CHAPTER II
In this chapter on literature review, greater stress has been given to the earlier work done on important organic biocides and their effects on microalgae. Even though very few literature are available on this aspect during 1960s, Most of the earlier works done on this aspect are after 1970s, and hence literature was collected from that period onwards. For some organic biocides consistent data have been obtained by different workers with regard to their effects on microalgae. For a few biocides, the studies have included both pure culture and natural population. In view of the difference in chemical and toxicological properties of different biocides and the variation in experimental procedures of different workers (eg: axenic and non-axenic culture), caution is advised in comparing some of the data.

The biocides used for the present study come under three groups; insecticides, herbicides and fungicides.

2.1. INSECTICIDES

Based on their chemical nature, the insecticides are classified into three groups; (a) Organochlorines (b) Organophosphates and (c) Carbamates.
a) Organochlorines:

As the organochlorines are widely used for the past four decades, the toxicological effects of these insecticides have been studied by various investigators.

Raghu and Macrae (1967) reported that Y-BHC used for insect control in rice fields, promoted algal growth than in untreated fields, and this result was established in pot experiments. The insecticides selectively stimulated the indigenous blue green soil algae.

Wurstur (1968), who emphasized the important contribution of phytoplankton to global photosynthesis and the consequences of interference with this process, found that concentration of DDT below 10 ppb inhibited photosynthesis in marine planktonic algae. The toxicity of DDT to the diatom Skeletonema costatum, increased with decreasing cell concentration and it was concluded that low levels of DDT in natural waters might have deleterious effects on the phytoplankton. A similar conclusion was reached by Sodergren (1968) who reported that growth of Chlorella sp. was affected by less than 0.3 ppb DDT.

A study was carried out to determine what affect the entry of three broad spectrum insecticides may have on algal population (Christie, 1969). The organochlorine tested was DDT. It was found that the three pesticides, varied in their degree of toxicity to algae and also in the extent to which they were degraded in the presence of algae.
DDT uptake and metabolism by marine diatom were reported (Keil and Priester, 1969), and it was concluded that the diatom was capable of absorbing and concentrating DDT above the level in sea water. DDT was metabolised by this organism to DDE.

Algal species isolated from various marine environments responded differentially to DDT (Menzel et al., 1970) whereas the estuarine naked green flagellate Dunaliella tertiolecta was insensitive to 1000 ppb DDT. Photosynthetic $^{14}$C uptake by the marine diatom Skeletonema costatum and the coccolithophorid Coccolithus huxleyi was significantly reduced to above 10 ppb DDT. Menzel et al., (1970) further showed that cell division in the diatom, but not in Coccolithus, was prevented by 100 ppb DDT. The most DDT sensitive organism tested by Menzel et al., (1970) was also a marine diatom, Cyclotella nana, in which $^{14}$C uptake and growth were affected at DDT levels of 1 ppb and 100 ppb respectively.

Growth and $^{14}$C assimilation in low-density population of Scenedesmus quadricauda were inhibited by 0.1 ppm DDT. (Stadnyk et al., 1971). But, contrary to this report Morgan (1972) reported that growth of green algae Chlamydomonas reinhardii was not appreciably affected by DDT at levels upto 20 ppm and there was no significant effect on $^{14}$C uptake.

Mosser et al., (1972 a) confirmed that Dunaliella tertiolecta was unaffected by 1000 ppb DDT and that other organisms, including Euglena gracile and Chlamydomonas reinhardii were also relatively resistant.
Mosser et al., (1972 b) investigated the effects of DDT on mixed cultures of marine algae, containing a 'sensitive', diatom (Thalassiosira pseudonana) and a resistant green alga (Dunaliella tertiolecta) in equal proportions. It was found that the growth of D. tertiolecta was not inhibited at any DDT level tested. However, T. pseudonana grew faster and soon out numbered D. tertiolecta. D. tertiolecta in control cultures, was affected by 100 ppb of DDT to the extent that its competitive success was significantly diminished, eventhough T. pseudonana was unaffected by 10 ppb DDT in the pure culture. Mosser et al., (1972 b) pointed out that DDT can occur in natural waters at levels equivalent to those which caused a marked change in species ratio in their experiments and considered the ecological implication of such a pesticide induced alternations in phytoplankton populations, via effects on the selectively grazing zooplankton.

Photosynthetic $^{14}$C fixation by Scenedesmus quadricauda was not significantly inhibited by DDT at concentrations upto 1 ppm, although the metabolite DDE was inhibitory (Luard 1973).

Recognising that in nature, organisms may be exposed to several toxicants simultaneously, Mosser et al., (1974) investigated the effects of mixture of organochlorines on the growth of marine diatom Thalassiosira pseudonana. Because of their ubiquity, chlorinated hydrocarbons were considered to be likely environmental contaminants. Results indicated that interaction between the organochlorines may occur, causing their toxicity to phytoplankton. DDE, the universal pollutant derived from
DDT, and polychlorinated biphenyls (PCB's are industrial organochlorines) were far more inhibitory to T. pseudonana in combination than they were individually. In complete contrast, the addition of DDT (500 ppb) to cultures, whose growth had been prevented by PCB's (50 ppb) substantially restored growth (Mosser et al., 1974).

Butler et al., (1975a) reported the effects of various concentrations of insecticides on the growth of 36 isolates of planktonic algae. Among the different insecticides tested, organochlorine insecticide was found to be more toxic. Powers et al., (1975) reported the toxicity of DDE to a marine dinoflagellate and it was found to be highly toxic to Exuvieilla baltica. Concentrations as low as 0.1 parts per thousand million (10^9 ppm) significantly inhibitd growth for at least 24 hur as measured by cell number and growth rate.

DDT and PCB's have been observed to reduce the rate of cell division, in marine phytoplankton, thereby indirectly reducing the total photosynthetic carbon fixation in treated cultures (Fisher 1975). Total marine photosynthesis will likely remain undiminished by these compounds, although alteration in phytoplankton communities through selective toxicity could affect herbivore population.

The effect of endrin on primary production in a pond ecosystem was reported (Nassar, 1976). The results clearly indicated that endrin did affect the primary production of phytoplankton, while its higher concentration affected the rate of oxygen consumption of phytoplankton. Dieldrin
induced destruction of marine algal cells was reported by Powers et al., (1977). Treatment of axenic cultures of the marine dinoflagellate *Exuviella baltica* with 10 parts per thousand million (10^9 ptm) of dieldrin inhibited growth rate and caused large number of cells to disintegrate within 12 hours of exposure.

Subramanian et al., (1979) reported the effect of low concentration of DDT on the growth and production of marine diatom *Skeletonema costatum*. At 1 ppb concentration there is no marked change. Above 4 ppb a marked reduction in cell number was noticed. Concentrations above 10 ppb DDT impaired growth rate.

The effect of low concentration of organochlorine insecticide DDT was compared with organophosphate and carbamate insecticides (Rama-chandran et al., 1980). Results clearly indicated that organochlorine was toxic than other insecticides as reported by other workers.

An organochlorine insecticide permethrine and its carrier solvent was found to affect microalgae additively, synergistically and antagonistically when tested for photosynthesis in green algae, as reported by Stratton and Corke (1981). Interaction of DDT with two species of fresh water algae was reported (Goulding and Ellis, 1981). The amount of inhibition varied with time and with the method of growth assessment. Bioaccumulation studies showed that both the algae accumulated ^14^C DDT.

Field studies were also conducted by several workers. Rajendran and Venugopalan (1983) conducted *in situ* studies with natural phytoplankton
to find the effect of pesticides on them. In additions to organochlorine insecticides, organophosphates and carbamates were also tested. As observed by various other workers Rajendran and Venugopalan (1983) also found that the primary production was inhibited considerably by these pesticides.

By using a mortal stain — Evan’s blue, Walsh (1983) reported the cell death of marine diatom exposed to the pesticides. All the pesticides tested, particularly organochlorine insecticides caused death of cells, but significant mortality occurred only at concentrations higher than the EC$_{50}$ values calculated from population growth studies.

Regarding the effect of biocides on cellular composition or microalgae, only very little reports are available. Goulding et al., (1984) reported the effect of DDT on the cellular composition of Chlorella fusca. Results showed that DDT caused a decrease in cell size by 46%, when measured by biovolume, and 43% when based on dry weight. Patil et al., (1985) studied and discussed the effect of DDT on parimary productivity of phytoplankton of a pond ecosystem. It has been observed that at higher concentrations of DDT, the rate of oxygen consumption increased and the reserve food material was metabolised.

A number of organochlorine insecticides were found to inhibit the growth of Ankistrodesmus fusiformis, in vitro (de Fatia celeste, and Caceres, 1986). It was found that at 2 ppm concentration of gamma BHC the growth of the algae was inhibited. The results clearly suggested that gamma BHC interferred with the physiology of this organism on concentrations lower than 1 ppm.
Lal et al., (1987) reported the effect of DDT, fenitrothion and chlorpyrifos on growth, photosynthesis and nitrogen fixation in Anabaena (Arm 310) and Aulosira fertilissima. DDT inhibited the growth of Anabaena whereas it was stimulatory to Aulosira. Fenitrothion and chlorpyrifos were quite toxic even at concentrations of 100 times less than DDT.

The effect of pesticides on the filamentous forms of algae is very rare. One such report was published by Awasti and Singh (1987). They reported on the effect of DDT and BHC on filamentous green algae Spirogyra cylindrica. As in the case of unicellular microalgae, this alga was sensitive to both the insecticides even at low concentrations. The effect of DDT was more severe than BHC.

Field studies were conducted to find out the combined effects of chloro organic pesticides on the primary production of phytoplankton communities (Savinova and Savinov, 1987). Only short term experiments were possible in the field and the results showed that the concentration of each toxicant brought the level of primary production down to 53-77% of the control.

Regarding the study of the effect of toxicant on biochemical compounds, Yu Huzhao (1987) reported the effect of DDT and BHC on amino-acid contents of Chlorella vulgaris. Experimental results show that the treatment with 0.1 ppm of DDT and BHC had very little effect on amino acid content. However, after being treated with 1-10 ppm of DDT and BHC, the aminoacid content increased.
Yasuno et al., (1987) were not able to find out any toxic effect of the organochlorine insecticide permethrin. They conducted a field study in an enclosure system containing phytoplankton and zooplankton. The insecticide addition did not significantly change the primary production, respiration, chlorophyll a concentration and sedimentation.

Piska and Waghray (1990) compared the toxic effect of an organochlorine insecticide endosulfan with two other insecticides and found that endosulfan was most toxic than the other insecticide tested.

Fate and biological affects of lindane and deltamethrin in fresh water mesocosms were reported (Caquet et al., 1992). The phytoplanktonic and periphytic communities were positively affected by the treatment.

Polychlorinated biphenyls:

Polychlorinated biphenyls which are similar in structure, persistence and biological effects to some organochlorine pesticides have been widely detected as environmental pollutants. Although they are not considered as pesticides, the PCB's find numerous industrial uses and they may be analytically confused with DDT (Keil et al., 1971). It is important to investigate their reaction with the biota. Several workers have reported that PCB's are toxic to algae and some evidence shows that they are being more toxic than DDT.

The marine diatom Cylindrotheca Closterium took and concentrated the PCB mixture (Aroclor, 1242) which at 10 ppb reduced growth, RNA
and chlorophyll levels in this organism (Keil et al., 1971). Growth rates of marine diatoms *Thalassiosira pseudonana* and *Skeletonema costatum* were reduced in the presence of 25 ppb PCB's and severely inhibited by 100 ppb, at which level DDT was only slightly inhibitory (Mosser et al., 1972a). By comparison with the diatoms, other algae were less sensitive to PCB's and DDT, suggesting that selective inhibition of sensitive phytoplankton species by organochlorines might alter the species composition in natural algal communities. This view was endorsed by Mosser et al., (1972 b), who found that at 1 ppb PCB's affected the species ratio in a mixture of *T. pseudonana* (sensitive) and *D. tertiolecta* (relatively resistant).

Moore and Harriss (1972) reported on the first *in situ* bioassay of short term effects of polychlorinated biphenyl compounds on natural phytoplankton communities. The effect of PCB's reported in these studies suggested that the toxic effects of organochlorines were more acute *in situ* at a community level than for single species laboratory cultures. This view was further recommended by Moore and Harriss (1974), that natural, mixed phytoplankton communities were more sensitive to PCB's than pure or mixed laboratory cultures. It was found that the greatest sensitivity to PCB was in net plankton and not in nannoplankton.

Uptake of PCB's by marine phytoplankton was reported by Harding and Philips (1978 b) who found that there was a definite relationship between cell density and accumulation of PCB's. Effects of PCB on marine phytoplankton photosynthesis and cell division was also reported.
by Harding and Phillips (1978b). PCB concentration as low as 1.0 \( \mu g/l \) reduced cell division of *Thalassiosira pseudonana* and *Isochrysis galbana*. A similar observation was reported by Michaels *et al.*, (1982).

Responses of marine diatom *Thalassiosira rotula* to PCB's was reported by Reiriz *et al.*, (1983). The results of this experiments concluded that, the growth phase from which the inoculum came, had a determining influence on the sensibility of microorganisms to the toxic substance. This sensitivity was highest in exponential phase.

Regarding the biochemical aspects of microalgae, Bazulic *et al.*, (1988) reported the PCB effects on production of carbohydrates, lipids and proteins in marine diatom *Phaeodactylum tricornutum*. 'Acorl 1254' had an influence on biochemical composition of phytoplankton.

b) Organophosphates:

Of the wide range of pesticides screened, organophosphorus compounds were found to be relatively non-toxic to marine phytoplankton (Ukeles, 1962), Christie (1969) observed that 100 ppm malathion had little significant effect on the green alga *Chlorella pyrenoidosa*. Moore (1970) found that 'malathion' and 'parathion' were relatively non-inhibitory to the flagellate *Euglena gracilis*.

It is seen that organophosphorus insecticides are relatively non-toxic to microalgae. However, the finding that some of the "newer" organophosphorus insecticides, as reported by Derby and Ituber (1971),
were found to depress the oxygen evolution in algal cultures of four species of marine phytoplankton and signified the need to obtain further data on the antialgal activity of this group of insecticides. Indeed, at 2.5 ppb, Dursban had been found to stimulate growth of blue green algae in artificial ponds (Hulbert et al., 1972).

Butler et al., (1975b) reported on the effects of various concentrations of diazinone, an organophosphate and the results showed that diazinon was toxic at concentrations of 10 ppm. They also reported the capability of diazinon being absorbed by the several isolates of microalgae. Organophosphate was found to be less toxic than other insecticides tested (Butler et al., 1975b). But contradictory to this report, Ramachandra et al., (1980) reported that organophosphates were more toxic than carbamate insecticides but less toxic than organochlorines. Murray and Guthrie (1980) reported that, eventhough, the organophosphates showed an initial inhibition of growth, at a later stage the algal population approached or exceeded those of control by some measures.

There was an interesting report that algae could utilize the elements of insecticides or its biodegraded metabolites (Rath and Misra, 1981). 'Dimecron - 100', an organophosphorus insecticide was tested for its nutritional value in relation to growth of Oscillatoria obscura at 400 ppm. A significant increase in growth in relation to optical density, dry weight and total chlorophyll content was noted in the Dimecron treated algae (Rath and Misra, 1981).
The effects of methyl parathion on the growth, cell size, pigment and protein content of Chlorella protothecoides was reported (Saroja and Bose, 1982). Slight or moderate inhibition with respect to cell number, packed cell volume, pigment content and protein content was noticed at 10 ppm and 20 ppm. 30 ppm and higher concentrations inhibited the growth severely. Chlorophyll was found to be the most sensitive parameter. There was another report about the effect of methyl parathion (Rajendran and Venugopalan, 1983). Field study was conducted to find out the effect of methyl parathion, malathion - two organophosphates and other insecticides. Primary production was found inhibited considerably by these pesticides.

It is important to know that the insecticide 'malathion' can have a toxic effect on unicellular chlorophyllous marine phytoplanktonic organisms and that it is concentrated by the first link of the trophic chain (Prevot and Soyer, 1985). Malathion was found toxic to the dinoflagellate Prorocentrum micans even at 10 ppm, causing a clear decrease of chlorophyll and an increase of the oxygen consumption of phytoplankton population at 1.27 to 5.07 ppm of malathion. At this concentration of toxicants they reported a significant reduction in dissolved oxygen and free CO₂ of water.

Influence of the organophosphorus insecticide DDVP on aquatic ecosystem was reported by Paland Konar (1985). DDVP at 0.014 to 1.424 ppm did not alter dissolved oxygen, pH, temperature, colour and odour of water primary productivity of water and populations of phytoplankton and zooplankton were significantly reduced at these concentrations. Results
of these experiments concluded that frequent spillage of DDVP into fish pond or in natural water would be detrimental to basic ecosystem parameters which are responsible for high fish yield.

In a field study conducted by Pal and Konar (1989) it was found that continuous drainage of phosphamidon into water hampered the growth and production of food chain organisms even at low concentration. They found that phytoplankton population decreased at 0.4257 ppm.

In a laboratory study, Raine et al., (1990) reported that Nuvan was toxic to phytoplankton at a concentration of 1 ppm. They compared the results of 'in vitro' study with that of natural populations. The effect of monocrotophos was reported by Piska and Wagnray (1990) and found that gross and net production decreased gradually in treated samples.

Physiological alterations induced by an organophosphorus insecticide 'trichlorfon' on Anabaena have been reported by Marco et al., (1990). The addition of trichlorfon to nitrate containing cultures of Anabaena resulted in a decrease in the content of all the main nitrogen compounds and an increase on the carbohydrate fraction per unit dry weight, cell division, and morphology were altered. All these trichlorfon induced alterations were noticeable from the first 24 hours of treatment, but inhibition of growth did not occur until the fourth day.

Pollution of aquatic ecosystem by the pesticide 'methyl parathion' was studied by Pal and Konar (1990). A 90 day observation was carried
out and found that primary productivity and phytoplankton population significantly decreased at all exposures. Toxicity studies were performed with three different species of fresh water microalgae, to study the direct effects of the insecticide. Dursban\textsuperscript{(R)} 4 E had no appreciable effect on the growth of non-limited algae at concentrations relevant for field situations. For phosphorus limited algae, however, significant and dissimilar effects were found (Van Donk \textit{et al.}, 1992).

c) Carbamates:

Microalgae were also susceptible to carbaryl insecticides, which was lethal to two species at 1 ppm and to all five species tested at 10 ppm (Ukeles 1962). Christie (1969) compared the toxicity to carbaryl sevin with two other insecticides and found that three pesticides varied in their degree of toxicity to algae and also in the extent to which they are degraded in the presence of algae. At a lower level i.e., 0.1 ppm, carbaryl was found to stimulate growth and $^{14}\text{C}$ assimilation in low density populations of the fresh water algae \textit{Scenedesmus quadricauda} (Stadynk \textit{et al.}, 1971). Bulter \textit{et al.}, (1975b) while testing the toxicity of various insecticides to 36 isolates of planktonic algae, found that carbaryl was toxic at a concentration of 25 ppm.

Ramachandran \textit{et al.}, (1980) compared the toxic effect of sevin with other insecticides and it was found that sevin was least toxic than the other two insecticides. In a field study conducted by Rajendran and Venugopal (1983) it was reported that primary production was inhibited
considerably by the insecticides including carbaryl sevin. The effect of Carbaryl on single species and on communities made up of three and five species has been reported by Maly and Ruber (1983).

Regarding the effect of insecticides on the reproductive stage of microalgae, Cain and Cain (1984) reported the effect of carbaryl and propoxur (both are carbamates) on zygospore germination and growth of green alga *Chlamydomonas moewusii*. Carbaryl produced significant growth inhibition over the concentration range tested. But one important conclusion they reported is that concentration which adversely affected growth had no significant effect on zygospore germination.

Kentzer *et al.*, (1984) reported on the toxic effects of carbaryl against laboratory strain of phytoplanktonic algae, *Chlorella* and *Anacystis*. Carbaryl in concentration of 10 ppm was found to inhibit the cell multiplication and total production of chlorophyll a. But contrary to this report, Khalil and Mostafa (1986) reported that, methomyl which is also a carbamate insecticide showed no significant effect on the growth of fresh water alga *Phormidium fragile* upto a concentration of 112.5 ppm. But there was a gradual decrease in all nitrogen fraction, total carbohydrate content, chlorophyll a and carotene content. The same carbaryl insecticide methomyl - $^{14}$C labelled, was found to inhibit the growth and total carbohydrate content of *Nostoc muscorum* and *Tolypothrix tenuis* at a concentration of 100 ppm (Kobbia *et al.*, 1991).
2.2. HERBICIDES:

There are many reports of the direct effect which herbicides can exert upon microalgae. Recognizing the importance of evaluating the tolerance of marine phytoplankton to pesticides, used on commercial shell fish beds Ukeles (1962) tested a wide range of toxicants, including phenyl urea herbicides, for effects on the growth of marine algae in an enriched sea water medium. Diuron, lethal to all but one species at 0.004 ppm was the most toxic of the phenyl ureas and the relative order toxicity was diuron > monuron > neburon > fenuron (Ukeles, 1962).

Available data on 2, 4 D and 2, 4, 5 T suggest that only at high concentration does 2, 4 D have adverse effects on algal population natural or cultured. Stimulation of algal growth and photosynthesis was observed with 10 ppm 2, 4 D (Walsh et al., 1970).

Evidence for atrazine inhibitory action on algal photosynthesis has come from Walsh (1972), and Hollister and Walsh (1973). Inhibition of algal photosynthesis has been established with monuron (Walsh, 1972). According to Hollister and Walsh (1973) the effects of O₂ - evolution of several different types of marine phytoplankton, the diatom, are generally less sensitive than other algal types to phenyl urea and triazine herbicides.

The effect of various concentrations of Atrazine and 2, 4, D was tested on the growth of 36 isolates of planktonic algae by Butler et al., (1975 b) and found that atrazine was more toxic than 2, 4, D.
Effect of pesticides on filamentous forms of algae is very rare. Singh et al., (1978) reported that two nitrogen fixing, filamentous species of cyanobacteria were sensitive than a unicellular non-fixing species to the herbicide, Alachlor.

By comparing the salt marsh edaphic algae in culture, microecosystems and in the field Plumley and Davis (1980) reported that at 2.2 ppm concentration of the herbicide atrazine, the rate of photosynthesis, chlorophyll content and cell numbers in unialgal cultures were reduced. But, the result with lower concentration indicated an ability to maintain chlorophyll production and cell division with reduced photosynthesis.

Bednarz (1981 a) reported the sensibility of a green algae and 3 blue green algae to six herbicides and 3 other insecticides. The effect of 2, 4, D acid on green and blue green algae in unialgal and mixed cultures was reported by Bednarz (1981 b). Low concentration of 2, 4, D usually stimulated the growth of algae, but higher concentrations inhibited or stopped the growth. It was concluded that tolerant species decreased the toxicity of the herbicide in the medium thus helping the more sensitive algae to survive. The evaluation of the adaptation ability of some green algae to 2, 4, D monuron, and diuron admixtures, under laboratory conditions was also reported by Bednarz (1981 c). The results of these experiments showed that, the toxicity action of monuron, diuron, simazine, atrazine and 2, 4, D upon Ankistrodesmus Chlorella, Dityosphaerum Scenedesmus and Hormidium was irreversible, even if the algae were transferred to media free of these substances.
In a field study, conducted by De Noyelles et al. (1982) comparison was made between atrazine treated ponds and control ponds. Atrazine at concentrations of 20 to 500 ppb inhibited photosynthesis and both concentrations depressed phytoplankton growth in the ponds within a few days. Laboratory tests verified the effects on other species at concentrations of atrazine as low as 1 to 5 ppb.

Impact of the herbicide Magnacide - H (2 propenal) on algae was studied by Fritz Sheridan (1982). The concentration of magnacide - H to effect a 50% reduction in photosynthesis was different for 3 algal species tested for different temperatures. Study on physiological response of the blue green algae to 5 herbicides was carried out (Mehta and Hawxby 1983).

The toxicity of the herbicide stam - f - 34 (propanil) on Nostoc calcicola was reported by Pandey et al., (1984). The herbicide caused an inhibition of the nitrogen fixing capability of alga which is concentration dependent and lethal at 30 ppm. Effects of Atrazine and its degradation products, alone and in combination on phototrophic microorganisms were reported by Stratton (1984). Atrazine was significantly more toxic than its degradation products.

Meyerhoff et al., (1985) compared the chronic toxicity of tebuthiuron to an alga, Selenastrum capricornutum, a cladoceran and the fat head minnow and found that the alga was the most sensitive among the three groups.
Regarding the effect of toxicant on biochemical compounds of microalgae, Salama et al., (1985) investigated the effect of herbicide 'Amitrole' on growth, carbohydrate and nitrogenous compounds in two blue green algae and found that lower and moderate levels i.e., upto 3.6 ppm induced more accumulation of carbohydrate.

In addition to laboratory studies, field studies were also conducted to find out the effect of atrazine, Moorhead and Kosinski (1986) reported on the effect of atrazine on the productivity of algal communities in artificial stream. Net community productivity was fairly low, indicating high respiration relative to oxygen production. Krieger and Baker (1986) also reported on the effects of herbicides on stream algal productivity and nutrient uptake. Detoxification of herbicides by blue green algae was reported by Chinnaswamy and Patel (1986).

Hersh and Crumpton (1987) conducted a study of naturally occurring atrazine tolerance to algae from different Iowa springs. The main purpose of this experiment were to develop a quick method of assessing the effects of an algal growth and also to investigate an ecologically meaningful end point for toxin growth experiments. Atrazine induced photosynthetic inhibition of Cyclotella meneghiniana (Bacillarophyta) has been reported by Millie and Hersh (1987). Mayasich (1987) also reported on the growth responses of Nannochloris oculata Droop and Phaeodactylum tricornutum Journin to the herbicide atrazine as influenced by light intensity and temperature in unialgal and bialgal assemblage.
algal assays were highly efficient in detection of biological potential.

Simple marine algal bioassay method has been described for short and long term studies on pesticides and industrial wastes (Walsh and Alexander, 1980). It can be used for rapid screening of a variety of substances with single species and multiple species tests which would give relative toxicities of the pollutants tested. Following this method, 96 hour EC_{50} values for some pesticides and diatom Skeletonema have been reported by the above authors.

Acute static toxicity tests were conducted with six insecticides (Ambush, Bux, Dursban, Fentrifanil, Larvin and Pydrin) and one herbicide (Borthwick et al., 1981), algal bioassays were conducted with marine algae to determine the concentration of pesticide that would inhibit population growth by 50% in 96 hour. It was found that the synthetic pyrethroids - Ambush and Pydrin were the most toxic of the seven pesticides tested.

Based on photosynthesis (oxygen evolution), EC_{50} was determined from short assays of 5 minutes duration. Green algae isolated at random from an atrazine contaminated stream exhibited similar tolerance to atrazine (range of EC_{50} 42-125 \mu g/l) compared to algae isolated at random from a non-contaminated stream (range of EC_{50} s : 35 - 152 \mu g/l) (Hersh and Crumpton, 1989).
Effects of herbicides on photosynthetic electron transport in algal systems were reported by Samuel and Bose (1987). Pyridazinone herbicides Sandoz 9785, Sandoz 9789 and Sandoz 6706, inhibited photosystem II electron transport in Chlorella protothecoides, when the herbicides were added to assay medium. The inhibitory efficiency varies with the algal species and the nature of substitution.

Characterisation of the adaptation responses of Anacystis nidulans to growth in the presence of sublethal doses of herbicide was reported by Hatfield et al., (1989) who found that the contents of accessory pigments phycoeyanin increases in relation to chlorophyll.

Mishra and Pandey (1989) reported on the toxicity of three herbicides to some nitrogen fixing cyanobacteria like Nostoc Linckia, N. calcicola and Anabean doliolium. These cyanobacteria were found to be tolerant to 2, 4, D than to Machete and Saturn. Price et al., (1989) reported that the run off from agricultural field treated with tebuthiuron above 2.24 kg/hectare concentration might adversely affect the green algal community of Playa lakes.

The primary and secondary effects of simazine and terbutryne on fresh water marsh periphyton have been studied and reported by Gurney and Robinson (1989).

The effects of the pyridinone herbicides fluridone on the growth, pigment content and composition and photosynthetic capability of Oscillatoria agardhii Gomont were investigated by Millie et al., (1990). Fluridone
concentration ranging from 0 to 100 ppb decreased biomass, chlorophyll \( a \) and total carotenoid contents with increasing fluridone concentration. Francois and Robinson (1990) examined the toxicity of three triazine herbicides (atrazine, simazine and terbutryn) in unialgal batch cultures of Chlamydomonas. Among the three herbicides, Terbutryn was the greatest inhibitor of growth and \( \text{CO}_2 \)-fixing.

Impact of an organophosphate herbicide (Glyphosate\textsuperscript{(R)}) on periphyton communities developed in experimental streams was reported by Austin et al., (1991). The addition of Glyphosate to a periphyton community appeared to have little effect on subsequent successional patterns. Abou-Waly (1991) investigated on the response of Scenedesmus sp., to three phenyl ureas, namely maloran, dicuran and patoran. Among these herbicides maloran was found to be highly toxic compared to other two. Abou Waly et al., (1991 a) conducted experiments with Atrazine and hexazinone to unialgal cultures of Anabaena flos-aquae and Selenastrum capricornutum. Reduction in growth was observed with an increase of atrazine concentration. Hexazinone treated cultures of \( S. \) capricornutum had substantially recovered by 7 days after treatment.

2.3. Fungicides:

Compared to insecticides and herbicides the literature on the effect of fungicides on microalgae is very little
Ethyl mercury phosphate was lethal to all marine phytoplankton species tested when incorporated at a level of 60 ppb in the culture media (Ukeles, 1962). Harriss et al. (1970) found that three organomercury fungicides, at less than 1 ppb, reduced growth and photosynthesis in the marine diatom Nitzchia delicatissima and also in a natural population of fresh water phytoplankton. These results indicated that at least some marine and fresh water phytoplankton species were sensitive to organomercury compounds at levels below those proposed for water quality standards and hence suggested that entry of such compounds into natural waters should be prevented.

Somasekhar and Sreenath (1984) reported on the effect of two fungicides on primary production in a pond ecosystem. In comparison with the control the gross production and net production was found to decrease gradually with the increase in concentration of the toxicant, reaching a zero value at 1000 ppm. The effect of organic fungicide Dithane M45 and Cynkotox on some unicellular algae was reported by Kosawska and Falkowski (1984) Dithane M45 depressed 50% cell multiplication and total production of chlorophyll at 0.2 to 0.6 ppm in Chlorella and Scenedesmus and at 2 ppm in Anacystis nidulans.

The effect of mercuric chloride and 'Emisan 6' on the photosynthetic efficiency of Westiellopsis prolifica was studied by Rath et al., (1985) and found that the mercury based pesticide toxicity was totally concentration dependent. At lower concentration, mercury acted as a growth regulator and at higher concentration it acted as a growth inhibitor.
Rath et al., (1986) reported the effect of the insecticide Emisan-6 on the nitrogen metabolism of the alga _W._ prolifica. They concluded that the effect was totally dependent upon the dose and duration of the experiment.

2.4. BIOASSAY STUDIES TO DETERMINE EC$_{50}$ VALUES.

Biological assays are essential for evaluating the toxicity of chemicals and are an important tool in detecting and quantifying environmental pollutants such as persistent pesticide residues. A wide range of toxicity tests have been developed in the recent decades utilizing different organisms such as algae, crustaceans, molluscs and fishes to predict the probable effect of new chemicals and effluents on aquatic ecosystem (Sprague, 1973; Walsh et al., 1980; Reish and Oshida 1986).

It was reported that (Walsh, 1972, Hollister and Walsh 1973) green algae are very susceptible to one herbicide – atrazine. Its EC$_{50}$ value ranges from 0.06 to 0.16 mg/l (ppm). Hollister and Walsh, (1973) stated that when bioassay analyses are conducted for the effects of herbicides on marine unicellular algae, two factors are particularly important

1. The response in relation to taxonomic position and

2. The wide range of responses by individual species within a given family.

The marine unicellular algae _Skeletonema costatum_, _Amphiprora paludosa_ and _Phaeodactylum tricornutum_ were exposed to dimethoate and bayluside in laboratory bioassays (Ibrahim, 1983). There was variation
in the growth response of the species and metabolism of metabolic products. Walsh (1983) reported the cell death and inhibition of population growth of marine unicellular algae by pesticides. All the pesticides caused death of cells, but significant mortality occurred only at concentrations higher than EC\(_{50}\) calculated from population growth studies.

Walsh and Merrill (1984) conducted short term static tests which estimated the growth rate responses by measurements of change of biomass, chlorophyll content, cell number or fluorescence. Rates of primary production have also been estimated by measurement of oxygen evolution or uptake of \(^{14}\text{C}\).

The 96 hour EC\(_{50}\) limits of five species of diatoms to the commonly used organochlorine pesticides, DDT and Heptachlor was studied in laboratory cultures by Rajaretanm et al., (1987). The impact of pesticides on the diatom was measured in terms of population growth, chlorophyll-a content and carbon content. Statistical characterization of atrazine (herbicide) induced photosynthetic inhibition of *Cyclotella meneghiniana* was reported by Millie and Hersh, 1987).

In the static algal toxicity tests, toxicants are usually added to cultures with low numbers of algae and population growth is measured over a period of time; often 96 hour. Wright (1978) compared the toxicity of Atrazine herbicide on fresh water algae to other triazine herbicides. Cain et al. (1979) compared results of fresh water algal tests to chemical analyses of effluent from a sewage treatment plant and concluded that...
Toxicity testing with fresh water algae in river Periyar in Kerala was reported by Joy (1990). The effect of effluent from a fertilizer factory was studied on two species of unicellular algae to predict the probable effect of continued discharge of this complex waste on the microphytic flora. EC$_{50}$ values of the effluent on growth of *Nitzschia palea* and *Oocystis pusilla* were reported.

The acute toxicity of commercial herbicide, Paraquat was determined by 96 hour static bioassay on the fresh water chlorophytes (Ibrahim, 1990). The 96 hour EC$_{50}$ values of Paraquat for reducing growth and metabolic products of three algae were determined. The three algae and their test parameters responded differentially to Paraquat and it was observed that Paraquat has inhibitory effect on the primary producers.

Abou Waly *et al.*, (1991 a) reported the growth responses of fresh water algae *Anabena flos-aquae* and *Salenastrum capricornutum* to Atrazine and Hexazinone. The EC$_{50}$ values were calculated for each herbicide at 3, 5 and 7 days after treatment. The EC$_{50}$ values generally increased with time.

2.5. BIOACCUMULATION STUDIES:

The introduction of gas chromatography in 1952 for residue analysis made it possible to detect and determine the concentration of organochlorine compounds in very small quantities. The presence of many pesticides can be detected at the parts per trillion level. Residues of pesticides occur in biological and physical components of coastal and oceanic environments and some of the residues have been implicated in degradation of
portions of these environments. Analysis of pesticide residues indicates that pesticides can reach non-target organisms in the environment and give indication of biological reservoirs of pesticides in the environments.

Activated by Rachel Carson's 'Silent Spring', and growing public anxiety about pollution residue analysts have carried out a large number of surveys since 1960.

Some algal species show a marked capacity to concentrate DDT from the surrounding medium, although the degree of accumulation varies with DDT concentration and the algal species (Sodergren, 1968; Vance Drummond, 1969; Keil and Priester, 1969; Cox, 1970; Rice and Sikka, 1973 b).

Sodergren (1968) studied the mechanism of DDT uptake by Chlorella sp., and found that $^{14}$C DDT at a concentration of 0.6 ppb was rapidly taken up i.e., within 15 seconds by Chlorella cells. The author concluded that the rate of penetration of DDT into algal cells was probably equal to its rate of diffusion in water and noted that DDT accumulation in the algae in continuous culture induced morphological changes and cell clumping.

Vance and Drummond (1969) incorporated higher pp-DDT concentration up to 20 ppm in cultures of green and blue green algae. The algae were generally quite resistant to the toxic effects, even though DDT was concentrated at least 100 fold from the medium. They also degraded the pesticide to a slight extent. These authors considered that whereas
algae are generally more resistant than higher members of the food chain to the effects of chlorinated pesticides, they are very efficient potentiatitors of pesticide residues within the food web. Christie (1969) reported the uptake of DDT, Sevin and malathion on algal populations. Results showed that degradation of \( ^{14} \text{C} \) DDT occurred only in the presence of live algae. Uptake of \( ^{14} \text{C} \) malathion (100 ppm) was reported by axenic cultures of Chlorella pyrenoidosa. This species also took up \( ^{14} \text{C} \) Sevin Carbaryl from the medium, but the compound was not altered appreciably by the alga in acidic medium and above 0.1 ppm inhibited the growth (Christie, 1969).

Keil and Priester (1969) reported that the marine diatom *Cylindrothoea closterium* concentrated DDT 190 fold from a medium containing 0.1 ppm of insecticide. In view of their tendency for intracellular storage of oil, it was considered possible that diatoms might serve as "pick-up" organisms for oil soluble pesticides, which might then be detoxified (Keil and Priester, 1969). Like DDT, its metabolite pp-DDE was accumulated to a high degree by Chlorella cells, only a small part of added DDE remaining in the aqueous medium (Sodergren, 1971).

Regarding the uptake of pesticides from the culture medium, Krikwood and Fletcher (1970) reported the absorption of MCPB by a unicellular alga to a greater extent than MCPA. Greatest uptake occurred in *Chlamydomonas globosa* an alga of relatively large size and thin cell wall. They concluded that the uptake of herbicide was optimal at pH values favouring their movement as undissociated molecules.
It was reported that DDT residues in marine phytoplankton increased from the year 1955 to 1969 (Cox, 1970). DDT and its metabolites were noticed in all the samples analysed.

Six species of axenic marine algae, representing four taxonomic division, rapidly took up $^{14}$C dieldrin but formed no detectable metabolites of the insecticide (Rice and Sikka, 1973 b). This indicated, as had been suggested by Menzel et al., (1970), that the algae might either incorporate dieldrin as small particles or that saturation was maintained while the algae concentrated the pesticide from solution. Total dieldrin uptake increased with increasing cell concentration (Rice and Sikka, 1973 b), but uptake was not correlated with number of cells per unit mass. However Rice and Sikka (1973 b) did not detect any algal metabolites of dieldrin. Photodieldrin has been identified as product of $^{14}$C dieldrin in some microbial cultures isolated from dieldrin contaminated lake water (Matsumura et al., 1970).

Uptake of methoxychlor (methoxy-DIJT) by actively growing green algae and diatoms was reported by Butler et al., (1975b). They also reported the uptake of diazinon, another phosphorothoniate insecticide and acaricide by several algae. The cultures of algae removed significant amount of 2, 4, D from a medium containing 0.01 ppm 2, 4, D. But algal degradation of atrazine was not detected by Butler et al. (1975 b). However, under optimal light conditions of Scenedesmus absorbed amitrole and metabolised.

Schauberger and Wildman (1977) investigated the bioconcentration of dieldrin and aldrin, both organochlorine insecticides, in fresh water blue green algae and found that there was a great difference in uptake
blue green algae and found that there was a great difference in uptake among three species. Moreover, they found that the pattern of bioconcentration ratios of dieldrin and aldrin was different among these three species. However, these differences in uptake could not be related to difference in toxic effects on the alga.

Polychlorinated biphenyls are considered to be industrial organochlorines. PCB uptake by marine phytoplankton has also been reported (Harding and Phillips, 1978b) The relationship between cell density and accumulation of PCB was also investigated.

Glooschenko et al., (1979) showed that Scenedesmus quadricauda accumulated chlordane at the same rate alive or when heat killed and that bioaccumulation was essentially complete in 24 hours. These studies suggest that bioaccumulation in algae is not affected by rate of cell growth or metabolism at least for organic compounds.

Using a model laboratory ecosystem Miyamoto et al., (1979) showed that, the bioaccumulation ratio of algae was much greater than for other representative aquatic organisms for DDT and total DDT residue, which included the degradation products DDD and DDE. These investigators concluded that the bioaccumulation ratio may increase with time and algae may represent a chronic reservoir of contamination by the prolonged addition of a chemical through food chain to fish.

Adsorption of an organophosphorus insecticide 'Fenitrothion' by planktonic and benthic algae was reported by Lakshminarayana and Bourque
These studies showed that fenitrothion will be absorbed or actively taken up by plankton, particularly the phytoplankton.

In a comparative study of the uptake of the organochlorine, DDT and the organophosphate Fenvalerate by Daphnia, snails, fish and algae in a model ecosystem, Ohkawa et al. (1980) showed that algae had the highest bioaccumulation ratio for DDT and a subsequently high ratio for Fenvalerate. The interaction of DDT with two species of fresh water algae was studied by Goulding and Ellis (1981). Both the algae, Anabaena variabilis and Chlorella fusca accumulated $^{14}$C DDT. Neither alga significantly metabolized DDT although cells of C. fusca contained a small amount of DDE after 480 hour incubation with the insecticide.

The bioconcentration of two polychlorinated biphenyls such as Aroclor 1232 and Aroclor-1248 by live and dead cells of diatom Thalassiosira rotula has been reported (Murado et al., 1984). PCBs were concentrated in T. rotula and metabolic transformation of PCBs were also noticed.

Accumulation, degradation and biological effects of lindane on Scenedesmus obliques was reported by Lin-Yi-Xing and Sun-Bo-Zen (1987). This particular species possess certain accumulating capacity for γ-BHC, which is higher at 1 mg l$^{-1}$ than at 10 mg l$^{-1}$.

Compared to organochlorine insecticides, organophosphates are considered to be easily degradable. The capabilities of five algal species to degrade two organophosphate insecticides, such as monocrotophos and
quinalphos have been determined (Megharaj et al., 1987). It is evident that green and blue green algae are equally potential in detoxifying these insecticides.

Dhanraj et al., (1989) reported on the bioconcentration and metabolism of aldrin and phorate two blue green alga, Anabaena sp. and Aulosira fertilissima. They showed marked ability to bioconcentrate aldrin and phorate from culture medium. Aldrin was metabolized to dieldrin by both blue green algae, but no metabolism was noticed in the case of phorate.

There are reports about the concentration of different organochlorine pesticides in the sediments of the Arabian sea and in the sediments along the east coast of India (Sarkar and Sen Gupta, 1987, 1988). Chlorinated pesticide residues in sediments from the Arabian Sea along the Central West Coast of India indicated that the residue levels of all the organochlorine pesticides detected were in the order : Dieldrin < pp - DDE < DDD < DDT < pp - DDE < aldrin < BHC (Sarkar and Sen Gupta, 1987). The same investigators reported on the pesticide residues in the sediments of east coast of India also (Sarkar and Sen Gupta, 1988) and they reported that apart from DDT and its isomers, residues of gamma-BHC, aldrin and dieldrin were recorded from a number of places. Their concentration values are high mainly in river mouths which indicate that these pesticides are of land origin.