Bull. Inst. Oceanogr. & Fish., ARE, 13 (2), 1987: 103 - 110.

EFFECTS OF SALINITY AND PESTICIDES ON THE MORPHOLOGY OF SOME MICROSCOPIC ALGAE

EZZAT A. IBRAHIM.

National Institute of Oceanography and Fisheries, Aquatic Plants Lab., Zamalek Research Station, Cairo, Egypt.

ABSTRACT

The effects of salinity, Bayluscide and Dimethoate on the morphology of the freshwater chlorophytes **Scenedesmus dimorphus** (Trup.) Kuetzing, **Scenedesmus quadricauda** (Trup.) de Brebisson, **Ankistrodesmus falcatus** (Corda) Ralfs, and **Staurastrum boreale** W. West and the marine diatom **Amphiprora paludosa** W. Smith were investugated. Salinity as represented by NaCl caused significant increase in cell size accompanied by lysis in the cytoplasm. The insecticide Dimethoate increased cell volume and size by rupturing the cell wall, while the cytoplasm was obviously shrinking. The molluscicide Bayluscide slightly reduced the cell size and volume via rupture in the cell wall; phenomena enhanced at high concentrations.

INTRODUCTION

Little is known dealing with the morphological responses of phytoplankton cells to salinity and pesticides. The morphological aberrations of some freshwater green and blue-green algal cells under the influence of some pesticides have been reported by Ibrahim (1978). On the other hand, there is no available information on the effect of salinity on the morphology of the freshwater phytoplankton. The most commonly observed morphological responses is due to the effects of heavy metals. This has been studied in a wide variety of organisms including representatives from chlorophyceae (Rosko and Rachlin, 1977; Ibrahim, 1978) chrysophyceae (Davies, 1974), bacillariophyceae (Nuzzi, 1972; Sunda; Sunda & Guillard, 1976; Berland et al., 1977; Morel et al., 1978; Thomas et al., 1980; Sicko-Goad, 1982) and cyanobacteria (Ibrahim, 1978; Rachlin et al., 1982).

In a series of experiments on the effects of salinity and pesticides on growth and metabolic activities of some phytoplankters. I have noticed morphological aberrations of the treated cells to these materials. The present study, however, was initiated in order to indicate these phenomena.

The present work deals with the effects of salinity as represented by NaCl, the molluscicide Bayluscide and the insecticide Dimethoate on the morphology of the freshwater chlorophytes **Scenedesmus dimorphus, Scenedesmus quadricauda, Ankistrodesmus falcatus** and **Staurastrum boreale** and the diatom **Amphiprora paludosa.** The results of these observations are presented below.

MATERIAL AND METHODS

The test algae used in this study were isolated from Lake Wadi El-Rayan for **S. dimorphus, S. quadricauda, A. falcatus** and **St. boreale** and from Lake Quarun for **Amphiprora paludosa.** The method of Pringsheim (1946) was used to obtain pure unialgal cultures.

The chlorophytes **S. dimorphus, S. quadricauda, A. falcatus,** and **St. boreale** were grown and tested in a medium prepared by dissolving the following major salts in 1 l glass distilled water: 467 mg $NaNO_3$, 31 mg K_2HPO_2, 18.5 mg $CaCl_2 . 2H_2O$, 10.5 mg $NaHCO_3$ and 56 µg Fe^{2+} as $FeSO_4$. In case of **A. paludosa,** artificial sea water of 30 % salinity was prepared using the formula reported by Kester et al., (1967) in glass distilled water. The medium was enriched with 55 mg/l KNO_3, 6.95 mg/l KH_2PO_4, 20 mg/l Na_2SiO_3, 56 µg/l Fe^{2+} as $FeSO_4$ and 0.5 µg/l vitamin B_{12}. For trace elements of both media, 39.6 mg $CuSO_4$, 43 mg $ZnSO_4$, 13 mg KI, 15 mg KBr, 40.6 mg $MnSO_4$, 56 mg $CoCl_2$, 10 mg H_3BO_3, 18.4 mg H_2MoO_4, 16.7 mg Na_2WO_4 and $Na_2B_4O_7$ were dissolved in 1 l glass distilled water and 1 ml was added to each liter.

Algae were grown in 250-ml Erlenmeyer flasks with 100 ml medium. Each flask was inoculated with 5000 cells/ml of test algal cells in logarithmic growth phase. The cultures were incubated with hand shaking once a day under 4000 lux illumination from "cool white" fluorescent tubes; lighting cycle, 14 h light followed by 10 h darkness (14 L : 10 D), temperature 25±1°C.

Salinity

Salinity of the external medium of **S. dimorphus, S. quadricauda, A. falcatus** and **St. boreale** was increased by appropriate amount of analytical grade NaCl ranged from 0 to that inhibits population growth of each of these algae by more than 50 %.

Dimethoate

Dimethoate is the common name of dimethyl S-(N-methyl carbomyl methyl) phosphothioate. It is widely used in Egypt against cotton pest. It was tested with **St. boreale** and **A. paludosa.**

Bayluscide

The registerted trade mark (bayer 73), a 70 % formula of ethanol amine

salt of 5,2'-dichloro-4'-nitro salicylic anilide. This molluscicide is used extensively to eradicate the snail vectors of **Bilharriziasis,** namely **Biomophalaria alexandrina** and **Bulinus truncatus.** Bayluscide was investigated with **St. borcale** and **A. paludosa.**

After 96 h incubation, aliquots from control and cultures were examined and photographed using an inverted microscope equipped with photographic camera attachment.

RESULTS

Plate I shows the effect of salinity as NaCl on the morphology of **S. dimorphus, S. quadricauda, A. falcatus** and **St. boreale.** The above three photos represent normal cells grown in absence of NaCl. When **S. dimorphus** grown in 1 ppt NaCl, the cells remained weakly attached to each other in the coenobium and the cells showed a slight swelling. At higher concentrations of 2, 3, 4 and 5 ppt, a severe aberration accompaneid by increasing in cell volumes was noticed. The frequency of aberrant coenobia had increased with increasing NaCl concentrations.

For **S. quadricauda,** a slight increase in cell size was found at 2 ppt NaCl. With increasing NaCl concentrations to 4 and 6 ppt, the cells had significantly increased in volume accompaneid by longitudinal spleting the cytoplasm into two portions. At high doses of 8 and 10 ppt, swelling of the cells had increased, the spines of the terminal cells were absent and the cytoplasm divided into numerous parts. The frequency of aberrant coenobia increased with increasing NaCl doses.

In case of **A. falcatus,** there was no obvious aberration in the cells at 0.7 ppt NaCl. At high doses of 1.4, 2.1 and 2.8 ppt, the cell volumes had significantly increased compared with the control cells. At the highest dose of 3.5 ppt, the frequency of aberrant cells increased and eventually lysed.

Plate II shows the effects of the insecticide Dimethoate, molluscicide Bayluscide and NaCl on the morphology of **St. boreale.**

At 15 and 20 ppm Dimethoate, the cell volumes increased and the arms showed an obvious aberration. At the highest dose of 25 ppm, the frequency of aberrant cells increased and eventually lysed. Each cell was destroyed into two parts.

Bayluscide at concentrations of 300 and 400 ppb, caused bulging and bending in the treated cells. The arms showed a great aberration at one half of the cell, while they were completely disappeared at the other. At the highest dose of 500 ppb, the cells were destroyed into two parts at the isthmus. The majority of treated cells lost their arms and the cytoplasm lysed.

Plate III indicates the effects of Dimethoate and Bayluscide on the morphology of the diatom **A. paludosa.**

Dimethoate formed aberrant cells that were abnormally swollen with a granular central cytoplasm. At high doses of 12 and 15 ppm, the treated

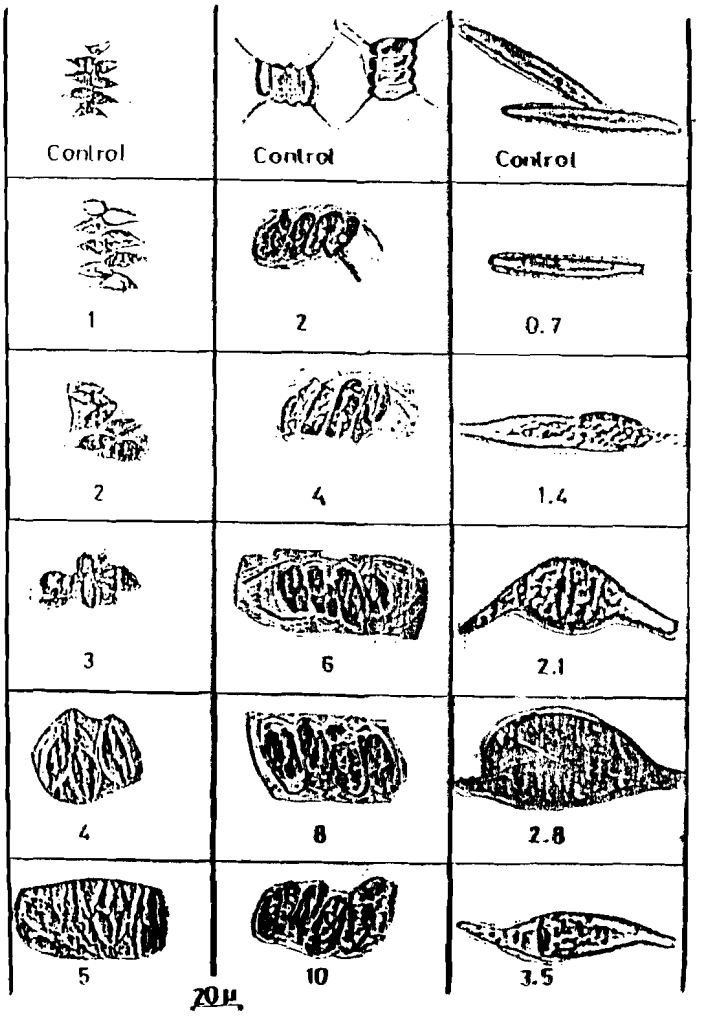

PLATE I

Morphological responses of the test algae to selected concentrations of Na Cl ($g.l^{-1}$, ppt).

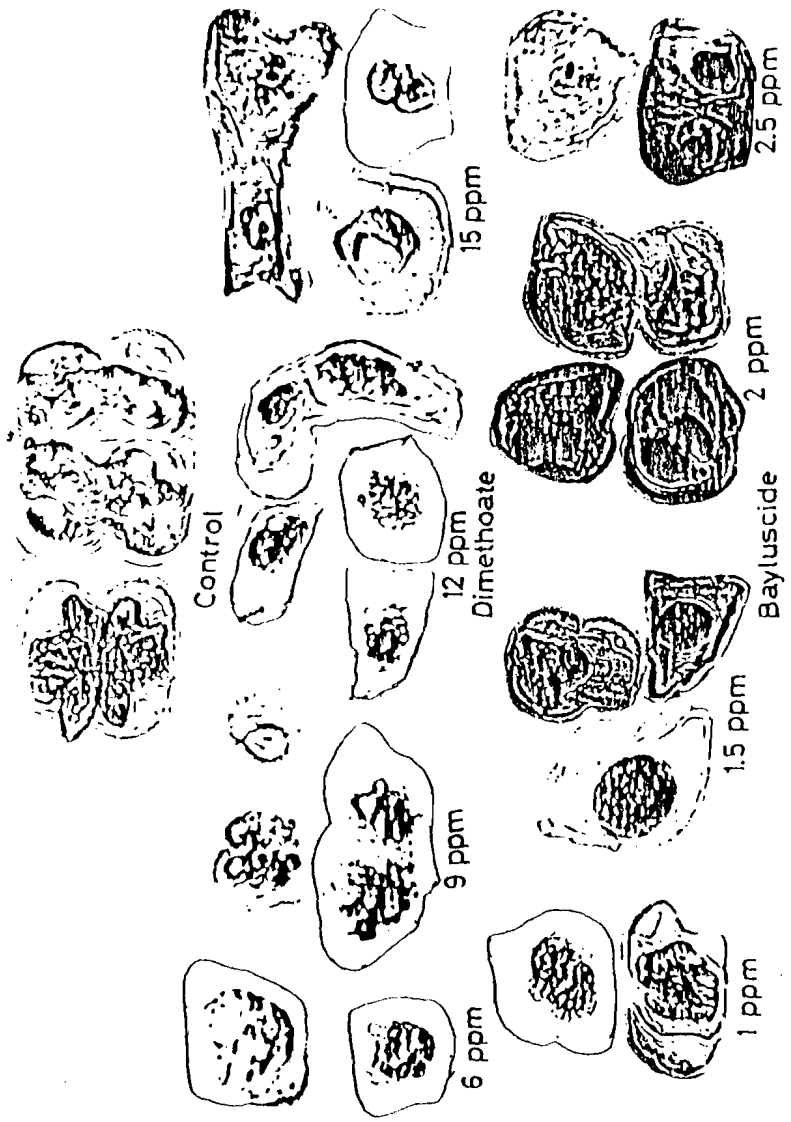

PLATE II

Effects of Dimethoate and Bayluscide on the morphology of **Amphiprora paludosa.**

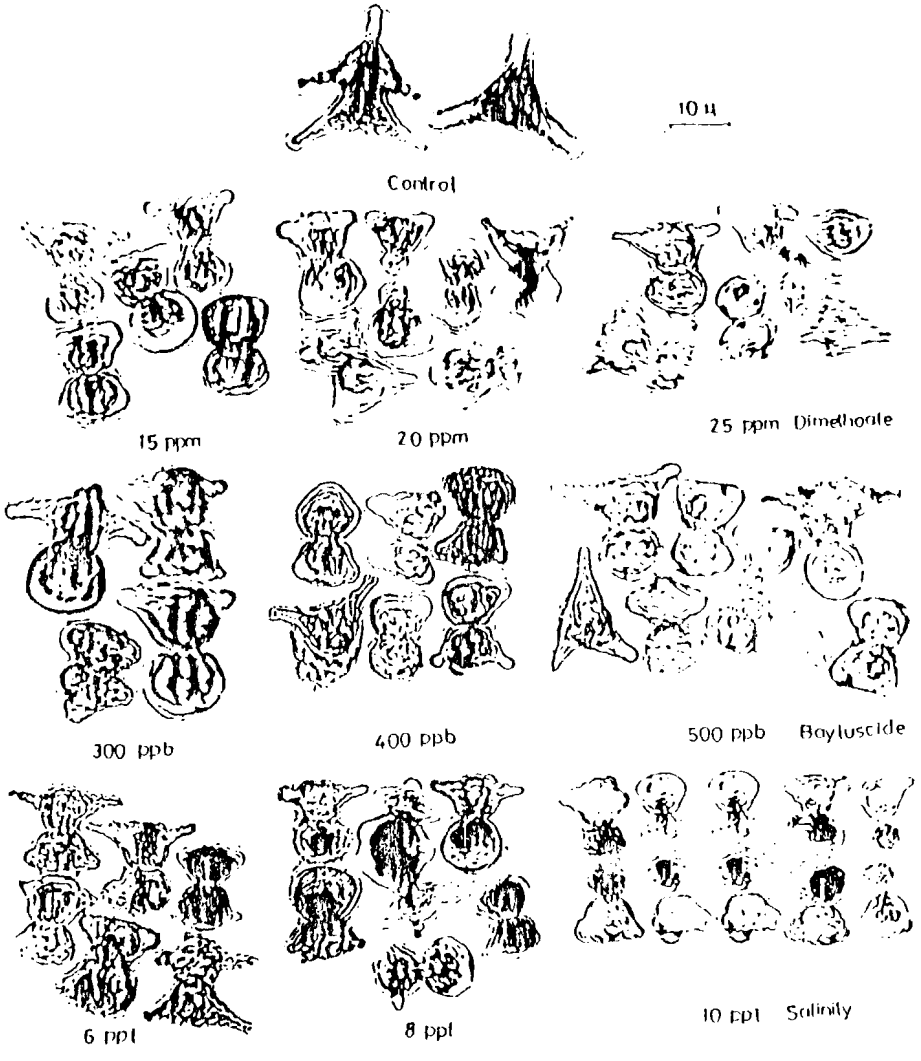

PLATE III

Effect of Na Cl, Dimethoate and Bayluscide on the morphology of the chlorophyte **Staurastrum boreale.**

cells showed abnormal elongation with a reduced central cytoplasm. The frequency of distorted cells increased with increasing Dimethoate concentrations.

On the other hand, Bayluscide had an obvious inhibitory effects on cell contents without alteration in the cell structure even at the highest dose. The cytoplasm of the treated cells became granular and eventually lysed.

DISCUSSION

The present investigation revealed the morphological responses of the test algal cells brought about by short term exposure to the concentrations of NaCl and pesticides. The quantification of these changes can be used as a clue towards either the direct physiological understanding of the changes themselves or as means of focusing attention on the key structural cellular changes induced by salinity and pesticides.

It has been demonstrated that **S. dimorphus, S. quadricauda, A. falcatus** and **St. boreale** cells showed an obvious increase in size with increasing NaCl concentrations. In order for the cells to have increased in size during the exposure period (96-h), either there was an active synthesis of cellular material including the cell wall, or there was depolymerization of mucopolymers in the wall matrix allowing the wall to stretch. Since there was no evidence of wall rupture, the swelling of the treated cells was mainly due to changes in the cellular metabolites. It was found that carbohydrate content of the test algae decreased with increasing NaCl concentrations (Ibrahim et al., 1985). This is mainly due to the increase in the indogenous inorganic phosphate level which plays an important role in controlling carbon distribution between starch and glycerol under the influence of NaCl (Gimmler and Moller, 1981). They also stated that the actively growing algal cells posses sufficient amounts of storage dopts that can be converted into osmotically active materials under NaCl stress. Also the permeability of the treated algal cell wall had increased to a limit which permits the exchange between the cellular and extracellular ions to equiliberate the differences in osmotic pressure.

On the other hand, **A. paludosa** cells showed an obvious increase in size under the influence of Dimethoate. This was mainly due to rupturing the cell walls. In the meantime, the cell contents are shrinking. These aberrations were also observed with **St. boreale** cells when treated with Dimethoate. The rupture in the cell wall was obviously noticed at the highest dose (Plate II).

Bayluscide reduced the cytoplasm of the treated **A. paludosa** and **St. boreale** cells without obvious increase in cell size. Its high doses caused a reduction in cell size and volume. This can be realized to shrinking of cytoplasm and a pulling away of plasma lemma from the cell wall.

This study has demonstrated the efficacy of utilizing the morphological changes for understanding the physiological mechanisms of phytoplankton cells grown under the influence of salinity and pesticides. The reported morphological responses can be extended to other pesticides and other phytoplankters in order to obtain the key structure changes and therefore places the investigator in a better position to understand the physiological mechanisms.

REFERENCES

Berland, B.R., D. J. Bonin, O.J. Guerin-Ancey, V.I. Kapkove and D.P. Arlhac, 1977. Action de metaux lourds a des doses sublethales sur les caracteristiques de la croissance chez la diatomee **Skeletonema costatum. Mar. Biol.**, 42: 17 - 30.

Davies, A.G., 1974. The growth kinetics of **Isochrysis galbana** in cultures containing sublethal concentrations of mercuric chloride. **J. Mar. Biol. Ass., U.K.**, 54: 157 - 169.

Gimmler, H. and E.M. Moller, 1981. Salinity dependent regulation of starch and glycerol metabolism of **Dunaliella parva. Cell and Environment**, 4:367 - 375.

Ibrahim, E.A., 1978. **Effect of some pollutants on growth and metabolism of some freshwater planktonic organisms.** Ph.D. Thesis, Cairo University, 173 pp.

Ibrahim, E.A., A.S. Shaaban and O.E. Taha, 1985. Growth and metabolic response of the freshwater unicellular algae **Scenedesmus dimorphus, Scenedesmus quadricauda** and **Ankistrodesmus falcatus. Bull. Inst. Oceanogr. and Fish., ARE**, 11:183 196.

Kester, D.R., I.W. Dredall. D.N. Connors and R.M. Pytokowicz, 1967. Preparation of artificial seawater. **Limol. Oceanogr.**, 12:176 - 179.

Morel, N.M., J.G. Renter and F.M. Morel, 1978. Copper toxicity to **Skeletonema costatum. J. Phycol.**, 14:43 - 48.

Nuzzi, R., 1972. Toxicity of mercury to phytoplankton. **Nature**, 237:38 - 39.

Pringsheim, E.G., 1946. **Pure cultures of algae, preparation and maintenance.** Cambridge Univ. Press, 119 pp.

Rachlin, J.W., T.E. Jensen, M. Baxter and V. Jani, 1982. Utilization of morphometric analysis in evaluating response of **Plectonema boryanum** (Cyanophyceae) to exposure to eight heavy metals. **Arch. Environm. Contam. Toxicol.**, 11:323 - 333.

Rosko, J.J. and J.W. Rachlin, 1977. The effect of cadmium, copper, mercury, zinc and lead on cell division, growth and chlorophyll-a content of the chlorophyte **Chlorella vulgaris. Bull. Torry Bot. Club**, 104: 226 - 233.

Sicko-Goad, L., 1982. A morphometric analysis of algal response to low dose short-term heavy metal exposure. **Protoplasma**, 110:75 - 86.

Sunda, W. and R.R.L. Guillard, 1976. The relationship between cupric ion activity and toxicity of copper to phytoplankton. **J. Mar. Res.**, 34:511 - 529.

Thomas. W.H., J.T. Hollibough and D.L.R. Seibert, 1980. Effects of heavy metals on the morphology of some marine phytoplankton. **Phycologia**, 19, (3):202 - 209.