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TOXICITY OF 2,4-D ACID TO PHYTOPLANKTON

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Abstract—The toxic effects of 2,4-D on *Phaeodactylum tricornutum* (Bohlin) and *Dunaliella tertiolecta* (Butcher), two species of phytoplankton well suited to bioassay studies and responsive to pollutants, were studied by monitoring changes in growth in terms of cell populations, chlorophyll fluorescence and the rate of $^{14}CO_2$ assimilation. Short term bioassays, batch and continuous cultures were studied. Pure 2,4-D acid appeared more toxic than the commercial amine form of the herbicide but this may have been due to small quantities of acetone present in the solvent. Concentrations of amine herbicide in excess of 100 mg l^{-1} extended the duration of the lag phase and inhibited growth but smaller concentrations stimulated growth, the amine being consumed by phytoplankton in preference to nitrate. Continuous culture confirmed the ability of phytoplankton to adapt slowly to herbicide concentrations even as high as 500 mg l^{-1}. It is suggested that green algae adapt more rapidly to environmental change than do diatoms.

Key words—algae, batch culture, chemostat, 2,4-D acid, toxicity

INTRODUCTION

There is a need to minimize the use of pesticides and to eliminate their residues from the environment. Pesticides are, of course, very beneficial in agriculture but they create significant environmental hazards especially in natural waters.

2,4 Dichlorophenoxyacetic acid (2,4-D) and its salts are widely used as herbicides, especially in cereal, hay and pasture crops and also as growth regulators of citrus fruits (Chamarro and Esplugas, 1993). 2,4-D is a selective herbicide which kills broad-leaved plants but not grasses or conifers. Its chemical structure is a modification of a naturally occuring plant hormone (WHO, 1989). The herbicide is available as the free acid but is used in agriculture and forestry in formulations as a salt or ester. 2,4-D amine salts are water soluble and as such have a high potential to contaminate surface waters.

Environmental pollution by 2,4-D may occur as a result of the production or disposal of 2,4-D or of its by-products. Such pollution will generally become localized at the production site and more dispersed at areas of waste dumping and pollution if disposal or leaching occurs into neighbouring water courses. Disposal of unused 2,4-D in agriculture and the washing of equipment may result in localized pollution of water supplies through direct contamination or through leaching from the soil.

Previous investigations of the effect of 2,4-D on aquatic organisms (Lembi and Coleridge, 1975; Hawxby *et al.*, 1977; Boyle, 1980; Wong and Chang, 1988; Ünsal, 1991) produced somewhat confusing results. The observations indicate that low concentrations of 2,4-D, perhaps 1–5 mg l^{-1} depending on conditions, may stimulate the rate of photosynthesis but that larger concentrations inhibit growth, the magnitude of the inhibition apparently depending markedly on the species of phytoplankton studied, the nature of the bioassay and especially on the duration of the test.

2,4-D acid is amongst the herbicides produced industrially by factories bordering the Bay of Izmit (lattitude: 40°N; longitude: 29°E). Pollution of the Bay is a cause for concern (Orhon *et al.*, 1984). The present paper reports the effects of 2,4-D acid on two species of marine phytoplankton as part of a programme assessing the possible impact of industrial waste discharged into the sea. The aim has been to obtain a coherent survey of the physiological response of the phytoplankton rather than to determine precise values of toxicity. It will be found that the relationship between phytoplankton growth and 2,4-D is complex and the present study does much to clarify a confusing literature. It describes the effect of 2,4-D on both batch and continuous (long term) cultures of phytoplankton. Batch cultures are most commonly studied because of their simplicity in predicting the ecological impact of contamination but observation of continuous cultures is also essential to understand long-term steady state responses (Rhee, 1989). Growth has been followed by observations of cell numbers, chlorophyll fluorescence and carbon dioxide assimilation. Few studies have previously

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been conducted concerning the effects of 2,4-D using these three monitoring methods simultaneously and no report exists on the toxicity of 2,4-D in continuous cultures.

MATERIALS AND METHODS

Test algae

Cultures of *Phaeodactylum tricornutum* (diatom) and *Dunaliella tertiolecta* (green algae) were utilized in this study. Both species are widely used in, and known to be well suited to, algal bioassay studies (Maestrini *et al.*, 1984). They were obtained from the Marine Science Institute, Middle East Technical University, Turkey.

Culture media

Species were cultured in (a) modified f medium (fully enriched); The constituents of f medium (Guillard and Ryther, 1962) were modified slightly. (Okay *et al.*, 1994), (b) a nitrate limited medium and (c) a phosphate limited medium. The nitrate concentration was decreased to 0.3 mg N l^{-1} and the phosphate concentration to 0.1 mg P l^{-1} to obtain limited nutrient conditions in batch culture experiments. In order to detect the precise change of nitrate concentration the inflowing nitrate concentration in chemostat experiments without limiting the medium, was adjusted to 5 mg N l^{-1}.

Dilution water

Sea water (20‰) collected from the surface waters of the Marmara Sea which is an extension of the Mediterranian, was filtered, sterilized in an autoclave at 120°C for 20 min and stored frozen for further batch and chemostat experiments.

Preparation of test solutions

Modified f medium was prepared as five concentrated stock solutions instead of one, to prevent precipitation since the solutions were 1000 fold concentrated. Test solutions were prepared by diluting these stock solutions with sea water. The appropriate concentrations of 2,4-D acid and test alga were added.

A concentrated stock solution of the amine salt of 2,4-D acid was prepared in distilled water from the emulsifiable concentrate form and diluted with culture medium to provide additional test solutions.

Pure 2,4-D acid crystals were dissolved in acetone and then added to a culture medium to provide test solutions. Acetone is the most commonly used solvent for this type of bioassay but care must be taken to restrict the solvent level to about 1% or below (Tandon *et al.*, 1988). The acetone concentration in test solutions did not exceed 1.5% under our experimental conditions. The same amount of acetone was added to controls.

Phaeodactylum cultures were incubated in a series of dimethyl amine salt solutions with and without additions of nitrate.

Apparatus

1000 ml Erlenmeyer flasks used for batch culture experiments were placed on a rotary shaker. In chemostat experiments, a 600 ml culture vessel with an overflow was placed on a magnetic stirrer. Feeding was performed by a peristaltic pump (Masterflex).

Experimental conditions

The Erlenmeyer flasks and chemostat culture chamber were illuminated by cool-white fluorescent lamps placed horizontally over the systems. The light intensity, measured with a LICOR 185 Quantameter at the surface of the culture media, was 3500–4000 lux. Experiments were performed in an isolated air-conditioned room. The temperature was held constant at 20 ± 1°C.

Preculture and inoculation

Inocula for batch cultures were taken from the preincubated phytoplankton. Phytoplankton species were added to the test solutions of batch culture to give concentrations of 10^4 cells ml^{-1}. The chemostat culture was inoculated in the same way and continuous feeding was started after the growth of sufficient biomass.

Monitoring algal growth

The measurement of *Phaeodactylum tricornutum* biomass was achieved by counting cells under a microscope, by measuring the *in vivo* fluorescence of chlorophyll (Fluorescence spectrophotometer Shimadzu Model RF-540) and by monitoring the ^{14}C uptake rate simultaneously. *In vivo* fluorescence and cell counting were utilised to determine the growth of *Dunaliella tertiolecta* cultivated in the amine salt of 2,4-D acid solutions. Pure 2,4-D acid biossays were conducted by measuring only *in vivo* fluorescence.

In chemostat experiments, steady state conditions were monitored daily by counting cells, by measuring *in vivo* fluorescence and by analyzing for nitrate and phosphate in the outflow. Flow rates and inflow nitrate and phosphate concentrations were also controlled daily.

To estimate the toxicity of 2,4-D acid, a short-term (4 + 2 h) ^{14}C uptake incubation test was performed both with diatoms and with green algae (Damgaard and Nyholm, 1980). Radioactivities were counted with a Packard 1550 Tri-Carb Liquid Scintillation Counter.

The short-term and batch tests were performed in duplicate.

Other analyses

Nutrient analyses were performed by a Technicon Autoanalyzer II. Standard Methods (APHA, AWWA, WPCP, 1985) modified for continuous

analysis (Technicon Industrial Method a., 1977; Technicon Industrial Method b., 1977) were used.

A series of batch experiments using herbicide and *Phaeodactylum tricornutum* was also carried out to investigate the possible degradation of herbicide. For this purpose, u.v. absorbances of filtered solutions were measured at 289 nm. A linear calibration was obtained up to 500 mg l^{-1}.

RESULTS AND DISCUSSION

(*I*) *Short-term bioassays*

Figure 1 shows the result of short-term toxicity tests in the presence of herbicide. Such short-term tests are frequently used as the basis for control of the toxic effects of effluents and new products (Kusk and Nyholm, 1991). They are generally evaluated as the concentration of toxic substance which inhibits 50% of phytoplankton growth (LC_{50} values); These values were derived from a straight line fit on transformed data and 95% confidence limits for the LC_{50} values were calculated. LC_{50} values are 185 ± 11 mg l^{-1} for *Dunaliella tertiolecta* and 362 ± 9 mg l^{-1} for *Phaeodactylum tricornutum*. Presumably chemicals are transported through the cell walls of green algae more readily than those of diatoms and therefore, in short-term experiments, *Dunaliella tertiolecta* is more sensitive to herbicide than *Phaeodactylum tricornutum*. The obvious non-linearity of the inhibition-concentration graphs indicates the herbicide to have at least two effects on the phytoplankton cells. Moreover, these tests may have little relevance to the long-term effects of the herbicide. These themes underly our discussion of the results of many of the other bioassays.

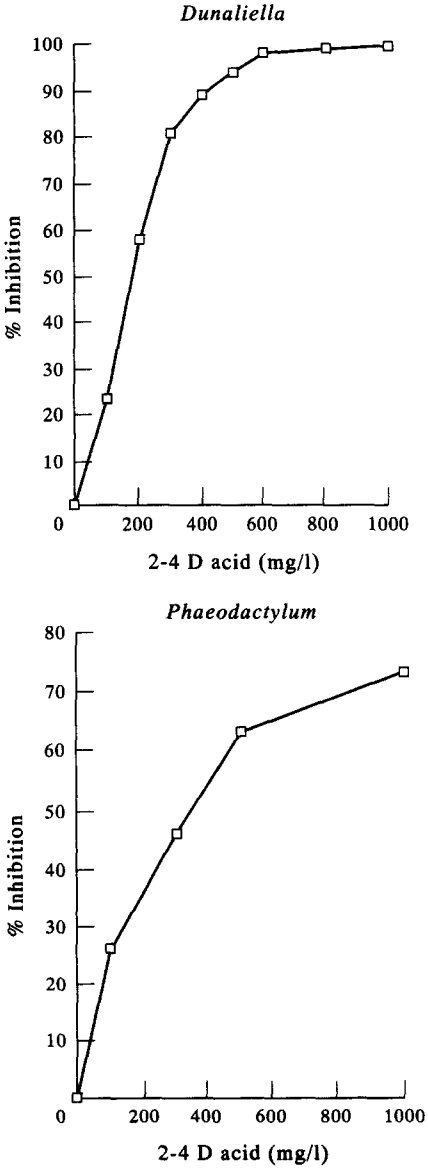

Fig. 1. The short-term toxicity tests.

(*II*) *Batch bioassays*

(*a*) *Bioassays in modified f medium.* Figure 2 (a–c) show the effects of the amine salts of 2,4-D on the chlorophyll fluorescence, population and rate of carbon dioxide assimilation of *Phaeodactylum tricornutum*. The results are replotted in Fig. 3 normalized to the maximum values recorded in each assay. Figure 4 shows the effects of increasing concentrations of the herbicide on the chlorophyll fluorescence of *Dunaliella*.

Phaeodactylum tricornutum exhibited a lag phase of ~2 d under the conditions of the bioassay. The duration of the lag phase increased markedly in the presence of the herbicide extending to 11 d in a concentrations of 500 mg l^{-1} of the herbicide. In other words, increasing lengths of time were required for cell biochemistry to overcome the increased concentrations of herbicide. Walsh *et al.* (1982) has suggested that the duration of the lag phase is the period in which the medium loses its toxicity but we have seen no evidence of this, the lag phase appears to be the time needed for cells to adapt to their environment.

Figures 2 and 3 show that the variations of fluorescence and cell numbers with time are very similar. Increase in herbicide concentration appears to diminish the maximum population of *Phaeodactylum tricornutum*. However, the increase in the duration of the lag phase clearly delays the development of the log phase and it remains possible that, had the assays been continued for longer than 20 d, all the final populations would have been similar. The effect of the herbicide on the rate of assimilation of carbon dioxide, i.e. on the rate of photosynthesis, was more complex. The maximum rates of assimilation occurred approximately on the same day as the maximum rates of increase of fluorescence and cell population. Accordingly, maximum rates of assimilation were delayed by increasing concentrations of herbicide. The magnitudes of the

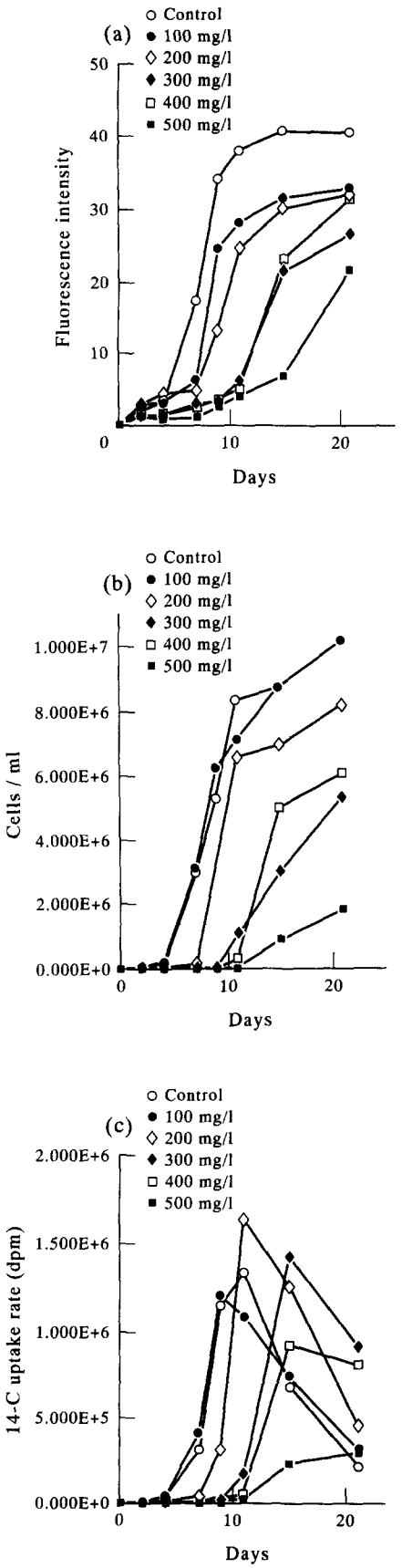

Fig. 2. The results of 2,4-D herbicide on *Phaeodactylum tricornutum*.

maximum rates, however, increased when herbicide was added. It was not until concentrations of herbicide exceeded 300 mg l^{-1} that the herbicide inhibited the rates of photosynthesis. (This suggests that even had the bioassays been prolonged further, populations of *Phaeodactylum tricornutum* at these high concentrations would not have reached the values attained in the absence of herbicide.)

Phaeodactylum tricornutum, a diatom, possessing a cell wall composed predominantly of silica, had a natural (i.e. in the absence of herbicide) lag time of 2 d in these assays and, presumably, the passage of chemicals into the cell is delayed by the cell wall. *Dunaliella*, a green algae, possesses organic cell membranes rather than a cell wall and Fig. 4 shows that, under assay conditions, the duration of the lag phase was negligible. We made similar observations with work on the effects of herbicidal wastewaters on *Phaeodactylum tricornutum* and *Chlorella* sp. (Okay *et al.*, 1994).

(*b*) *Bioassays in N-limited medium*. The effects of toxic substances on phytoplankton species vary depending on the different environmental factors, one of which is the composition of the growth medium. As a general rule, it is easier to resist toxic effects if all other living conditions are optimum. The nitrogen concentration of the medium was therefore reduced in the expectation that the *Phaeodactylum tricornutum* cultures would be less able to resist toxicity compared to cultures in f medium. The surprising results of this bioassay are shown in Fig. 5. A clear difference was seen when control and herbicide containing samples were compared. Contrary to expectations, *Phaeodactylum* increased its biomass in the presence of herbicide. The diatoms were able to use the dimethyl amine salt of 2,4-D acid as a nitrogen source, thus compensating for the limited nitrate. It is well known that dimethyl amine salts (ammonium) can easily be used as a nitrogen source by phytoplankton (Parson and Bruce, 1993) and the present investigations support this.

100 mg l^{-1} herbicide increased growth greatly compared to the control sample though the lag phase was prolonged by about 2 d. In the presence of 300 mg l^{-1} herbicide, although the lag phase was again increased, the growth rate, the rate of ^{14}C uptake and the fluorescence intensity were rather high, almost equal to the values achieved by *Phaeodactylum* in modified f medium. When 500 mg l^{-1} of herbicide was added the lag phase of growth was prolonged to approx. 20 d and the growth rate decreased. This high concentration of herbicide had obvious negative effects on growth in the absence of nitrate.

(*c*) *Bioassays in P-limited medium*. Figure 6 shows the effect of the herbicide on *Phaeodactylum tricornutum* cultures growing in a phosphate deficient medium. Comparison with the effects in fully enriched medium shown in Fig. 2 indicates first that the rates of growth as measured by fluorescence,

cell counts and by assimilation of carbon dioxide decreased in the phosphate limited medium. Secondly, growth was more strongly inhibited by the addition of herbicide. This, of course, was the behaviour one had anticipated in the nitrogen limited medium and it is consistent with the explanation of the enhancement of growth by the herbicide in the nitrogen limited medium as being due to the utilisation of amine as a source of nitrogen.

(*d*) *Bioassays with pure 2,4-D acid.* It is more

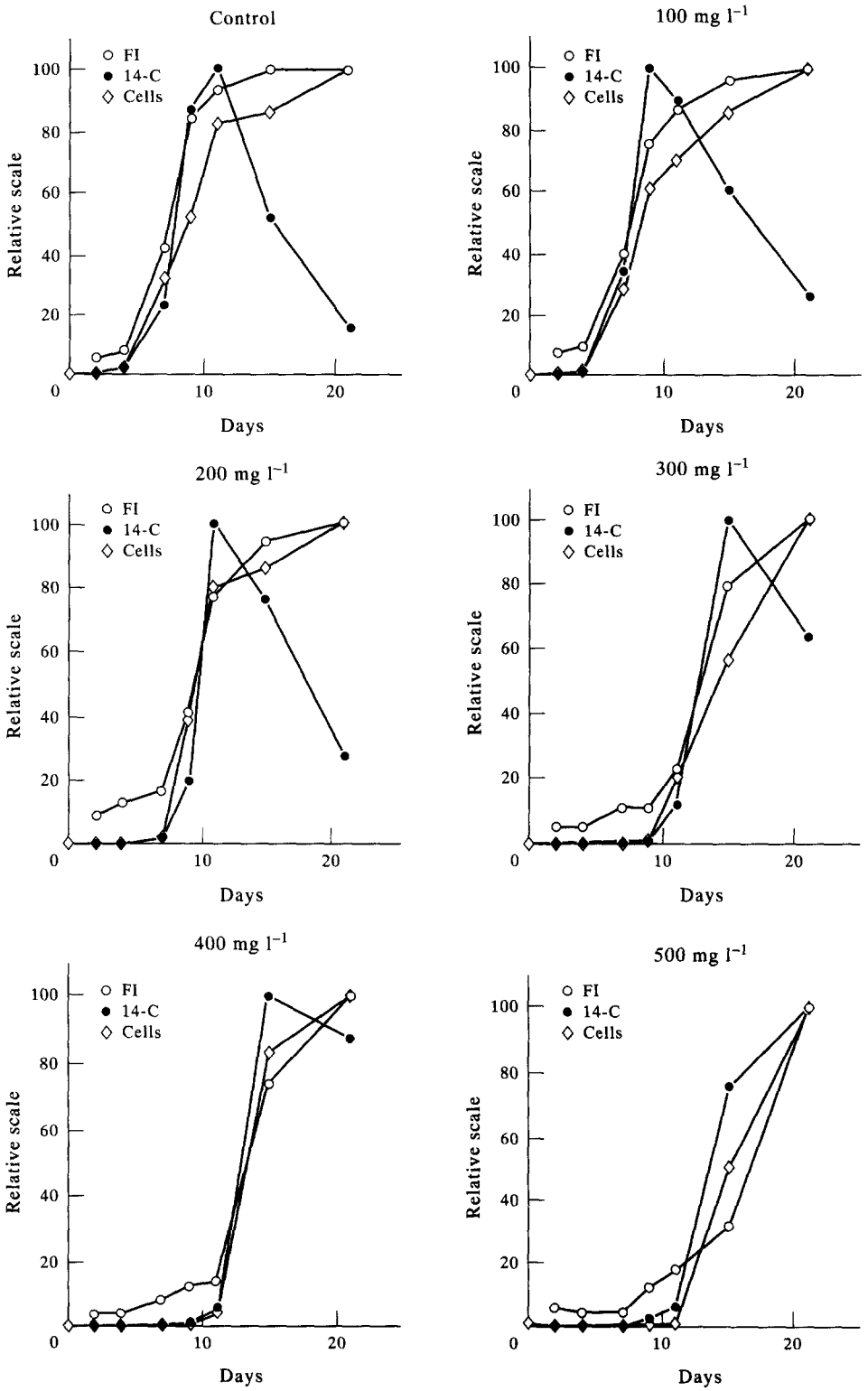

Fig. 3. The demonstration of the relative effects of 2,4-D herbicide.

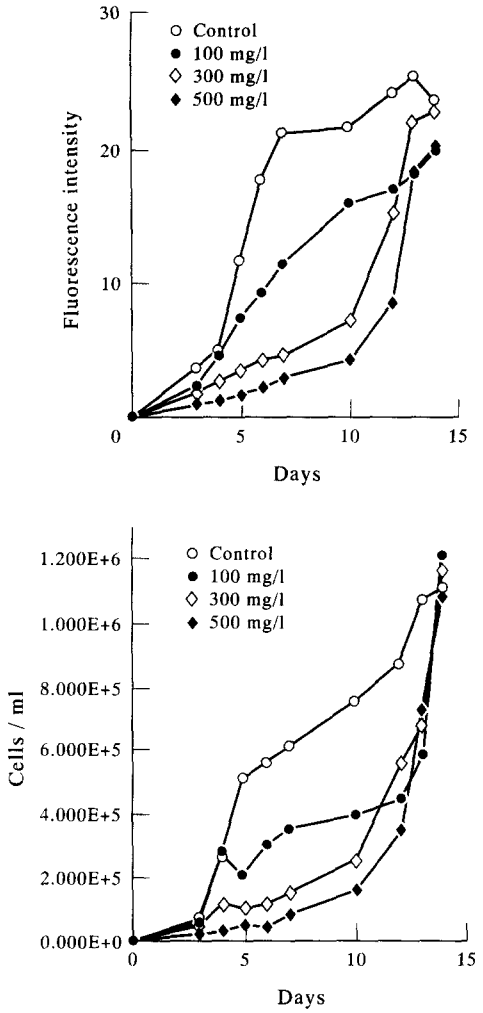

Fig. 4. The effects of 2,4-D herbicide on *Dunaliella tertiolecta* in modified f medium.

important to know the toxic effects of the amine salt of 2,4-D acid than those of pure 2,4-D acid because it is the amine salt which is mainly used in agriculture and which dissolves in natural waters. However, knowledge of the toxicity of pure 2,4-D acid is also important due to the fact that it can be found in nature as a degradation product or as production waste from industry.

The solubility of pure 2,4-D acid in water is very low. For this reason one needs a solvent to obtain application concentrations. Acetone has frequently been used for this purpose. Since acetone is toxic to phytoplankton, the same concentration of acetone as used to dissolve 2,4-D acid, 1.5%, must also be added to the control. The effect of adding 2,4-D acid in 1.5% acetone to both *Phaeodactylum tricornutum* and to *Dunaliella tertiolecta* is shown in Fig. 7. Again, it was observed that *Phaodactylum* shows a lag phase and *Dunaliella* does not. In fact, in the control experiments, *Phaeodactylum* exhibited a lag phase of 7 d rather than the usual 2 (compare Fig. 2) and this was presumably due to the presence of acetone. The presence of a 100 mg l^{-1} herbicide increased the lag phase to 13 d. Further increase in herbicide concentration inhibited growth completely through

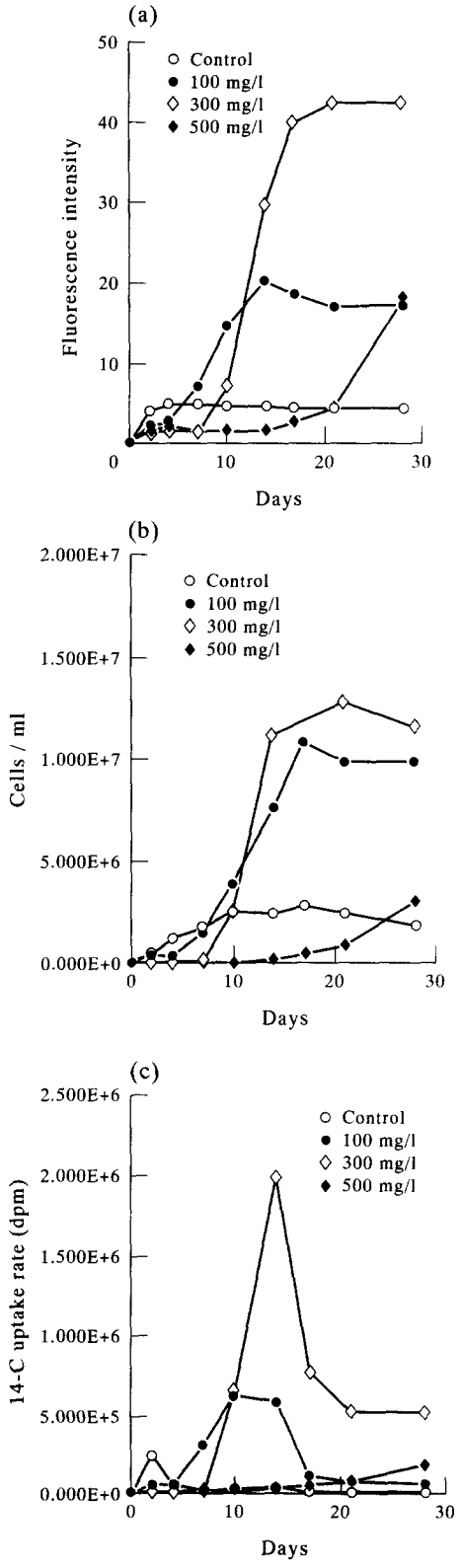

Fig. 5. The effects of 2,4-D herbicide on *Phaeodactylum tricornutum* in N-limited medium.

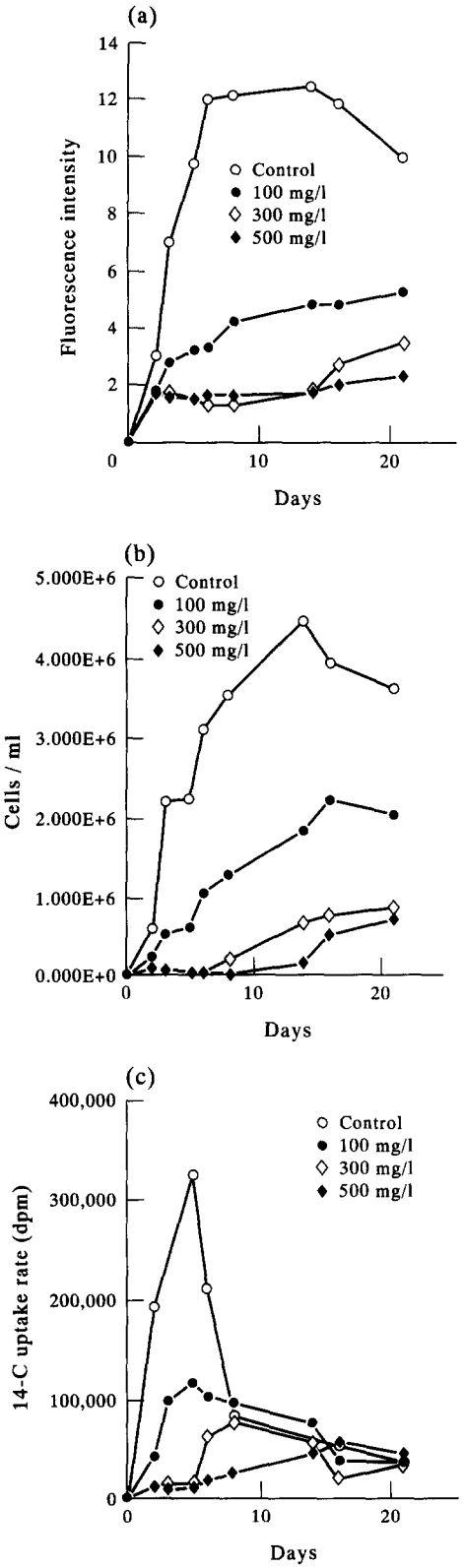

Fig. 6. The effects of 2,4-D herbicide on *Phaeodactylum tricornutum* in P-limited medium.

out the period of observation. Comparison of these experiments and those with cultures of *Dunaliella* with the results obtained previously with the amine salt of 2,4-D suggests that the pure acid is the more toxic and inhibits growth more strongly than the amine salt. It should be remembered, however, that acetone was present in all the experiments with pure acid and the toxicity of the acetone may have weakened the resistance of the cells to the herbicide.

(III) Continuous culture (chemostat) bioassays

Continuous culture bioassays have previously been performed in order to estimate the long term effects of pollutants, wastewaters and toxic substances on living organisms in natural waters (Rhee *et al.*, 1988; Kayser, 1976). The chemostat results with *Phaeodactylum* in modified f medium with the amine salt of 2,4-D are shown in Fig. 8. The nitrate concentration in the medium was adjusted to 5 mg N l^{-1} in order to be able to detect the

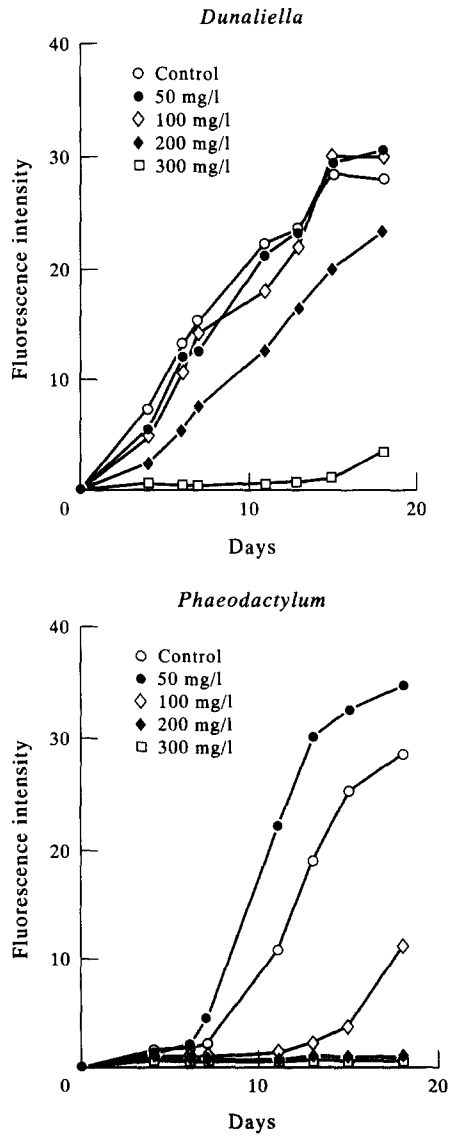

Fig. 7. The effects of pure 2,4-D acid on cultures of phytoplankton.

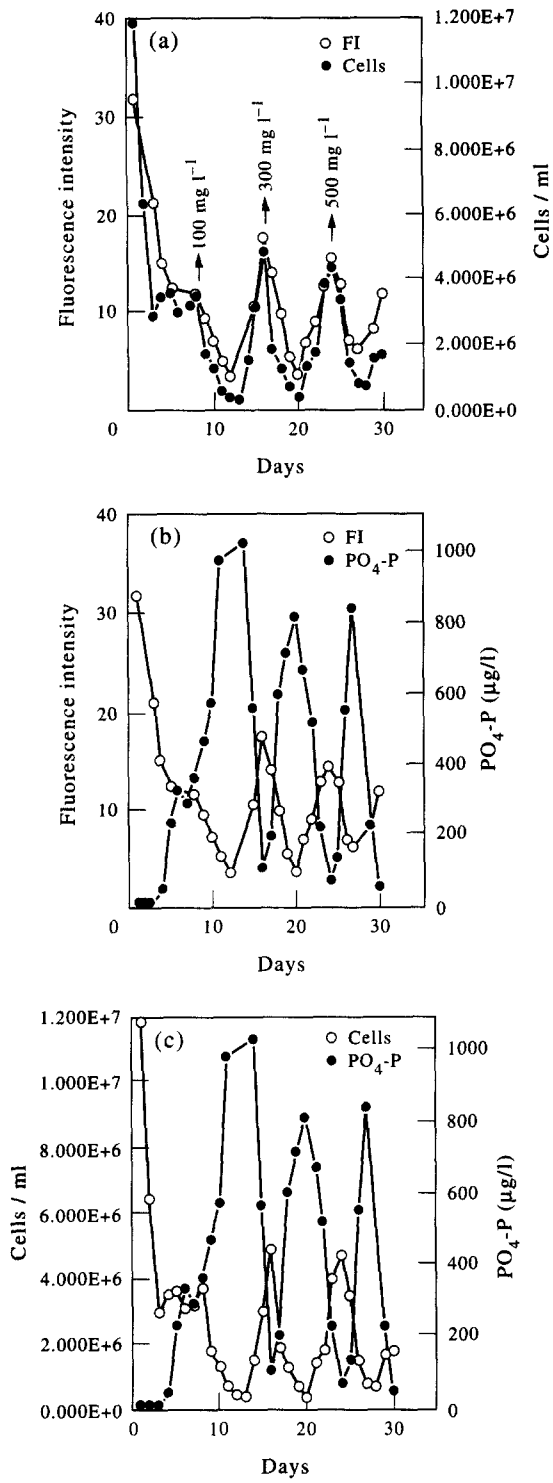

Fig. 8. The chemostat bioassay results of *Phaeodactylum tricornutum* in the presence of 2,4-D herbcide.

concentrations which were consumed by phytoplankton. The monitoring of growth was performed by analysing daily for NO_3–N, PO_4–P, cell concentrations and fluorescence intensity. Figure 8 a shows changes in cell concentrations and fluorescence intensity with time. Both parameters are exactly in accord throughout the experiment. Figure 8 (b, c) show changes in fluorescence intensity, cell concentrations and phosphate uptake with respect to time. After the growth of *Phaeodactylum* batchwise, the culture medium was fed at a dilution rate of 0.715 d^{-1}. The cell concentration in the culture chamber decreased and reached a steady state value in about 5 d. At that point, 100 mg l^{-1} of herbicide was introduced into the culture medium. Figure 8 shows that as a result the cell concentration in the culture chamber was suddenly affected by herbicide and decreased to a very low level. After an adaptation period (corresponding to the lag phase in batch cultures), the culture became restored. The concentration of herbicide was now increased to 300 mg l^{-1} and subsequently to 500 mg l^{-1}. Each time the cell population decreased and showed an adaptation period before they were restored. Figure 8 shows that as expected, minimum cell populations corresponded to maximum phosphate concentrations in the outflow and vice versa. Nitrate concentrations were also measured but no difference could be observed between concentrations in the inflow and outflow. This suggests that the phytoplankton preferentially consumed dimethyl amine salt. The chemostat toxicity bioassay results are consistent with the batch culture results and there are two important points to be noted:

(1) Because the culture was single specied, there was no interspecies competition. One sees that, when the dilution rate was low enough (no "wash out") the culture had time to adapt. Thus the dilution rate is an important parameter both in this type of bioassay and in natural waters.

(2) The culture was fed, starting with a low concentration of herbicide which was increased slowly. This means that the culture had time to become acclimatized to toxic material. In natural waters a sudden input of toxic substances could cause different and more dramatic results. In other words it is expected that the concentration of 500 mg l^{-1} herbicide may have more pronounced toxic effects on unacclimatized algal cultures as found in natural environments.

The continuous toxicity bioassays confirm the lag phase in batch cultures to be the result of the adaptation period of phytoplankton and not the loss of toxicity by the herbicide.

CONCLUSIONS

(1) Bioassays of the growth of the diatom *Phaeodactylum tricornutum* and the green algae *Dunaliella tertiolecta* confirm that the herbicide 2,4-D has significant effects on the growth of phytoplankton at concentrations of the order of 100 mg l^{-1}. At the much lower concentrations likely to be found after dilution in natural waters, the effects of the herbicide should be negligible.

(2) 2,4-D is most frequently used in the form of its amine salt. Whereas, in the short-term this appeared to halve the growth of the phytoplankton at concentrations of 362 ± 9 mg l^{-1} and 184 ± 11 mg l^{-1} for *Phaeodactylum tricornutum* and *Dunaliella tertiolecta* respectively, bioassays conducted in a continuous flow of fresh herbicide show clearly that phytoplankton adapt to the presence of the herbicide and can establish a stable culture of *Phaeodactylum* even in concentrations of 500 mg l^{-1}. The period of time needed for adaptation, a matter of days, is reflected in the bioassays of batch cultures by an increase in the period of the lag phase. Subsequently, concentrations of more than 300 mg l^{-1} of the herbicide in batch cultures reduce the rate of assimilation of carbon dioxide. However, the amine moiety of the herbicidal salt can act as a preferential source of nitrogen for phytoplankton cells and the presence of concentrations of the amine salt of 2,4-D up to the order of 100 mg l^{-1}, depending on the conditions, increases the rate of growth of phytoplankton in N deficient (N limited) medium and may increase the rate of assimilation of carbon dioxide even in f medium.

(3) The herbicide is more toxic to diatoms in P deficient medium than in fully enriched medium.

(4) The pure 2,4-D acid appears to inhibit the growth of phytoplankton more strongly than the amine salt but the enhanced inhibition may be a result of the toxicity of the acetone added to ensure the solubility of the acid.

(5) Throughout this study it has been observed that the diatom, *Phaeodactylum tricornutum*, assimilates chemicals and adapts to changes in its aqueous environment more slowly than the green algae, *Dunaliella tertiolecta*, presumably as a consequence of the silica cell wall surrounding the diatom cells.

(6) Although in short term bioassays *Dunaliella tertiolecta* was less resistant to herbicide than *Phaeodactylum tricornutum*, in batch bioassays the tolerance of *Dunaliella* to both the herbicide and the pure form of 2,4-D was much greater than that of *Phaeodactylum*. The batch bioassays are more reliable than short-term bioassays in predicting environmental effects and it can be concluded that 2,4-D acid is more toxic to diatoms than green alga.

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REFERENCES

APHA, AWWA, WPCP (1985) *Standard Methods for the Examination of Water and Wastewater* (Edited Greenberg A. E., Trussel R. R., Clesceri L. S. and Franson M. A. H.), 16th edn. APHA, Washington, D.C.

Boyle T. P. (1980) Effects of aquatic herbicide 2,4-D DMA on the ecology of experimental ponds. *Environ. Pollut.* **21,** 35–49.

Chamarro E. and Esplugas S. (1993) Photodecomposition of 2,4-Dichlorophenoxyacetic acid: Influence of pH. *J. Chem. Tech. Biotechnol.* **57,** 273–279.

Damgaard B. M. and Nyholm N. (1980) Ecotoxicity tests for aquatic environments. In *Comparative Ring-Test with Microorganisms*. Nordic Council for Applied Research.

Guillard R. R. L. and Ryther J. H. (1962) Studies of marine planktonic diatoms I. *Cyclotella nana* Hustedt and *Detonula confervaceae* (Cleve). *Gran. Can. J. Microbiol.* **8,** 229–239.

Hawxby K., Tubea B., Ownby J. and Basler E. (1977) Effects of various classes of herbicides on four species of algae. *Pestic. Biochem. Physiol.* **7,** 203–209.

Kayser H. (1976) Wastewater assay with continuous algal cultures: the effect of mercuric acetate on the growth of some marine dinoflagellates. *Marine Biol.* **36,** 61–72.

Kusk O. and Nyholm N. (1991) Evaluation of a phytoplankton toxicity test for water pollution assesment and control. *Arch. Environ. Contam. Toxicol.* **20,** 375–379.

Lembi C. A. and Coleridge S. E. (1975) Selective toxicity of detergents and herbicides to phytoplankton. Purdue University, Water Resources Research Center, West Lafayette, Indiana, Tech. Rep. No. 71.

Maestrini S. Y., Bonin D. J. and Droop M. R. (1984) Phytoplankton as indicators of sea water quality: bioassay approaches and protocols. In *Algae as Ecological Indicators* (edited by Shubert L. E.), pp. 71–132. Academic Press, New York.

Okay O. S., Morkoç E. and Gaines A. (1994) Effects of two herbicidal wastewaters on *Chlorella* sp. and *Phaeodactylum tricornutum*. *Environ. Pollut.* **84,** 1–6.

Orhon D., Gönenç E., Tünay O. and Akkaya M. (1984) The prevention and removal of water pollution in ijzmit Bay (in Turkish). Tech. Rep. ITU, Civil Engineering Department.

Parson J. M. and Bruce C. P. (1993) Seasonal pattern of ammonium (methylamine) uptake by phytoplankton in an oligotrophic lake. *Hydrobiologia* **250,** 105–117.

Rhee G. Y., Shane L. and DeNucci A. (1988) Steady-state effects of 2,5,2,5'-tetrachlorobiphenyl on growth, photosynthesis, and P uptake in *Selenastrum capricornutum*. *Appl. Environ. Microbiol.* **54,** 1394–1398.

Rhee G. Y. (1989) Continuous culture algal bioassays for organic pollutants in aquatic ecosystems. *Hydrobiologia* 188/189, 247–257.

Tandon R. S., Lal R. and Rao V. V. S. N. (1988) Interaction of endosulfan and malathion with blue-green algae *Anabaena* and *Aulosira fertilissima*. *Environ. Pollut.* **55,** 1–9.

Technicon Industrial Method a. (1977) Nitrate and nitrite in water and wastewater. No. 158-71W/A.

Technicon Industrial Method b. (1977) Phosphate analysis in water seawater. No. 253-80E.

Ünsal M. (1991) Effects of herbicides on the growth of marine phytoplankton. *FAO/UNEP/IOC Workshop on the Biological Effects of Pollutants on Marine Organisms*, Malta.

Walsh G. E., Duke K. M. and Foster R. B. (1982) Algae and crustacean as indicators of bioactivity of industrial wastes. *Wat. Res.* **16,** 879–883.

WHO (1989) Environmental Health Criteria 84. 2,4-Dichlorophenoxyacetic acid (2,4-D), Environmental Aspects, Geneva.

Wong P. K. and Chang L. (1988) The effects of 2,4-D herbicide and organophosphorus insecticides on growth, photosynthesis, and chlorophyll a synthesis of *Chlamydomonas reinhardtii* (m +). *Environ. Pollut.* **55,** 178–189.