CHAPTER VI
SUMMARY AND CONCLUSIONS

The impact of some commonly used biocides on three microalgal cultures was investigated. The two unicellular algae selected are of importance for mariculture as larval and post larval food in hatchery systems. The fresh water algae collected from paddy fields, formed a mixture representing a natural population.

In order to find out the effective concentration of biocides that would inhibit the growth of algae by 50%, 96 hour bioassay tests were conducted. The number of cells, and rates of carbon production were determined after 24, 48, 72 and 96 hour and they were compared with that of control. The variations in pigment contents were also estimated. The significance of using three parameters is that one parameter does not give accurate results.

The results of 96 hour bioassay tests revealed that there was variation in growth responses of microalgal species with three biocides. In almost all cases the rate of carbon production was found to be more affected than other parameters. It was also revealed that EC50 values varied with the duration of experiment.

The 96 hour bioassay tests did not give a real picture of the impact of various biocides. Therefore, long term experiments were conducted to
understand the effect of different biocides on physiological and biochemical characters of three microalgal cultures. Each experiment was of 20 days duration. A total 21 sets of experiments were conducted.

Among the physiological parameters, growth, rate of carbon production, pigments such as chlorophyll a and b in green algae and chlorophyll a and c in brown algae as well as carotenoids were estimated during the period of experiments lasting 20 days. Biochemical parameters such as protein and carbohydrate contents were also estimated.

The five different biocides selected were of common use in agricultural fields. Hence their residues may be ultimately reaching the aquatic ecosystem. In nature mixtures of biocides occur and so combinations of five different biocides were also tested.

Due to the low water solubility in the case of ‘B.H.C’ and ‘Carbaryl sevin’, acetone was used as the solvent. Results showed that acetone has no stimulatory or inhibitory effect on microalgal culture.

The growth of algae was found to be affected by the presence of biocide. The effect of different concentrations was specific for each biocide. The lower concentration of most of the biocides caused a stimulatory effect especially in *T. gracilis*.

The percentage inhibition of algal growth was found to increase with increased concentration of biocide. It was also observed that almost all
biocides showed maximum toxic effect in the culture at the commencement of application. With the ageing of culture the toxic effect decreases. So it can be concluded that most of the biocides show an immediate shock response followed by a habituation response.

The results of experiments with mixed culture of fresh water algae showed that species composition was affected. In the natural population of phytoplankton, the chlorophycean members were found dominating, *Chlorella ovalis* was the dominant species. The other species especially *Nitzchia longissima* was only nominally represented and this species was found seriously affected by biocide treatment.

The rate of carbon production was found to be affected by biocide treatment. In this case also lower concentration of some of the biocides showed stimulation in carbon production. But the percentage stimulation was less compared to that observed for growth of microalgae.

With the increase in the concentration of biocide, the carbon production was inhibited. The maximum inhibition was observed at the beginning of the experiment. Compared to gross carbon production, net carbon production was less. The difference in gross and net carbon production may be due to the fact that, at higher concentrations of biocide treatment, due to increased respiratory rate, the reserve food material is completely metabolized and that leads to decrease in net production. The percentage inhibition in carbon production was found decreased with the age of culture.
The pigment content was also affected by biocide treatment. At lower concentrations the chlorophyll pigment was stimulated especially in _T. gracilis_ and mixed culture of fresh water algae. The higher concentrations showed very low values for pigments especially at the beginning of the culture.

In the case of control cultures, after an exponential phase, the chlorophyll pigments decreased but carotenoid content increased. But a reverse condition was observed in treated cultures especially at higher concentrations. In the case of _D. inornata_ the pigment content was very much affected.

Another peculiarity observed was that even though the treated cultures show inhibitory response with respect to pigment, the percentage inhibition was less compared to photosynthetic rate. It shows that chlorophyll activity was inhibited. Among the estimated pigments, dead chlorophyll may also come.

The biochemical products like proteins and carbohydrate of algae were found affected by biocide treatment. In the case of control cultures, the protein content was found to decrease with age of culture and carbohydrate content was found to increase. The lower concentrations of some of the biocides tested showed stimulation of proteins and carbohydrates. But the percentage stimulation was high with respect to carbohydrate content. This showed that carbohydrate was resistant to biocides than protein. With the increase in the concentration of biocides, the protein
and carbohydrate content was found inhibited. One peculiarity observed with 'Nuvacron' treatment is that in the case of _T. gracilis_ the protein synthesis was not much affected even at 100 ppm concentration of 'Nuvacron. This may be because of the fact that, the 'Nuvacron' undergo degradation and these metabolites may act as nutrients in the medium. These increased nutrient uptake may ultimately leads to increased protein content. Thus it can be concluded that there was a relationship between the available nutrients and protein synthesis in microalgae.

Morphological deformities like cell well breakage, chloroplast disarrangement, gigantism of some of the cells, and even clumping of cells were also observed as a result of biocide treatment.

The results of bioaccumulation studies revealed that the estuarine form of an alga, _T. gracilis_, had an ability to absorb the organochlorine insecticide from the medium and got accumulated in it. The degree of accumulation was found to increase with the concentration of toxicant in the medium and lengthened duration of contact. It was observed that nearly 86% of the toxicant present in the medium was concentrated by the alga at 4 ppm concentration.

Statistical analysis of data was done using analysis of variance. In most cases the variations in growth responses of algae as a result of biocide treatment were highly significant.

Out of the three microalgal cultures investigated the estuarine form _T. gracilis_ was found to be a resistant species. Because of this character
and also due to the capacity to accumulate toxicant this alga can be recommended for waste water treatment as scavengers.

Among the different biocides, the fungicide - 'Cuman L(R) was found most toxic. The organophosphate insecticide 'Nuvacron' was less toxic and because of its highly degradable nature it can be recommended for field use.

The combination of biocides showed more toxicity than individual biocides, except the fungicide 'Cuman L(R).

It was observed that the lethal limit of five different biocides tested here was low as compared to the concentrations of biocides that farmers are applying in the field. So caution is advised in applying different biocides in the field.

The above findings revealed that the interaction between different biocides and the microalgae could play a significant role in the alterations in aquatic ecosystem.

Further research is needed especially in the case of biodegradation and bioaccumulation characters of microalgae. This area should focus on the identification of kind of algae that take up different pollutants into their cells and how the pollutants are degraded or are passed on to consumer organisms.
With the liberalization of global trade and tariff it is possible that several toxic pesticides might reach the country in the name of plant protection and increasing agricultural production. Some of this algae can remain as very useful sentinels for protecting the ecosystem from superfluous toxicity and bioaccumulation.

The protection of aquatic resources requires the quantities of biocides used to be held to the minimum possible. It is hoped that the use of natural biocides and also by adopting the biological control of pests would provide an answer for a safer environment in future years.