate a faster growth in the unknown at the 5th day of culturing.
4. The final values are the same but the growth was slower in the sediment extract.
5. Although the integrals would wrongly indicate an analogous growth in both cases, the 5th day value shows a slacking off in the unknown towards the end of the experiment.

6-7. The block diagrams clearly indicate a growth stimulating, respectively growth inhibiting effect of the sediment extracts.

8. A shorter initial stationary phase with a subsequent faster growth in the exponential phase has led to a higher value of the integral. However, at the end of the experiment the plateau-value is lower than in the control.
9. A longer initial stationary phase resulting in a lower integral value; as the growth rate was, however, very fast in the exponential phase of growth, the 5th day value exceeds that of the control.

## 3. Results and discussion

In Fig. 2 we have diagrammed the procent organic matter (as ash-free dry weight) of the sediments sampled at the 25 sites of the Mathematical Model.

From these results it appears that the sediments of the coastal and offshore region South-West of the Scheldt estuary are much richer in organic matter than the other prospected sites.

We remind the reader that all the culturing experiments have been started from samples with a different volume but containing exactly the same amount of organic matter.

So no attempt shall be made to correlate the composition of the sediment with the growth of the algae.

The data of the daily algal counts and the computed integrals are given in Tables 1 and 2, for the series with respectively addition of extra culture medium and that without.

The ratios of the integral to the control and of the 5th day value to the control are given in Table 3.

Due to the breaking of the extraction bottle, tests on points M09 and M10 could not be carried out.

It will be seen from these tables that the experiments have been run in series of 6 (5 unknown samples and 1 control) with extracts taken at random from the 25 samples.

As the growth curves of the controls in the different series are somewhat different, (the 5 series could not be run simultaneously) the data obtained from each unknown sample were therefore only related to the control of their own series.

Comparing Table 1 and 2, it is clear that the addition of the usual culture medium to the sediment extracts has influenced the growth curves tremendously. In 5 days, the number of algae has increased a 100-fold, whereas on sediment extracts solely the increase is only a 10-fold.

Table 3 on the contrary clearly reveals the beneficial effect of sediment extracts as a food source for algae.

Indeed, in most of the samples cultured on the extracts as sole food source, growth was usually much better than in the

controls, not rarely reaching a 2-fold increase at the end of the experiment.

In the series with addition of usual culture medium, this growth stimulation was of course much lower to non-existing, since from the start there was no factor limiting the development of the algae.

Looking through the data, it appears that in the case of addition of culture medium, the influence of the sediment extracts on the ratios of the 5th day values (sample to control) for the whole area covered, ranges from 29 % growth inhibition to 26 % growth stimulation averaging 2 % growth stimulation.
This average is of course to be considered as no effect, if one takes the roughness of the methodology into consideration.

Looking at the ratios of the growth curves (integrals), the extremes are 34 % inhibition and 27 % stimulation, with an average of 2 % growth stimulation i.e. exactly the same figure as for the 5th day values.

In other words, if one considers the area covered by our experiments as a whole, it appears that the influence of sediment extracts on the growth of algae in "good" culture media averages 0, thus neither a growth stimulation, nor a growth inhibition.

If one plots these data on the maps (Fig. 3) some interesting "local" characteristics emerge. For example point M05 at the mouth of the Western Scheldt, is quite toxic since its sediments inhibit the growth of "well-fed" algae for about 30 %.

The comparison of the two zones : 16 through 20 and 21 through 25 reveals some curious facts.

In the first zone all the results show a slight growth-stimulating effect of the sediment extracts, decreasing from the vicinity of the coast towards the open sea, in the latter zone on the contrary they are mostly negative, and the algal growth decreases from the coast to offshore, reaching a 25 % growth reduction at point M25.

Let us now turn to the series run on sediment extracts solely.

From Table 3, it appears that the growth stimulation, respectively inhibition, based on 5th day values, ranges from 58% inhibition to 383 % stimulation, with an average of 65 % growth stimulation.

Considering the integrals, the highest inhibition was 38 %, the highest stimulation 233 % and the average growth stimulation 83 %.

From these results we can conclude that extracts of North Sea sediments definitely stimulate the growth of algae, and contain all the nutritive substances necessary for a good algal growth, exactly as the previously cited "Erdschreiber media" do.

Plotting the results as diagrams on the map (Fig. 4) reveals the following[1] : only in a few cases the final ratios are negative; the most obvious is point 5 where the growth inhibition averages about 50 % (58 % and 38 % for the two ratios respectively).

This result corroborates the one obtained for that particular point in the series with addition of extra culture medium where we found 30 % toxicity. On the other hand it also clearly demonstrates, once the more, that the influence of toxicants increases when the environmental conditions (including availability of food) become worse.

The results at the other points in front of the Scheldt estuary are relevant too, since the growth stimulations are all quite low when compared to those obtained elsewhere; the beneficial effect of the nutrients present in the extracts is thus countered by the presence of pollutants.

Point MO6 seems to be different to the adjacent sites, a finding which was already detected in the series with addition of culture medium.

The "good quality" of the sediments at points 16 through 20, mentioned in the series on well-fed algae, was confirmed in the present one too. We again could observe a positive gradient from the offshore to the coast.

[1] we should like to emphasize the different scales of Figs. 2 and 3 (5-fold difference).

For the transect 21 through 25, on the contrary, only site M24 points to a negative influence, while M22, M23 and M25 are definitely growth stimulating.

## 4. Conclusions

The capital role of sediments in aquatic ecosystems as nutrient traps or reservoirs is known since a long time.

More recently, the adsorption capacity of some sediments for many if not all types of pollutants has been emphasized.

The following question thus naturally arose : "what would be the resulting effect of a simultaneous 'leaching out' or release of minerals, necessary for the growth of primary producers and of pollutants adsorbed to the sediments?"

The preliminary experiments which we have run to find an answer to this very important question, are quite satisfying since they clearly showed the negative influence of the Scheldt on the adjacent area of the North Sea sediments.

So this new type of bioassays, as well as that previously carried out on the water itself (PERSOONE and UYTTERSPROT, 1972) seem to be worth to be considered as detection tools in the scope of pollution research in the marine environment.

Although it is clear that the methodology and the choice of the test organisms should be further improved, we should like to strongly support the opinion of AUBERT who since years claims that we are in an urgent need of bioassays in pollution research complementary to chemical analyses.

According to the latter author (AUBERT et al., 1970) : "l'imperfection majeure de l'étude des pollutions par l'analyse chimique réside dans le fait que si les résultats permettent de mesurer la dilution et la diffusion de l'agent chimique "in situ" ils n'apportent aucun renseignement sur la toxicité effective de ces corps, c.-à.-d. sur l'aspect biologique du problème et sur les conséquences effectives de cette pollution tant pour l'homme que pour les animaux et les végétaux marins".

## 5. Literature cited

AUBERT, M., J. AUBERT, B. DONNIER and M. BARELLI. 1972.

Utilisation de la chaîne trophodynamique dans l'étude de la toxicité des rejets d'eaux chimiquement polluées. p. 208 - 212
*In : Marine Pollution and Sea Life*
*Fishing News (Books) Ltd., London, 624 p.*

PERSOONE, G. and G. UYTTERSPROT. 1972.

Invloed van de kwaliteit van zeewater op de groeisnelheid van mariene eencellige wieren.
*I.C.W.B.-C.I.P.S. Mathematical model of pollution in the North Sea, Technical Report*
*Physiol.-Synthese 04*

Table 1. Growth curve data and integral for the series with addition of usual culture medium

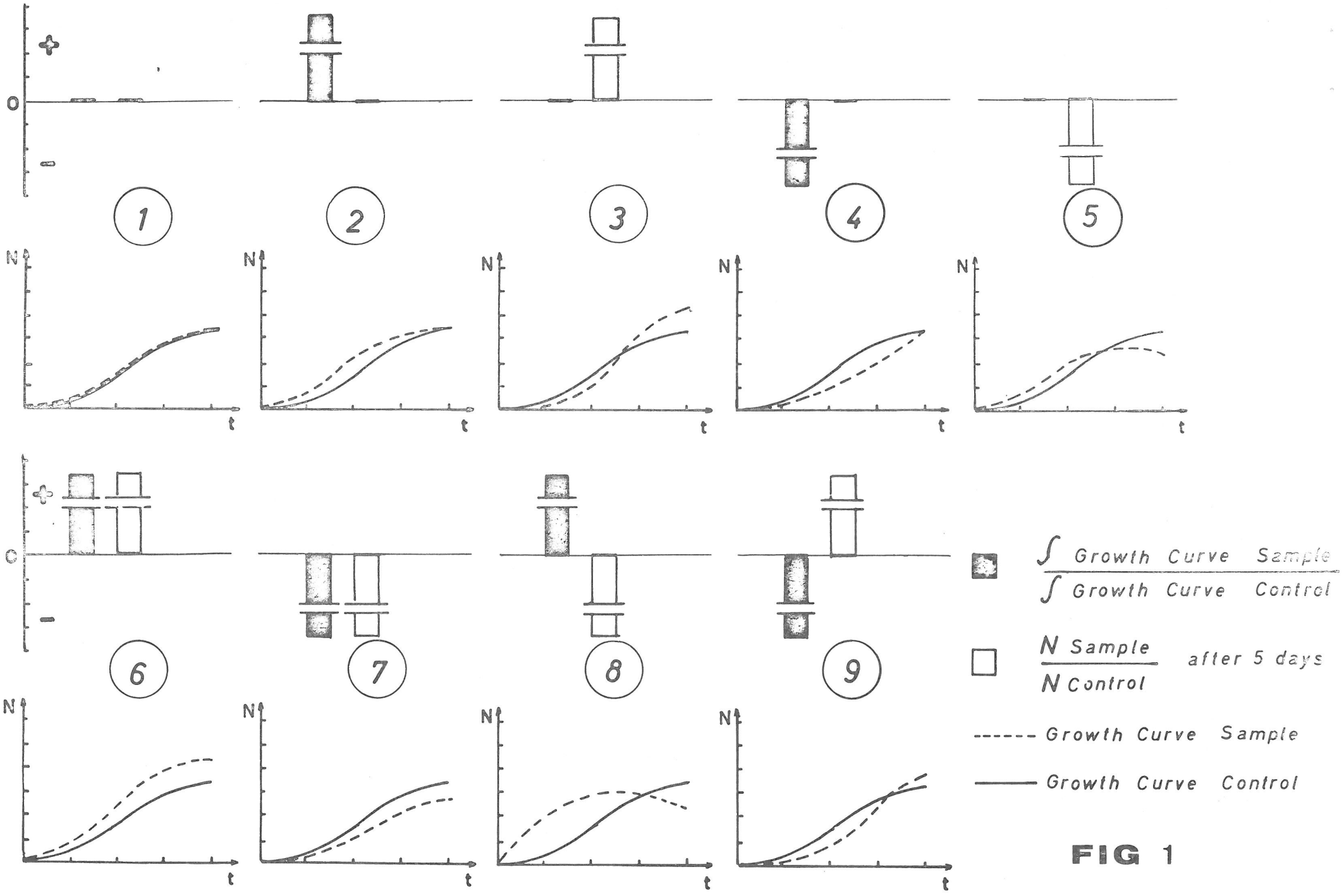
| Identity of sample | Concentration of algae in $10^6$ cells/ml | | | | | | Integral of growth curve |
|---|---|---|---|---|---|---|---|
| | Start | 1 day | 2 days | 3 days | 4 days | 5 days | |
| CONTROL 1 | 0,10 | 0,68 | 2,05 | 6,50 | 9,50 | 13,6 | 25,20 |
| M01280671 | 0,10 | 0,90 | 2,37 | 7,75 | 10,8 | 17,1 | 29,69 |
| M03010771 | 0,10 | 0,78 | 2,27 | 7,50 | 10,8 | 14,2 | 28,28 |
| M04290671 | 0,10 | 0,91 | 2,35 | 7,25 | 10,8 | 16,0 | 28,89 |
| M06 | 0,10 | 0,78 | 2,42 | 7,50 | 10,5 | 15,2 | 28,35 |
| M08050771 | 0,10 | 0,82 | 2,42 | 7,50 | 10,5 | 15,0 | 28,33 |
| CONTROL 2 | 0,10 | 0,58 | 2,40 | 5,62 | 10,2 | 12,7 | 25,22 |
| M02300671 | 0,10 | 0,57 | 2,56 | 5,25 | 8,20 | 12,0 | 22,19 |
| M05020771 | 0,10 | 0,63 | 2,40 | 3,25 | 6,00 | 9,00 | 16,54 |
| M07070172 | 0,10 | 0,57 | 2,59 | 5,62 | 9,80 | 13,7 | 25,18 |
| M11070771 | 0,10 | 0,58 | 2,62 | 5,62 | 9,65 | 13,0 | 24,79 |
| CONTROL 3 | 0,10 | 0,90 | 1,77 | 2,40 | 5,60 | 11,8 | 16,01 |
| M16160871 | 0,10 | 1,10 | 1,87 | 3,15 | 6,58 | 14,8 | 19,27 |
| M17170871 | 0,10 | 1,25 | 1,77 | 3,20 | 7,70 | 13,2 | 20,36 |
| M18180871 | 0,10 | 1,52 | 1,83 | 3,02 | 7,00 | 12,8 | 19,56 |
| M19150871 | 0,10 | 0,90 | 1,83 | 3,15 | 7,20 | 12,8 | 19,12 |
| CONTROL 4 | 0,10 | 0,63 | 2,44 | 4,51 | 10,4 | 12,5 | 24,55 |
| M12080771 | 0,10 | 0,66 | 2,68 | 5,30 | 10,0 | 11,2 | 24,40 |
| M13080771 | 0,10 | 0,80 | 2,90 | 5,90 | 10,4 | 12,5 | 26,38 |
| M14090771 | 0,10 | 0,66 | 2,75 | 5,60 | 10,5 | 13,2 | 26,17 |
| M20190871 | 0,10 | 0,72 | 2,98 | 5,60 | 10,2 | 13,2 | 26,05 |
| M21260871 | 0,10 | 0,60 | 2,76 | 5,10 | 9,30 | 12,0 | 23,70 |
| CONTROL 5 | 0,10 | 0,91 | 1,22 | 3,48 | 7,00 | 10,1 | 17,74 |
| M15090771 | 0,10 | 0,62 | 0,65 | 2,88 | 5,80 | 8,80 | 14,36 |
| M22250871 | 0,10 | 0,52 | 0,84 | 3,34 | 6,80 | 10,5 | 16,66 |
| M23250871 | 0,10 | 0,60 | 0,78 | 3,00 | 6,30 | 9,30 | 15,37 |
| M25240871 | 0,10 | 0,60 | 0,82 | 2,56 | 5,40 | 7,70 | 13,32 |

Table 2. Growth curve data and integral for the series on sediment extracts solely

| Identity of sample | Concentration of algae in $10^6$ cells/ml | | | | | | Integral of growth curve |
|---|---|---|---|---|---|---|---|
| | Start | 1 day | 2 days | 3 days | 4 days | 5 days | |
| CONTROL 1 | 0,10 | 0,32 | 0,38 | 0,42 | 0,50 | 0,88 | 2,09 |
| M01280671 | 0,10 | 0,98 | 1,18 | 1,54 | 1,74 | 2,12 | 6,62 |
| M03010771 | 0,10 | 0,53 | 0,41 | 0,44 | 0,58 | 1,12 | 2,59 |
| M04290671 | 0,10 | 0,62 | 0,31 | 0,46 | 0,60 | 1,12 | 2,66 |
| M06 | 0,10 | 0,60 | 0,69 | 0,82 | 0,88 | 1,38 | 3,73 |
| M08050771 | 0,10 | 0,66 | 0,80 | 1,26 | 1,42 | 1,88 | 5,15 |
| CONTROL 2 | 0,10 | 0,50 | 0,66 | 0,83 | 0,90 | 0,86 | 3,43 |
| M02300671 | 0,10 | 0,75 | 1,26 | 1,45 | 1,70 | 1,70 | 6,14 |
| M05020771 | 0,10 | 0,24 | 0,44 | 0,50 | 0,66 | 0,36 | 2,14 |
| M07070172 | 0,10 | 0,39 | 0,62 | 1,05 | 0,99 | 0,76 | 3,53 |
| M11070771 | 0,10 | 0,61 | 1,20 | 1,30 | 1,38 | 0,90 | 5,09 |
| M24240871 | 0,10 | 0,44 | 0,61 | 0,85 | 0,97 | 0,66 | 3,35 |
| CONTROL 3 | 0,10 | 0,45 | 0,73 | 1,35 | 1,32 | 1,50 | 4,64 |
| M16160871 | 0,10 | 0,65 | 1,50 | 2,70 | 4,05 | 7,25 | 12,19 |
| M17170871 | 0,10 | 0,55 | 1,16 | 1,57 | 2,58 | 3,12 | 7,51 |
| M18180871 | 0,10 | 0,55 | 1,16 | 1,42 | 2,58 | 2,62 | 7,21 |
| M19150871 | 0,10 | 0,55 | 1,11 | 1,35 | 2,42 | 3,12 | 7,06 |
| CONTROL 4 | 0,10 | 0,37 | 0,66 | 0,82 | 0,95 | 0,88 | 3,33 |
| M12080771 | 0,10 | 0,51 | 0,96 | 1,05 | 1,01 | 0,97 | 4,08 |
| M13080771 | 0,10 | 0,66 | 2,20 | 2,50 | 1,10 | 1,05 | 6,75 |
| M14090771 | 0,10 | 0,51 | 1,85 | 2,08 | 1,11 | 1,05 | 5,90 |
| M20190871 | 0,10 | 0,69 | 1,75 | 2,08 | 1,34 | 1,00 | 6,33 |
| M21260871 | 0,10 | 0,57 | 1,30 | 1,55 | 1,16 | 0,94 | 5,07 |
| CONTROL 5 | 0,10 | 0,25 | 0,28 | 0,31 | 0,34 | 0,64 | 1,52 |
| M15090771 | 0,10 | 0,57 | 0,62 | 1,08 | 1,20 | 1,14 | 4,19 |
| M22250871 | 0,10 | 0,75 | 0,57 | 0,98 | 1,10 | 1,05 | 4,13 |
| M23250871 | 0,10 | 0,85 | 0,82 | 1,06 | 1,20 | 2,20 | 5,06 |
| M25240871 | 0,10 | 0,55 | 0,52 | 1,16 | 1,34 | 1,32 | 4,39 |

Table 3.

| Sampling sites | Series with addition of usual culture medium | | | | Series on sediment extract solely | | | |
|---|---|---|---|---|---|---|---|---|
| | % N sample / N control after 5 days | Growth stimul.(+) Growth inhib.(-) | % ∫ growth curve sample / ∫ growth curve control | Growth stimul.(+) Growth inhib.(-) | % N sample / N control after 5 days | Growth stimul.(+) Growth inhib.(-) | % ∫ growth curve sample / ∫ growth curve control | Growth stimul.(+) Growth inhib.(-) |
| M01 | 126 | +26 | 118 | +18 | 241 | +141 | 317 | +217 |
| M02 | 94 | - 6 | 88 | -12 | 191 | + 91 | 179 | + 79 |
| M03 | 104 | + 4 | 112 | +12 | 127 | + 27 | 124 | + 24 |
| M04 | 118 | +18 | 115 | +15 | 127 | + 27 | 127 | + 27 |
| M05 | 71 | -29 | 66 | -34 | 42 | - 58 | 62 | - 38 |
| M06 | 112 | +12 | 113 | +13 | 157 | + 57 | 178 | + 78 |
| M07 | 106 | + 6 | 100 | 0 | 88 | - 12 | 103 | + 3 |
| M08 | 110 | +10 | 112 | +12 | 214 | +114 | 246 | +146 |
| M09 | - | | - | | - | | - | |
| M10 | - | | - | | - | | - | |
| M11 | 102 | + 2 | 98 | - 2 | 105 | + 5 | 148 | + 48 |
| M12 | 90 | -10 | 99 | - 1 | 111 | + 11 | 122 | + 22 |
| M13 | 100 | 0 | 107 | + 7 | 121 | + 21 | 203 | +103 |
| M14 | 106 | + 6 | 107 | + 7 | 120 | + 20 | 177 | + 77 |
| M15 | 87 | -13 | 81 | -19 | 178 | + 78 | 216 | +116 |
| M16 | 125 | +25 | 120 | +20 | 483 | +383 | 263 | +163 |
| M17 | 112 | +12 | 127 | +27 | 208 | +108 | 162 | + 62 |
| M18 | 108 | + 8 | 122 | +22 | 175 | + 75 | 155 | + 55 |
| M19 | 108 | + 8 | 119 | +19 | 208 | +108 | 152 | + 55 |
| M20 | 106 | + 6 | 106 | + 6 | 114 | + 14 | 190 | + 90 |
| M21 | 96 | - 4 | 97 | - 3 | 107 | + 7 | 152 | + 52 |
| M22 | 104 | + 4 | 94 | - 6 | 164 | + 64 | 212 | +112 |
| M23 | 92 | - 8 | 87 | -13 | 234 | +134 | 333 | +233 |
| M24 | 91 | - 9 | 83 | -17 | 77 | - 23 | 98 | - 2 |
| M25 | 76 | -24 | 75 | -25 | 206 | +106 | 289 | +189 |


+
0
−
1
2
3
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5
N
t
+
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6
7
8
9
N
t
∫ Growth Curve Sample / ∫ Growth Curve Control
N Sample / N Control after 5 days
Growth Curve Sample
Growth Curve Control

**FIG 1**

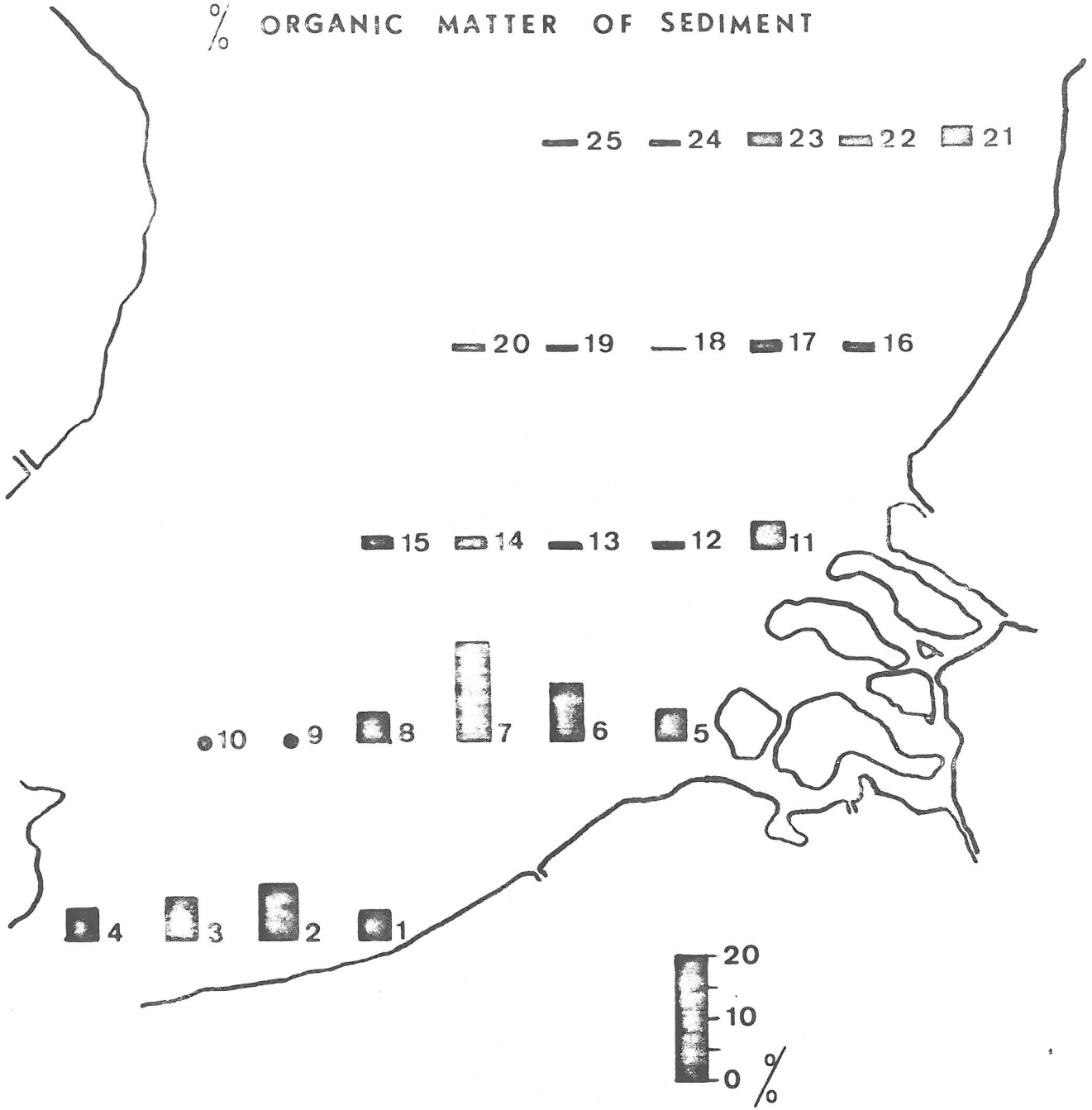
% ORGANIC MATTER OF SEDIMENT
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
20
10
0 %

FIG 2

GROWTH OF *DUNALIELLA VIRIDIS* IN USUAL CULTURE MEDIUM WITH EXTRACT OF SEDIMENT

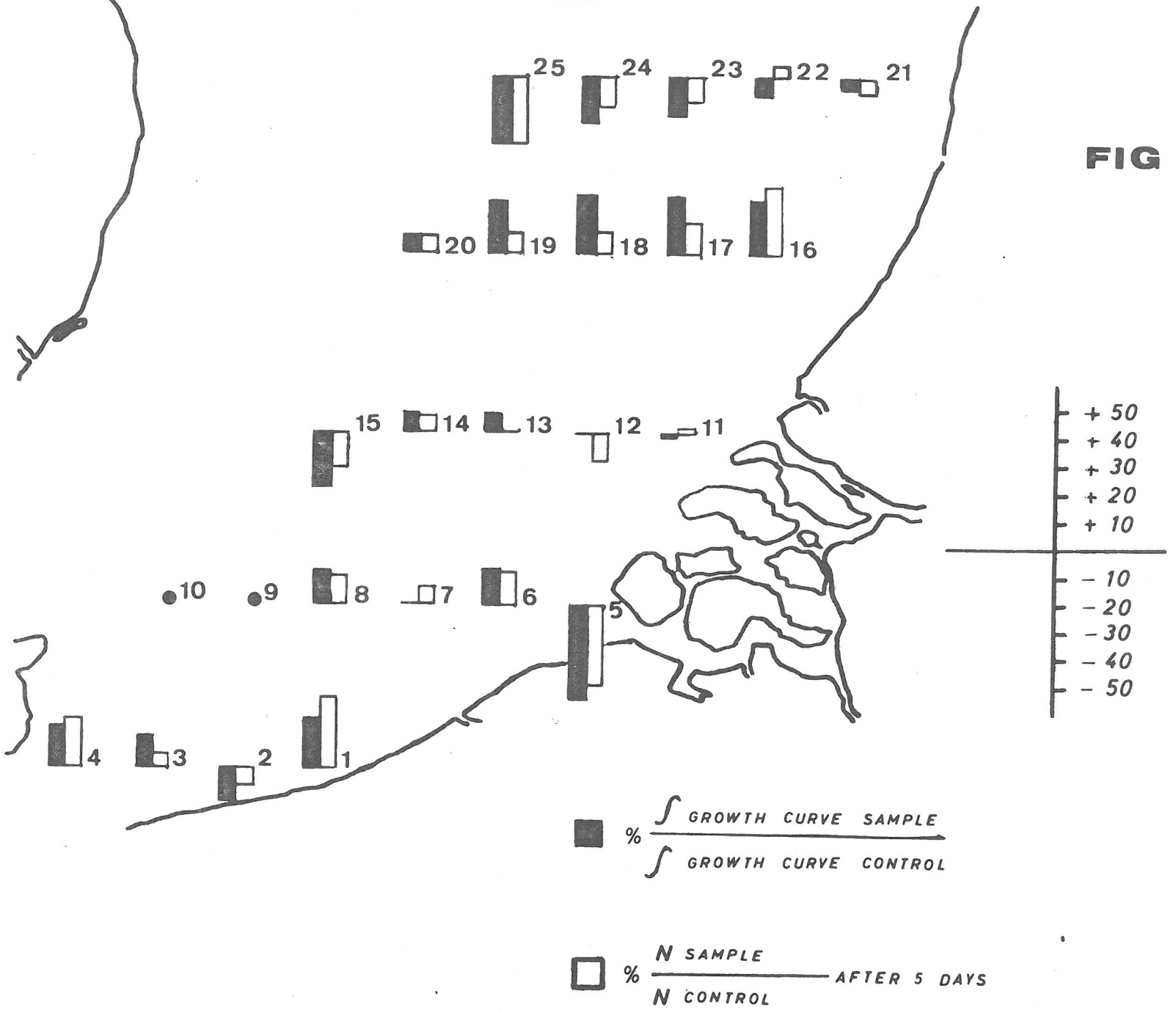


FIG 3


FIG 4

GROWTH OF DUNALIELLA VIRIDIS

ON EXTRACT OF SEDIMENT SOLELY