STUDIES ON THE TOXICITY OF DDT AND ALDRIN ON A BLUE GREEN ALGA : *SPIRULINA PLATENSIS*

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This dissertation entitled "Studies on the Toxicity of DDT and Aldrin on a Blue Green Alga: Spirulina platensis" embodies the work carried out at the School of Environmental Sciences, Jawaharlal Nehru University, New Delhi. This work has not been submitted in part or full for any degree or diploma of any University.

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C O N T E N T S

I N T R O D U C T I O N

Use of chemicals in pest management is not new. Over a long period, the chemicals used for this purpose were a comparatively small range of copper or arsenic compounds or naturally occuring insecticides, such as pyrethrum and rotenone. However, with the advent of DDT in 1940s, a new era in pest management emerged. Its cheapness and general toxicity stimulated the search for new chemicals with similar properties. These chemicals were so successful in controlling pests that today about 8000 such pesticide formulations are in commone use; and over five hundred of these contain two or more "active ingredients" (Barik, 1984). These chemicals were given the general name "pesticides" (to cide = to kill) which means "any organism, substance, or thing that is manufactured, represented, sold or used as a means of directly or indirectly controlling, preventing, mitigating, attracting or repelling any pest or altering growth, development or characteristics of plant life, that is not a pest" (Chagu and Afghan, 1982).

However, shortly after their wide acceptance as "wonder drugs" (Heckman, 1982) a general awareness about their possible harmful side effects started arising during 1950's and 1960's. Carson's (1962) classic "Silent Spring" was instrumental in shaping public opinion on possible side effects of pesticides. It was noted that some of these compounds, especially of organochlorine group,

were very slow to degrade and were toxic to nontarget organisms. Morever, when people came to know about decreasing effectiveness of some pesticides on target organism, the conclusion drawn was that "each new pesticide is little more than a means of short term control than a permanent solution" (Heckman, 1982).

Pesticides are classified either on the basis of their chemical nature or on the basis of target organisms. On the basis of target organisms, pesticides are classified as :-

(1) Insecticides : To control insects (e.g., DDT, malathion etc)

(2) Herbicides : To control herbs (e.g, 2-4-D, 2-4, 5-T, DMPA etc.)

(3) Rodenticides : To control rodents (e.g., Dicumarol, Coumachlor etc.)

(4) Molluscides : To control snails (e.g., Diquat, etc.)

(5) Fungicides : To control fungi (e.g., Theran, Beramyle etc)

(6) Piscides : To control undesirable fish population (e.g., Rotenone, Toxaphone, etc.).

On the basis of chemical nature, pesticides are classified into organochlorines (e.g., DDT, aldrin, etc.), organophosphates (e.g., malathion, parathion, etc.) and carbamates (e.g., carbofuran, baygon, etc.).

Presence of pesticides in aquatic environment may originate from surface runoffs from agricultural land, industrial waste discharge, accidental spills, deliberate direct application, sewage effluents, air drift, dead animals and animal excreta,

food chain etc. (Gerakis and Sficas, 1974). Relatively small proportions of pesticides reach aquatic systems through agricultural runoffs. Water soluble pesticides are transported in dissolved state, while nonsoluble pesticides are carried as suspended particles or get adsorbed onto particulate matter and then carried to water bodies. This may be one of the reasons why some organochlorine pesticides are in much higher concentrations in sediments than in the overlying water. According to Edwards (1977) surface runoff may be important source of pesticide pollution of lakes or local water bodies but for rivers and estuaries, industrial discharge is more important. Contamination of water bodies may also arise due to fall out from accidental spray from large scale spraying of forests or agricultural lands. Wind may carry drifts to large distances.

Direct entry of pesticides into aquatic systems to control undesirable organisms is well known. In the following table, some of the insecticides which have been intentionally applied to aquatic systems are presented (Service, 1977).

Insecticides	: Uses
(1) Chlorinated hydrocarbons	
(a) DDT	Mosquito and black fly control
(b) Aldrin	Rice culture

(c) Endrin	Rice culture, use is limited due to its toxicity.
(d) Dieldrin	Contrl of DDT resistant mosquitoes
(e) Telodrin	Rice culture
(f) Lindane	Limited use in rice culture.
(2) Carbamates.	
(a) Hopicides	Rice culture
(b) Propoxur	Control of DDT and dieldrin resistant mosquitoes.
(c) Carbofuran	Control of insects and nematode in rice culture.
(3) Organophosphates	
(a) Temephas	Mosquito larvicide
(b) Fenthion	Mosquito control
(c) Chlorpyrifos	- do -
(d) Fenthrothion	- do -
(e) Chlorthion	- do -

Of these pesticides, some of the compounds, especially of organochlorine group, have a general toxicity and are very slow to degrade. This property was once considered to be beneficial because of long lasting effects. However, there was a little anxiety about ecological hazards caused by them. It was only in late 1950s that reports regarding their occurrence in ecological samples started appearing in the literature. Now, there is

little doubt that organochlorine pesticides are one of the most important groups of chemicals which cause considerable ecological hazards. Due to these reasons, use of these chemicals have been banned in western countries but their use is still continued in India. The following table gives an insight regarding status of organochlorine pesticides in EEC, India and USA (Nanda, 1986).

S. No.	Pesticide	Status in USA	Status in EEC	Status in India
1.	BHC	Banned	Approved for beet seeds and orchids only	Approved for general use
2.	Dieldrin	- do -	Approved for individual trees only	used in storage and quarantine locust control in scheduled areas.
3.	Chlordane	Restricted	Approved as a dip for conifer seedlings	approved for general use.
4.	Aldrin	- do -	Approved for strawberries, ornaments and vineyards; and in U.K. and Ireland for potatoes	approved for general use
5.	Endrin	Banned	Approved as a caricide on strawberries	phased out
6.	Heptachlor	Restricted	-	approved for general use.

7.	DDT	- do -	Approved for sugarbeets, turf for sports, ornaments and potatoes	approved for general use
8.	Lindane	- do -	-	approved for general use

Organochlorine pesticides are still used in India in significant amounts as is shown in the following table (Nanda, 1986) :

Pesticide	Projected demand for 1984-85 in M.tonnes	Projected demand for 1989-90 in M. tonnes
BHC	42500	47000
DDT	17750	19750
Aldrin	200	300
Chlordane	50	100
Heptatchlor	50	100
Lindane	20	50

Algae play an important role in aquatic ecosystems. They are the main primary producers and some algae (of cyanophyceae family) play very important role in nitrogen economy of paddy fields. According to Venkatraman (1983) about 20 to 30 kg/hectare of biologically fixed nitrogen can be made available from blue-green algae. They have the capacity to accumulate large amount of

toxicants present in the aquatic system. Some of these algae can be used as indicators of DDT pollution, i.e., Chladophora and Oscillatoria (Ware et al., 1968). Venkatraman (1983) has reported that Spirulina sp. can be used as a second stage in treatment of sewage effluents after growing water hyacinth in them.

Some of the algae can be used as potential sources of food and/or feed. Spirulina platensis and Scendesmus obliqueus have been recognized to be important sources of protein (Venkatraman, 1983; Becker, 1984; Ciferri, 1983). Besides its use as a protein source, presence of carotene in Spirulina sp. is valuable for vitamin A nutrition. The presence of vitamin B complex in Spirulina sp. particularly combalamine and minerals are also of significant interest (Ciferri, 1983).

According to Venkatraman (1983) blue green algae may be the "technically advantageous candidate" for transfer nif genes from prokaryotes to higher plants.

When pesticides enter the aquatic systems, they come in direct contact or indirect contact with components of aquatic environment. Algae being primary producers of this ecosystem are also affected. They interact with pesticides in various ways and as a result, either change in their physiological processes is observed or the pesticides themselves get detoxified. Thus, it

is important to study the various kinds of stress factors that could affect productivity of algae.

The present study aims to monitor toxicity of two organochlorine pesticides, viz., DDT and aldrin on a cyanobacterium (Spirulina platensis). Effect of temperature on pesticide toxicity has also been studied. This study seeks to investigate the effects of the two pesticides mentioned above on Spirulina sp. in terms of:

1. Biomass, growth rate, specific growth rate and doubling time;
2. LC 50 values;
3. Chlorophyll content;
4. Protein content, and
5. SO_4^{2-} uptake from medium.

L I T E R A T U R E R E V I E W

Organochlorine pesticides are a group of very persistant compounds. These compounds remain unaltered for many years in aquatic systems (Edwards, 1973). Their residues have been found in farm soils (Harris et al., 1977; Miles and Harris, 1978), oligotrophic lakes and sediments (Veith et al., 1977; Kueseth, 1981), aquatic environment (Bjerk and Brevik, 1980), marine ecosystems (Young et al., 1976) and even in Antartican environment (Woodwell et al., 1971), although their use in the West has ceased since the early 1970's.

General toxicity of these pesticides and their insoluble nature in water were once considered to be beneficial to users but with the publication of Carson's "Silent Spring" in 1962, studies regarding their side effects started.

A chemical is toxic to an organism only when it comes in the surroundings of that organism. Therefore, it is imperative to consider, in general, the behaviour of pesticides in the aquatic environment.

The behaviour of pesticides in aquatic environment is mainly governed by their solubility in water. Since, most of the pesiticides are organic compounds, they are almost insoluble in water. Gunther et al. (1967) have reviewed water solubility of many pesticides. DDT is almost insoluble in water. Its water

solubility ranges from 1 ppb to 100 ppb depending upon technique applied (Bowman et al., 1976), while the lowest value of 1.2 ppb at 25°C is accepted as the most correct (Haque and Freed, 1974).

Many of these pesticides form a cluster and remain dispersed in aquatic system in the form of colloids (Bigger et al., 1967). It is also probable that pesticide residues may be accumulated near sediments. Studies of Glooshenko et al. (1976) show that DDT and its metabolites, HEOD and mirex constitute reservoirs of stable organochlorine residues in the lake sediments. Frank et al. (1979) have shown the presence of DDT and its metabolites and hepatchlor in the sediments of lake Huron and Georgian Bay.

Pesticides, due to their low solubility in water and higher hydrofobic character tend to adsorb onto solid surfaces. Extent of adsorption of these pesticides on solid surfaces depends on a large number of physical and chemical characteristics of both, solid particle and adsorbing material. Important properties of adsorbing compound that will affect adsorption include water solubility, size, shape, configuration and charge distribution of molecule; and in case of adsorbent, its surface area, the exposed functional group, composition at surface, porosity, size, shape, charge distribution, polarity and polarizability will have an effect on adsorption (Bedding et al., 1983).

Adsorption of neutral organic pesticides to particulate matter follows a physical type of adsorption and amount of pesticides

adsorbed is inversely related to its water solubility (Leopold et al., 1960). Presence of electrolytes in the aqueous system has a pronounced effect on adsorption of pesticides (Hurle and Freed, 1972).

Adsorption processes are generally represented by adsorption isotherms (Connell and Miller, 1984). Two widely used isotherm are:

(1) Langmuir Isotherm: Moles of solute adsorbed per gm of adsorbent (X) are expressed as a function of equilibrium concentration of solute in solution (C)

$$X = \frac{X_m . bC}{1 + bC}$$

where X_m is the number of moles of solute adsorbed per gm of adsorbent in forming a complete monolayer; C is the equilibrium concentration of the chemical and b is a constant related to energy of adsorption.

(2) Freundlich Isotherm: It is an empirical relationship and is expressed as :

$$\frac{X}{m} = KC^{1/n}$$

where X is the amount of chemical adsorbed per gm of adsorbent, C is equilibrium concentration of the chemical; K is the equilibrium constant indicative of the strength of the adsorption

and 1/n is a constant describing the degree of nonlinearity and m is numbr of moles.

Temperature plays an important role in the solubility of pesticides. In general solubility increases with an increase in temperature. However, this is not the case with thiocarbamate pesticides where pesticide solubility decreases with increasing temperatures (Haque and Freed, 1974), which may be due to hydrogen bond formation between thiocarbomate and water molecules.

High lipid solubility of organochlorine pesticides contributes to their accumulation in biotic communities in aquatic environment. Hemelink et al. (1971) have suggested that water insolubility of highly lipid soluble insecticides provides the driving force in producing lipid storage through a series of simple partitioning from water to lipid.

Most of the organochlorine pesticides are extremely persistent and slow to degrade in natural conditions. However, they may undergo degradation in sunlight or under UV light; for example, photoproducts from chlorinated cyclodienes are formed by action of sunlight. The residues of aldrin and dieldrin under sunlight form photoaldrin and photodieldrin, respectively (Reddy and Khan, 1975). Photoaldrin is further converted to photodieldrin (Khan et al., 1972). Under UV light photodieldrin was reported to form two metabolites, metabolite I and metabolite II (Reddy and Khan,

1975). These metabolites were formed within 12 hrs of exposure to UV light. Bensen (1971) reported two metabolites of photodieldrin under UV light irradiation in 68 hrs. Photochemical degradation of DDT was reported by Rosen et al. (1966) and Sheutz et al. (1971).

Toxicity of organochlorine pesticides to algae is reviewed by many workers (Lal, 1982, 1983, 1984; Butler, 1977; Cox, 1970; Ware and Roan, 1970; Lal and Saxena, 1980). In reviewing literature on this aspect, one may find conflicting results obtained by various authors. This may be due to different conditions under which experiments were carried out.

Effect of endrin on algae was studied by many workers (Vance and Drummond, 1969; Menzel et al., 1970; Batterton et al., 1971; Clegg and Koevenig, 1974, etc.). Menzel et al. (1970) have shown that growth of a marine alga Dunaliella tertiolecta was not affected at concentrations upto 1000 ppb of endrin. They have also shown that in the presence of 100 ppb of endrin, the early growth rate of Skeletonema costatum was reduced but it did not affect the final cell concentrations. 100 ppb endrin reduced growth rate of Coccolithus huxlyeii and completely inhibited growth of Cyclotella nana. Vance and Drummond (1969) have shown that endrin concentrations ranging from 5 ppm to 20 ppm eliminated growth of four unicellular algae. They have also shown that 20 ppm of endrin has no effect on Scendesmus

quadricauda, Oedogonium species and Anabaena cylindrica. However, 5 ppm of endrin inhibited cell division of Microcystis aeruginosa. Batterton et al. (1971) have shown that Agmenellum quadruplicatum and Anacystis nidulans were more tolerant to ketoendrin than endrin; the former was inhibited at all concentrations tested whereas the latter was inhibited only at higher concentrations. Cylindrospermum sp. has been shown to be tolerant to endrin concentrations as high as 600 ppm (Singh, 1973).

Effect of lindane on growth of algae was studied by several wokers (Ukeles, 1962; Borghii et al., 1973; Moore and Dorward, 1968; Sodergren, 1971; Ellis and Goulding, 1973; Hansen, 1979; Jeane-Levain, 1979, etc.). Hansen (1979) has shown 511 ppb of lindane to be lethal to Chlorella sp. whereas 2 ppm of lindane was lethal to Amphidinium carteri (Jeane-Levain, 1979). However, Singh (1973) has shown that 200 to 600 ppm of lindane has no effect on Aulosira fertilissima. Loosanoff et al. (1957) have shown that 1 ppm of lindane has no effect on Chlorella sp. and Chlamydomnas sp. Ukeles (1962) treated five unicellular algae with 1-9 ppm of lindane. He found that Protococcus sp. was the most sensitive. Lindane concentrations of 4 and 8 ppm were toxic to Navicula ostrearia and Phaeodactylum tricornutum (Daste and Neuville, 1974). Borghii et al. (1973) have shown that 5 ppm of lindane stopped cell growth in Acetabularia mediterranea.

Inhibition was generally reversible and recovery was quick once pesticide had been removed from medium. Young cells were more sensitive than older, regenerating algae. Moore and Dorward (1968) reported that 0.5 ppm of lindane inhibited growth of Endorina elegans by 60% of the control and alga was eliminated at 2.5 ppm whereas the same concentration reduced growth of Gonium pectorale by 84%. Chlorella pyrenoidosa accumulated 30% of lindane added to the medium but there was no effect on its growth (Sodergnen, 1971; Ellis and Goulding, 1973).

Effect of aldrin and dieldrin on algae was studied by several workers (Vance and Drummond, 1969; Clegg and Koevenig, 1974; Kopecek et al., 1976, etc.). Vance and Drummond (1969) have shown that aldrin at even high concentrations of 15 to 20 ppm has no effect on Scendesmus quadricauda, Oedogonium sp. and Anabaena cylindrica. Clegg and Koevenig (1974) have shown that 100 ppm of aldrin has no effect on Chlamydomonas sp. and Euglena gracilis. However, Poorman (1973) has shown that 50 and 100 ppm of aldrin inhibited growth of Euglena gracilis after a 24 hr treatment period and both concentrations stimulated growth after one week. In field studies, Lazaroff and Moore (1966) reported that dieldrin inhibited the growth of algae present in several samples of surface water of New York state. However, dieldrin did not stop flagellar movements of Euglena sp. (Moore, 1967). Growth of Navicula seminulum was reduced to 50% by 12.8 ppm dieldrin and growth was eliminated at 32 ppm (Cairns, 1968). Menzel et al.

(1970) have shown that upto 1000 ppb dieldrin did not effect growth rate of Dunalliela tertiolecta. Similarly, Cole and Plapp (1974) reported dieldrin to have little effect on growth of Chlorella pyrenoidosa. Stadnyk (1971) treated cultures of Scendesmus quadricauda with 0.1 and 1.0 ppm of dieldrin and it caused a decrease in cell number biomass and carbon assimilation. Microcystis aeruginosa was inhibited by less than 5 ppm of aldrin and dieldrin and many other algae resisted ever higher concentrations upto 100 ppm without any apparent effect on the population densities (Clegg and Koevenig, 1974). Powers et al. (197) reported that even 0.01 ppm of dieldrin inhibited growth of Exuviella baltica and caused distintegration of cells. Bousch and Matsumura (1975) have shown that susceptibility of Anacystis nidulans to pesticides was in the order: endrin > dieldrin > katoendrin $\gg$ photodieldrin.

Effect of DDT on algae has been extensively studied by many workers. Perhaps Wilson and Chaudhri (1946) were the first to study the effect of DDT on algae. They studied the effect of DDT on Chlorella sp. and five other unidentified algae and there appeared to be no injurious effect. Lazaroff and Moore (1966) and Moore and Dorward (1968) have studied effect of DDT on algae present in the samples from surface waters in New York state. Some of the strains were affected by 100 ppb of DDT while others were resistant to 5 ppm of DDT. Anacystis nidulans was shown to

be able to metabolize DDT. Ukeles (1962) treated cultures of marine phytoplankton with concentration of DDT from 0.02 to 0.60 ppm. The insecticide was generally non-toxic to growth of all algae except for Monocystis lutheria in which growth was inhibited. Concentrations of DDT from 0.01 to 100 ppm had no effect on the growth of Chlorella pyrenoidosa (Christie, 1969). Concentrations of DDT ranging from 0.2 and 20 ppm had no effect on the growth of Chlamydomonas reinhardtii (Morgan, 1972; Egloff and Partridge, 1972; Mosser et al., 1972) and on Coccolithus huxleyii at concentration upto 1 ppm (Menzel et al., 1970; Bowes, 1971, 1972; Fisher, 1975). Dekoning and Martimer (1971) and Mosser et al. (1972) have reported that 0.1 ppb, 1.0 ppb and 1 ppm DDT treatment for four days had no effect on the growth of Euglena gracilis. Poorman (1973) has found this growth to be stimulated two or three fold after 7 days exposure to 100 ppm DDT. Palmer and Malony (1955) reported no effect of 2 ppm DDT upto 21 days exposure on Chlorella variegata. Poorman (1973) exposed cells of Euglena gracilis to DDT concentration of 1, 5 and 10 ppm and found little effect on growth during 24 hrs period, while 50 and 100 ppm of DDT showed some inhibition. Treating cultures at 10, 50 and 100 ppm of DDT for 7 days caused growth to be stimulated.

Ellis and Goulding (1973) have shown that growth of Chlorella sp. was inhibited for four days by 1 ppm of DDT but after this period growth commenced at rates comparable to that of controls and the

final cell yield was the same as the control. Degree of inhibition was dependent on initial inoculum size and on the time of sampling. Bowes (1972) studied effect of DDT on several phytoplankton. DDT at 80 ppb had no effect on all but Skeletonema costatum which exhibited a 9-day lag before the cell division commenced. Further inoculation of Skeletonema sp. into 80 ppb of DDT gave another 9-day lag.

Bowes (1972) has shown that growth of Skeletonema costatum was completely inhibited for periods upto 9 days at 0.08 ppb DDT while Mosser et al. (1972) have shown that 0.1 ppm DDT caused only an initial inhibition of growth of Skeletonema which was resumed after four days. For the same species, results of Menzel et al. (1970), Fisher (1975) and Subramanian et al. (1979) are in accordance with those of Bowes (1972) growth being inhibited by 20% at less than 0.1 ppm DDT. In Thalassiosira pseudonana growth was inhibited by 70% by 0.1 ppm DDT (Manzel et al., 1970) after 7 days and by 30% by 0.05 ppm DDT after two days (Mosser et al., 1972). In Anabaena sp. growth was not affected by 1.0 ppm DDT (Goulding and Elles, 1981; Lal et al., 1982) and 10, 50 and 100 ppm of DDT stimulated growth (Lal et al., 1982) stimulation being maximum at 100 ppm after 35 days.

Cole and Plapp (1974) reported that DDT inhibition of the growth of Chlorella pyrenoidosa was inversely proportional to cell concentration and DDT was a weak inhibitor of photosynthesis.

In Anacystis nidulans DDT, DDD and DDE inhibited growth markedly (Boush and Matsumura, 1975). Toxicity was in the order: DDD > DDE > DDT. Mosser et al. (1974) reported growth inhibition of Thalassiosira pseudonana at 500 ppm of DDE. In Exuviella baltica DDE has been found to be highly toxic (Powers et al., 1979). Concentrations of DDE that affected this alga are those which have been reported in natural marine phytoplankton communities (Cox, 1972; Sodergren, 1971; Bowes, 1972).

Earlier studies on interaction of insecticides with algae revealed that insecticides have some form of damaging effect on algae (Ukeles, 1962). This has prompted further research on effects of insecticides on photosynthesis in algae (Chako et al., 1966; Wurster et al., 1968; Bowes and Gee, 1971; ;Bowes, 1972; Mosser et al., 1972a,b; Cole and Plapp, 1974; Butler, 1977). Mac Farlane et al. (1971) have shown that even 9.4 ppb DDT distorted chloroplast in Nitzschia delicatissima and at 100 and 1000 ppb morphology of chloroplast was completely distorted. Clegg and Koevenig (1974) have studied effects of DDT, aldrin, chlordane, dieldrin and diazinon on light reactions of photosynthesis by measuring production of ATP. Exposure of Chlorella sp. to 100 ppm of any one of the above pesticides reduced by half or more the amount of ATP as compared to that of the control, which was detected after 15 seconds of shaking. However, exposure of Chlamydomonas cultures to diazinon, aldrin, dieldrin, chlordane

and DDT reduced ATP levels by 20-25%. Exposure of Euglena sp. to chlordane reduced ATP level by 40% while aldrin and dieldrin were less effective. DDT and diazinon had no significant effect after 15 seconds, but after two minutes the amount of ATP was reduced significantly. Since population level of these algae was not affected by these insecticides, it was suggested by them that insecticides interfere with photphosphorylation during light reaction of photosynthesis.

Wurster (1968) has shown that fixation of $^{14}CO_2$ in four marine algae declined as the concentration of DDT was increased to 100 ppb. Menzel et al. (1978) observed that DDT affected $^{14}CO_2$ uptake differently in different marine algae and this was due to differential penetration of insecticide through the cell walls and membranes in different species. Fisher (1979) observed no decrease in $^{14}CO_2$ uptake per cell in Thallassiosira pseudonana and suggested that reduced photosynthesis in DDT treated cultures was only due to the presence of fewer photosynthesizing cells, suggesting thereby that primary effect of DDT is on cell division rate rather than on photosynthesis itself. However, Lee et al. (1976) reported that DDT concentrations between 3.6 ppb to 36 ppb inhibited photosynthetic CO_2 fixation in Selanastrum capricormutum and have shown that inhibition was dose dependent. The incorporation of ^{14}C from $^{14}CO_2$ indicated that DDT stimulated the incorporation of ^{14}C into glycolic acid, a major compound of photorespiration, and caused the concomitant suppression of flow

of $^{14}CO_2$ into aspartic acid, a major component of C-α-dicarboxylic acid pathway. This shift from an efficient pathway to a nonefficient pathway by DDT was interpreted as being caused by interruption of cyclic photophosphorylation (Lal and Shivaji, 1984; Lal and Saxena, 1982). Bowes (1972) has shown that electron transport in chloroplast particles of Dunaliella tertiolecta was sensitive to DDT and DDD. He has shown that DDD inhibits electron transport to the same extent as DDT. Concentration to cause 50% inhibition for both compounds was 20 uM. It indicated that, dechlorination process does not represent a detoxification process of DDT, at least, as far as this system is concerned.

Moore and Harris (1972) have shown that radioactive carbon uptake in marine phytoplankton community was inhibited by 5 ppb of DDT, uptake being reduced by 50% at 35 ppb concentration. Luard (1973) studied the effect of TDE, DDD and DDT on ^{14}C uptake by Scendesmus quadricauda. Concentrations of 0.1 to 1000 ppb of TDE were stimulatory. However, Sears and Yentsch (1977) were unable to detect any significant effect of DDT on photosynthesis of Fucus vesiculosus, Rhodymenia palmeta and Ulva lactuca.

DDT has been reported to inhibit Na^+ and K^+ ATPase activity in Anacystis nidulans (Boush and Batterton, 1971). Czeczuga and Grievasimow (1977) have shown that 1-5 ppm of DDT increased tyrosine content in Chlamydomonas nivialis; however, total amino

acid content is reduced. At 10 to 25 ppm, alanine, aspartic acid and threonine contents were also reduced.

Geike and Parashar (1976) studied the effect of BHC (0.001 to 10 ppm) on growth parameters of Chlorella pyrenoidasa. The chlorophyll content and total nitrogen content of the algae were inhibited while dry matter and carbohydrate content were less affected. BHC at 0.001 to 0.5 ppm also decreased dry weight and chlorophyll content of Tetrahymena pyriformes. Dieldrin at concentrations ranging from 0.1 to 100 ppm adversely affected the chlorophyll content, dry weight and photosynthetic activity in Ankistrodesmus braunii and Anacystis nidulans (Kopecek et al., 1976). Aldrin and dieldrin at 100 ppm were toxic to Anabaena cylindrica, Anacystic nidulans and Nostoc muscorum (Schauberger and Wildman, 1977). Parashar et al (1978) have reported alterations at ultrastructural level in cell morphology of Chlorella sp. treated with 10 ppm of BHC.

In Acetabularia mediterranea lindane slowed down morphogenesis (Puiseux-Dao et al., 1977) but DNA synthesis continued. It was also suggested that lindane acts at cellular membranes as indicated by osmotic shocks in Acetabularia and does not interact with cellular membrane of prokaryotes, in order to explain why lindane affects eukaryotes but not prokaryotes.

Simonis and Lee-Kaden (1979) have shown that in Anacystis nidulans lindane affects $^{14}CO_2$ fixation primarily resulting in suppression of protein synthesis caused by a depletion of intermediates of CO_2 fixation.

Lott (1977) using oxygen output as a measure of photosynthesis reported that chlordane at 0.1 to 100 ppb in Scendesmus quadricauda and 0.1 and 50 ppb in Chlamydomonas sp. stimulated respiration rate which increased with increasing concentrations of chlordane. Kaushik and Venkatraman (1983) have shown that 1 ppm of BHC had no effect on nitrogenase activity of Hapalosiphon welwitschii var. Vaginatus whereas in H. fontinalls, 1 ppm BHC produced 50% inhibition in nitrogenase activity. In Westiellopsis prolifica 1 ppm BHC enhanced nitrogenase activity.

Very few studies have been done regarding morphological changes induced by addition of insecticides in medium (Jeane-Levain, 1979; Borghi et al., 1973; Powers et al., 1977; Goulding and Ellis, 1981). Sodergren (1968) has shown that doses of DDT which were inimical to growth of Chlorella sp. caused cell clumping to occur. This indicates that under toxic doses cell properties may be changed. Bowes and Gee (1971) have shown that in marine algae DDT and DDE altered morphology of chloroplast. Powers et al. (1977) have shown that 0.01 ppb dieldrin caused large number of cells of Exuviella baltica to disintegrate within 12 hrs of exposure. The diameters of surviving cells were 11.2% smaller

than controls. This size reduction persisted to at least four generations after the cells had been transferred to dieldrin free medium. Goulding and Ellis (1981) observed that cells of Chlorella fusca and Anabaena cylindrica were smaller and more ovoid than those in the normal cultures. Borghi et al. (1973) have shown that due to lindane treatment in Acetabularia sp. in the basal part of plastid, lamellae were extended with one or more carbohydrate grains; whereas in apical parts small chloroplasts with numerous thylakoids and small polysaccharide granules were observed. Puiseux-Dao et al. (1977) have shown that lindane treatment markedly altered structure of plastids in Dunaliella and Amphidium spp. Jeane-Levian (1974, 1979) has shown that lindane at 10 ppm altered the number of cellular organelles and caused degeneration of nuclear apparatus. It also caused enlarged cells, increase in the number of Golgi bodies, endomultiplication of organelles and large vacuoles. He has also shown that lindane treatment in Dunaliella sp. inhibited cell division and synthesis of DNA and RNA. Synthesis of DNA was strongly inhibited during first cell cycle as compared to second cell cycle.

Some algae were reported to metabolize organochlorine insecticides. Over 80% of the lindane added to cultures of Chlamydomonas neinhardtii and Chlorella vulgaris was gone from the culture at the end of 2 weeks (Sweeney, 1968, 1969). A known metabolite was found in both cultures. Patil et al. (1972) have

shown a species of Dunaliella that was able to metabolize aldrin to dieldrin and aldrin-diol. Khan et al. (1972) have also shown several algae capable to epoxidize aldrin to dieldrin. Uptake and metabolism of DDT by six species of marine phytoplankton was studied by Rice and Sikka (1973). All species accumulated DDT and all converted a small amount of DDT to DDE. Tetraselmis sp. gave maximum conversion of DDT to DDE. Metabolism of DDT to DDE was shown by a marine diatom, Cylindrotheca closterium (Keil and Priester, 1969). Neudrof and Khan (1979) have shown that Ankistrodesmus amalloides metabolizes DDT to both DDD (0.8%) and DDE (3.5%). Bowes (1971) recovered small amounts of DDE (3.0 to 7.4%) of his cultures of Skeletonema costatum, Thalassiosira pseudonana, T. fluviatilis and Dunaliella tertiolecta after two or three weeks. He noticed that DDE was recoverable from Dunaliella tertiolecta cells if the cells were raptured.

Growth of algae is affeted by variations in temperature. Temperature may also affect toxicity due to pesticides.

Kremer (1978) has studied effect of temperature on photosynthesis of sea-weeds. He found that maximum photosynthesis occurred at 25°C in Lemnea annulata and at 35°C in Compsopogon hookeri. Temperature effect on growth and proliferation of Gymnodinium berve and Gomphospheria aponica was studied by Eng-Wilmot (1977). He found that G. breve showed optimal growth at 27°C and proliferated over a wide range of temperatures (17-30°C). At 4°C

and above 30°C alga started dying. G. aponica showed optimal growth between 24°C - 29°C with a maximum at 27°C and a minimum at 31°C.

Swarkznan and Adams (1979) have studied effects of increased temperature on seasonal dynamics of phytoplankton. They found that species with a lower phosphorus tolerance, a greater tolerance to nitrogen and lower optimal light intensity for growth would better survive in warmer habitats.

Hanisak and Harlin (1978) have shown that thalii of Codium fragile grew best at 25°C. Optimal temperature for photosynthesis of natural phytoplankton of blue green algae in lake Mendota was found to be between 20 - 30°C (Konapka and Brock, 1978).

Kruger and Eloff (1978) reported that lower temperature limit for growth of Microcystis sp. varies from 10.5 to 13.5°C, the thermal growth optimum was between 28.8 to 30.5°C, and upper temperature limit between 30.5 to 40.0°C.

Briand (1975) has studied temperature effect on membrane structure of Anacystis nidulans. He has shown that cells grown at 25°C before chilling, appeared unaffected; whereas those grown at 39°C before chilling showed significant morphological alterations.

Venkateswarlu (1970) studied thermal preference in river Mossi (India) and showed that Acananthus minutrssima and Cymbella microphalla were favoured by low temperature and high DO. Spirogyra sp., Cyclotella meneghiniana and Stigocloneium tenue were adversely affected by higher temperatures. Higher temperatures accelerated growth and multiplication of chlorococaless, blue-greens and desmids. In warm water high oxygen levels favoured green algae and high organic load favoured the blue greens.

Goldman and Ryther (1976) and Goldman (1977) have grown marine phytoplankton species in enriched continuous laboratory cultures. Competition among species was highly dependent on temperatures, although amount of organic matter produced was relatively independent. Below 10.8°C, Phaeodactylum tricosmutum was dominant species; at 27°C, Nitzschia sp. was predominating whereas above 27°C Oscillatoria species became increasingly dominant.

MATERIALS AND METHODS

Cultures of blue green alga, Spirulina platensis were obtained from Indian Agricultural Research Institute, New Delhi. Cultures were grown in 3 litre Erlenmeyer flat bottom flasks at 20±2°C, under artificial, fluorescent, cool, white light source with light intensity _ 2500 lux. Light and dark cycles were maintained at 16/8 hrs.

Stock cultures were maintained in log phase by adding a fixed amount of medium once in a week. To avoid sticking, cultures were shaken at least four times in a day.

Nutrient medium used to grow alga was as given by Menon (1981) excluding EDTA. The pH of the medium was maintained at 8.2.

The chemical composition of the medium as per reference quoted above was as under:-

$NaHCO_3$	=	18.0 g/l
K_2HPO_4	=	2.5 g/l
$NaNO_3$	=	2.5 g/l
NaCl	=	1.0 g/l
$MgSO_4.7H_2O$	=	0.2 g/l
$CaCl_2.2H_2O$	=	0.04 g/l
$FeSO_4.7H_2O$	=	0.01 g/l
A_5 soln.	=	1.0 ml/l

A_5 solution is a micronutrient solution and its composition was :

Boric acid	=	2.9 g/l
$MnCl_2$	=	1.81 g/l
$ZnCl_2$	=	0.11 g/l
$CuSO_4 \cdot 5H_2O$	=	0.08 g/l
$(NH_4)_2MoO_4$	=	0.18 g/l

Temperature studies were performed in thermally stable environment. Temperature was maintained thermostatically in glass aquaria, which were kept under artificial, cool, white, fluorescent light source. Algae were grown in these aquaria at $20 \pm 1^{o}C$, $30 \pm 1^{o}C$ and $40 \pm 1^{o}C$ for temperature studies.

Two organochlorine pesticides, namely DDT and aldrin, were selected for detailed study. DDT was obtained as p-p' DDT from Hindustan Insecticides Ltd., Delhi, whereas aldrin was obtained as 30 per cent EC solution from Northern Chemicals, Daulatabad Marg, Gurgaon. Pesticide solutions were made in absolute ethyl alcohol. Pesticide solutions were added in algal cultures so as to give final pesticide concentration 0.5 ppm, 1.0 ppm, 1.5 ppm, 2.0 ppm. Similar amounts of alcohol were added in control cultures. All studies were done in triplicate.

Various algal responses to these pesticides included measurements of (i) biomass as mg/l dry wt; (ii) chlorophyll content; (iii) protein content and (iv) sulphate uptake.

All studies regarding biomass and chlorophyll content were performed in 15 ml screw cap culture tubes whereas studies regarding protein content and SO_4^{--} uptake were performed in 250 ml conical flasks.

Biomass

Biomass was monitored spectrophotometrically at 490 nm, at every 24 hrs intervals upto a maximum of 120 hrs using a Bausch and Lomb "Spectronic-20" spectrophotometer (Stein, 1973). Biomass B represented as mg/l dry wt. was calculated from optical density, O.D. by the following equation :

$$B = 7.5696 + 800.7672 \times OD$$

Survival ratio was calculated by the following formula :

$$S.R. = \frac{B_{exp}}{B_{cont}} \times \frac{B_{cont}\,t_o}{B_{exp.}\,t_o} \times 100$$

where, B_{exp} = Experimental Biomass at time t

B_{cont} = Control Biomass at time t

$B_{exp}t_o$ = Experimental Biomass at time t_o

$B_{cont}t_o$ = Control Biomass at time t_o

Instantaneous growth rate and specific growth rate were calculated by the following formulae (Odum, 1971)

$$r = \frac{\ln N_2 - \ln N_1}{t_2 - t_1}$$

where, N_1 = No. of organisms at time t_1

N_2 = No. of organisms at time t_2

r = growth rate,

and $r\ sp = r/N_1$

Doubling time (time required for cells to multiply once) was calculated as (Turk, 1985):

$$t_D = 0.693/r$$

LC_{50} values were calculated as per Finney (1971) by regression analysis of survival ratio against ln concentrations.

Chlorophyll Estimation

Cultures were incubated with various pesticide concentrations for 6 hrs. A similar alcohol control was also incubated. After incubation period was over, contents were filtered with 2.5 cm Whatman GF/C glass fibre filter paper and were kept in dessicators which, in turn, were kept in a deep freezer for 24 hrs prior to extraction. 5 ml of 80 per cent acetone were added to each filter paper and extraction was continued for about 24 hrs in dark, cool conditions. After extraction period, the contents were transferred in cuvette and optical density was measured at 663 and 645 nm using a Bausch and Lomb "Spectronic

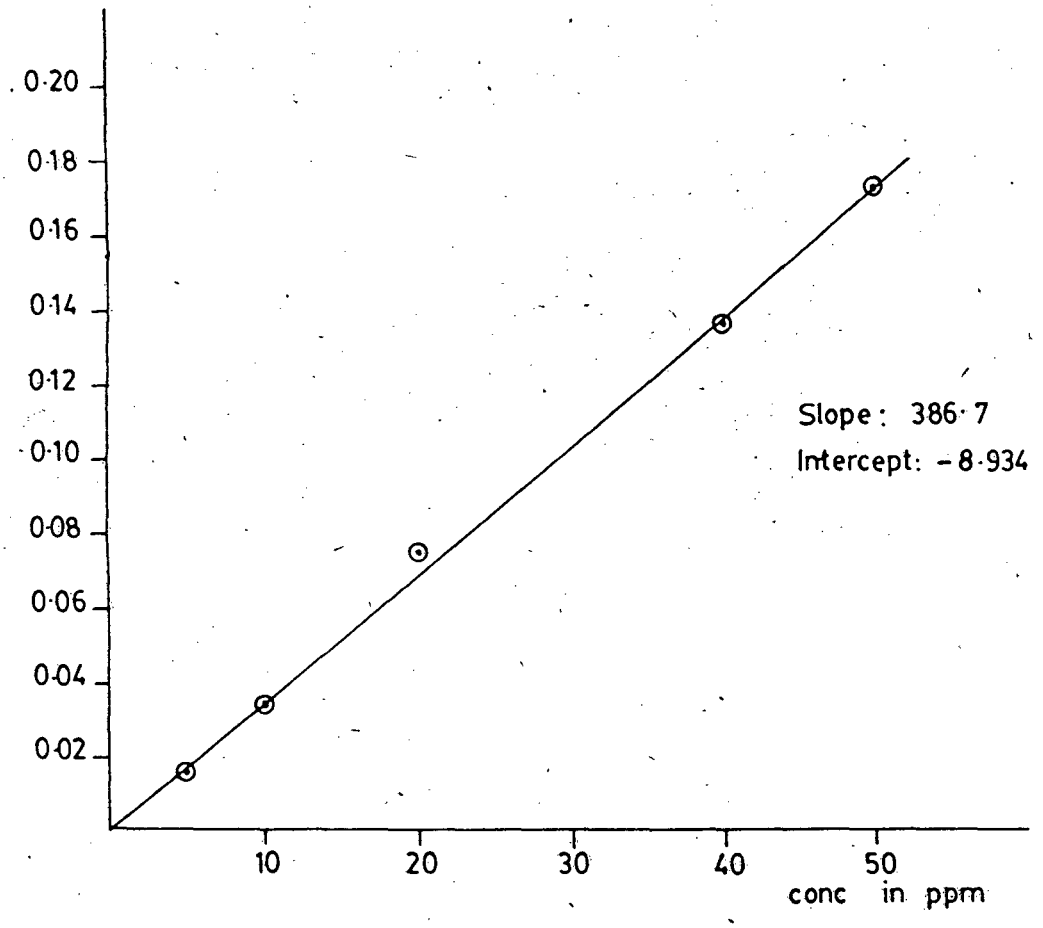


Fig-1 STANDARD CURVE FOR PROTEIN

1001" spectrophotometer. The chlorophyll content was calculated as per Venkatraman (1983) using the following equation

$$Chl^a \text{ (mg/ml)} = (0.127 \times A_{663}) - (0.00269 \times A_{645})$$

where, A is absorbance at a particular wavelength.

Protein Content

Protein content of the alga was measured initially and at 48 hrs, 72 hrs and 96 hrs. After above mentioned time intervals, 10 ml of culture was taken from each flask and after measuring biomass (spectrophotometrically at 490 nm) the contents were filtered by 2.5 cm Whatman GF/C glass fibre filters and these were kept in deep freezer for 24 hrs. After this time interval filters were kept in 5.0 ml of 0.5 N NaOH for 24 hrs in a dark, cool place. The protein content of the resultant extract was measured as per Lowry (1959).

To 1 ml of protein extract, 5 ml of reagent A (1 ml of 2.7 per cent Sodium Potassium Tartrate and 1 ml of 1 per cent $CuSO_4 \cdot 5H_2O$ added in 2 per cent Na_2CO_3 to make the volume 100 ml) and 0.5 ml of reagent B (Folin-Ciocalton reagent, Oser, 1975) were added. After half an hour OD was measured at 750 nm using a Bausch and Lomb "Spectronic 1001" spectrophotometer.

Protein standards were made from egg albumin. Protein content in the extract was calculated by the following equation :

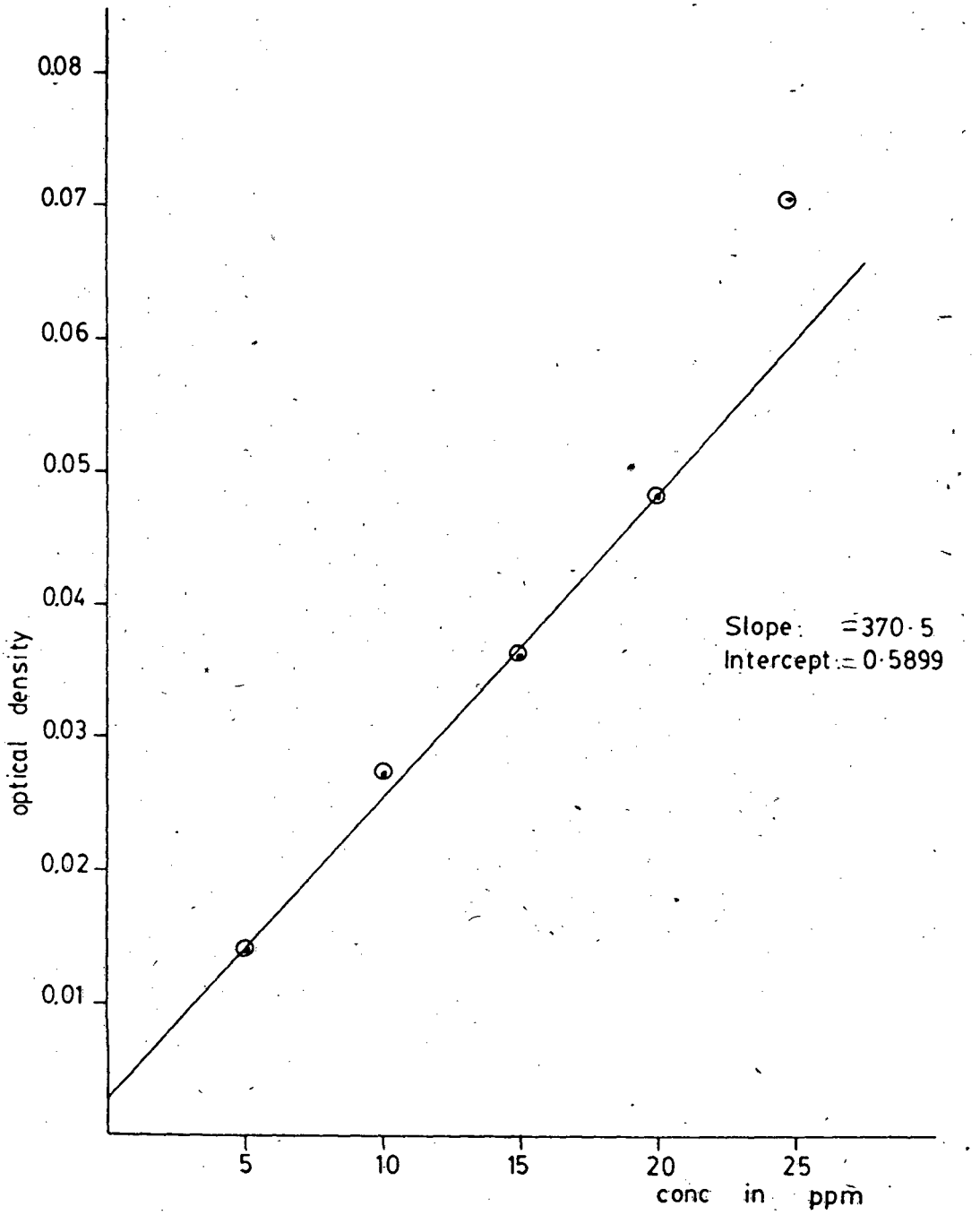


Fig 2 STANDARD CURVE FOR SO_4^{--}

Protein (mg/ml) = -8.934 + 386.7 x OD

SO_4^{--} Uptake

A SO_4^{--} free medium was prepared for SO_4^{--} uptake studies. In place of K_2SO_4, $FeSO_4$ and $MgSO_4$, similar amounts of KCl, $FeCl_3$ and $MgCl_2$ were added to provide these nutrients (Menon, 1981). Filtered algal sample was washed thrice with this SO_4^{--} free medium and then it was put in the SO_4^{--} free medium. Alga was allowed to grow in this medium for 24 hrs, so as to acclimatize the alga in this environment. Known concentrations of SO_4^{--} solutions were added in these cultures. Pesticide solutions were added in algal cultures to make final concentrations 1.0 ppm and 2.0 ppm.

Algal growth and amount of SO_4^{--} present in the medium were monitored at every 24 hrs interval. It was found that the SO_4^{--} uptake at 24 hrs interval was not much. So it was monitored at every 48 hrs intervals. Growth was monitored by the method described earlier. SO_4^{--} content in the filtrate was measured spectrophotometrically at 420 nm (APHA, 1977) by Bausch and Lomb "Spectronic 1001" spectrophotometer. SO_4^{--} content was calculated by the following equation :

SO_4^{--} (in ppm) = 0.5899 + 370.5 x OD.

Statistical Analyses

Simple correlations and regression equations for ln concentrations vs various parameters were computed. All statistical analyses were programmed on HP 1000/40 computer installed in the School of Computer and Systems Sciences, J.N.U., New Delhi.

Formulae used for calculating correlation coefficients were of Snedecor and Cochran (1967).

$$\text{Correlation coefficient } r = \frac{Sxy}{\sqrt{(Sxx) \cdot (Syy)}}$$

where,

$$\text{(i) } Sxy = \Sigma xy - \frac{(\Sigma x).(\Sigma y)}{n}$$

where, Σxy = sum of the products of x and y

x = experimental parameters

y = ln conc. of toxicant

n = no. of samples.

$$\text{(ii) } Sxx = \Sigma x^2 - \frac{(\Sigma x)^2}{n}$$

where, Σx^2 = sum of squares of x

$(\Sigma x)^2$ = square of total of x.

(iii) $Syy = \Sigma y^2 - \frac{(\Sigma y)^2}{n}$

where, Σy^2 = sum of squares of y

$(\Sigma y)^2$ = square of total of y.

Formula for regression equation :

$$y = mx + c$$

where, y = ln concentration of pesticides

x = experimental parameters

m = slope

c = intercept.

R E S U L T S

Biomass

Biomass of Spirulina platensis was monitored every 24 hrs spectrophotometrically at 490 nm upto a maximum of 120 hrs. It was represented as mg/l dry wt. Biomass of alga under various temperatures and pesticide concentrations are given in Tables 1-6. The maximum increase in algalbiomass was seen at 20°C, when after the end of the experiment (120 hrs) the final value was found to be 83.73 ± 2.49 mg/l dry wt. from an initial value of 34.06 ± 2.17 mg/l dry wt. At 30°C, maximum biomass observed at the end of the experiment was 166.45 ± 6.38 mg/l dry wt. from an initial value of 100.02 ± 6.95 mg/l dry wt. At 40°C, alga started dying and at the end of the experiment only 43.59 ± 6.79 mg/l dry wt. biomass was obtained from an initial value of 68.22 ± 4.14 mg/l dry wt. The growth rate at various temperatures was found to be 7.50×10^{-3}/hr, 4.24×10^{-3}/hr and -3.73×10^{-3}/hr at 20°C, 30°C and 40°C, respectively (Tables 7 and 10). Doubling time, i.e. the time required by cells to multiply once, was found to be 92.40 hrs, 163.44 hrs and -185.79 hrs at 20°C, 30°C and 40°C, respectively.

Addition of either of the two pesticides, namely, DDT and aldrin, to algal cultures alter the growth rate and this alteration was found to be temperature dependent also. Addition of DDT upto 1.5 ppm was found to increase growth rate at 20°C and 30°C, whereas,

when 2.0 pm DDT was added to cultures, growth rate was reduced in the algal cultures which were incubated at 20°C. Addition of 2.0 ppm DDT at 30°C to algal cultures has no inhibitory effect on growth rate. At 40°C, growth rate of alga was found to be in negative terms, indicating thereby a net death in algal cultures. This death process was found to be increased with increasing concentrations of DDT.

A gradual decrease in the growth rate of alga was noticed at 20°C with increasing concentrations of aldrin. At 30°C, aldrin upto 1.0 ppm was found to increase growth rate, thereafter a general decrease with increasing pesticide concentrations was seen. The same situation was observed when aldrin solutions were added to algal cultures incubated at 40°C. In this case also, growth rate was found to be more than controls upto 1.0 ppm whereas addition of 1.5 ppm or 2.0 ppm aldrin markedly reduced growth rate.

Doubling time for Spirulina sp. also showed the effect of pesticides and temperatures on algal biomass. Doubling time was found to be lesser than that of controls upto 1.5 ppm DDT added to cultures at 20°C, whereas addition of 2.0 ppm DDT at same temperatures was found to increase doubling time. At 30°C, doubling time of treated cultures was always found to be lesser than control cultures. This shows that DDT has no inhibitory effect on alga at this temperature upto 2.0 ppm. At 40°C, doubling time became negative, indicating thereby that after that

Table - 1

Effect of DDT on *Spirulina* sp. Biomass (mg/l dry wt) at 20°C

Time / Toxicant	Initial	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
Control	34.06 ±2.17	38.46 ±1.89	46.15 ±1.95	56.02 ±3.45	73.78 ±4.39	83.73 ±2.49
0.5 ppm	33.93 ±5.11	35.92 ±3.34	40.24 ±2.25	53.02 ±4.67	78.31 ±4.77	90.16 ±7.85
1.0 ppm	32.51 ±5.88	33.35 ±4.05	38.24 ±2.27	50.07 ±1.47	71.94 ±5.09	85.80 ±2.59
1.5 ppm	32.18 ±3.90	31.19 ±3.40	33.48 ±6.01	47.88 ±6.28	70.52 ±2.56	84.25 ±3.46
2.0 ppm	32.24 ±2.27	30.27 ±2.98	30.95 ±3.89	46.95 ±5.38	64.20 ±3.80	75.68 ±7.45

Table - 2

Effect of DDT on *Spirulina* sp. Biomass (mg/l dry wt) at 30°C

Time / Toxicant	Initial	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
Control	100.2 ±6.95	126.83 ±12.84	143.46 ±7.88	149.49 ±6.95	158.58 ±3.02	166.45 ±6.38
0.5 ppm	95.13 ±7.46	143.33 ±5.82	161.87 ±3.56	163.72 ±6.86	167.38 ±6.92	171.42 ±3.16
1.0 ppm	97.62 ±5.35	125.00 ±2.85	136.76 ±3.02	156.19 ±4.32	167.25 ±11.84	175.73 ±6.69
1.5 ppm	99.11 ±4.89	122.01 ±7.63	139.89 ±6.09	158.99 ±3.26	169.00 ±3.37	177.25 ±6.69
2.0 ppm	106.13 ±2.74	130.20 ±6.63	149.01 ±3.56	166.12 ±3.32	177.85 ±3.44	188.45 ±6.21

Table - 3

Effect of DDT on *Spirulina* sp. Biomass (mg/l dry wt) at 40°C

Time / Toxicant	Initial	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
Control	68.22 ±4.14	72.37 ±0.0	50.83 ±8.48	54.68 ±2.29	44.86 ±4.85	43.59 ±6.74
0.5 ppm	71.67 ±1.21	65.47 ±4.72	45.53 ±5.30	49.65 ±2.22	41.00 ±2.25	40.32 ±2.17
1.0 ppm	71.32 ±1.47	64.09 ±0.0	41.01 ±2.81	48.96 ±0.0	40.90 ±3.21	38.90 ±4.85
1.5 ppm	65.46 ±1.18	56.70 ±4.18	38.36 ±5.01	43.80 ±4.50	36.20 ±5.46	35.39 ±5.41
2.0 ppm	65.46 ±3.13	55.71 ±4.55	38.01 ±1.42	40.41 ±6.58	35.06 ±7.21	34.65 ±4.23

Table - 4

Effect of Aldrin on *Spirulina* sp. Biomass (mg/l dry wt) at 20°C

Time / Toxicant	Initial	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
Control	34.06 ±2.17	38.46 ±1.89	46.15 ±1.95	56.02 ±3.45	73.78 ±4.39	83.73 ±2.49
0.5 ppm	36.57 ±0.0	40.01 ±2.93	45.51 ±2.96	53.36 ±5.01	75.73 ±2.49	86.68 ±5.05
1.0 ppm	39.10 ±2.19	40.37 ±1.91	45.50 ±2.24	50.06 ±0.0	73.88 ±1.22	86.01 ±2.16
1.5 ppm	42.28 ±1.98	40.18 ±1.37	42.96 ±5.58	50.75 ±5.99	69.10 ±4.90	79.87 ±1.21
2.0 ppm	44.86 ±2.33	40.86 ±1.12	43.53 ±5.63	42.46 ±0.0	61.35 ±6.12	71.73 ±2.35

Table - 5

Effect of Aldrin on *Spirulina* sp. Biomass (mg/l dry wt) at 30°C.

Time / Toxicant	Initial	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
Control	100.02 ±6.95	126.83 ±12.84	143.46 ±7.88	149.49 ±6.95	148.58 ±3.02	166.45 ±6.38
0.5 ppm	106.89 ±7.39	150.87 ±6.09	164.60 ±3.16	170.16 ±1.68	165.01 ±1.73	199.06 ±6.93
1.0 ppm	96.70 ±0.0	129.73 ±9.27	138.87 ±4.21	136.87 ±2.62	132.90 ±8.36	162.04 ±6.58
1.5 ppm	92.68 ±1.32	121.89 ±6.03	132.09 ±6.08	128.19 ±4.24	129.90 ±3.26	150.53 ±9.95
2.0 ppm	100.02 ±6.95	122.47 ±5.34	129.40 ±5.48	127.39 ±3.11	124.27 ±0.0	142.29 ±9.51

Table - 6

Effect of Aldrin on Spirulina sp. Biomass (mg/l dry wt) at 40°C

Time / Toxicant	Initial	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
Control	68.22 ±4.14	72.37 ±0.0	50.83 ±8.48	54.68 ±2.29	44.86 ±4.85	43.59 ±6.74
0.5 ppm	71.00 ±4.84	68.78 ±3.13	47.29 ±1.16	50.24 ±8.33	43.11 ±3.90	46.82 ±4.51
1.0 ppm	73.77 ±1.21	70.22 ±2.07	48.20 ±0.0	49.99 ±2.26	44.29 ±0.0	48.35 ±4.61
1.5 ppm	75.88 ±1.22	70.97 ±2.40	48.83 ±2.37	50.06 ±7.04	44.25 ±2.90	47.06 ±2.87
2.0 ppm	68.42 ±3.12	58.04 ±3.50	40.02 ±5.99	40.83 ±5.93	36.85 ±3.91	38.26 ±6.73

much time, population of alga would remain at half of the initial value. This time was found to be decreasing with increasing concentrations of DDT which shows that toxicity increases with increasing concentrations of pesticides.

Doubling time for Spirulina sp. was found to be more with increasing concentrations of aldrin at 20°C and 30°C indicating thereby an increase in toxicity with increasing concentrations. At 40°C, doubling time was found to be lesser than required by controls upto 1.0 ppm whereas at higher concentraions its value was seen to increase with increasing concentrations.

Effects of pesticides on algal biomass can also be explained in terms of survival ratio. Toxicity of aldrin at 20°C in terms of survival ratio, increases with time upto 72 hrs. Maximum reduction in percentage of living cells at 72 hr interval was found to be 88.71%, 77.74%, 73.38% and 57.55% for 0.5 ppm, 1.0 ppm, 1.5 ppm and 2.0 ppm, respectively. After this time interval, alga started tolerating the pesticide and percentage of living cells was found to be more with increasing time intervals.

Addition of aldrin upto 1.0 ppm to algal cultures incubated at 30°C has a stimulatory effect till 48 hrs. However, survival ratio was found to be lesser with increasing time intervals. Higher concentrations of aldrin showed toxic effects just after first observation (24 hrs). Maximum reduction in percentage of

Table - 7

Growth Rate of Spirulina sp. at varying temperatures and DDT concentrations (x 10^{-3}/hr)

Temperature	Toxicant				
	Control	0.5 ppm	1.0 ppm	1.5 ppm	2.0 ppm
20°C	7.50	8.14	8.09	8.02	7.11
30°C	4.24	4.90	4.90	4.84	4.78
40°C	-3.74	-4.79	-5.06	-5.13	-5.30

Table - 8

Specific Growth Rate of Spirulina sp. at varying temperatures and DDT concentrations (x 10^{-4}/mg/hr)

Temperature	Toxicant				
	Control	0.5 ppm	1.0 ppm	1.5 ppm	2.0 ppm
20°C	2.20	2.24	2.49	2.49	2.20
30°C	0.42	0.52	0.50	0.49	0.45
40°C	-0.55	-0.67	-0.71	-0.78	-0.81

Table - 9

Doubling Time for Spirulina sp. at varying temperatures and DDT concentrations (in hrs)

Temperature	Toxicant				
	Control	0.5 ppm	1.0 ppm	1.5 ppm	2.0 ppm
20°C	92.40	85.13	85.66	86.41	97.47
30°C	163x44	141.43	141.43	143.18	144.98
40°C	-185.29	-144.68	-136.96	-135.09	-130.76

Table - 10

Growth Rate of Spirulina sp. at varying temperatures and Aldrin concentrations (x 10^{-3}/hr)

Temperature	Control	Toxicant 0.5 ppm	1.0 ppm	1.5 ppm	2.0 ppm
20°C	7.50	7.19	6.57	5.30	3.91
30°C	4.24	5.18	4.30	4.04	2.94
40°C	-3.73	-3.47	-3.52	-3.98	-4.84

Table - 11

Specific Growth Rate of Spirulina sp. at varying temperatures and Aldrin concentrations (x 10^{-4}/mg/hr)

Temperature	Control	Toxicant 0.5 ppm	1.0 ppm	1.5 ppm	2.0 ppm
20°C	2.20	1.97	1.68	1.25	0.87
30°C	0.42	0.49	0.45	0.44	0.29
40°C	-0.55	-0.49	-0.48	-0.53	-0.71

Table - 12

Doubling Time for Spirulina sp. at varying temperatures and Aldrin concentrations (in hrs)

Temperature	Control	Toxicant 0.5 ppm	1.0 ppm	1.5 ppm	2.0 ppm
20°C	92.40	96.38	105.48	130.75	177.24
30°C	163.44	133.78	161.16	171.54	235.71
40°C	-185.79	-199.71	-196.88	-174.12	-143.18

biomass treated with 2.0 ppm aldrin was found at 96 hrs when only 83.64 percent of cells survived. In this case also, after this period alga started tolerating toxic effect of the pesticide and survival ratio was found to be more than 100 per cent for lower concentrations of pesticides. Higher concentrations, however, still have some toxic effect, although survival ratio in this case also was found to be more than that for lesser time periods.

At 40°C, toxicity was found to increase with increasing exposure time upto 72 hrs when maximum reduction in living cells was noticed. After this time period, pesticide toxicity became lesser with increasing time intervals and at the end of the experiment, treated cultures with lower concentrations showed better growth than control cultures. Higher concentrations still had some toxicity but this toxicity was found lesser as compared to toxicity at 96 hrs.

Survival ratios for alga incubated at 20°C with DDT also showed similar trend. Toxicity was found to increase with increasing concentrations and with increasing time intervals upto 48 hrs. Maximum decrease in survival ratio was noted to be 71.09 per cent in case of 2.0 ppm DDT at 48 hrs. After this time interval, alga started tolerating pesticide and growth was found to be more than for lesser time periods. At 96 hrs survival ratio was found to be more than 100 per cent upto 1.5 ppm of DDT, whereas, 2.0 ppm DDT was still toxic to the alga.

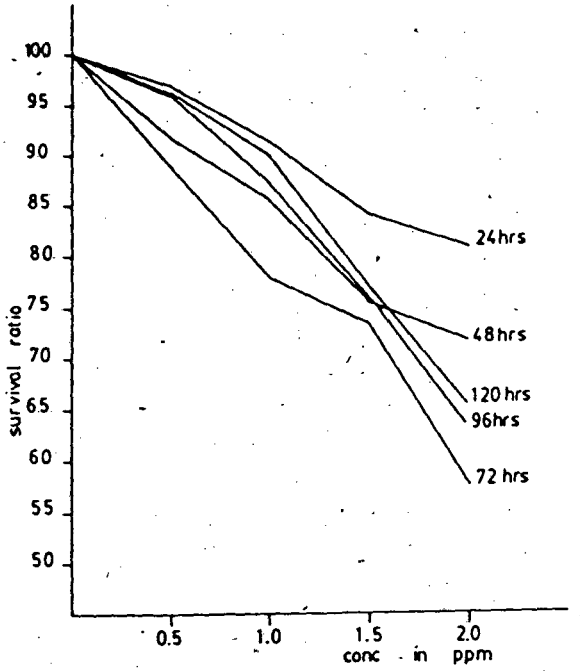


Fig 3 SURVIVAL RATIO OF SPIRULINA PLATENSIS TREATED WITH ALDRIN AT 20°C

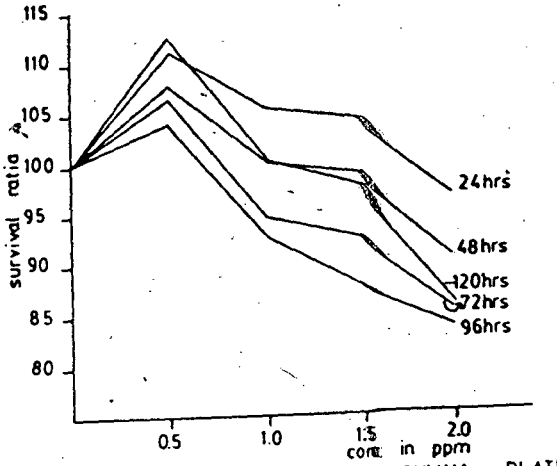


Fig 4 SURVIVAL RATIO OF SPIRULINA PLATENSIS TREATED WITH ALDRIN AT 30°C

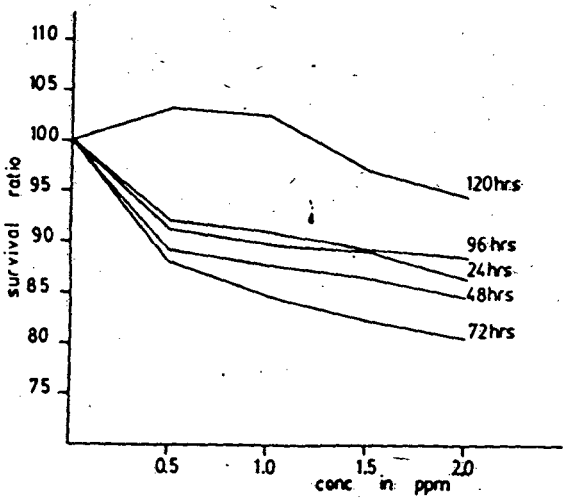


Fig 5 SURVIVAL RATIO OF SPIRULINA PLATENSIS TREATED WITH ALDRIN AT 40°C

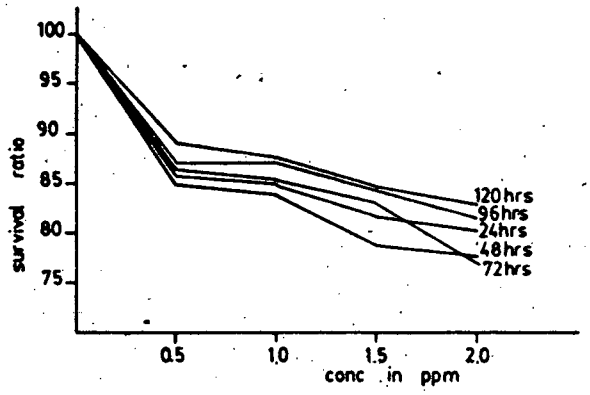


Fig 8 SURVIVAL RATIO OF SPIRULINA PLATENSIS TREATED WITH DDT AT 40°C

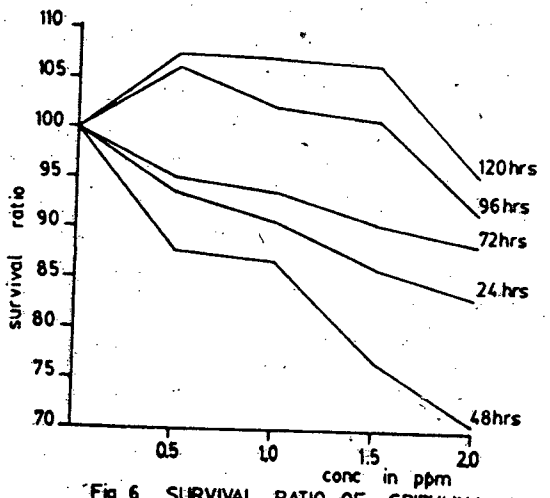


Fig 6 SURVIVAL RATIO OF SPIRULINA PLATENSIS TREATED WITH DDT AT 20°C

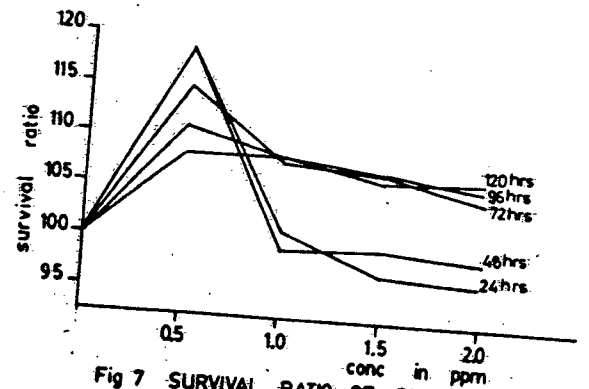


Fig 7 SURVIVAL RATIO OF SPIRULINA PLATENSIS TREATED WITH DDT AT 30°C

When the alga was incubated at 30°C, 0.5 ppm DDT had no toxicity at a time interval. 1.0 ppm DDT caused a marginal decrease in survival ratio at 48 hr, thereafter, growth was resumed and treated cultures showed better growth than control cultures. Addition of 1.5 ppm and 2.0 ppm DDT caused a decrease in survival ratio upto 48 hrs, thereafter, pesticide was not found to be toxic.

DDT at 40°C was found to be toxic at all concentrations and all time intervals. Toxicity increased upto 72 hrs, after that a sign of alga becoming tolerant to pesticide was observed, since survival ratio was found to be more with increasing time.

LC_{50} values, concentrations of toxicants needed to kill 50 per cent of the population was calculated according to Finney (1971) by regression analysis of survival ratio against ln concentrations.

LC_{50} values of both pesticides to Spirulina sp. were found to be high which shows resistance of algae to these pesticides. At 20°C, there was a decrease in LC_{50} value of aldrin with increasing time intervals upto 96 hrs. After that LC_{50} values were found to be more. This indicates that toxicity increased upto 96 hrs, after that alga showed resistance to pesticide. At 30°C, LC_{50} value of aldrin was seen to decrease with increasing time intervals and 120 hrs LC_{50} value was found to be 14.59 ppm. At 40°C, LC_{50} value was found to be very high upto 48 hrs,

Table - 13

LC_{50} values of aldrin for Spirulina platensis

Time / Temperature	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
20°C	32.79	7.17	3.25	5.26	10.28
30°C	379.94	92.76	23.34	17.64	14.59
40°C	-	-	354.25	-	-

Table - 14

LC_{50} values of DDT for Spirulina platensis

Time / Temperature	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
20°C	188.67	17.46	383.75	-	-
30°C	29.08	29.94	-	-	-
40°C	2164.62	454.87	376.16	-	-

whereas, 72 hrs LC_{50} was found to be 354.25 ppm. After this time period, pesticide was not found to be toxic to the alga.

LC_{50} values of DDT showed a similar trend. At 20°C, 48 hrs LC_{50} value was 17.46 pm. After that LC_{50} value was found to be more with increasing time intervals. At 30°C, toxicity of DDT was found only at the initial observation (24 hrs), after that LC_{50} value increased and it became very high after 72 hrs. At 40°C, toxicity increased with increasing time intervals upto 72 hrs. This was shown by decreasing values of LC_{50} with increasing time. However, after 96 hrs toxicity became very less and LC_{50} value was found to be immeasureable.

Chlorophyll-a

Chl a is the main photosynthesizing pigment of blue green alga, e.g., Spirulina platensis. Unlike higher plants and green algae they do not have chl b. Accessory pigments found in these algae are p-hycocyanin, phycoerythrin, β-contene, myxoxanthin and myxoxanthophyll (Desikachary, 1959).

Chl a of Spirulina sp. was monitored spectrophotometrically at 663 and 645 nm (Venkatraman, 1983).

Chl a in control cultures was found to be $4.79 \pm 0.07 \times 10^{-3}$ mg/mg and $4.18 \pm 0.43 \times 10^{-3}$ mg/mg under light and dark

conditions, respectively, when the alga was incubated at 20°C. At 30°C, the corresponding amounts of Chl a was found to be 4.97 $\pm$ 0.42 x 10^{-3} mg/mg and 4.33 $\pm$ 0.26 x 10^{-3} mg/mg. At 40°C, amount of Chl a was found to be much less. It was 4.20 $\pm$ 0.19 x 10^{-3} mg/mg under light and 4.10 $\pm$ 0.19 x 10^{-3} mg/mg under dark conditions, respectively.

Addition of either of the two pesticides to cultures had a profound effect on Chl a content of alga. Chl a content was found to decrease and this decrease was found to be concentration and temperature dependent. Thus, toxicity was found to increase with increasing concentrations and temperatures. Toxicity was found to be maximum with 2.0 ppm DDT at 40°C when Chl a content was only 56.90 per cent of control. The corresponding values for 20°C and 30°C, were 85.60 per cent and 74.46 per cent respectively. The Chl a content under dark conditions at 2.0 ppm were found to be 77.27 per cent, 73.23 per cent and 62.26 per cent of the control at 20°C, 30°C and 40°C respectively. Similar trend was also observed with other concentrations of DDT.

At 40°C, addition of 0.5 pm DDT did not cause much difference in Chl a content under light and dark conditions. It was found to be 4.00 $\pm$ 0.09 x 10^{-3} mg/mg and 3.99 $\pm$ 0.04 x 10^{-3} mg/mg under light and dark conditions respectively. However, with increasing concentrations of pesticide, cultures under light showed more decrease in Chl a content than under dark. Under light

Table 15

Effect of DDT on Chl a content of *Spirulina platensis*

Temperature		Toxicant: Control	0.5 ppm	1.0 ppm	1.5 ppm	2.0 ppm	n	K
20°C	L	4.79 ±0.07	4.29 ±0.03	4.27 ±0.21	4.20 ±0.08	4.10 ±0.15	0.13	-4.23
	D	4.18 ±0.43	3.99 ±0.0	3.96 ±0.49	3.80 ±0.49	3.23 ±0.06	0.47	-3.79
30°C	L	4.97 ±0.42	4.87 ±0.87	4.60 ±0.0	4.28 ±0.09	3.85 ±0.79	0.70	-4.47
	D	4.33 ±0.42	4.20 ±0.0	4.15 ±0.0	3.27 ±0.54	3.17 ±0.19	0.82	-3.78
40°C	L	4.20 ±0.19	4.00 ±0.09	3.79 ±0.0	2.60 ±0.06	2.39 ±0.05	1.29	-3.34
	D	4.10 ±0.19	3.99 ±0.04	3.90 ±0.0	3.21 ±0.09	2.55 ±0.08	1.00	-3.51

Table 16

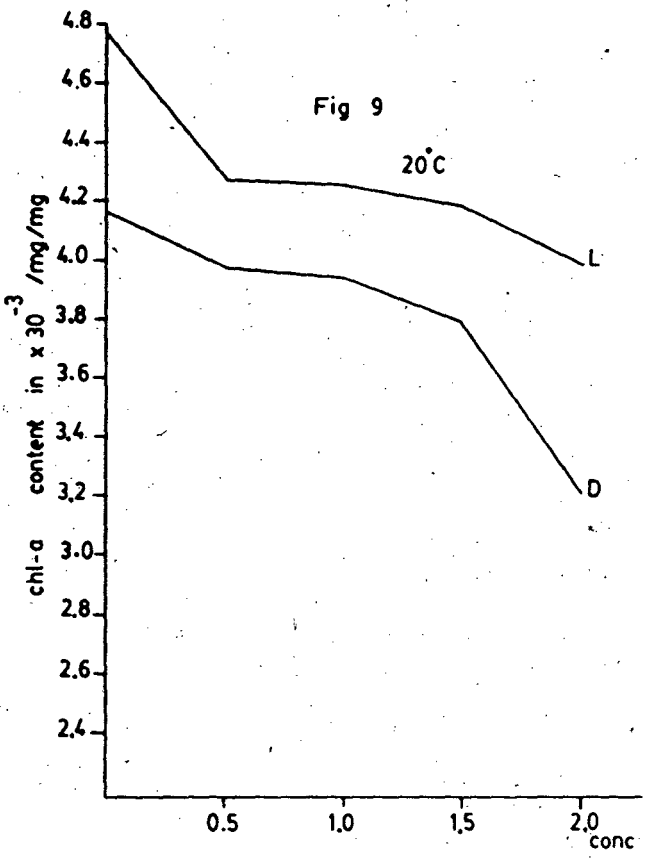
Effect of Aldrin on Chl a content of *Spirulina platensis*

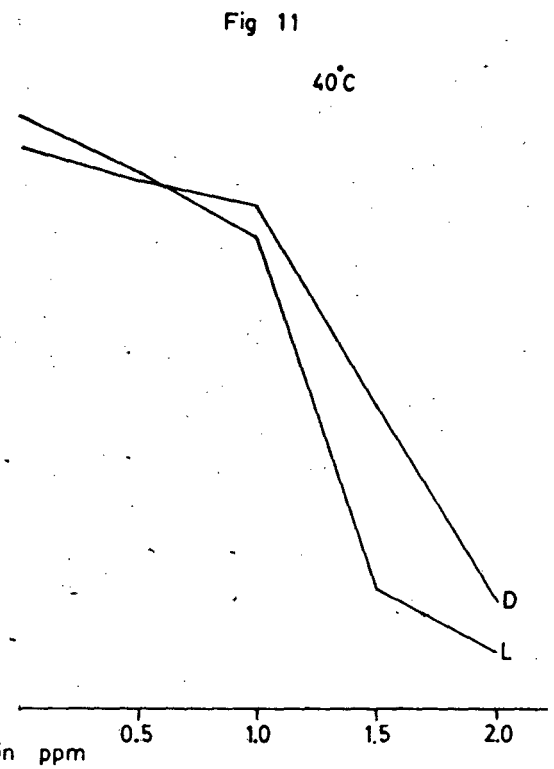
Temperature		Control	Toxicant 0.5 ppm	1.0 ppm	1.5 ppm	2.0 ppm	n	K
20°C	L	4.79 ±0.07	3.58 ±0.0	3.50 ±0.04	2.93 ±0.0	2.78 ±0.09	0.61	-3.26
	D	4.18 ±0.43	3.25 ±0.10	3.03 ±0.06	2.93 ±0.25	2.80 ±0.11	0.32	-3.03
30°C	L	4.97 ±0.42	4.23 ±0.15	4.10 ±0.21	3.98 ±0.44	3.66 ±0.09	0.37	-4.03
	D	4.33 ±0.26	3.96 ±0.30	3.91 ±0.35	3.80 ±0.28	3.45 ±0.78	0.32	-3.81
40°C	L	4.20 ±0.19	3.62 ±0.12	3.37 ±0.02	3.30 ±0.03	2.49 ±0.02	0.68	-3.27
	D	4.10 ±0.19	3.81 ±0.07	3.53 ±0.06	3.46 ±0.0	3.22 ±0.20	0.39	-3.55

conditions, it was found to be 4.00 ± 0.09, 3.79 ± 0.0; 2.60 ± 0.06 and 2.39 ± 0.05 x 10^{-3} mg/mg at 0.5, 1.0, 1.5 and 2.0 ppm concentrations. Corresponding values under dark conditions were 3.99 ± 0.04; 3.90 ± 0.0; 3.21 ± 0.09 and 2.55 ± 0.08 x 10^{-3} mg/mg.

Addition of aldrin to algal cultures showed a similar response. Maximum reduction in Chl a content yielding 58.04 per cent of the control was found to be with 2.0 ppm aldrin at 20°C under light conditions. Under dark conditions the same concentration reduced Chl a content to 66.99 per cent. When algae were incubated at 30°C, Chl a was found to decrease gradually with increasing concentrations of pesticide. Under light conditions, Chl a was found to be 85.11 per cent, 82.50 per cent, 80.08 per cent and 73.64 per cent of the control at 0.5 ppm, 1.0 ppm, 1.5 ppm and 2.0 ppm DDT concentrations. The corresponding values for 'dark' conditions were 91.46 per cent, 90.30 per cent, 87.76 per cent and 79.68 per cent, respectively.

At 40°C also, addition of aldrin caused a gradual decrease in Chl a. It was found to be 3.62 ± 0.12; 3.37 ± 0.02; 3.30 ± 0.03 and 2.49 ± 0.02 x 10^{-3} mg/mg at 0.5 ppm, 1.0 ppm, 1.5 ppm and 2.0 ppm respectively under light conditions. Corresponding values for dark conditions were, 3.81 ± 0.07; 3.53 ± 0.06; 3.46 ± 0.0 and 3.22 ± 0.20 x 10^{-3} mg/mg.





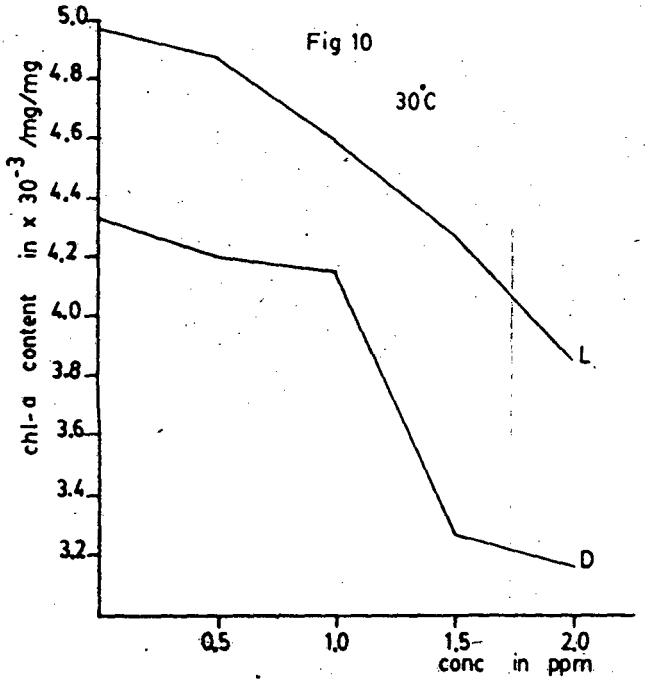
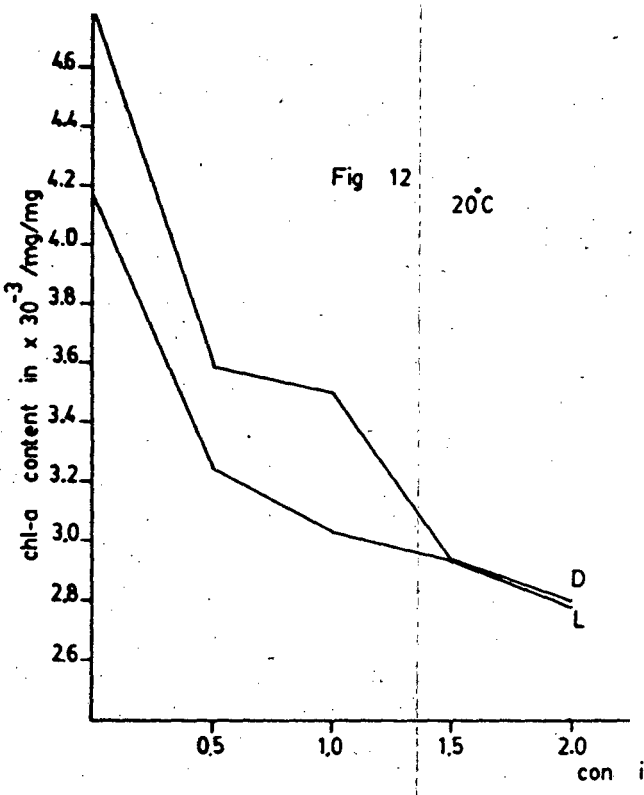
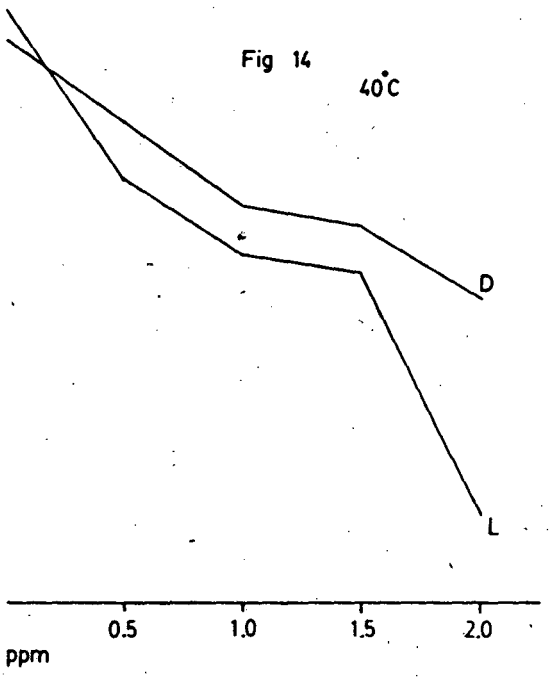


Fig 9-11 EFFECT OF DDT ON Chl-a CONTENT OF SPIRULINA PLATENSIS





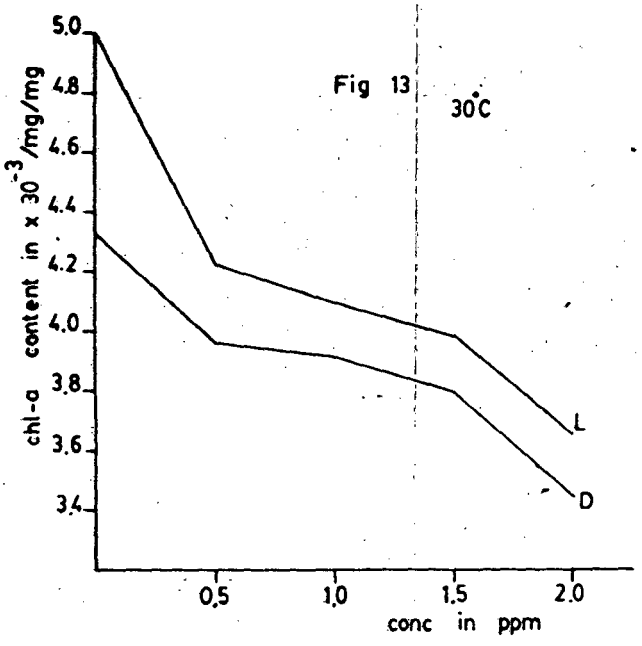


Fig 12-14 EFFECT OF ALDRIN ONChl-a CONTENT OF SPIRULINA PLATESIS

Table 17

Increase/Decrease at % of control in Chl a content of *Spirulina platensis* due to DDT

Temperature		Control	Toxicant 0.5 ppm	1.0 ppm	1.5 ppm	2.0 ppm
20°C	L	100	89.56	89.14	87.68	85.60
	D	100	95.46	94.74	90.90	77.27
32°C	L	100	97.09	92.56	86.12	77.4
	D	100	96.99	95.84	75.52	73.23
40°C	L	100	95.24	90.21	61.91	56.90
	D	100	97.32	95.12	78.29	62.20

Table 18

Increase/Decrease at % of control in Chl a content of *Spirulina platensis* due to Aldrin

Temperature		Control	Toxicant 0.5 ppm	1.0 ppm	1.5 ppm	2.0 ppm
20°C	L	100	74.74	73.07	61.17	58.04
	D	100	77.75	72.49	70.10	66.99
30°C	L	100	85.11	82.50	80.08	73.64
	D	100	91.46	90.30	87.76	79.68
40°C	L	100	86.19	80.24	78.57	59.29
	D	100	92.93	86.10	84.39	78.54

Generally, pecentage reduction in Chl a content of alga was found to be lesser under "dark" condition as compared to light conditions (Tables 17 and 18). This showed that pesticides are more toxic under light than under dark.

Protein Content

Protein is one of the main constituents of Spirulina platensis. It forms upto 65% of total dry wt. (Venkatraman, 1983).

In the present study total protein was estimated as per Lowry (1951). Initially, protein content at 20°, 30° & 40°C was found to be 0.47 ± 0.008 mg/mg. 0.480 ± 0.003 mg/mg and 0.473 ± 0.008 mg/mg dry wt, respectively. When pesticides were added to cultures they had profound effect on the protein content (Tables 21 to 26). This was found to be time, temperature and concentration dependent.

At 20°C, addition of 0.5 ppm aldrin caused an initial increase in protein content to 0.472 ± 0.008 at 48 hrs whereas exposure for more time caused a reduction in protein content. At 48 hrs protein content changed to 0.468 ± 0.009; 0.462 ± 0.002 and 0.457 ± 0.007 mg/mg dry wt. under 1.0 ppm, 1.5 ppm and 2.0 ppm aldrin respectively from an initial value of 0.470 ± 0.008 mg/mg dry wt. As the alga was allowed to be with pesticides for longer times, inhibition was found to be more. After 96 hrs only 0.463 ± 0.003; 0.452 ± 0.007 and 0.449 ± 0.008 mg/mg dry wt. protein was

obtained when alga was exposed to 0.5 ppm, 1.0 ppm, 1.5 ppm and 2.0 ppm aldrin, respectively.

Addition of DDT to algal cultures at this temperature showed a similar trend. 0.5 ppm DDT caused an initial increase in protein content followed by a decrease with increasing time intervals. Toxicity was found to be dose dependent. Protein content was found to be lesser either with increasing time intervals at a particular concentration or with increasing concentrations at a particular time interval.

When alga was incubated at 30°C, the protein content was found to be 0.480 $\pm$ 0.003 mg/mg dry wt. There is a general decrease in protein content of alga with increasing concentration of aldrin and DDT. This can be seen at each time specific exposure for a particular concentration as well with a particular time period for each concentration. DDT at 0.5 ppm increased protein content initially (at 48 hrs) but after that, a reduction in protein content was noticed. 1.0 ppm DDT had no effect on protein content of alga upto 48 hrs, after that a reduction in protein content with increasing time intervals was seen. Higher concentrations of DDT caused a reduction in protein content just after 48 hrs.

When alga was incubated at 40°C, the temperature effect manifested itself. Protein content in control cultures decreased

Table - 19

Effect of Aldrin on protein content of Spirulina platensis at 20°C

Time / Toxicant	Initial	48 hrs	72 hrs	96 hrs
Control	0.470 ±0.008	0.470 ±0.006	0.470 ±0.009	0.471 ±0.008
0.5 ppm	"	0.472 ±0.008	0.465 ±0.007	0.463 ±0.003
1.0 ppm	"	0.468 ±0.009	0.465 ±0.006	0.458 ±0.003
1.5 ppm	"	0.462 ±0.002	0.455 ±0.008	0.452 ±0.007
2.0 ppm	"	0.457 ±0.007	0.451 ±0.008	0.449 ±0.008

Table - 20

Effect of DDT on protein content of *Spirulina platensis* at -20°C

Time / Toxicant	Initial	48 hrs	72 hrs	96 hrs
Control	0.470 ±0.008	0.470 ±0.006	0.470 ±0.009	0.471 ±0.008
0.5 ppm	"	0.473 ±0.004	0.468 ±0.007	0.465 ±0.010
1.0 ppm	"	0.469 ±0.011	0.468 ±0.006	0.461 ±0.013
1.5 ppm	"	0.466 ±0.0	0.460 ±0.007	0.456 ±0.007
2.0 ppm	"	0.459 ±0.009	0.455 ±0.0	0.450 ±0.009

Table - 21

Effect of Aldrin on protein content of Spirulina platensis at 30°C

Time / Toxicant	Initial	48 hrs	72 hrs	96 hrs
Control	0.480 ±0.003	0.480 ±0.006	0.482 ±0.002	0.481 ±0.008
0.5 ppm	"	0.476 ±0.006	0.474 ±0.009	0.470 ±0.008
1.0 ppm	"	0.473 ±0.005	0.468 ±0.0	0.464 ±0.007
1.5 ppm	"	0.474 ±0.0	0.466 ±0.009	0.460 ±0.010
2.0 ppm	"	0.470 ±0.007	0.462 ±0.002	0.454 ±0.006

Table - 22

Effect of DDT on protein content of Spirulina platensis at 30°C

Time / Toxicant	Initial	48 hrs	72 hrs	96 hrs
Control	0.480 ±0.003	0.480 ±0.006	0.482 ±0.009	0.481 ±0.006
0.5 ppm	"	0.483 ±0.003	0.475 ±0.009	0.470 ±0.007
1.0 ppm	"	0.480 ±0.007	0.472 ±0.006	0.468 ±0.006
1.5 ppm	"	0.477 ±0.005	0.472 ±0.004	0.465 ±0.009
2.0 ppm	"	0.472 ±0.009	0.464 ±0.006	0.458 ±0.0

Table - 23

Effect of Aldrin on protein content of Spirulina platensis at 40°C

Time / Toxicant	Initial	48 hrs	72 hrs	96 hrs
Control	0.473 ±0.008	0.470 ±0.006	0.468 ±0.007	0.465 ±0.006
0.5 ppm	"	0.463 ±0.006	0.460 ±0.0	0.458 ±0.005
1.0 ppm	"	0.458 ±0.008	0.451 ±0.008	0.441 ±0.007
1.5 ppm	"	0.453 ±0.009	0.442 ±0.0	0.430 ±0.006
2.0 ppm	"	0.451 ±0.010	0.438 ±0.0	0.425 ±0.0

Table - 24

Effect of DDT on protein content of *Spirulina platensis* at 40°C

Time / Toxicant	Initial	48 hrs	72 hrs	96 hrs
Control	0.473 ±0.008	0.470 ±0.006	0.468 ±0.007	0.465 ±0.006
0.5 ppm	"	0.468 ±0.002	0.464 ±0.0	0.460 ±0.007
1.0 ppm	"	0.460 ±0.007	0.456 ±0.006	0.453 ±0.007
1.5 ppm	"	0.456 ±0.010	0.449 ±0.0	0.449 ±0.009
2.0 ppm	"	0.455 ±0.010	0.441 ±0.012	0.431 ±0.019

with increasing time intervals, but this decrease was not that much fast as compared to cultures containing pesticides. Addition of 0.5 ppm aldrin caused a reduction in protein content to 0.458 ± 0.005 mg/mg dry wt. at 96 hrs from an initial value of 0.473 ± 0.008 mg/mg dry wt. The corresponding values for 1.0 ppm, 1.5 ppm and 2.0 ppm aldrin were 0.441 ± 0.007; 0.430 ± 0.006 and 0.425 ± 0.0 mg/mg dry wt, respectively.

DDT at this temperature showed a similar trend. However, the magnitude of inhibition was not that much as that with aldrin. Protein contents observed at 96 hrs were 0.460 ± 0.007; 0.453 ± 0.007; 0.449 ± 0.009 and 0.431 ± 0.019 mg/mg dry wt. at DDT concentrations of 0.5 ppm, 1.0 ppm, 1.5 ppm and 2.0 ppm, respectively.

2.0 ppm aldrin at 40°C was found to be most toxic in terms of percentage reduction in protein content. After 96 hrs, protein content was found to be 89.59 per cent of the control. The toxicity was found to be in the following order - aldrin: 40°C DDT: 40°C aldrin 30°C DDT 30°C aldrin 20°C DDT 20°C.

SO_4^{--} Uptake

Sulphate is a macronutrient required by all plants. It is the constituent of amino acids, vitamin A, coenzyme A, etc.

In this study algae were grown at 20°C in a medium containing less SO_4^{--} than normal media. Pesticides were added to study

Table - 25

Spirulina sp. Biomass (mg/l) under 15 ppm SO_4^{--}

Time / Toxicant	Initial	48 hrs	96 hrs	144 hrs
Control	93.98 ±4.14	126.61 ±0.0	136.67 ±5.28	173.82 ±4.39
1.0 ppm DDT	93.98 ±4.14	146.84 ±3.97	173.82 ±4.19	221.86 ±6.28
2.0 ppm DDT	93.98 ±4.02	141.69 ±4.85	179.47 ±6.10	209.21 ±3.92
1.0 ppm Aldrin	89.55 ±3.33	112.34 ±4.82	100.98 ±3.11	106.09 ±5.88
2.0 ppm Aldrin	91.00 ±2.65	98.46 ±2.00	74.36 ±2.27	66.46 ±2.17

Table - 26

Spirulina sp. Biomass (mg/l) under 20 ppm SO_4^{--}

Time / Toxicant	Initial	48 hrs	96 hrs	144 hrs
Control	89.55 ±3.95	136.61 ±4.27	146.84 ±3.25	183.82 ±3.44
1.0 ppm DDT	93.98 ±2.56	141.69 ±5.82	197.01 ±4.32	234.99 ±3.37
2.0 ppm DDT	93.98 ±2.56	152.07 ±3.80	191.06 ±3.82	215.48 ±6.81
1.0 ppm Aldrin	91.75 ±4.06	112.28 ±2.24	107.62 ±3.85	117.02 ±3.11
2.0 ppm Aldrin	97.62 ±5.35	118.18 ±3.91	71.17 ±4.02	73.17 ±2.36

their effect on the growth of and SO_4^{--} uptake by algae. Uptake was measured indirectly by estimating SO_4^{--} remaining in the medium at fixed time intervals.

Growth of alga was found to be more when 20 ppm SO_4^{--} was present in the medium. In this case at the end of experiment 183.82 $\pm$ 3.44 mg/l dry wt. biomass was obtained from an initial value of 83.55 $\pm$ 3.95 mg/l dry wt. With 15 ppm SO_4^{--} growth was not that much fast; 173.82 $\pm$ 4.39 mg/l dry wt biomass was obtained at 144 hrs from an initial value of 93.98 $\pm$ 4.14 mg/l. Growth rate worked out to be 4.27×10^{-3}/hr and 4.99×10^{-3}/hr under 15 and 20 ppm SO_4^{--}, respectively.

Addition of DDT was found to increase growth of algae in lower SO_4^{--} concentration. At 1.0 ppm DDT concentration, when 15 ppm SO_4^{--} was present in culture, 221.86 $\pm$ 6.28 mg/l dry wt. biomass was obtained from an initial value of 93.98 $\pm$ 4.14 mg/l dry wt. Under the same SO_4^{--} concentration addition of 2.0 ppm DDT changed the biomass value to 209.21 $\pm$ 3.92 mg/l dry wt. from an initial value of 93.98 $\pm$ 4.02 mg/l dry wt.

When 20 ppm SO_4^{--} was added in the culture, addition of 1.0 ppm DDT produced 234.99 $\pm$ 3.37 mg/l dry wt. biomass from an initial value of 93.98 $\pm$ 2.56 mg/l dry wt. With 2.0 ppm DDT biomass grew to 215.48 $\pm$ 6.81 mg/l dry wt. from an initial value of 93.98 $\pm$ 2.56 mg/l dry wt.

Addition of aldrin to these cultures reduced growth of alga

Table 27

Growth rate of Spirulina sp. under low SO_4^{--} stress and pesticide concentrations (x 10^{-3}/hr).

(in parentheses are doubling time)

TOXICANT SO_4^{--}	Control	DDT		Aldrin	
		1.0 ppm	2.0 ppm	1.0 ppm	2.0 ppm
15 ppm	4.27 (162.30)	5.96 (116.28)	5.57 (124.42)	1.17 (592.31)	-2.19 (-316.44)
20 ppm	4.99 (138.87)	6.36 (108.96)	5.76 (120.31)	1.69 (410.06)	-2.00 (-346.5)
normal concentration	7.50 (92.40)	8.09 (85.66)	7.11 (97.47)	6.57 (105.48)	3.91 (177.24)

Table - 28

SO_4^{--} concentration in algal cultures at various time intervals initially containing 15 ppm

Time / Toxicant	Initial	48 hrs	96 hrs	144 hrs
Control	15	11.69	11.02	10.00
1.0 ppm DDT	"	12.10	11.85	10.65
2.0 ppm DDT	"	13.89	12.10	11.19
1.0 ppm Aldrin	"	13.36	14.38	14.64
2.0 ppm Aldrin	"	13.96	14.69	16.01

Table - 29

SO_4^{--} concentration in algal cultures at various time intervals initially containing 20 ppm

Time / Toxicant	Initial	48 hrs	96 hrs	144 hrs
Control	20	17.16	14.76	11.72
1.0 ppm DDT	"	18.57	17.11	14.76
2.0 ppm DDT	"	19.61	17.86	15.00
1.0 ppm Aldrin	"	17.67	18.52	19.88
2.0 ppm Aldrin	"	19.00	18.89	20.52

Table - 30

Survival Ratio (SO_4^{--} conc. 15 ppm)

Time / Toxicant	Initial	48 hrs	96 hrs	144 hrs
Control	100	100	100	100
1.0 ppm DDT	"	115.98	127.18	127.64
2.0 ppm DDT	"	111.91	131.13	120.36
1.0 ppm Aldrin	"	88.73	73.89	61.03
2.0 ppm Aldrin	"	77.74	54.41	38.24

Table 31

Survival Ratio (20 ppm SO_4^{--})

Time / Toxicant	Initial	48 hrs	96 hrs	120 hrs
Control	100	100	100	100
1.0 ppm DDT	"	98.82	127.84	121.81
2.0 ppm DDT	"	106.06	123.98	111.70
1.0 ppm Aldrin	"	80.22	71.53	58.40
2.0 ppm Aldrin	"	79.37	47.31	36.52

markedly when 15 ppm SO_4^{--} was present in the medium, 1.0 ppm aldrin produced 106.09 ± 5.88 mg/l biomass from an initial value of 89.55 ± 3.33 mg/l dry wt. The corresponding values for 2.0 ppm aldrin were 66.46 ± 2.17 and 91.0 ± 2.65 mg/l dry wt., respectively. This shows that under 2.0 ppm aldrin, a net death process started and alga started dying.

In the presence of 20 ppm SO_4^{--}, addition of 1.0 ppm aldrin changed the biomass to 117.02 ± 3.11 mg/l dry wt. from an initial value of 91.75 ± 4.06 mg/l dry wt. In the presence of 2.0 ppm aldrin in the culture, alga started dying and only 73.17 ± 2.36 mg/l dry wt. biomass was obtained from an initial value of 97.62 ± 5.35 mg/l dry wt.

Uptake of sulphate was measured indirectly by measuring the concentration of sulfate in the medium after a fixed time interval. In control cultures, uptake was found to be very fast. After 144 hrs 10.00 ppm SO_4^{--} remained out of the initial value of 15 ppm. When 20 ppm of SO_4^{--} was initially present in the culture, after 144 hrs 11.72 ppm SO_4^{--} was recovered.

In the presence of DDT, SO_4^{--} uptake was suppressed and at the end of the experiment, the remaining SO_4^{--} content in the medium was always found to be more than what remained in control cultures. In the presence of aldrin, when alga started dying, SO_4^{--} content found in the medium was not much less than initial

Fig 15 20°C

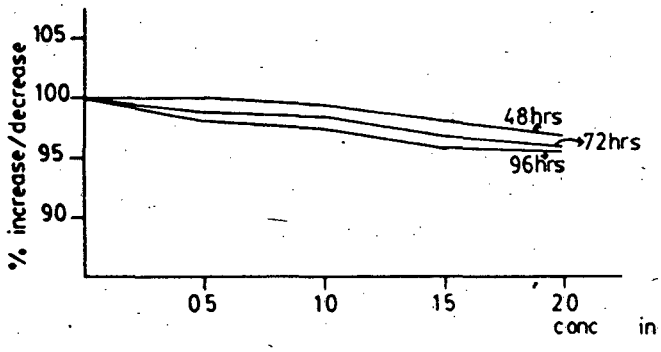


Fig 16 30°C

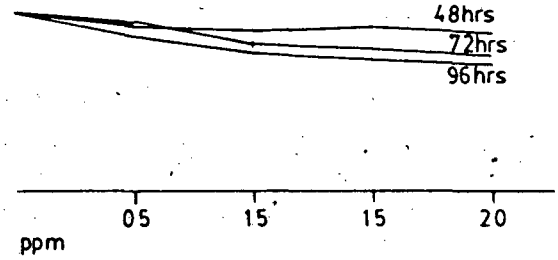


Fig 17 40°C

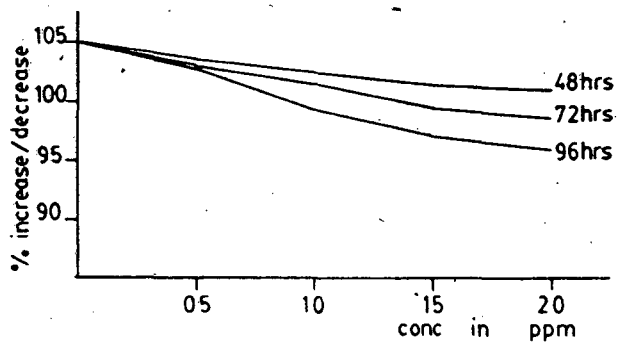
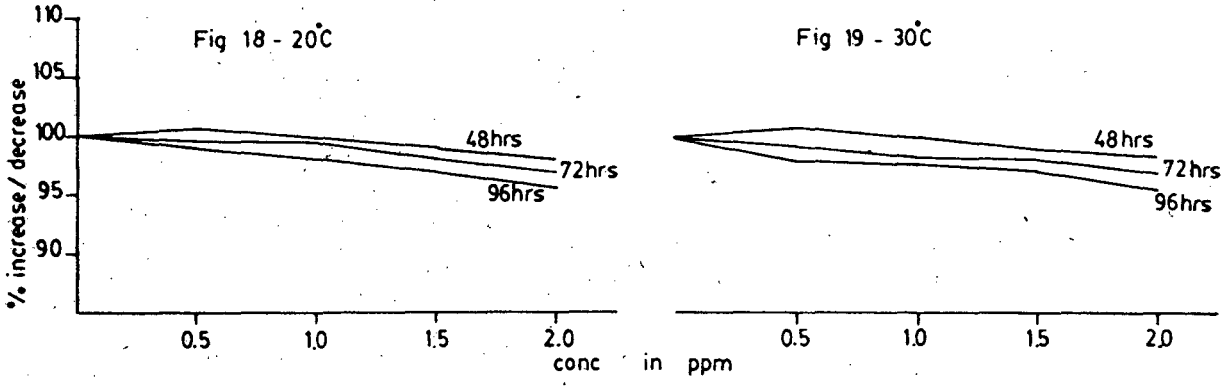


Fig 15-17 % INCREASE/DECREASE IN PROTEIN CONTENT OF SPIRULINA DUE TO ALDRIN



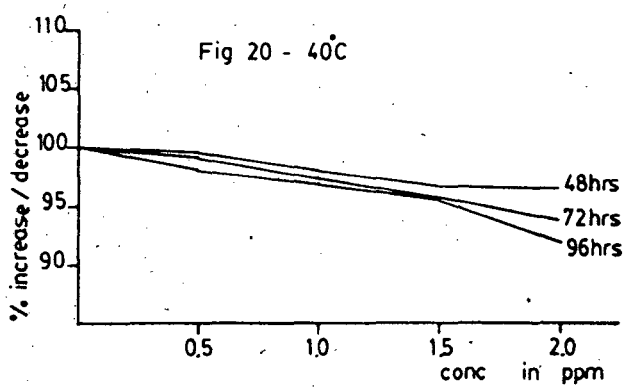


Fig 18-20 % INCREASE/DECREASE IN PROTEIN CONTENT OF SPIRULINA DUE TO DDT

value. With 2.0 ppm aldrin at the end of the experiment SO_4^{--} content in the medium was more than the initial SO_4^{--} concentration.

DISCUSSION

Presence of organochlorine pesticides in aquatic system has an effect on the productivity of phytoplankton. They may change the species composition of the ecosystem (Mosser et al., 1972b), alter the growth of species (Moore, 1970), or alter the morphology of cells (Powers et al., 1977; Puiseux-Dao et al., 1973).

As reported by earlier workers (Poorman, 1973; Ellis and Goulding, 1973; Mooser, 1972), in the present study also, growth of Spirulina platensis was found to be inhibited by both the experimental pesticides upto a certain period of time. Thereafter, growth started resuming. As shown in the results, toxicity of aldrin to alga incubated at 20°C increased upto 72 hrs. After that growth (in terms of survival ratio) was found to be more with increasing time intervals. At 30°C, alga could tolerate aldrin upto 1.0 ppm whereas higher concentrations were still toxic. In this case also, alga started developing resistance to the experimental pesticide. At 40°C, the temperature itself is deleterious to alga. At this temperature, with lower concentrations of aldrin the algal growth was more than in control cultures, but only after 72 hrs.

In case of DDT contaminated alga incubated at 20°C, toxicity tolerance was found to increase after 48 hrs. After that, algal

growth was found to be at faster rates. At 30°C, lower concentrations of DDT had no toxic effect at any time interval, whereas higher concentrations had toxic effets only upto 48 hrs, thereafter, growth became apparent. At 40°C, DDT was found to be toxic at all concentrations, indicating thereby that toxicity increased due to increased temperature.

The explanation of the question why pesticides are becoming lesser toxic after certain time interval may be offered by the metabolism of pesticides by algae (Sweeney, 1968, 1969; Patil et al., 1972; Khan et al., 1972; Keil and Priester, 1969). The development of resistance by algae to these pesticides may also be one of the reasons (Poorman, 1976; Borghi et al., 1973; Powers et al., 1977). Deposition of pesticides together with dead cells of algae at the bottom of culture tubes may cause lesser availability of pesticides for the remaining cells. With increasing time intervals, cell numbers increase whereas, lesser amount of pesticides remains in the medium. This may also lead to lesser toxicity at increased time intervals.

LC_{50} values of both the pesticides were found to be high for Spirulina sp. This shows the relatively high tolerance of algae to these pesticides (Clegg and Koevenig, 1974; Poorman, 1973; Morgan, 1972, etc.). Christie (1969) has explained this due to very low water solubility of these pesticides, thereby minimizing the probability of their getting into surroundings of these

algae. He has also ascribed this high tolerance to rapid degradation of pesticides by micro-organisms.

LC_{50} values for both the pesticides were found to be decreasing with increasing time intervals. This shows increasing toxicity with increasing time intervals. But after a certain time interval, the LC_{50} values were found to increase with time. This shows the development of resistance in algae or the metabolism of the pesticides by algae. Christie (1969), Gregory (1969) and Poorman (1973) have shown that DDT is not toxic to Euglena gracilis even at very high concentrations. In the present study, DDT was found to be only initially toxic to algae, LC_{50} values being 29.08 ppm at 24 hrs interval and 29.94 ppm at 48 hrs interval but after that time interval, there was a sharp increase in LC_{50} values.

Chl a content of Spirulina platensis under the influence of these two pesticides was found to decrease with increasing concentrations and temperatures. In control cultures, it was found to be $4.79 \pm 0.07 \times 10^{-3}$; $4.97 \pm 0.42 \times 10^{-3}$ and $4.20 \pm 0.19 \times 10^{-3}$ mg/mg dry wt. under 20°, 30° and $40^{\circ}C$ when cultures were grown under light. Thus, under normal conditions Chl a was found to be maximum at $30^{\circ}C$, the temperature which has been found to be suitable to most of the enzymatic activities of algae. At $40^{\circ}C$, when the temperature effect manifests itself, Chl a content

was found to be much lesser. Kirt et al. (1967) have also shown that prolonged exposure to supra-optimal temperatures by Euglena gracilis resulted in bleaching of the cells, Chl a at these temperatures being 25% lower than dark grown cells developed at optimal temperatures.

In the present study, Chl a content of Spirulina sp. in control cultures was always found to be lesser under 'dark' conditions than under 'light' conditions. It was found to be $4.18 \pm 0.43 \times 10^{-3}$; $4.33 \pm 0.26 \times 10^{-3}$ and $4.10 \pm 0.19 \times 10^{-3}$ mg/mg dry wt. at 20^{o}, 30^{o} and 40^{o}C respectively.

Synthesis of Chl a involves many light mediated processes and only in the presence of light the complete synthesis of Chl a can take place. The precursor of Chl a biosynthesis, i.e., protochlorophyllide is accumulated in the dark conditions and this protochlorophyllide is converted to Chl a in the presence of light (Baker, 1984). Under dark conditions, conversion of protochlorophyllide to Chl a does not take place, so new Chl a synthesis is stopped. This is why Chl a content is found to be more in light grown cultures than under dark grown cultures.

Presence of the two experimental pesticides, viz., DDT and aldrin in algal cultures caused a reduction in Chl a content of alga and this reduction was found to be concentration dependent. With increasing concentrations of pesticides, reduction in Chl a

content was found to be more. That DDT caused a reduction in Chl a content of algae has been reported by many workers (Mac Farlane et al., 1972; Goulding and Ellis, 1984, etc.). This decrease in Chl a content of alga by pesticide is interpreted to be due to their inhibitory effect on synthesis and activity of some enzymes responsible for Chl a synthesis. Geike (1974) has reported that higher concentration of BHC above 1.0 ppm inhibited activity of some enzymes including ALA dehydrogenase, the enzyme which plays most important role in Chl a synthesis. ALA dehydrogenase is the first enzyme in the synthesis of Chl a from ALA to PBG (Porphobilinogen). Similar inhibition of other enzymes by other pesticides may also take place which may result in the reduced content of Chl a in the alga.

Light and dark conditions have been found to influence the toxicity of pesticides. In the present study, cultures under light showed more reduction in Chl a content (in terms of % reduction) as compared to cultures under dark. This shows that pesticides are more toxic under light conditions than under dark conditions. This may be due to the higher toxicity of degraded products of the pesticides than the parent compound. Photoproducts of DDT have been reported by many workers to be more toxic than the parent compound. Bousch and Matsumura (1975) have shown that in the case of Anacystis sp. toxicity is in the order of DDD > DDE > DDT. Mosser et al. (1974) reported growth inhibition of Thalassiosira pseudonana, more by DDE than DDT.

Powers et al. (1979) have shown DDE to be highly toxic to Exuviella baltica.

Protein content of organisms depends on many factors, including temperature, since enzymes responsible for protein synthesis are sensitive to higher temperatures. At 40°C, protein content of Spirulina sp. was found to be lesser as compared to that at other temperatures. Presence of alga at 40°C for longer times resulted in the death of cells and inactivation of enzymes and inactivation of protein. This is the reason why protein content in control cultures was found to decrease with increasing time intervals at 40°C.

Presence of pesticides in algal cultures had a profound effect on the protein content of algae. Whereas, lower concentrations produced slightly higher protein content initially, higher concentrations of pesticides were found to decrease protein content of alga at all time intervals and this toxicity was found to be dose dependent. Slightly higher protein content found at lower concentrations of pesticides may be due to the stimulatory or hormatic effect of some of the enzymes related to amino acid synthesis or polypeptide synthesis. Geike (1978) also has reported that BHC at 0.001 ppm stimulted the activity of glutamic dehydrogenase, the enzyme that is responsible for synthesis of glutamic acid from -ketoglutaric acid.

However, when alga was allowed to be with pesticides for longer time intervals, even lower concentrations had adverse effects in terms of protein content. At higher concentrations pesticides showed their toxic effect at first observation itself (48 hrs).

Inhibitory effect of pesticides on protein content of algae may be explained on the basis of their inhibitory action on DNA or RNA synthesis also. Organochlorine pesticides are reported to interfere with the uptake of nucleic acid precursors (French, 1976). This inhibition of synthesis of nucleic acid is due to the interaction of DDT with complex regulatory processes associated with the transfer of precursors necessary for nucleic acid synthesis (Lal and Saxena, 1982). Lal et al. (1980) and Lal and Saxena (1980) have also shown inhibition of nucleic acid synthesis in DDT treated organisms. DDT is also reported to inhibit the synthesis of RNA-transcription (French, 1976). Thus, it can be assumed that inhibitory effect of DDT and aldrin at transcription and translation level of protein synthesis may be responsible for lower protein content in pesticides treated cultures.

Reduction in the protein content of treated cultures may also be attributed to the decreased uptake of amino acids, as reported by Czeczuga and Gierasimov (1977) for Chlomydomonas sp. and Scendesmus basilliensis. Lower concentrations of pesticides are reported to either having no effect or slightly increase the

uptake of amino acids which may also be one of the reasons of higher protein content when algae are exposed to lower concentrations of pesticides. Saxena et al. (1981) have reported that uptake of leucine was strongly inhibited in the presence of higher concentrations of pesticides.

In the presence of lower than normal SO_4^{--} concentrations in the culture medium the growth rate was lowered. It was found to be 4.27×10^{-3}/hr and 4.99×10^{-3}/hr under 15 and 20 ppm SO_4^{--} concentrations as compared to 7.50×10^{-3}/hr in normal medium as per Menon (1981). Addition of DDT to the medium increased the growth rate which may be explained by the fact that DDT is lesser toxic to alga and under lower concentrations of DDT alga may be acclimatizing itself to tolerate the toxicant. Addition of aldrin decreased the growth rate and at 2.0 ppm aldrin, net death in culture indicated its higher toxicity.

Sulphate uptake as measured indirectly by measuring sulphate content in the medium was found to be markedly influenced in the presence of pesticides. In control cultures, when sulphate was added, it was removed from the medium very fast. This is similar to the results reported earlier (Dreyfus, 1964; Vallee, 1969). In treated cultures uptake was not that much fast.

Uptake of sulphate by living cells is known to be an active process mediated by permease enzyme system (Utikein et al., 1976; Wedding and Black, 1960; Vallee, 1969). Uptake of sulphate is

also mediated by enzyme ATP sulphurylase which catalyzes sulphurylation of ATP to form APS.

DDT and other organochlorine pesticides are known to inhibit ATP synthesis (Clegg and Koevenig, 1974). The reduced assimilatory power may be one of the reasons why less sulphate is absorbed by algae in the presence of pesticides.

In the aquatic medium, nutrients are absorbed by general cell surface of algal cells and since algal morphology is changed in the presence of organochlorine pesticides (Sodergren, 1968; Bowes and Gee, 1971; Puiseux-Dao et al., 1977; French and Roberts, 1976, etc.), the uptake of sulphate is altered due to altered cell surface. Since for given mass of cells, maximum surface area will be in dispersed conditions of cells, if cells are forming clumps (as is reported by Sodergren, 1968; in Chlorella sp. in the presence of DDT) this may reduce their surface area, thereby adversely affecting their ability to absorb sulphate efficiently.

In case of aldrin, cells started dying due to its higher toxicity and concomittent decay of dead cells result in the release of sulphate along with other nutrients into the medium. Simultaneously, there are lesser number of cells present in the medium to absorb sulphate. This is one of the reasons why very limited amount of sulphate is removed from the medium in the case of aldrin treated cells.

SUMMARY AND CONCLUSIONS

Presence of organochlorine pesticdes in aquatic environment has a profound effect on aquatic organisms, including algae. Earlier they were considered as a boon to users but soon after their wide acceptance, data regarding their resistance and toxicity to non-target organisms started appearing in literature.

Toxic effect of organochlorine pesticide on algae is reviewed by many workers (Butler, 1977; Lal, 1980, 1983, 1984, 1985; Ware and Roan, 1970).

In the present study toxicity of two organochlorine pesticides, viz., DDT and aldrin to a cyanophycean alga, Spirulina platensis is monitored. The parameters selected were biomass, Chl a, protein and SO_4^{--} uptake. Biomass was monitored spectrophotometrically at 490 nm (Stein, 1973). Chl a content was measured as per Venkatraman (1983). Protein content was measured according to Lowry (1951). Growth under limited SO_4^{--} oncentrations in the presence of pesticides was also studied. SO_4^{--} was measured indirectly by measuring amount of SO_4^{--} present in the medium after a fixed time interval. SO_4^{--} content in medium was measured spectrophotometrically at 420 nm (APHA, 1980).

On the basis of ressults obtained in the present study, following conclusions can be drawn :

1) Based on experimental parameters selected, aldrin is found to be more toxic than DDT.

2) Toxicity increases upto a certain period of time, thereafter alga starts tolerating adverse effects which is due to development of resistance towards or due to metabolism of pesticides by alga.

3) LC_{50} values of both the pesticides are found to be very high.

4) Toxicity of pesticides in terms of Chl a is found to be more under light than under dark conditions.

5) Cultures with lower concentrations of pesticides produce slightly higher protein content initially at $20^{o}C$, thereafter a reduction in protein content is noticed.

6) Higher concentrations of pesticides cause reduction in protein content uniformly.

7) Growth of alga is reduced under limited SO_4^{--} concentration.

8) DDT is found to increase the growth rate under limited SO_4^{--} concentration whereas aldrin causes a reduction in the growth rate.

9) Uptake of SO_4^{--} by ;alga is reduced in the presence of pesticides, reduction being more in the case of aldrin.

For future work, studies regarding C^{14} uptake and metabolism of pesticides and mechanism of action are proposed to be undertaken.

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APPENDIX I

Regression Equations Between ln Concentration of Aldrin (y) and Survival Ratio (x)

20°C	24 hrs	$y = -0.08x + 7.49$
	48 hrs	$y = -0.07x + 5.47$
	72 hrs	$y = -0.05x + 3.76$
	96 hrs	$y = -0.04x + 3.65$
	120 hrs	$y = -0.05x + 4.83$
30°C	24 hrs	$y = -0.10x + 10.94$
	48 hrs	$y = -0.09x + 9.03$
	72 hrs	$y = -0.07x + 6.65$
	96 hrs	$y = -0.07x + 6.37$
	120 hrs	$y = -0.06x + 5.68$
40°C	24 hrs	$y = -0.29x + 25.52$
	48 hrs	$y = -0.31x + 26.80$
	72 hrs	$y = -0.18x + 14.87$
	96 hrs	$y = -0.15x + 15.43$
	120 hrs	$y = -0.32x + 28.91$

APPENDIX II

Regression Equations between ln concentration of DDT (y) and Survival Ratio (x)

20°C	24 hrs	$y = -0.13x + 11.74$
	48 hrs	$y = -0.08x + 6.86$
	72 hrs	$y = -0.11x + 11.45$
	96 hrs	$y = -0.22x + 20.41$
	120 hrs	$y = -0.14x + 15.07$
30°C	24 hrs	$y = -0.06x + 6.37$
	48 hrs	$y = -0.07x + 6.90$
	72 hrs	$y = -0.14x + 15.57$
	96 hrs	$y = -0.28x + 30.28$
	120 hrs	$y = -0.95x + 102.67$
40°C	24 hrs	$y = -0.24x + 19.68$
	48 hrs	$y = -0.17x + 14.20$
	72 hrs	$y = -0.17x + 14.43$
	96 hrs	$y = -0.28x + 24.04$
	120 hrs	$y = -0.25x + 22.10$