Chapter 3

Soil algalization – interaction with pesticides

3.1 Introduction

Repeated addition of pre-cultured microalgae – algalization - into agricultural soil can lead to the establishment of the algae with favorable modification of the physical and chemical conditions of the soil. Algalization has been promoted to increase the productivity of tropical paddy fields. Purposive and deliberate introduction of cyanophycean members reduce the nitrogenous fertilizer consumption. Substantial quantities of amino acids like alanine, aspartic acid and glutamic acid, vitamins like B$_{12}$ and, auxin like substances are liberated by algae. These extra metabolites form a source of directly available nitrogen as well as accelerate crop growth enabling the crop plants to utilize more of the applied nutrients. Oxygen produced during algal photosynthesis reduces the oxidizable matter content in the soil. Algalization was shown to reduce iron toxicity by creating oxidizing conditions in the root zone of rice plants, which converted Fe$^{2+}$ to Fe$^{3+}$ making it insoluble thereby reducing the iron
content of the water (Goyal and Goyal, 1998). Growth of micro algae has a modifying effect on soil pH bringing it to almost neutrality from the range of 6.5-8.5 (Ansaveni and Kannaiyan, 1995). Algae have long been accredited as tools for reducing the salinity in the soil (Goyal, 1997). They create microenvironments in the root zone of rice plans with highly reduced salinity, which leads to better crop response.

Repeated algal application for 4-5 consecutive seasons ensures algal establishment, which sustains the algalization effect in the absence of fresh inoculation. The need of the hour is only to identify ‘super’ strains, performing the desired functions, grow them on large scale and make available quality inocula on demand. In view of the tremendous potential of algae, systematic survey of the autochthonous algae and their screening will provide the germplasm to choose from.

As algalization is a recommended practice of tropical paddy cultivation, so also pesticide application to control pests. The persistence of pesticides and its rates of entry into aquatic systems have been worked out in many instances (Ammato et al., 1992). Mesocosm studies of pesticide use experimental ponds and in situ enclosures to which pesticides are applied (Touart, 1988). Using this definition, small laboratory sized chambers and microorganisms used for studies may be referred to as microcosms (Nimmo and Mc Even; 1994). Use of microcosm appears to be excellent for controlling physical aspects of systems to study processes including pesticide degradation or transformation, acute effects or interactions of two species (Kersting and Van, 1992; Stay et al., 1989; Lewis et al., 1985; Pourtier, 1985 and Stay et al., 1985). Therefore in the present study,
microcosm based studies were conducted to quantify pesticide residues and its leaching from the system. Pesticide residue was estimated by gas chromatography.

Several popular soil-applied herbicides can be commonly detected in surface water following run off events (Fawcett et al., 1997). Leistra and Boesten (1989) have reported measurements of residues of pesticides in shallow and deep ground water – mainly some triazine herbicides and their transformation products, ranging between 0.1 µg l⁻¹ and 0.5 µg l⁻¹. Hernandez et al. (1998), in their study on pesticide mobility in the soil had predicted that atrazine and metribuzin (herbicides) were probable leachers while organophosphorus pesticides (ef namiphos and chloipyriphos) should be considered as improbable leachers. Pesticide leaching is inhibited by fine grained soil because of either low vertical permeability or high surface area; both enhance adsorption on the solid phase (Domagalski, 1992). However pesticides might leave the surface zone of soil by a variety of mechanisms (Yaron, 1989); they may leach downward with flowing water, volatilize to the atmosphere or chemically or biologically transform to new form. In an analysis of 270 ground water samples collected from the vicinity of rice fields, it was found that the mean concentrations of six commonly used pesticides ranged from 0.002 ppb for chlorpyriphos to 0.209 ppb for monochrotophos (Castaneda, 1996).

How do the rice field pesticide residues affect the soil algae? Does algalization alter the pesticide leaching from the fields? These questions were addressed through a soil microcosm study using S. elongatus as test organism.
3.2 Methodology

3.2.1 Soil algalization

*Synechococcus elongatus* was inoculated into a soil microcosm, and its effect on soil properties was studied. Soil from paddy field was collected and filled into nine pots of similar size. Fertilizer (urea) was added to three of these pots and was inoculated with *Synechococcus elongatus* suspension in BG11 culture medium. Another set of three pots were directly inoculated with *Synechococcus elongatus* to serve as control for fertilizer effect. The remaining three pots were kept as blank controls. The experimental pots were incubated outdoor with regular watering for 15 days. The soil samples were analysed after incubation for the following attributes.

1) Soil pH: 20g of soil sample was shaken with 40ml distilled water for half an hour in a rotary shaker. The slurry was decanted and then filtered using Whatman No 1 filter paper and the pH of the filtrate was measured with glass electrode.

2) EC: The filtrate from the above was used to measure the electrical conductivity with the help of a conductivity meter.

3) Chlorophyll *a*: The amount of chlorophyll *a* in the soil was measured as an indication of total algal biomass. Chlorophyll *a* was extracted with 90% acetone (APHA, 1992) and estimated by spectrophotometry.
3.2.2 Pesticide residue and algal growth

Paddy field soil was collected and filled into six similar sized pots. They were enriched with urea as in the previous experiment. Chlorpyriphos was added to all of these to a final concentration equivalent to 375 g ha⁻¹. To three of pots were inoculated with a suspension of *Synechococcus elongatus*. The initial concentration of chlorpyriphos was determined by gas chromatography. The soil microcosm was kept in day light with regular watering so that algal growth was promoted. The period of incubation was 15 days upon which the soil was sampled and analysed for chlorpyriphos. The soil sample was dried at room temperature and sieved through 1mm mesh. 15 g sieved soil sample was mixed with 0.3 g activated charcoal, 2 g floristil and 10 g anhydrous sodium sulfate.

Glass column 22 mm X 60 cm was clamped vertically at 2 places. A cotton plug was inserted at the bottom of the column with the help of a glass rod and 3 cm layer of anhydrous sodium sulfate was packed over it. Soil sample mixture was transferred into the column. After filling, the column was tapped gently to get a uniform packing. Pesticide from the column

<table>
<thead>
<tr>
<th>GC parameters used.</th>
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<tbody>
<tr>
<td><strong>Gas chromatography</strong></td>
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<tr>
<td>Column</td>
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<tr>
<td>Column temperature</td>
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<td>Detector</td>
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was eluted with 10% acetone in hexane (100 ml) and the elutant was collected drop wise in 4-5 h in the pear shaped 600 ml rotary evaporation flask. Finally the elutant was concentrated to about 1-2 ml. The final volume was made up to 10ml with hexane in a graduated stoppered test-tube and pesticide was quantified in gas chromatography

A similar set of experiment was carried out using monochrotophos; the concentration of monochrotophos addition was equivalent to 500 g ha⁻¹ in the experimental pots.

3.2.3 Pesticide leaching and algal growth

The effect of pesticide leachates from soil was studied using laboratory microcosm. Paddy field soil was collected in similar sized pots and enriched with urea as in the previous experiments. Four pesticides were selected for the study. They were fenvalarate, malathion, 2,4-D and glyphosate. Each pesticide was applied to triplicate pots in concentrations equivalent to the EC₅₀ of each pesticide towards *S. elongatus* as reported in chapter 2. Another set amended with pesticides as above was algalized with *S. elongatus*. The rest of the samples free of pesticides but fortified with fertilizer were kept as control. After incubating the microcosms for 15 days, soil samples were collected, weighed and shaken with distilled water for half an hour in a rotary shaker. The filtrate was taken as leachate. This was amended with nutrients so as to attain the level of BG11 medium.

The leachates enriched with nutrients were inoculated with *S. elongatus* at a cell density of 2x10⁵ cell ml⁻¹. After 96 h the cells were
harvested by centrifugation. Chlorophyll $a$ of the cell mass was estimated following the method of Becker (1994).

3.3 Results

3.3.1 Soil properties upon algalization

The soil supported the growth of *S. elongatus* upon enrichment with urea producing nearly three times biomass as measured by chlorophyll $a$ (Table 3.1). The unfortified soil had only marginal growth of algae. In nitrogen enriched samples, the pH increased from 4.4 to 5.2. There was no significant change in electrical conductivity.

Table 3.1: Effect of algalization on the soil properties and algal biomass.

<table>
<thead>
<tr>
<th>Soil attributes</th>
<th>Control</th>
<th>Algalized soil</th>
<th>Nitrogen enriched algalized soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.48</td>
<td>4.91</td>
<td>5.21</td>
</tr>
<tr>
<td>EC (m mohs cm$^{-1}$)</td>
<td>16.88</td>
<td>16.05</td>
<td>16.99</td>
</tr>
<tr>
<td>Chlorophyll $a$ ($\mu$g g$^{-1}$)</td>
<td>423.667±14.295</td>
<td>569.667±22.502</td>
<td>1228±93.402</td>
</tr>
</tbody>
</table>

3.3.2 Fate of pesticide upon algalization

The initial concentration of chlorpyriphos taken from control soil samples was 14.17 mg g$^{-1}$ of dry soil. Upon algalization and incubation for 15 days the pesticide concentration was estimated as 13.57 mg g$^{-1}$ of dry soil (Table 3.2). The pesticide concentration in the un-inoculated cultures was 13.63 mg g$^{-1}$ of dry soil.
Table 3.2 Concentration of pesticide residues in paddy field soil amended with chlorpyriphos

<table>
<thead>
<tr>
<th>Category</th>
<th>Pesticide concentrations (mg g⁻¹)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>Nitrogen enriched, pesticide amended soil</td>
<td>14.17 ± 0.960</td>
</tr>
<tr>
<td>Nitrogen enriched, pesticide amended, algalized soil</td>
<td>14.17 ± 0.681</td>
</tr>
</tbody>
</table>

In similar experiment using monochrotophos, the concentrations of pesticide in control soil was 18.94 mg g⁻¹. After 15 days of incubation the monochrotophos residue in non algalized soil samples was 18.18 mg g⁻¹ and 17.28 mg g⁻¹ in algalized soil samples (Table 3.3).

Table 3.3: Concentration of residues in paddy field soil fortified with mochrotophos

<table>
<thead>
<tr>
<th>Category</th>
<th>Pesticide concentrations (mg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>Nitrogen enriched, pesticide amended soil</td>
<td>18.94 ± 1.170</td>
</tr>
<tr>
<td>Nitrogen enriched, pesticide amended, algalized soil</td>
<td>18.94 ± 0.550</td>
</tr>
</tbody>
</table>
3.3.3 Leachate toxicity

The leachate from fenvalarate amended soil reduced the growth of *S. elongatus*. The biomass produced upon 96 h exposure was 125 μg l\(^{-1}\) and 99 μg l\(^{-1}\) chlorophyll a in non algalized and algalized soils. The respective control produced 232 μg l\(^{-1}\) chlorophyll a (Fig. 3.1).

Figure 3.1: Chlorophyll a (μg l\(^{-1}\)) of *S. elongatus* cultured in leachate prepared from soil added with various pesticides

![Chlorophyll a graph](image)

Malathion was added to soil at a concentration of 200 mg l\(^{-1}\) and the leachate gave 11 μg l\(^{-1}\) chlorophyll a, which was 95% growth inhibition compared to control; whereas leachate from algalized soil added with pesticide produced 135μg l\(^{-1}\) chlorophyll a.

Leachate from soil added with 2,4-D (450 mg l\(^{-1}\)) also showed inhibition of growth. Leachate from algalized, pesticide added soil microcosm gave 160 μg l\(^{-1}\) chlorophyll a, which was about 31% inhibition. And leachate from non-algalized, pesticide added soil gave 182μg l\(^{-1}\) chlorophyll a.
Microcosm experiment with glyphosate was done with 5 mg l\(^{-1}\) concentration. Leachate from the three test groups did not show any significant variation in the growth of *S. elongatus* in them. Analysis of variance on the data gave F=1.916 against a critical value of 5.143 at 5% level of significance and 8 degrees of freedom.

### 3.4 Discussion

**Algalization**

Soil microalgae are recognized to have a vital role in the balancing of soil nutrients and making them available to the plants. Soil properties are found to be ameliorated to suit plant life by the soil flora. Algalization of soil at microcosm level with *S. elongatus* was monitored in the present study. Soil pH of the control soil was found to be 4.48. Growth of *S. elongatus* has increased the pH from 4.48 to 5.21. Addition of fertilizer has enhanced algal growth and hence further rise of pH. A good growth of unicellular alga can change acidic soil to neutral, making soil conditions more conducive for crops (Amsaveni and Kannaiyan, 1995). The F value obtained is 12.987 whereas critical value of F at 5% level of significance is 5.143. Therefore the differences in pH observed in the experiment were significant.

Conductivity is a measure of the total electrically charged ions in the system. The conductivity of control soil is 16.81 m mohs cm\(^{-1}\), which has come down to 16.05 m mohs cm\(^{-1}\) in algalized soil and increased to 16.99 m mohs cm\(^{-1}\) in algalized-fertilized soil. It is evinced by the reading that algal growth may reduce conductivity by the absorption of mineral nutrients by the algae. However, on a later phase in the life cycle, these nutrients may be
secreted of excreted back to soil or may be returned by the death and decomposition of the algae; thus making the nutrients more readily absorbable and assimilable by the plants. Addition of urea in soil has increased the conductivity, which might take some more days of algal growth to consume all the fertilizer.

Chlorophyll $a$ measurement has shown presence of microalgae in control soil as well. However the quantity of the natural flora is comparatively poor. Upon inoculation with *S. elongatus* the chlorophyll $a$ level has increased from 0.423mg l$^{-1}$ to 0.569mg l$^{-1}$. Further increase of chlorophyll $a$ to 1.228mg l$^{-1}$ by addition of fertilizer in the soil could be observed which indicate that algalization with *S. elongatus* becomes effective only upon addition of nitrogenous fertilizer.

**Pesticide Residue**

The nature and microbial degradation of organophosphorus insecticide by bacteria and fungi have been well documented. Many gaseous, solid and liquid recalcitrant pollutants including those of natural and xenobiotic origins *viz.* carbon dioxide, nitrogen, phosphorus, phenolics, pesticides, antibiotics, lignin and detergents are detoxified or metabolized by cyanobacteria (Subramanian and Uma, 1999). Friensen-Pankratz *et al.* (2003) observed that the presence of algae (*Selenastrum capricornutum*) decreased the aqueous persistence of pesticides (atrazine and lindane), and speculated that algae either provided sites for pesticide sorption or facilitated pesticide degradation.
A specific report of cyanobacteria assimilation of organophosphorus pesticides as phosphorus nutrition has come from Subramanian et al. (1994). They have suggested the degradation of organophosphorus pesticides by acid phosphatase and subsequent metabolization, which was indicated by the enhancement of growth and other parameters in the absence of phosphate in the medium. There are earlier reports also that cyanobacteria assimilate phosphorus in excess of their requirements (Batterton and Van Baalen, 1968; Volk and Phynney, 1968; Stewart and Alexander, 1971). Healey (1982) had suggested that greater acid phosphatase activity and absence of detectable level of alkaline phosphatase in the presence of pesticides may be connected to the role of acid phosphatase in polyphosphate degradation.

However the present study shows residues of chlorpyriphos at a concentration of 13.63 mg l\(^{-1}\) of dry soil and 13.57 mg l\(^{-1}\) of dry soil after 15 days of algalization. Both have shown an initial concentration of 14.14 mg l\(^{-1}\). Similarly, monochrotophos, another organophosphorus insecticide, has shown more or less same pattern of residue concentration; 18.18mg l\(^{-1}\) and 17.28mg l\(^{-1}\) in control and algalized soil respectively, and the initial concentration was 18.94 mg l\(^{-1}\). It is evident that only a minor fraction has degraded in 15 days. One probability is that 15 days of incubation may not be just sufficient to enable algal degradation of organophosphates.

A second probability might be attributed to the number of extra cellular compounds released by the organism, which might interfere with the pesticides or their degraded products in direct gas chromatography (Subramanian et al., 1994). Cyanobacteria are known to release a large number of extra cellular substances (Fogg, 1962; Whitton, 1965;
Subramanian and Shanmugasundaram, 1986), which might hinder with the monitoring of pesticide degradation.

**Leachate toxicity**

Leaching of pesticides from agricultural fields to nearby areas and even up to deep ground water table are being studied ardently. Leaching is one of the sure ways of pesticide transport in the soil. However many changes that might take place to a pesticide in the soil, might as well change it to a less toxic residue. An indirect method of evaluation the toxicity of residual molecules of pesticide from soil is adopted in the present study.

The soil leachates of fenvalarate, malathion and 2,4-D reduced the growth of *S. elongatus* when exposed to *in vitro* cultures. The test species was tolerant to glyphosate leachate. The toxicity of the leachates depends upon its water solubility as well as the chemical degradability and the toxicity of transformation products. In the present study the toxicity of the leachates upon algalization increased in the order fenvalarate > malathion > 2,4-D. The pattern of toxicity of leachates from non-algalized system deviated from the above, indicating interaction between algae, soil properties and pesticides.

The agricultural industry and urban pesticide uses are increasingly relying upon pyrethroid insecticides and shifting to more potent members of the class, yet a little information is available on residues of these substances in aquatic systems (Weston *et al.*, 2004). Fenvalarate (a pyrethroid) leachate from algalized microcosm was found to be more toxic than leachate from non-algalized system. An increase in toxicity of degradation products of the
pesticide was observed by Sinclair and Boxall (2003) as well in many cases they have studied; and they have put forward an explanation that the phenomenon may be due to either (1) presence of pesticide toxicophore in the degraded products; (2) the product may be the active part of the pesticide; (3) the product is accumulated to greater extend than parent compound; or (4) the product has a more potent mode of action than the parent. Yet another possibility is that algalization facilitates the leaching of the added pesticides and hence becoming more available in the leachate.

Leachate from algalized soil fortified with malathion was found to be remarkably less toxic than leachate form non-algalized soil. Evidently the organophosphate was degraded by *S. elongatus* into nontoxic products or utilized the same for metabolic consumption. In the previous study (Chapter 2) *S. elongatus* had shown a higher level of malathion tolerance and gave the highest EC$_{50}$ of all insecticides (EC$_{50}$ 199.84 mg l$^{-1}$). Moostafa and Helling (2001) observed a 25% faster degradation of isoproturon by cyanobacteria (*Anabaena*) than *Chlorella*. A similar case of pesticide detoxification was reported by Hoagland *et al.* (2002) in which metabolites of atrazine *viz.* deethylatrazine and deisopropylatrizine were found to be 16 to more than 300 times less toxic to diatoms and chlorophyte taxa. Leachate from the 2,4-D microcosm showed a similar detoxification by *S. elongatus*. However the difference in toxicity was not as vivid as that of malathion microcosm.

Leachate from glyphosate microcosm did not show any significant difference from that of control. Huang *et al.* (2004) reported that very small amount of glyphosate molecules were mobilized and only its transformed products were detected in their study. It seems in the present study that
glyphosate molecules are adhered to the soil particles and are not available for algal toxicity or leaching. Hence the leachate from pesticide added soil and pesticide added algalized soil had produced algal growth equivalent to that of control.