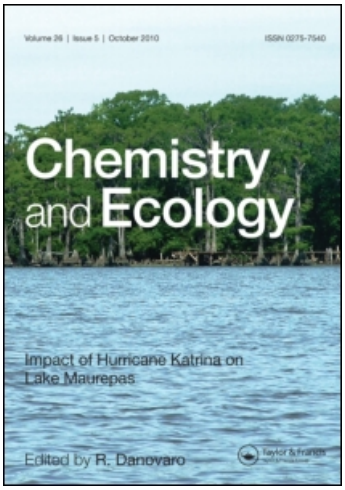

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

Determination of Toxic Pollutants in Water Using A Marine Phytoplankton Dunaliella Bioculata and Doppler Laser Velocimetry

Sushila Krishnaswamy Chang[a]
[a] Department of Biotechnology, Ngee Ann Polytechnic, Singapore

To cite this Article Chang, Sushila Krishnaswamy(1995) 'Determination of Toxic Pollutants in Water Using A Marine Phytoplankton Dunaliella Bioculata and Doppler Laser Velocimetry', Chemistry and Ecology, 10: 1, 87 — 95
To link to this Article: DOI: 10.1080/02757549508035332
URL: http://dx.doi.org/10.1080/02757549508035332

Chemistry and Ecology, 1995, Vol. 10, pp. 87–95
Reprints available directly from the publisher
Photocopying permitted by license only

DETERMINATION OF TOXIC POLLUTANTS IN WATER USING A MARINE PHYTOPLANKTON *DUNALIELLA BIOCULATA* AND DOPPLER LASER VELOCIMETRY

SUSHILA KRISHNASWAMY CHANG

Department of Biotechnology, Ngee Ann Polytechnic, 535 Clementi Road, Singapore 2159

(Received 8 October 1993)

This paper reports an experimental study of the response of the unicellular flagellated algae *Dunaliella bioculata* to toxic pollutants in water using the 'Doppler laser velocimetry' method. The response to toxic pollutants was encouraging and indicates that *Dunaliella bioculata* is a good biological model for the detection of toxic pollutants in water.

KEY WORDS: *Dunaliella*, bioassay, water quality, toxicity, lindane, Doppler laser velocimetry

INTRODUCTION

The limitations in water resources in areas of the developing world and the limited availability of water supplies to many of the rapidly expanding cities of Asia, and their expanding demands for potable water, emphasises the need to protect water resources from pollution. In recent years the water resources of many developing nations are increasingly subject to pollution from toxic discharges from factories and from agricultural run-off. Many synthetic organic chemicals resulting from industrial contamination of surface water pose a potential health risk to consumers.

The extensive use of pesticides in the agricultural and domestic environments has resulted in the contamination of ground and surface water resources. Many surface water sources are found to have a number of pesticides such as ethylene dibromide, and dibromochloropropane.

In the recent years many ground water sources have been found to contain measurable concentrations of volatile organic chemicals. These volatile organic chemicals are potentially dangerous and pose a possible health risk because some are probable or known human carcinogens.

One of the most difficult tasks in water supply engineering is to ensure that raw water entering a water treatment plant is free form toxic pollutants as treatment plants are not designed to remove them and they could be carried through into drinking water supply. The traditional method used in most water treatment plants is to employ a fish bioassay station to monitor toxic chemicals in water. Although this method is sensitive to toxic agents, it has several drawbacks; sophisticated electronic equipment to monitor the physiological activities of the fish is needed and fish bioassay systems are costly to install and to maintain.

This paper presents an alternative method for the detection of pollutants by monitoring the motility/speed of a unicellular marine biflagellate phytoplankton (algae) *Dunaliella bioculata*, using the 'Doppler laser velocimetry' method.

THE OBJECTIVES

This paper discusses a laboratory study with the following objectives:

1) to propose an alternative method to the conventional fish bioassay, to monitor the toxic chemicals in raw water;
2) to highlight the rapidity of response of the unicellular algae *Dunaliella* to toxic chemicals;
3) to formulate a method based on the statistical performance of a large number of organisms;
4) to advocate the economy of large scale cultivation and maintenance of the biological organism *Dunaliella* in comparison to other methods.

MATERIALS & METHODS

Dunaliella is grown in an aqueous medium fortified with nutrients (Shepard, 1970). The medium can be sterilized by autoclaving for 20 minutes at 100°C and at 1.5 atm. pressure, or by the use of millipore filtration at 0.22 μm. The algae may be grown in large transparent tanks equipped with paddle stirrers to provide constant agitation of the medium. They are photosynthetic organisms and thrive well in tropical temperatures and normal daylight conditions. *Dunaliella* is ovoid with an approximate length of 30 μm. It is armed with a pair of flagellae at its anterior pole. *Dunaliella* moves with the aid of the flagellae; they are approximately 50 μm in length and resemble the cilia lining the bronchial tubes in human lungs (Marano *et al.*, 1985). *Dunaliella bioculata* is our chosen biological model as it has been used previously to analyse the toxic effects of the gaseous phase of cigarette fumes (Izard, 1967). *Dunaliella bioculata* is also preferred to *Chlamydomonas* (another algal species) because it lacks the pectocellulosic cell wall.

THE EQUIPMENT

Doppler laser velocimetry uses a beam of monochromatic light from a helium-neon laser directed on to the population of algae. The laser utilised is the helium-neon laser of 6 mw ($\gamma = 6320$ A) and the scattering angle is equal to 8°. The schematic diagram shown in Figure 1 illustrates the operation of the Doppler laser velocimetry apparatus. The helium – neon laser beam is reflected at the mirror M1 and is separated into two parts by L1. One part of the laser beam is polarised by 'P' and is directed through a cuvette containing the algal population. The length of the cuvette is limited to 2 mm to minimise the effect of multiple diffusion. At L2, the emerging laser beam from the cuvette meets the beam which has not passed through the cell, i.e. the beam separated into two parts at L1 meets again, and converges towards a photomultiplicater. The photomultiplicater compares the two beams and the frequency of the beam diffused by the algae is analysed using a spectrum analyser.

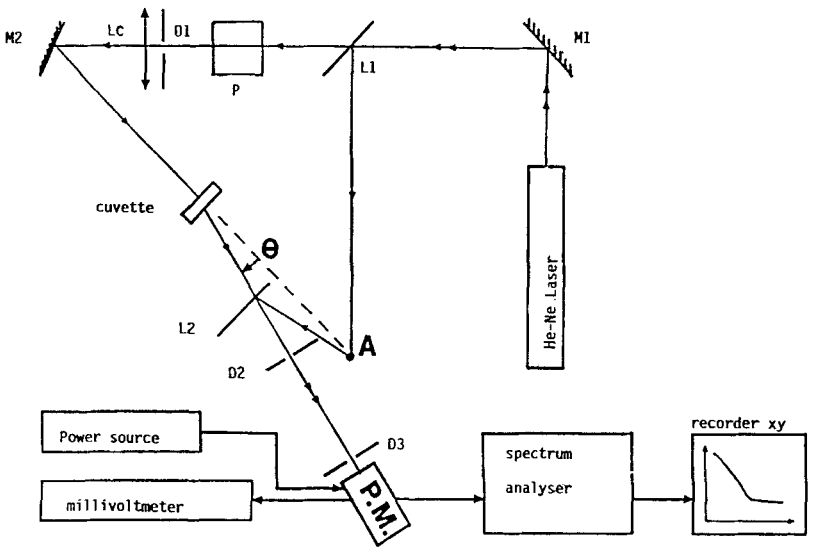

Figure 1 Experimental set-up of Doppler laser velocimetry
M1 and M2 : mirrors
L1 and L2 : separators of diffused light
P : polariser
PM : photomultiplicater
A : oscillator
Θ : scattering angle

As the algae are in continuous motion the re-emitted laser beam has a frequency that differs from that of the initial incident light. This difference in frequency is due to the Doppler effect and is proportional to the speed of algal displacement. It can be measured using the classical formula for the Doppler effect. Hence any variations in the motility of *Dunaliella* due to the presence of chemicals or toxic agents in aqueous medium can be quantified instantaneously (Boon and Nossal, 1974).

Our data are based on experiments conducted on *Dunaliella* using three different contaminants:

1) lindane, an organochlorine insecticide used in agricultural and domestic environments;
2) copper sulphate, a common algicide used in the control of algal blooms in reservoirs;
3) acrolein, an aldehyde and potential carcinogen to some animals.

EFFECTS OF LINDANE ON MOTILITY OF *DUNALIELLA*

Lindane (γBHC) is the name of a commercial product containing 99% of the γ isomer of hexachlorocyclohexane. It is a lipophilic molecule and has a molecular weight of 290 g. The other isomers, α, β, δ, ε of hexachlorocyclohexane, are less used as insecticides.

Lindane is one of the most effective insecticides. It is five to twenty times more toxic than DDT for certain insects. The activities of the isomers are less well known. Microscopic observation permitted us to identify the effective concentrations of lindane and its δ isomers as these concentrations provoked visible alterations in the movement of the algae. Observations of the modal algal velocity of lindane concentrations of 5, 10, 15, 20 and 30 μgml^{-1} were made. Similarly, the observation of the modal velocity of the algae for the δ isomers at 3, 15, 10, 20 and 30 μgml^{-1} were made. These observations are illustrated graphically in Figure 2 (lindane) and Figure 3 (δ isomer). The graphs in Figures 2 and 3 show that within the first twenty minutes of contact with the contaminant, the velocity ratios vary with concentration. After twenty minutes or less, the modal velocity achieves a steady state. The graphs also show that with 5 to 10 μgml^{-1} of lindane, and also the δ isomer, there appears to be a transient 'excited' stage which lasts approximately five minutes when the velocity actually increases. At higher concentrations of contaminant, the algae have a notably marked reduced velocity and the decrease is proportional to the increase in the concentration of the contaminant (Krishnaswamy, 1985).

Figures 4 and 5 show video images of the trajectory of *Dunaliella* cells under normal conditions (control) with no added contaminant and *Dunaliella* cells after 30 minutes of exposure to 30 μgml^{-1} of lindane. The cells were filmed with a Zeiss

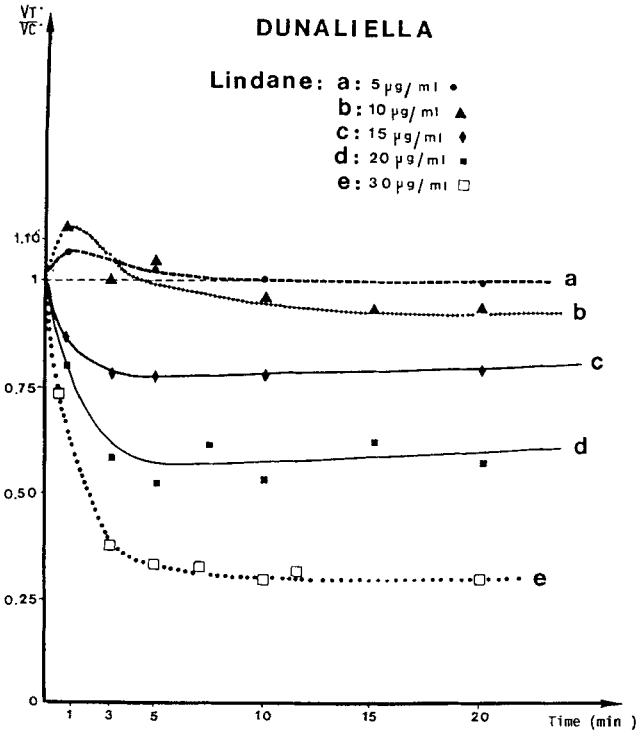

Figure 2 Effect of lindane (γ HCH) on the motility of *Dunaliella* during the first 20 minutes of action. All measurements were done by Doppler laser velocimetry. Note change of scale. VT· is the modal velocity of treated cells. VC· is the modal velocity of control cells. (5 μgml^{-1}) = 1.5×10^{-5}M, 10 μgml^{-1} = 3.4×10^{-5}M, 15 μgml^{-1} = 5.2×10^{-5}M, 20 μgml^{-1} = 6.8×10^{-5}M, 30 μgml^{-1} = 1×10^{-4}M).

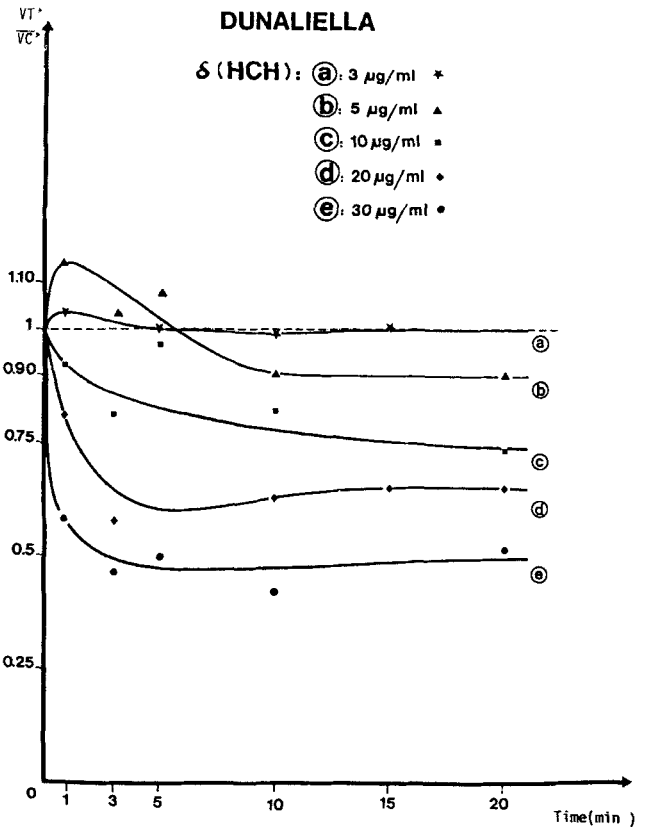

Figure 3 Effects of δ HCH on the motility of Dunaleilla during the first 20 minutes of action. All measurements were done by Doppler laser velocimetry. VT' is the modal velocity of treated cells. VC' is the modal velocity of control cells. (3 μgml^{-1} = 1 × 10^{-5}M, 5 μgml^{-1} = 1.5 × 10^{-5}M, 10 μgml^{-1} = 3.4 × 10^{-5}M, 20 μgml^{-1} = 6.8 × 10^{-5}M, 30 μgml^{-1} = 1 × 10^{-4}M).

microscope equipped with a dark field condenser and connected to the AVEC/VIM system (Hamamatsu).

The control cells (Figure 4) demonstrate a slim, direct sinusoidal trajectory. The cells treated with lindane (Figure 5) have a thicker trajectory as the *Dunaliella* tend to vibrate on their axes.

EFFECTS OF COPPER SULPHATE ON THE MOTILITY OF *DUNALIELLA*

The curves shown in Figure 6 indicate the effect of copper sulphate on the motility of Dunaliella. A decrease in velocity during the first hour of contact is observed. This decrease in velocity is proportional to an increase in the concentration of copper sulphate from 10 to 100 μgml^{-1}. After three hours exposure to the higher concentration, the inhibition is complete and the velocity is almost zero. On observing the algae microscopically the algae appeared dead. However, for copper sulphate

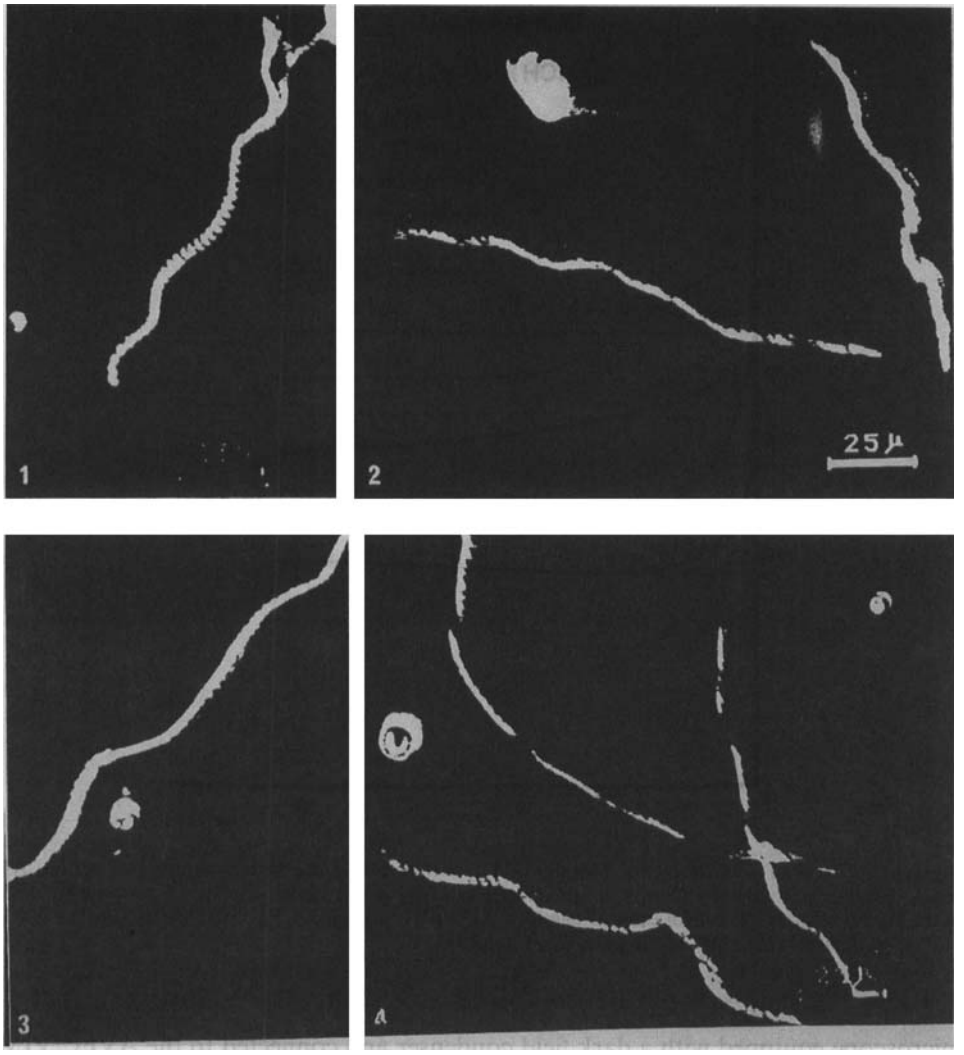

Figure 4 Trajectory of control cells. Video images were obtained with a video camera fixed on to a Zeiss microscope equipped with a dark-field condenser and connected to the AVEC/VIM system (Hamamatsu).

concentrations of 10, 20 and 50 μgml^{-1} the algae appear to recover from inhibition and return to their original activity within 24 hours.

EFFECTS OF ACROLEIN ON THE MOTILITY OF *DUNALIELLA*

Acrolein is an unsaturated aldehyde. It is an industrial pollutant present in synthetic textiles and in plastic materials. Acrolein causes bronchial irritations and is known to be carcinogenic in some animals. The experimental effects on *Dunaliella* are

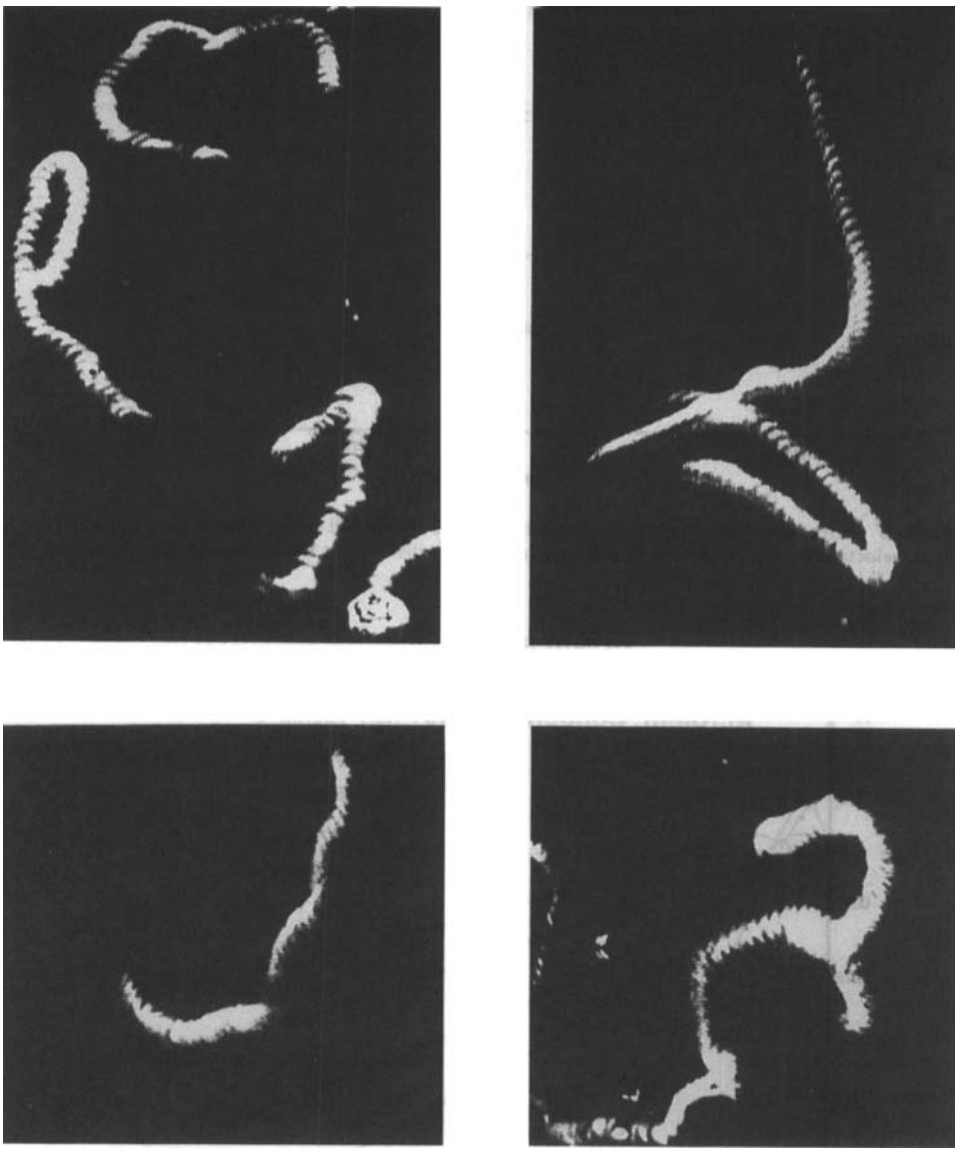

Figure 5 Trajectory of *Dunaliella* cells aftere 30 minutes exposure to lindane (30 $\mu g ml^{-1}$ or $1 \times 10^{-4} M$). Video images were obtained with a video camera fixed on to a Zeiss microscope equipped with a dark-field condenser and connected to the AVEC/VIM system (Hamamatsu).

illustrated in Figure 7. The concentrations of liquid acrolein used were 1, 2, 5, 10 and 20 $\mu g ml^{-1}$. Experiments indicate that during the first twenty minutes of exposure the effects of acrolein are very pronounced. However, at all concentrations there appears to be a transient 'excited' stage during the first two minutes exposure when velocity actually increases as seen for lindane. After 15–20 minutes, no visible movement was noticed for concentrations of 10 and 20 $\mu g ml^{-1}$.

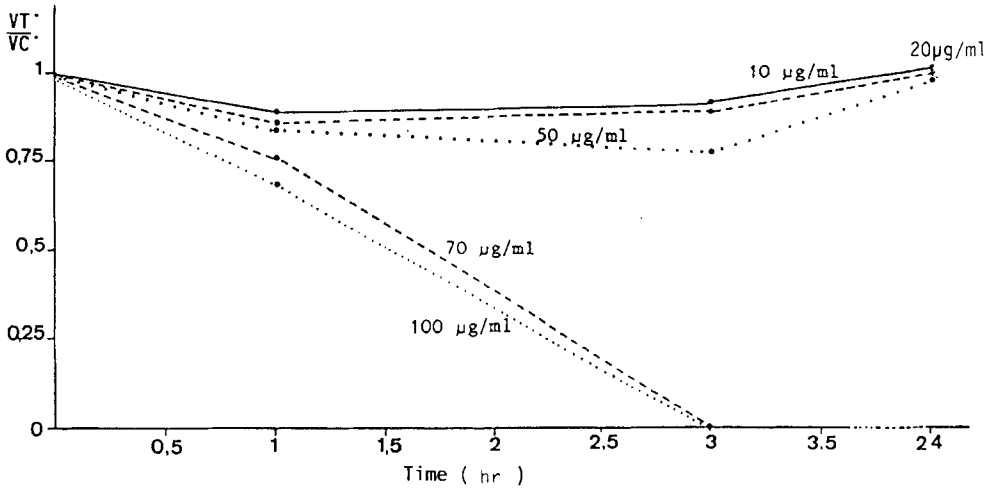

Figure 6 Effect of copper sulphate on the motility of *Dunaliella*. All measurements were done by Doppler laser velocimetry. VT' is the modal velocity of treated cells. VC' is the modal velocity of control cells.

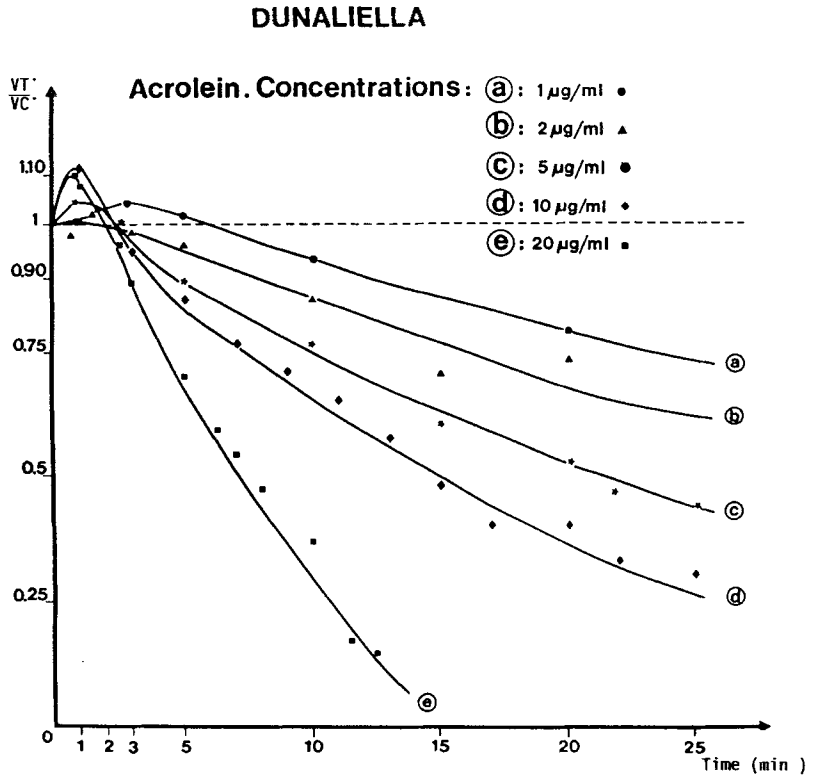

Figure 7 Effect of acrolein on the motility of *Dunaliella*. All measurements were done by Doppler laser velocimetry. VT' is the modal velocity of treated cells. VC' is the modal velocity of control cells.

CONCLUSIONS

The findings of our experimental studies are encouraging. Experiments have indicated that *Dunaliella* is sensitive to toxic pollutants in water. The responses to high concentrations of toxic chemicals could be obtained within two minutes. However, even with lower concentrations the results can be obtained within fifteen minutes to half an hour.

Dunaliella can be grown in small scale tanks in a tropical climate. The costs of such cultivation are low. The cost of Doppler laser velocimetry equipment is also relatively low. The remarkable advantage of this method is that the response to chemical toxins are based on large number of organisms compared to fish monitoring tests where it is usual to use relatively few fish for bioassay and a probit transformation to analyse the toxic effects. The Doppler laser velocimetry method is based on the statistical performance of a large number of organisms.

Current research shows that the Doppler laser velocimetry method can be upgraded to identify different forms of algal movements. Correlations could be established between the types of pollutants and the specificity of the different movements of the algae treated with the pollutants. *Dunaliella* could also be used as an indicator organism for detection of toxic pollutants in water either by using Doppler laser velocimetry or by other methods.

References

Boon J.P. and Nossal R. (1974) Inelastic light scattering by large structural particles. *Biophysical Journal* **4:** 865–880.

Krishanaswamy S. (1985) Quantification de la motilité flagellar chez *Dunaliella* par velocimetrie Doppler laser et par microcinematographie. Mise au point d'un test de cilio – inhibition. Doctorate thesis, Universite de Paris VII, France.

Izard C. (1967) Sur la multiplication du *Dunaliella bioculata* en presence de phase gazeuse de fumée de cigarette et sur l'obtention de mutations en presence d'acroleine. *C.R. Acad. Sci. Paris*, **265**, 1799.

Marano F., Santa-Maria A. & Krishnaswamy S. (1985) The flagellar apparatus of *Dunaliella bioculata*: isolation of basal body flagellar root complex. *Protoplasma*, **127**, 82–92.

Shepard D.C. (1970) Axenic cultures of *Acetabularia* in synthetic media. In: *Methods in Cell Physiology* Prescott D. (Ed), Academic Press New York and London, **4**, 46–69.