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GROWTH OF SOME GREEN ALGAE FROM RIVER NILE IN POLLUTED CULTURES AND THE POSSIBILITY OF THEIR USE AS WATER POLLUTION INDICATORS

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ABSTRACT

Four chlorophycean species namely: **Pandorina morum** (Volvocales), **Chlorella vulgaris**, **Scendesmus quadricauda** (Chlorococcales) and **Cosmarium** sp. (Zygnematales) were isolated from water samples collected from Damietta Branch, River Nile. The four species have been grown in liqiud cultures under the presence of one pollution factor pH changes (acidic and alkaline), natural and autoclaved sewage concentrations, commercial detergent concentrations and nitrite concentrations, one at a time. The growth rates have been studied for all cultures. The polluting factors used proved to be quite different in their effects on the growth rates of every species, with acid pollution and natural sewage as the most drastic ones. The four test species also showed big fifferences in their response. **Cosmarium** was the most susceptible species, in general, and could be considered as a real oligotrophic one. On the other hand, **Scenedesmus quadricauda** was the strongest in withstanding pollution and could be considered as a real eutrophic species. **Pandorina morum** showed to be a kind of mesotrophic to oligotrophic species, while **Chlorella vulgaris** showed to be a mesotrophic with tendency towards the eutrophic species.

INTRODUCTION

It is well established that the environmental disturbances, such as pollution, induce changes in the structure and function of biological systems. As a result, many biologists have attempted to judge the degree and severity of pollution by analysing changes in biological systems (Kofoid, 1903; Forbes and Richardson, 1913; Forbes, 1928; Purdy, 1930 and Patrick, 1949).

Algae are perhaps the most suitable and convenient biological community for monitoring pollution effects. For a number of years, there has been a series of proposals indicating that one or more algae could be used as organism indicative of water quality.

Fjerdingstad (1964) and Williams (1964) considered diatoms to be the algal group that commonly used as indicative organisms for trophic state and saprobity of water. Palmer (1969) listed 80 species of fresh water algae according to their tolerance to pollutants. His list included species of Cyanophyceae, Euglenophyceae, Chlorophyceae, Cryptophyceae and Bacillariophyceae. The use of phytoplankton as biological indicators for water pollution was discussed by Fjerdingstad (1971) who pointed out that biological assessment for water quality is preferable, rapid and accurate. Patrick (1971) examined a number of streams in U.S.A. and suggested the use of a frequency of algae as an indicator for water quality. Phillips (1977) showed the significance of the use of algae as biological indicators to define areas of trace metal pollution. Round (1981) recommended the use of indicator species or indicator communities of algae for the assessment of water quality. Wu (1984) and Wu and Suen (1985), working on Hsin Dien River in Taiwan, have concluded that the change of the relative abundance of diatoms, green algae and Flagellates in general, was revealed to be a good indication to water pollution.

The use of phytoplankton as biological indicators of water quality is probably new to River Nile and other Egyptian water bodies. Based on a previous study (Zahran et al., 1988) on the water pollution of Damietta Branch of the River Nile through physico-chemical properties and their translation in algal populations; it was decided to isolate some species from water samples collected from our stations on Damietta Branch and check their tolerance against several pollution factors that the River is actually subjected to, like: sewage, detergent, nitrite, alkaline and acid pollutions; and the possibility of the use of such species as water pollution indicators.

EXPERIMENTAL

A- Culture media:

Two types of media were used for isolation and culturing the experiment algae. The first is Woods Hole MBL pH 7.2 medium (Nichols, 1973). The second one is Desmid Agar (Star, 1964).

B- Isolation and purification of test algae:

Water samples were collected from Damietta Branch of the River Nile (Egypt) at Mansoura and Farskour stations during fall 1984, centrifuged at 3000 rpm for 10 minutes, supernatant was then carefully decanted and the residual algal pellets were washed with sterile liquid media and recentrifuged. By means of a sterilized needle, algal pellets were streaked over sterilized agar plates of both above mentioned media; plates were then sealed by means of tape, kept in culture room at 25 $\pm$ 1^{o}C and light intensity approximately 3700 lux. Plates were examined microscopically every 3 days. Restreaking into fresh plates was done every 10 days. Pure algal colonies that started to appear (1-1.5 month from the strait) were carefully picked up by means of sterile Pasteur pipettes, restreaked over agar plates and incubated as above mentioned. Finally, we were able to get unialgal cultures of Scenedesmus quadricauda, Chlorella vulgaris, Cosmarium sp. and Pandorina morum. The first two algae were found to grow best in Woods Hole MBL pH 7.2 medium, the third in desmid agar medium while the fourth one grew best in desmid agar supplemented with 50 ml/1 soil extract.

To get axenic cultures, the test algae were first grown in liquid media for about 12 days to attain vigorous growth. 20 ml of culture medium were centrifuuged at 3000 rpm for about 10 minutes, algal pellets were then treated with an antibiotic solution prepared by dissolving 100 mg penicillin G (Na salt) and 50 mg streptomycin-SO_4 in 100 ml distilled water. After 30 minutes, algae were centrifuged and the excess antibiotic solution was decanted. The algal pellets were washed using sterile liquid media used for culturing. We were successful to get axenic cultures of the four test algae through streaking that was repeated every 10 days.

C- Treatments:

I- Sewage

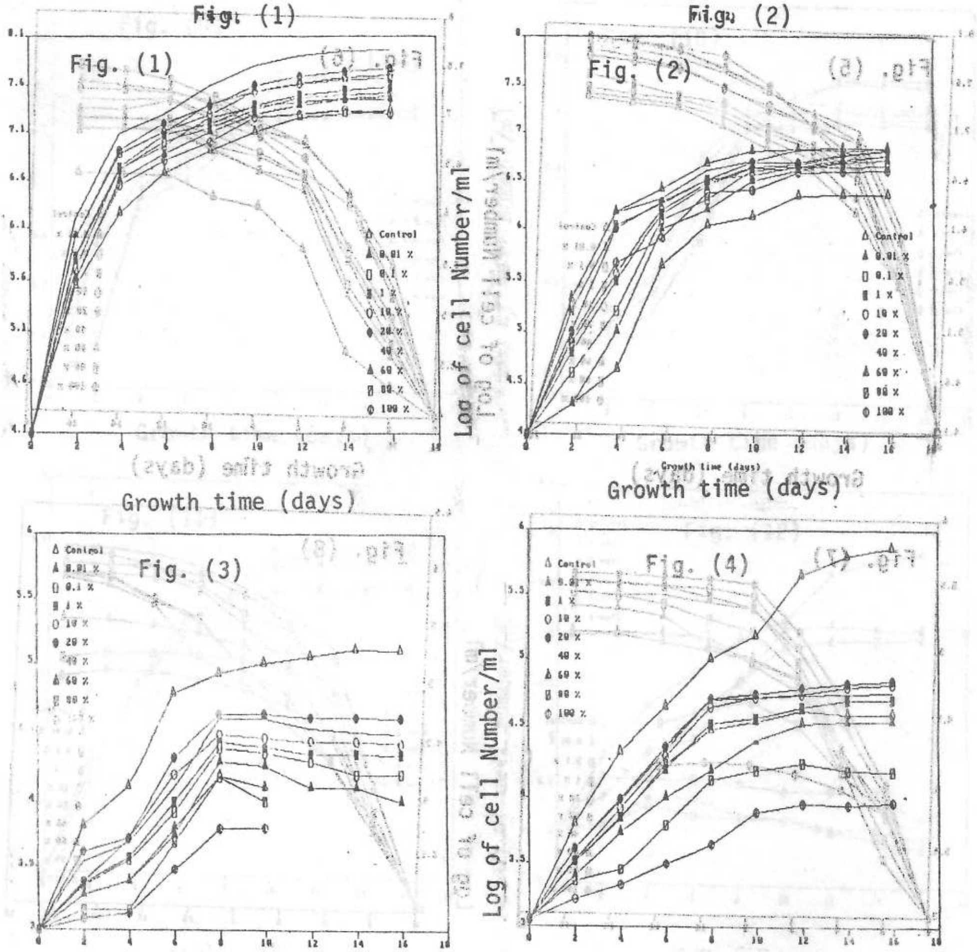
Domestic sewage was collected from the main sewage station of Mansoura City at intervals of time, mixed thoroughly, filtered through Whatman No. 1 filter paper and volumes of filtrate were added to culture media to make them 0.01%, 0.1%, 1.0%, 20.0%, 40.0%, 60.0%, 80.0% and 100.0% in sewage. Same concentrations have been made up with another group of cultures using sewage that has been autoclaved for 30 minutes.

II- Detergents

One gm of a mixture (of equal amounts w:) of commercial detergents namely: Randy, Savo, Santo, Abeer, Nana and Fomo was dissolved in 1.0 liter of glass-distilled, deionized water, thus each 1.0 ml would contain 1.0 mg detergent. Culture media were supplemented with various volumes of detergent solution to make up the following concentrations: 1.0 mg/l, 2.0 mg/l, 3.0 mg/l, 4.0 mg/l and 5.0 mg/l.

III- Nitrite

Stock solution of $NaNO_2$ was prepared by dissolving 0.492 g $NaNO_2$ (AR) in one liter glass distilled water. 10.0 ml of the stock solution were, then, diluted to one liter, thus each one ml would contain 1.0 gm of NO_2-N. Volumes of the final solution were added to culture media to make the concentrations of 1.0 gm, 2.0 gm, 3.0 gm, 4.0 gm and 5.0 g/l in NO_2-N.



Effect of various concentrations of unautoclaved (natural) sewage on growth of **Chlorella vulgaris** (Fig. 1), **Scenedesmus quadricauda** (Fig. 2), **Pandorina morum** (Fig. 3) and **Cosmarium** sp (Fig. 4).

IV- pH changes

By means of dilute solution of H_2SO_4, KOH and standardized PYE-UNICAM pH-meter, the pH of culture media was adjusted to obtain the desired pH (pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0). The pH of the control culture was 7.2. All cultures were readjusted daily for the exact pH. Before transferring the electrode from one culture to another, it was immersed in Lougl's solution (algal killing agent) for 2 minutes in addition to usual washing.

D- Counting:

Cell counting was made every couple of days uptil 16 days from the start, by means of a haemocytometer for three times after shaking, and an overall average was made up in every case. The growth rate was then estimated by calculating the log of cell number/ml.

RESULTS

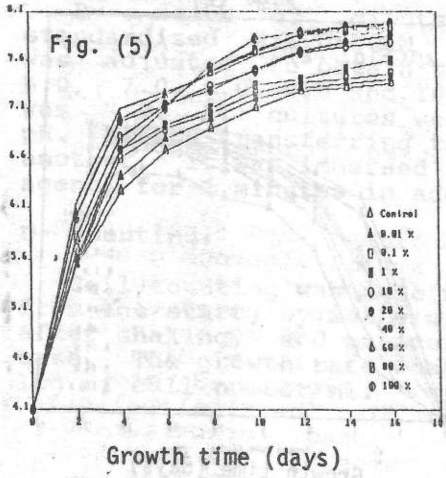
The growth curves representing the four test organisms treated with different concentrations of natural and autoclaved sewage are represented in Figs. 1-4 and 5-8, respectively. Those for treatments with different concentrations of detergent mixture, nitrite and acidic and alkaline cultures are represented in Figs. 9-12, 13-16 and 17-20, respectively.

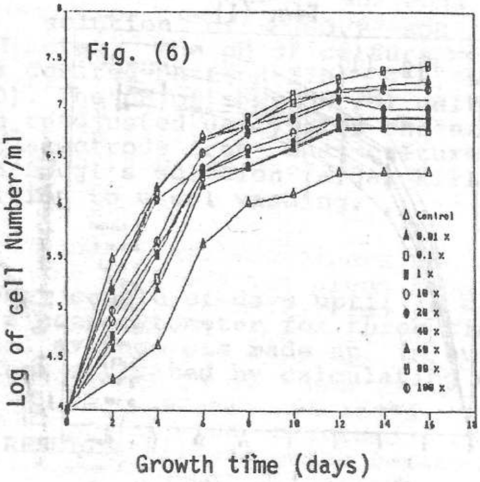
Cosmarium had growth rates that were lower that of the control ones for all the treatments used except for that with autoclaved sewage. On the other hand, all the growth curves of **Chlorella vulgaris** with all pollutants showed to be higher than or similar to the control except for the acidic treatment. **Scenedesmus quadricauda**; another species of Chlorococcales showed to be quite similar to **Chlorella vulgaris** except for the cultures with detergent where it had growth curves lower than that of the control one. As for **Pandorina morum**, its growth curves for sewage treatment whether natural or autoclaved were very similar to those of **Cosmarium**, but quite different from it (with growth curves higher than the control) concerning the detergent and nitrite treatments. As for pH deviations from neutral, **Pandorina morum** had always lower growth rates whether for alkaline or acidic ones.

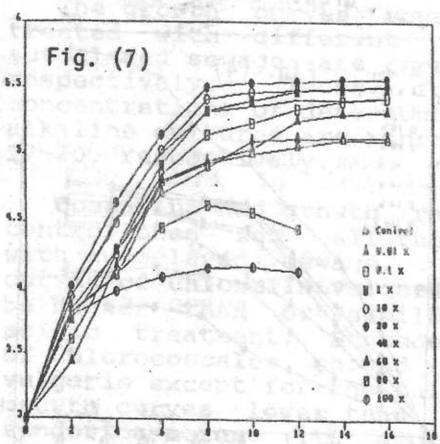
DISCUSSION

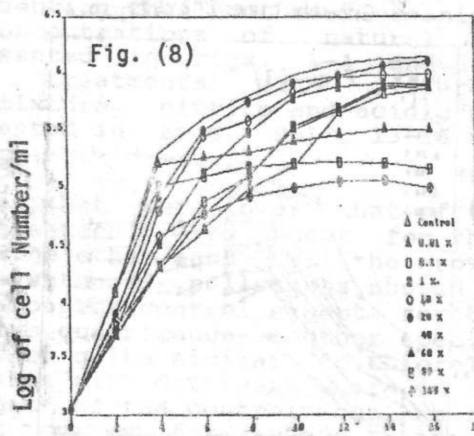
The four test algal species showed to be different in their tolerance against the different pollutants used with their different concentrations.

As for natural (unautoclaved) sewage, Cosmarium proved to be the most susceptible one. All its growth curves with different concentrations of sewage were much lower than the control one, with those for cultures with higher concentrations at the bottom (Lowest growth rate). The log

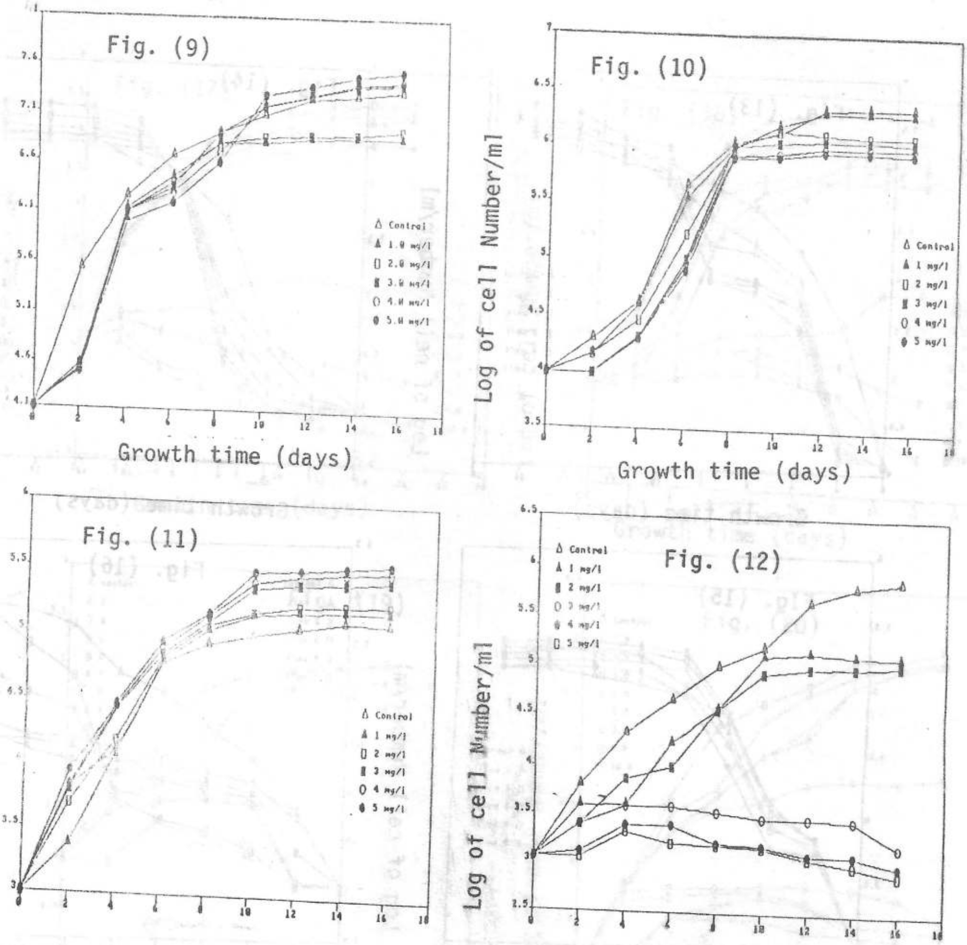




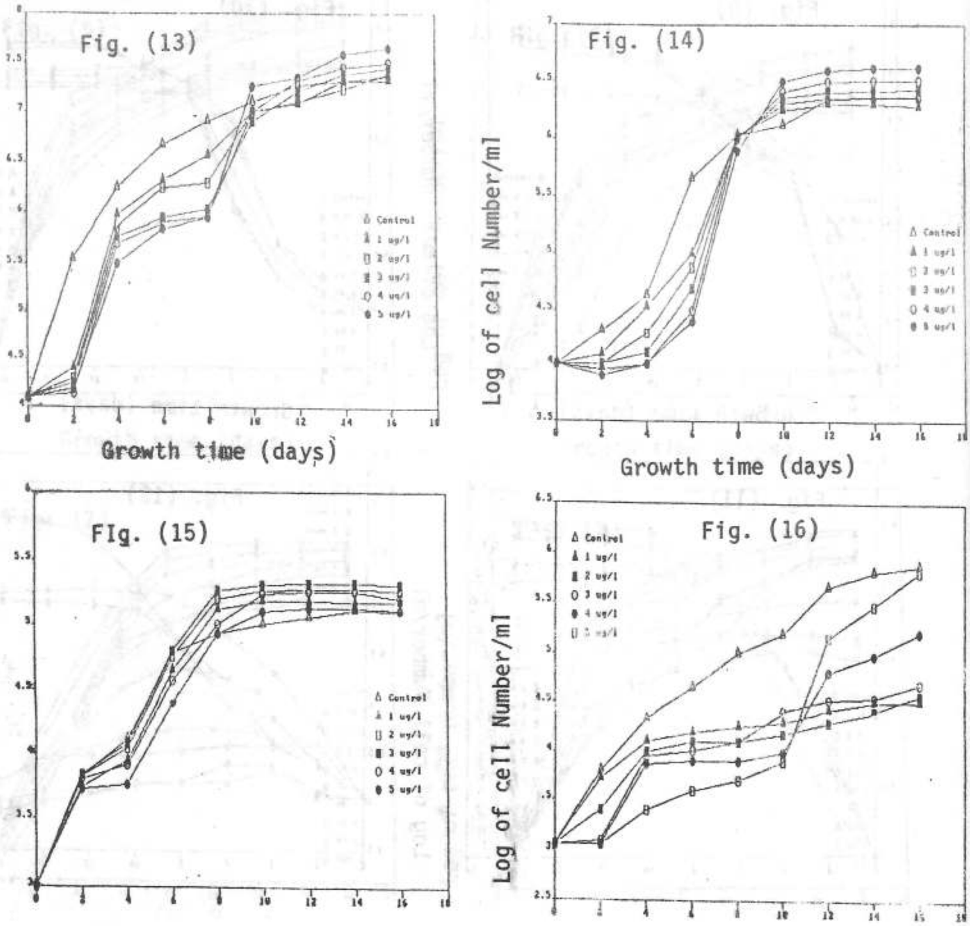




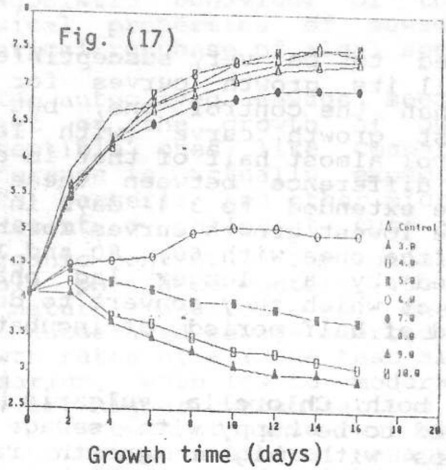
Effect of various concentrations of autoclaved sewage on growth of **Chlorella vulgaris** (Fig. 5), **Scenedesmus quadricauda (Fig. 6)**, **Pandorina morum** (Fig. 7), and **Cosmarium** sp (Fig. 8).

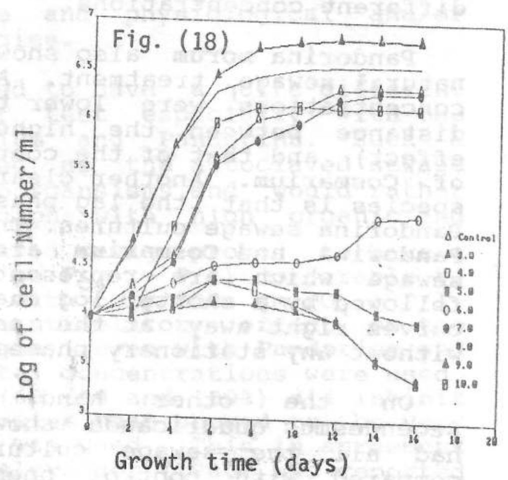


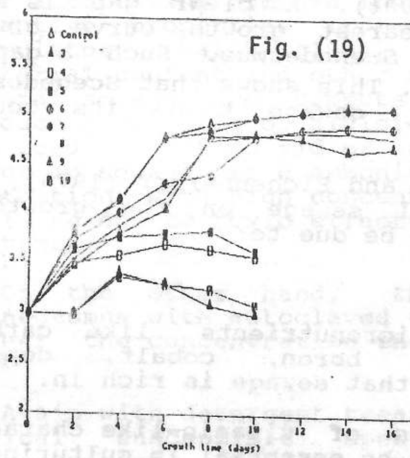
Effect of various concentrations of a mixture of commercial detergents on growth of **Chlorella** (Fig. 9), **Scenedesmus quadricauda** (Fig. 10), **Pandorina morum** (Fig. 11) and **Cosmarium** sp (Fig. 12).

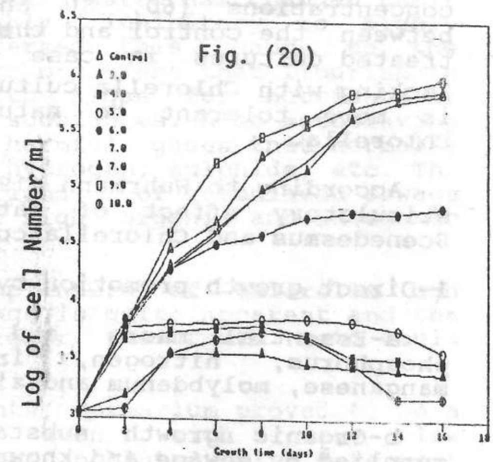


Effect of various concentration of $No_{-2}N$ on growth of **Chlorella vulgaris** (Fig. 13), **Scenedesmus quadricauda** (Fig. 14), **Pandorina morum** (Fig. 15) and **Cosmarium** sp (Fig. 16).









Effect of various pH values on growth of **Chlorella vulgaris** (Fig. 17), **Scenedesmus quadricauda** (Fig. 18), **Pandoring morum** (Fig. 19) and **Cosmarium** sp (Fig. 20).

phase has been reduced to almost half-way where it started a kind of stationary phase whose cell density differed with different concentrations.

Pandorina morum also showed to be very susceptible to natural sewage treatment. All its growth curves for all concentrations were lower than the control one, but the distance between the highest growth curve (with least effect) and that of the control almost half of that in case of **Cosmarium**. Another clear difference between these two species is that the lag phase extended to 3-4 days in all **Pandorina** sewage cultures. The lowest growth curves for both **Pandorina** and **Cosmarium** are the ones with 60, 80 and 100% sewage which are represented by a longer lag phase, followed by a shorter log one at which they convert to death curves right away at the end of half period of incubation without any stationary phases.

On the other hand, both **Chlorella vulgaris** and **Secendesmus quadricauda** showed to be happy with sewage and had all the sewage cultures with higher growth rates compared with control ones, even with the high sewage concentrations (60, 80 and 100%). A clear gap is seen between the control and the nearest growth curve of the treated cultures in case of **Scenedesmus**. Such a gap is lacking with **Chlorella** cultures. This shows that **Scenedesmus** is more tolerant to natural sewage than its cousin **Chlorella**.

According to Wuhrmann (1975) and Eichenberger (1979), the stimulatory effect of natural sewage on the growth of **Scenedesmus** and **Chlorella** could be due to:

1-Direct growth promotion by:

a-Essential macro and micronutrients like carbon, phosphorus, nitrogen, iron, boron, cobalt, copper, manganese, molybdenum and zinc that sewage is rich in.

b-Organic growth substances of vitamin-like character supplied by sewage and known to be essential in culturing of some algae (Shwartz, 1965).

2- Indirect effects like:

a- Solubilization of metals by naturally occurring or man-made complexing agents, thus increasing the supply of essential microelements.

b- Precipitation and co-precipitation of inhibitory metals and organic substances by different compounds, possibly iron hydroxides, phosphates or carbonates (Stumm, 1972).

However, Walsh (1984) concluded that the effects of sewage upon algal growth can not be predicted from a more chemical composition. He suggested that the effect would rather be the result of additive, synergetic and antagonistic behaviour of the chemicals in relation to physical properties of sewage and physiological and/or genetical response of algal species.

The autoclaved sewage seemed to have a quite different effect on the growth of our test especially with the susceptible ones like **Cosmarium** and **Pandorina**. Such a difference is actually expected as the autoclaved sewage lacks bacteria and other microorgansisms and would rather represent a pollutant suspension with high organic and inorganic contents. Our autoclaved sewage proved to have a double phosphate content (450 gm P_{O4} - P/l) comparable to the natural (unautoclaved) one with only 200 gm PO_4 - P/l. The autoclaved sewage exerted a stimulatory effect on the growth rates of all the test algae, even with **Pandorina** and **Cosmarium**, when low to moderate concentrations were used. However, higher concentrations (60, 80 and 100%) did inhibit the growth of these two species that showed to be very susceptible when natural sewage was used. This in agreement with the findings of Walsh and Alexander (1980) who reported that autoclaving of some industrial wastes has changed their bioactive properties from highly inhibitory to highly stimulatory. Walsh (1984) referred this change to the bacterial content and other micro-organisms that flourish in natural sewage and compete with algae for nutrients or produce algistatic or algicidal substances. Also autoclaving may lead to getting rid of some harmful gases that normally occur in sewage like ammonia, hydrogen, sulphide, etc. The inhibition with high concentrations of autoclaved sewage could be due to toxic effects of high organic and inorganic content.

On the other hand, the pleasure of **Chlorella** and **Scenedesmus** with autoclaved sewage is quite apparent and the higher the concentration the higher the growth rate would be.

Again with detergent treatments, **Cosmarium** proved to be a typical susceptible species even with very low concentrations. All its cultures polluted with detergent mixture had growth rates lower than the control. The growth curves for cultures treated with 3 mg of commercial detergent mixture are almost flat, while those for 4 and 5 mg treatments resemble death curves rather than growth ones. **Pandorina**; a companion of **Cosmarium** in case of sewage; behaved differently with detergent. It showed higher growth rates with all concentrations used (1-5 mg/l). Yamane (1984) reported that nonionic and anionic washing agents may exhibit an inhibitory effect upon algal growth and the inhibition is mainly species specific. The stimulatory effect of detergents on the growth of **Pandorina** could be due to its ability to make use of the phosphate content of the detergents. It is well established that a major ingredient of most detergents is phosphate; and according to Ryther (1971) and Kumar (1981); the discharges of detergents into water-ways may support luxuriant growths or blooms of some algal species.

Scendesmus and Chlorella, the two species that were quite happy with sewage proved to be less tolerant with detergent mixture. They are here again more or less similar with growth rates a little bit lower than the control ones. In this sence they are nearer to Cosmarium than Pandorina is.

Concerning the nitrite pollution, Cosmarium is still conservative in being the least tolerant among our test algae. All its cultures with different concentrations had their growth curves completely underneath the control ones all the way from the beginning of the experiment until its end. Attractive features of the treated cultures are extending the lag phase, shortening the log one and break through of the curves with highest concentrations (4 and 5 g/l) after 10 days of the start from being the most susceptible ones with least growth rates to a position much higher than other treated cultures and very near to the control ones. Nitrite had similar effects on the growth of Scenedesmus and Pandorina. It exerted an inhibitory effect with growth curves lower than the control ones uptil 6-8 days, then converted to a stimulation that made the curves of the treated cultures jumb over the control. The inhibition effect of a pollutant or a toxic substance followed by stimulation could be; according to Walsh (1984); due to the development of resistance by algae against the toxicity after being subjected to, for sometime. Another reason that we may suggest is the possibility that nitrite ions be oxidized into nitrate ones by active oxygen resulting from algal photosynthesis.

Concerning pH changes, all our test algae except Pandorina were able to withstand the deviations from neutral towards alkaline. Both Chlorella and Scenedesmus had growth rates higher than the control in alkaline cultures with pH up to 10.0. Chlorella was more tolerant in this sence as the growth curve of Scenedesmus started to go down right after pH 10.0. Cosmarium, the alga that showed to be very susceptible to all kinds of pollution treatments so far, was able to withstand pH 9.0, but pH 10.0 expectedly had a great inhibitory effect on its growth.

Acid pollution; represented in our treatments by cultures with pH adjusted to 5, 4 and 3; had the most drastic effect on all test algae used. None of our species could show any tolerance against this treatment. All growth curves of all species are either stationary or death curves. Unexpected result here is that both Chlorella and Scenedesmus (the chlorococcalean species) proved to be more susceptible to acid pollution than Pandorina and Cosmarium and had death curves with pH 5, 4 and 3.

It is well established that the availability of CO_2 and bicarbonate for algal photosynthesis is highly pH dependent. The increase in pH decreases the free CO_2 level, thus oligotrophic algae confined to free CO_2 as a carbon for photosynthesis would be unable to grow well under such conditions.

At high pH values (above pH 8), the growth of the most oligotrophic species ceased or was greatly reduced. On the other hand, most of the eutrophic algal species are able to use bicarbonate ions directly and their growth would continue undiminished up to pH values above 9.0 (Fogg, 1965; Raven, 1968 and 1970 and Moss, 1972 and 1973).

According to our findings we may classify our test algae based on the saprobic zones as follows:

a-Cosmarium to be an oligotrophic species.

b-Pandorina morum to be a kind of mesotrophic to oligotrophic one.

c-Chlorella vulgaris to be a mesotrophic with tendency towards the eutrophic zone.

d-Scenedesmus quadricauda to be a real eutrophic species.

The idea that flagellates, in general, are to be considered as species of the eutrophic zone is not a straight rule, and should be dealt with care. Some eutrophic specie supposed to be tolerant against several pollutants, could be more susceptible to a specific pollutant (especially acid pollution) than some oligotropic ones.

In general, the classification of algae concerning saprobic zones and their use as eutrophication and\ or water pollution indicators must be specific on the species level; as the one established on the class or generic levels could be dangerously misleading.

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