CHAPTER 2

• REVIEW OF LITERATURE
2.1 Fate of pesticide-toxicity on cyanobacterial community

Both the structure and function of the microbial communities may be impaired by pesticide toxicity. Proteins are the primary molecules that can be affected by environmental, physiological or pathological conditions. Pesticides may also be metabolized or bioaccumulated by microorganisms. Mechanisms of toxicity vary, depending on the type of pesticide and the microbial species.

Cyanobacteria showed varied responses to pesticides from complete inhibition to initial suppression, followed by gradual recovery and satisfactory growth (Ravindran et al., 2000). However, there is scanty information available about the effect of these pesticides on algal communities (Choudhury and Kennedy, 2004) and about the susceptibility of cyanobacteria to toxicants such as herbicides, fungicides, and heavy metals (Lee et al., 2002).

Widenfalk et al. (2004) exposed microbial communities of natural sediments to concentrations of the phenylurea herbicide isoproturon that are considered to be environmentally safe. It was shown that isoproturon inhibited the bacterial activity and negatively affected the microbial biomass. However, the observations did not show a consistent, inverse relationship with pesticide concentration, indicating the difficulty with proposing the effects of low pesticide doses to microorganisms. Furthermore, Widenfalk et al. (2004) also showed that environmentally safe concentrations of the phthalimide fungicide captan affected the bacterial activity in natural sediment. In contrast, microbial biomass, respiration and denitrification were not affected by captan. Surprisingly, the negative effect on bacterial activity, observed at environmental safe concentrations, disappeared at a higher test concentration, indicating a non-linear relationship of the toxicity.

Herbicides are generally most toxic to phototrophic microorganisms, exhibiting toxicity by disrupting photosynthesis. Herbicides therefore affect various mechanisms associated with photosynthesis, respiration, growth, cell and nucleus division or synthesis of proteins, carotenoids or lipids (Ecobichon, 1991) which eventually kill or injure plants.

Kobbia and El-Sharouny (2007) employed cyanobacteria — *Nostoc muscorum*, *Tolypothrix lanata* and *Aulosira laxa* to assess different responses against 2,4-D herbicide at...
different concentrations. About one half of the herbicides used in agricultural act by inhibiting the light reaction of photosynthesis, mostly by targeting photosystem II (PSII) complex (Schreiber et al., 2002). A series of algal and cyanobacterial PSII based whole cell (Campanella et al., 2000) and tissue (Rizzuto et al., 2000) biosensors have therefore been developed for detection of the class of herbicide which inhibit photosynthetic electron transport.

Cyanobacteria have developed natural methods of responding to pesticides through passive accumulation in cells and surface binding to various functional groups. The mats have also been shown to degrade many organic compounds including trinitrotoluene, chrysene, naphthalene, hexadecane, phenanthrene, polychlorinated biphenyl, trichloroethylene and the pesticides chlordane, carbofuran and paraquat (Kuritz and Wolk, 1995). Perchloroethylene (PCE) and trichloroethylene (TCE) are common xenobiotic contaminants that are recalcitrant to degradation (O’Niell et al., 1999).

2.1.1 LC50 value and effect of pesticide on morphological characteristics as well as heterocyst frequency

Schafer et al. (2007) studied the change of the community structure which was detectable at a concentration range as low as 1/100 to 1/1000 the acute 48 h-LC50 of Daphnia magna. Aslim and Ozturk (2009) examined the effects of three herbicides on the growth of 10 threatened aquatic cyanobacterial isolates by using 9-day exposure experiments at concentrations of 50-200 mg l⁻¹ for trifluralin and 2,4-D and 0.05-1 mg l⁻¹ for linuron. Concentrations of herbicides that elicited a 50% growth reduction over 9 days (EC50) were 136-882 mg l⁻¹ trifluralin, 122-747 mg l⁻¹ 2,4-D and 0.002-0.714 mg l⁻¹ linuron. Synechocystis sp. Hs was more tolerant to the three herbicides than the other isolates of cyanobacteria. Chroococcus sp. S27, Microcystis sp. S17, and Synechococcus sp. S24 were the most sensitive to trifluralin, 2,4-D, and linuron, respectively.

In microorganisms, pesticides had shown to interfere with cell growth, division as well as biosynthetic reactions and molecular composition (DeLorenzo et al., 2001). When the herbicides were present in low concentrations at the cellular site of action, growth by cell division and elongation was usually stimulated. However, with increasing concentrations, a variety of growth abnormalities were induced within 24 h of treatment (Grossmann, 2000). The study of Singh et al. (2008) demonstrated that the differences in length and width of cells in comparison with normal conditions might be due to sodic stress. Besides the morphological observations, authors
have also recorded several heterocysts which were in the form of chain. The presence of heterocyst chains in sodic stress soils certainly fix high atmospheric N\(_2\) in comparison to vegetative ones because heterocysts are important site of nitrogen fixation and thus increase the soil fertility. Morphological examination conducted by Hu et al. (2008) showed that the *Aphanizomenon* filaments disintegrated and the cells lysed gradually after 48 h of toxin exposure. Transmission electron microscopy revealed that cellular inclusions of stressed cells leaked out completely and the cell membranes were grossly damaged. In addition to other changes, clear alteration in morphological shape was reported by Salwa et al. (1999) for tolerant species belonging to the three algal groups i.e. green algae, blue-green algae and diatoms.

Microscopic examination of the cells revealed that the heterocyst frequency in the filamentous forms in both free and immobilized state cultures had increased at the 8\(^{th}\) day of growth. However, the increase was higher in the immobilized state when compared with free cultures (Singh and Datta, 2006). Mahmoud et al. (1992) calculated the survival of five genera of N\(_2\)-fixing cyanobacteria under salt and drought stress in clay and sandy soil. These conditions considerably decreased the survival of *Nostoc microscopicum* and *Rivulara natans*. Nitrogenase activity was also decreased and this could be attributed to the reduction of heterocyst frequency under the experimental conditions. El-Enany and Issa (2000) noticed the impact of sewage water on some physiological activities of cyanobacteria. Metal-tolerant (*Nostoc linckia*) and metal-sensitive (*Nostoc rivularis*) cyanobacteria were grown at three levels of sewage water (25, 50 and 75\%). The growth rate showed significant stimulation in low and moderate levels (50\% for *Nostoc linckia* and 25\% for *Nostoc rivularis*). The heterocyst frequency as well as nitrogenase activity was increased in cyanobacteria grown at low and moderate levels (25\% and 50\% sewage). The effect of indole acetic acid (IAA) on growth, dinitrogen fixation and heterocyst frequency of *Anabaena* PCC 7119 and *Nodularia* sp. had been investigated. Concentrations of IAA ranging from \(10^{-10}\) to \(10^{-4}\) M did not changed the growth of *Anabaena* PCC 7119. Concentrations higher than \(10^{-4}\) M were inhibitory. Similar results were found in *Nodularia* sp. although in this case the inhibitory effect appeared with \(10^{-3}\)M of IAA. Neither the nitrogenase activity nor the heterocyst frequency was enhanced by IAA treatment (Leganes et al., 1987). Assessment of the influence of carbofuran on the nontarget cyanobacterium *Anabaena doliolum* revealed that treatment with 100 ppm carbofuran for 48 hours resulted in no significant change in heterocyst frequency (Ola, 1999).
2.1.2 Effect of pesticide on pigments

The herbicide affects many physicochemical and physiological processes like the reduction in photosynthesis and the degradation of chlorophyll, as well as inhibits transport of the plant growth hormone auxin and enhances the oxidation of auxin (Cole, 1985). However, DeLorenzo et al. (1999) showed that the herbicide atrazine induced functional and structural changes of estuarine microbial communities. Atrazine, in the concentrations of 40 and 160 μg l⁻¹, was found to reduce chlorophyll a. Xue et al. (2005) emphasized that the enhanced UV-B generally decreased the chlorophyll content whereas it increased UV-B absorbing compounds in many algae and cyanobacteria. The fungicide karathane seemed to be inhibitory to the growth of Anabaena oryzae whereas all levels of the fungicide exposure stimulated the growth of Nostoc muscorum. Trifluralin at all applied doses inhibited the growth of both organisms. However the magnitude of inhibition was always more pronounced in Anabaena oryzae. The lowest concentrations of dursban and dimethoate favored chlorophyll a synthesis in the cells of both cyanobacteria. Contrary to chlorophyll, total carotenoid accumulation was significantly suppressed. Moderate and higher doses of karathane and trifluralin increased chlorophyll a biosynthesis in Nostoc and to lesser extent in Anabaena, a phenomenon that was accompanied by significant increase in total carotenoid content (Kobbia et al., 1991). Marco and Orus (1993) reported the ultrastructural analysis of Anabaena nitrate-grown cells. The result showed that the trichlorfon does not damage thylakoid membranes, but brings about the accumulation of enlarged cyanophycin granules and the increase of carboxysome number. Nitrate uptake rate and chlorophyll and phycobiliprotein contents are also reduced by trichlorfon treatment in the cyanobacteria Synechococcus UAM 211, GloeothecePCC 6501, Plectonema calothricoides, Nostoc UAM 205 and Chlorogloeopsis PCC 6912.

Ahluwalia et al. (2002) proved that the administration of relatively higher doses (> 5 μg ml⁻¹) of Diquat into Nostoc muscorum and Cylindrospermum sp. cultures could be highly toxic, thereby reducing their chlorophyll a (Chl a) content and contributing to a progressive decrease in growth which culminates incomplete lysis of the cells with increasing level of the herbicide. The highest concentration tested (15 μg ml⁻¹) has been found to be algicidal for both the cyanobacteria. At this concentration, the same authors demonstrated that paraquat supplemented into culture medium containing Cylindrospermum sp. also had an algicidal effect (Kaur et al.,
2002). Ghadai et al. (2010) studied the effect of commercial organophosphorous insecticide Diazinon on two cyanobacterial isolates i.e. *Anabaena cylindrica* and *Oscillatoria tenue* in terms of the chlorophyll *a* for every four days up to twenty eight days. Cyanobacterial isolates were treated with different concentrations of Diazinon i.e. 1 ppm, 3 ppm, 5 ppm, 7 ppm and 10 ppm. Finally Maximum Allowable Concentration (MAC) of Diazinon on cyanobacterial isolates was determined.

Regulation of growth of diazotrophic cyanobacterium *Anabaena doliolum* by nitrogen sources was reported by Singh (1991). The author noticed that maximum growth of *A. doliolum* in terms of pigments content was supported by nitrate-nitrogen while ammonium nitrogen supported poor growth of both in terms of pigment and cell number. Nirmal Kumar and Rita Kumar (2002) reported effect of fluchloralin on *Nostoc muscorum* which indicated an inhibitory effect on phosynthesis affecting the total chlorophyll irrespective of the treatment of 40, 60 and 80 µg ml⁻¹ herbicide. Parikh and Datta (2005) carried out textile dye decolourization using cyanobacteria *Gloeocapsa pleurocapsoides* and *Phormidium ceylanicum* to decolourise Acid red 97 and FF sky blue dyes by more than 80% after 26-days. It was found that chlorophyll *a* synthesis in all the cultures was strongly inhibited by the dyes.

Nirmal Kumar (1991) studied the effect of herbicide isoproturon on chlorophyll *a* content of *Anabaena* sp. It was observed that chlorophyll *a* content was decreased in response to increasing concentration of herbicide. Nirmal Kumar et al. (2008) proposed that *Westiellopsis prolifica* Janet when treated with different concentrations (2.5, 5.0 and 7.5%) of Atlas dye industry effluent showed the gradual increase of chlorophyll *a*, whereas for carotenoids it was observed at 2.5% dose of Atlas dye effluent. In *Tolypothrix scytonemoides*, photosynthetic rates decreased with decreasing water potentials as in the case of *Chroococcidiopsis* strains. There was a sharp decrease in the rate of photosynthesis between −2000 and −3500 kPa as a result of loss of chlorophyll *a* (Uma and Anand, 2003).

The herbicide tolerance was tested by growing the cyanobacterial cultures in BG-11 medium supplemented with varying concentrations of the commonly used rice herbicide, viz butachlor under *in vitro* conditions. The phycobiliproteins and chlorophyll *a* were assessed at periodic intervals. *Westiellopsis* showed the maximum tolerance followed by *Anabaena*, *Nostoc* and *Oscillatoria* (Selvakumar et al., 2002). *Anabaena variabilis* when treated with two
photosynthetic inhibitor herbicides, atrazine (both purified and formulated) and [3-(3,4-dichlorophenyl)-1,1-dimethyl urea] (DCMU) showed highest inhibitory effects on phycobiliproteins, chlorophyll $a$ and heterocyst differentiation as compared to carotenoids (Singh et al., 2011).

Growth and photosynthetic pigments, i.e., phycocyanin, carotenoids and chlorophyll $a$ were adversely affected by endosulfan treatment and the inhibition was found to be dose dependent. The toxic effect of endosulfan was more pronounced on phycocyanin; however, a considerable reduction in chlorophyll $a$ and carotenoids was also noticed (Prasad et al., 2005).

2.1.3 Effect of pesticide on metabolites

Among cyanobacteria, extensive research shows that UV-B has negative effects on cell physiology, nucleic acids, proteins and lipids (Unsal-Kacmaz et al., 2002). Six days after the application of monosulfuron at 0.03 to 0.3 nmol $l^{-1}$ under laboratory conditions, growth of the nitrogen-fixing cyanobacteria *Anabaena flos-aquae*, *Anabaena azollae*, and *Anabaena azotica* was stimulated but at higher concentrations (30 to 300 nmol $l^{-1}$) protein synthesis was inhibited. The production of 16 amino acids in *A. flos-aquae* was reduced from 7 to 69% with increasing monosulfuron concentration (Shen et al., 2009).

Bentazon and molinate are selective herbicides recommended for integrated weed management in rice. Their toxicity on growth and some biochemical and physiological parameters of *Nostoc muscorum*, an abundant cyanobacterium in Portuguese rice fields, was evaluated under laboratory conditions during time- and concentration-dependent exposure for 72 h. Results showed that toxic concentrations (0.75-2 mM) of both herbicides had pleiotropic effects on the cyanobacterium. Protein content was increased by both herbicides although the effect was particularly evident with higher concentrations of molinate (1.5-2 mM). The herbicides had contrasting effects on carbohydrates content i.e. molinate increased this organic fraction whereas bentazon decreased it (Galthano et al., 2010).

Vargas et al. (2002) determined the biochemical composition and fatty acid content of twelve strains of filamentous, heterocystous, nitrogen-fixing cyanobacteria. These cyanobacteria when grown under diazotrophic conditions, protein, carbohydrate, lipid and nucleic acids were comprised by 37-52%, 16-38%, 8-13%, and 8-11% of the dry weight, respectively. The
presence of a combined nitrogen source resulted in an increase in the protein content of the cells and a decrease in the levels of lipids and carbohydrates, although biomass productivity was not affected significantly. Biochemical composition also changed during culture growth, with the highest levels of proteins and lipids occurring as the culture entered stationary phase whereas the highest levels of carbohydrate and nucleic acids were found during the exponential phase.

The acute toxicity was determined for soil algae *Chlorella kessleri* and *Anabaena inaequalis*, exposed to pesticides lindane, pentachlorophenol (PCP), isoproturon (IPU) and methyl parathion (MP). Toxicity markers included growth inhibition, chlorophyll biosynthesis, and total carbohydrate content, as a function of dose and time. Carbohydrate production responses were similar to those for cell density (growth) and chlorophyll biosynthesis, with MP having the lowest adverse impact. The overall relative toxicity among the four tested pesticides was: for *Chlorella*, lindane>IPU>PCP>MP; and for *Anabaena*, PCP>IPU>lindane>MP. The results confirm that toxicants such as these pesticides may affect individual (though related) species to significantly different degrees (Fadwa and Helling, 2002).

*Synechocystis* PCC 6803 and *Anabaena variabilis* ATCC 29413 showed a high degree of tolerance to the herbicide glyphosate, applied as the free acid, the monoisopropylamine salt or the commercial formulation (Roundup > R). Differential toxicity between herbicide formulations was observed (Roundup > isopropylamine salt > free acid) and correlated with their rates of uptake. There was no evidence of glyphosate degradation. Shikimate accumulation, together with partial alleviation of inhibition by aromatic amino acids, suggests that the target site for glyphosate is in the pathway of aromatic amino acid biosynthesis (Powell et al., 1991).

A comparative study on the cyanobacterial isolates, *Anabaena ambigua* Rao (A100) and *Oscillatoria foreaui* Fremy (A1340) were carried out by Lakshmi and Annamalai (2007) to investigate the response of an organophosphorus insecticide Divap 100 at different concentrations of 1, 5 and 10 ppm on the growth and release of extracellular products such as proteins, nitrogen, ammonia, carbohydrates, amino acids and phenols. The experiment was conducted up to 28 days and observed at intervals of 4 days, which showed to be deleteriously affecting the release of these extracellular products in both the organisms. However, in *Anabaena ambigua*, the release of extracellular products was affected at higher concentrations (5
and 10 pm) of Divap 100. It also revealed that these products were released in higher amounts only at late exponential phases even in untreated control conditions in both the isolates.

A reduction in the amount of sugar content and peroxidation of membrane lipids as well as qualitative and quantitative changes in the phosphoglycolipids and neutral lipids of *Phormidium corium* when exposed to UV-B radiation was observed by Bhandari and Sharma (2006). However, the fatty acid profile did not exhibit any qualitative changes due to UV-B treatment. Nirmal Kumar et al. (1996) studied the impact of pesticides on nucleic acids of *Anabaena* sp.310. It was observed that bavistin, a fungicide, enhanced the nucleic acid content at lower doses while the same suppressed nucleic acid content at higher doses.

Krishnamurthi et al. (2006) determined the DNA damaging potential and the genotoxicity of individual compounds in pesticide contaminated soil. The contaminated soil sample showed 79% (P<0.001) of DNA strand break, whereas technical grade of major carbaryl and α-naphthol constituents of the contaminated soil showed 64% (P<0.01) and 60% (P<0.02) damage respectively. The results indicate that the toxicity caused by contaminated soil is mainly due to carbaryl and α-naphthol, which are the major constituents of the soil sample as analyzed by GC-MS. The effect of the rice field herbicide machete (2-chloro-2′6′-diethyl-N-(Butoxymethyl) acetonilide) on the growth and cell composition of *Anacystis nidulans*, *Nostoc muscorum* and *Anabaena dolioiulum* was investigated by Pandey and Kashyap (1986). The results showed complete inhibition of these cyanobacteria at 2.5, 5.0 and 20 μg/ml, respectively, while a slight stimulation of growth was observed at lower concentrations. Stimulation of cyanobacterial growth in the presence of low concentrations of machete was associated with an increase in the cellular levels of phycobilins and RNA while there was little impact on the levels of chlorophyll *a* and DNA.

**2.1.4 Effect of pesticide on enzymes**

Lower concentrations of bavistin supported the growth of *Tolypothrix scytonemoides*. Activities of nitrogenase and glutamine synthetase were affected in all the pesticide treatments but nitrogenase activity was enhanced in the presence of bavistin (Rajendran et al., 2007). A comparative study between the nitrate reductase (NR) activity of green and blue green algae in presence of heavy metals was conducted to present a situation where nitrate reductase process may be affected in presence of heavy metals (Awasthi, 2005).
The systemic morpholine fungicide tridemorph (known to exert its antifungal action through inhibition of ergosterol biosynthesis) can also inhibit certain enzyme activities of organisms. It was found to strongly inhibit the glucose and lactate dehydrogenase activities in cultures of four gram (+) bacteria and a gram (−) bacterium, *Rhizobium leguminosarum*. Growth of these bacteria was inhibited by tridemorph at concentrations between 7 and 60 mg L⁻¹. In contrast, similar dehydrogenase activities in other gram (−) organisms like *Escherichia coli* and *Azotobacter vinelandii* which showed no growth inhibition at 200 mg L⁻¹ tridemorph, were either not inhibited or slightly inhibited. Similarly, succinate dehydrogenase activity in *Rhodococcus* sp. AK 1 was strongly inhibited by tridemorph (Kalam and Banerjee, 1995).

The primary effect of the toxic pesticide trichlorfon was inhibition of nitrate uptake in cyanobacteria (Marco and Orus, 1993). A strong reduction in the rate of nitrate uptake was observed 3 h after the addition of the pesticide to batch cultures of *Anabaena PCC 7119*. Nitrate reductase (ferredoxin: nitrate reductase) activity was also lowered as a result of trichlorfon action.

Nitric oxide (NO) stimulated the activity of plasma membrane H⁺-ATPase, 5'-nucleotidase, peroxidase, ascorbate peroxidase and glutathione reductase in ultraviolet B (UV-B) irradiated *Chlorella pyrenoidosa*. It also boosted the activity of nitrogen-metabolism enzymes such as nitrate reductase, nitrite reductase and glutamine synthetase which were inhibited by UV-B irradiation (Chen et al., 2010). Two pre-emergence herbicides, butachlor and fluchloralin, did affect nitrogenase, nitrate reductase and glutamine synthetase activities in *Nostoc muscorum* but in *Gloeocapsa* sp., a stimulatory effect on nitrogenase and glutamine synthetase activity was observed (Singh and Tiwari, 1988).

The seven different species of phototrophic nonsulfur bacteria (namely *Rhodobacter capsulatus*, *R. sphaeroides*, *Rhodospirillum rubrum*, *Rhodopseudomonas acidophila*, *R. blastica*, *R. viridis* and *Rhodomicrobium vannielii*) were grown in the presence of butachlor to analyze the effect of herbicide on growth rate and nitrogen fixation abilities. An increase of 1-4% was observed in the growth rate and 2-10% in nitrogen-fixing abilities in case of *Rhodobacter capsulatus* and *Rhodospirillum rubrum*, when grown under nitrogen-fixing conditions while a reduction of 17-47% and 17-85%, respectively was observed for the other 5 species under
similar conditions. The finding that *Rhodopseudomonas acidophila*, *R. blastica*, *R. viridis* and *Rhodomicrobium vannielii* showed stronger inhibitions of nitrogenase activity seems to indicate
that species in genera *Rhodobacter* and *Rhodospirillum* were less influenced by butachlor than
those in *Rhodopseudomonas* and *Rhodomicrobium* in terms of nitrogen-fixing ability. Overall,
nitrogenase activity was closely correlated with both growth rate and glutamine synthetase
activity (representing nitrogen metabolism) (Lee et al., 2007).

Hazel and Greaves (1981) reported the influences of the herbicide butachlor (n-
butoxymethylchloro -2', 6'-diethylacetnilide) on microbial populations, respiration, nitrogen
fixation and nitrification and on the activities of dehydrogenase and hydrogen peroxidase in
paddy soil. Butachlor enhanced the activity of dehydrogenase at increasing concentrations. The
soil dehydrogenase showed the highest activity on the 16th day of butachlor application. The
hydrogen peroxidase could be stimulated by butachlor (Min et al., 2001). Effects of glyphosate,
paraquat, trifluralin and atrazine on the activities of dehydrogenase, phosphatase and urease in
soil were measured. Only glyphosate at 21.6 kg/ha was found to inhibit the enzyme activities and
generally the results were not statistically significant. Enzyme activity associated with micro-
organisms proliferating in soil supplemented was similarly not affected by the herbicides. It is
proposed that effects of natural stress can be used to judge the relative importance of herbicide
induced change.

The effect of urea fertilization on nitrogenase activity of heterocystous cyanobacteria was
determined by Irisarri et al. (2001). Treatments consisted of two levels of nitrogen, 0 (control)
and 70 kg ha\(^{-1}\), supplied as urea when flooding was established. It was found that maximal
nitrogenase activity was reached after 12 weeks of flooding in both the treatments, with an
average of about 20 \(\mu\)mol C\(_2\)H\(_4\) m\(^{-2}\) h\(^{-1}\). To improve the understanding of the environmental
factors that can limit nitrogenase activity in rice fields, two of the most abundant cyanobacteria
isolates were tested for tolerance to combined nitrogen and two herbicides. In both isolates 0.2
mM ammonium inhibited the nitrogenase activity after 24 h of growth. The composition of
culture-independent microbial communities and the change of nitrogenase activities under
butachlor application to paddy soil were investigated by Chen et al. (2009).
2.1.5 Biotransformation and functional group variation

Additive effects can be expected for compounds with a similar mode of action, while synergistic effects trigger a more than additive effect in the exposed organism due to changes in chemical biotransformation (Belden and Lydy, 2000). In addition to the parent compounds, we also need to consider that most pesticides applied in agriculture are transformed by physical, chemical and biological processes into one or more transformation products. Both parent pesticides and transformation products may exert a toxic action in aquatic organisms whenever the concentration is sufficient to trigger such an effect. In fact, the transformation product can sometimes be more toxic than the parent compound (Belden and Lydy, 2000).

Biotransformations using microorganisms such as fungi and bacteria have been reported by Ishihara et al. (2000). Although the chemical structure of 2,4-D is relatively complex, it is readily degraded and used as a carbon source by various environmental microorganisms (Lee et al., 2005). Audus (1964) reported the disappearance of 2,4-D from soil within 3-4 weeks. Similarly, a reduction of 90% of 2,4-D from its original concentration in 2 weeks by a bacterial culture isolated from sewage was reported by Rosenberg and Alexander (1980). A number of 2,4-D degrading bacteria belonging to genera Pseudomonas, Streptomyces, Alcaligenes and Achromobacter have been isolated and identified (Sinton et al., 1986).

The degradation of an organophosphorus pesticide, fenamiphos, by different species of five green algae and five cyanobacteria was studied. All the species tested were able to transform fenamiphos to its primary oxidation product, fenamiphos sulfoxide (FSO), while the majority of these cultures were able to hydrolyze FSO to fenamiphos sulfoxide phenol (FSOP). Fenamiphos sulfone phenol, FSOP, and FSO were detected in the culture extracts of these algae and cyanobacteria (Caceres et al., 2008).

Various metabolites of butyl benzyl phthalate (BBP) engendered by Arthrobacter sp. strain WY were isolated and identified by a combination of chromatographic and spectrophotometric analyses, which revealed a pathway involving monobutylphthalate (MBuP), monobenzyl phthalate (MBzP), phthalic acid and protocatechuic acid (Chatterjee and Dutta, 2008). The protocatechuic acid, in turn, was processed by ortho-cleavage dioxygenase to form β-carboxy-cis,cis-muconate, ultimately leading to the TCA cycle. Benzyl alcohol was found to be metabolized by the Acinetobacter sp. strain FW via benzaldehyde, benzoic acid and catechol.
Catechol was further degraded by ortho-cleavage dioxygenase to cis,cis-muconic acid and subsequently to muconolactone leading to β-ketoadipate pathway.

Amarante et al. (2003) developed a method for simultaneous extraction and determination of 2,4-D and its major transformation product, i.e., the 2,4-dichlorophenol (2,4-DCP) in soil samples. Eaton and Sandusky (2009) had identified many camphor-degrading bacteria that are able to transform 2-methylisoborneol (2-MIB). *Rhodococcus ruber* T1 metabolizes camphor through 6-hydroxycamphor but converts 2-MIB to 3-hydroxy-2-MIB. *Pseudomonas putida* G1, which metabolizes camphor through 5-hydroxycamphor, converts MIB primarily to 6-hydroxy-2-MIB. *Rhodococcus wratislaviensis* DLC-cam converts 2-MIB through 5-hydroxy-2-MIB to 5-keto-2-MIB. Together, these three strains produce metabolites resulting from hydroxylation at all of the three available secondary carbons on the six-member ring of 2-MIB.

The mixed culture (*Pseudomonas*, *Actinomycetes*, a species of white rot fungus and *Trichoderma harzanium*) degraded 2-, 3- and 4-chlorophenol (1.56 mM) via a meta-cleavage pathway. During the degradation of 2- and 3-chlorophenol by the mixed culture, 3-chlorocatechol production was observed. Further, metabolism was toxic to cells as it led to inactivation of the catechol 2,3-dioxygenase enzyme upon meta-cleavage of 3-chlorocatechol resulting in incomplete degradation. Inactivation of the meta-cleavage enzyme led to an accumulation of brown coloured polymers which interfered with the measurement of cell growth using optical density. Degradation of 4-chlorophenol by the mixed culture led to an accumulation of 5-chloro-2-hydroxymuconic semialdehyde, the metacleavage product of 4-chlorocatechol. The accumulation of this compound did not interfere with the measurement of cell growth using optical density. The 5-chloro-2-hydroxymuconic semialdehyde was further metabolized by mixed culture with a stoichiometric release of chloride, indicating complete degradation of 4-chlorophenol by the mixed culture via a meta-cleavage pathway (Farrell and Quilty, 1999).

The toxic effects of the herbicide atrazine, its degradation products deethyl-atrazine and deisopropylatrazine and the herbicide metolachlor were examined in unialgal cultures of *Chlorella fusca* var-fusca. The toxicity of a mixture of atrazine and metolachlor was also evaluated using the same bioassay system. Cell numbers were determined daily and growth rates...
were calculated for a period of 4 days. The order of toxicity of chemicals was atrazine>metolachlor>deethyl-atrazine>deisopropyl-atrazine (Kotrikla and Lekkas, 1999).

Lee et al. (2003) studied the role of the blue-green algal species present in the soil in the dissipation of endosulfan and its metabolites in the soil environment. Two *Anabaena* species, *Anabaena* sp. PCC 7120 and *Anabaena flos-aquae*, were used in the study. *Anabaena* sp. PCC 7120 produced three principal biotransformation compounds, chiefly endosulfan diol (endodiol), and minor amounts of endosulfan hydroxyether and endosulfan lactone. Trace amounts of endosulfan sulfate were detected. In comparison, the biotransformation of endosulfan by *Anabaena flos-aquae* yielded mainly endodiol with minor amounts of endosulfan sulfate. An unknown compound was produced up to 70% from endosulfan spiked in the medium inoculated by *A. flos-aquae* after 8 days of incubation. Therefore, fate of the endosulfan was dependent on the species used in the study. Within 1 day of incubation, two *Anabaena* species produced low amounts of β-endosulfan after application of α-endosulfan.

According to Davis et al. (2003), the ability of algae to adsorb and accumulate xenobiotics mainly depends upon its chemical composition and the algal samples were studied using Fourier Transform Infrared (FTIR) technique. During the last decade, FTIR spectroscopy has proven and accepted to be a powerful tool for the study of functional groups in biological samples (Dumas and Miller, 2003). Amino, carboxyl and phosphate groups present on the cell surface of the *Mucor rouxii* biomass are involved in chemical interaction with copper ion as revealed from FTIR and SEM-EDX study (Majumdar et al., 2008).

Based on FTIR study, the *Ecklonia* sp. was subjected to chemical modification of its amino and carboxyl groups, to examine their roles in the Cr (VI) removal from the aqueous phase. Methylation of the amino group significantly decreased the Cr (VI) removal rate whereas amination of the carboxyl group significantly increased it. Meanwhile, esterification of the carboxyl group and carboxylation of the amino group decreased the Cr (VI) removal rate, but the former showed a more negative effect than the latter. These findings indicated that the amino and carboxyl groups take part in the Cr (VI) removal from the aqueous phase (Park et al., 2005).
2.1.6 Protein profiling

Bhargava et al. (2006) determined the initial characterization of copper-induced changes in the global proteome (cuprome) of *Anabaena doliolum* subjected to short- and long-term treatments. PD Quest analysis revealed that out of 215 protein spots in the control, 79 showed alterations (26 up- and 36 down-regulation, and 5 up- and 12 down regulation respectively, after 24 and 168 h of Cu treatment) in their expression pattern. The short-term (24 h) and long-term (168 h) treatments induced 158 and 96 proteins respectively. Of the 158 newly induced proteins, 30 were found to sustain the long-term treatment. In view of the appearance of two sets of proteins, there appears a need to carry out short- and long-term proteome analysis for getting a holistic view of the proteome of cyanobacteria subjected to abiotic stress. Surosz and Palinska (2005) had studied the effect of different doses of copper on the SDS–PAGE protein profile of *Anabaena flos-aquae* and demonstrated down-regulation of the synthesis of a large number of proteins and up-regulation of only one protein of 55 kDa.

To face pesticide stress, the functioning of some proteins is altered or lost and that of others is enhanced or induced (Castielli et al., 2009). Geeta (2000) demonstrated the polypeptide pattern of blue-green algae grown in BG11 medium amended with different concentrations of NaN\textsubscript{3}. Gao et al. (2009) investigated different expression levels of proteins in the cytoplasm of *Synechocystis* sp. PCC 6803 under short-term and long-term UV-B stress by using a comparative proteomic approach. One hundred and twelve differentially expressed protein spots were identified by mass spectrometry to match 75 diverse protein species. They mainly focus on amino acid biosynthesis, photosynthesis and respiration, energy metabolism, protein biosynthesis, cell defence and other functional groups. The finding, showing that short-term response-proteins are quite different from long-term response-proteins, helps to identify the change in homeostatic mechanisms in *Synechocystis* sp. PCC 6803.

2.1.7 DNA Profiling

Randomly Amplified Polymorphic DNA (RAPD) was first employed by Williams et al. (1990) to examine human DNA samples from anonymous individuals. This method is better than Restriction Fragment Length Polymorphism (RFLP) as its simple and rapid molecular typing technique with high discriminatory power, require less DNA, easy to perform and inexpensive, only drawback is it exhibit low level of reproducibility because of the low stringency conditions.

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used in PCR and these conditions lead to mismatched pairing (Weising et al., 1995). DNA is one of the most critical cellular targets for hazardous chemicals and wastes which may get damaged by alteration of bases or disruption of the sugar phosphate backbone (Birnboim and Jevcak, 1981). Analyses of pesticide degradation, photosynthetic pigment content, isoenzyme variation or differentiated cell culture may also be misleading because of the variable expression of cyanobacterial gene products in culture (Kato et al., 1991). Taxonomic characters change so drastically that reliable result becomes difficult or even impossible to reproduce (Kumar et al., 2000). The RAPD assay has also been applied to detect genomic DNA alterations induced by several DNA damaging agents, such as benzo[a]pyrene (Castano and Becerril, 2004), heavy metals (Enan, 2006), mitomycin C (Becerril et al., 1999), 4- n-nonylphenol and 17- β estradiol (Atienzar et al., 2002), chrysotile asbestos (Yoshida et al., 2001), UV radiation (Kumar et al., 2004) or X-rays and radio nuclides (Theodorakis et al., 2001). Widenfalk et al. (2008) showed that community-level end points failed to detect changes induced by captan, glyphosate, isoproturon and pirimicarb at environmentally relevant and high concentrations, underpinning the need for application of molecular techniques in aquatic ecotoxicology.

Asadi et al. (2011) investigated the influence of microwave radiation on physiological behaviors of Phormidium sp. Kutzing ISC31 (Oscillatoriales). The organism grown in BG-11 medium was microwave-treated at a frequency of 2450 MHz using a microwave oven. Fifteen (15) microwave pretreatments were established, combining five intensities (180, 360, 540, 720 and 900 W/cm²) and three periods of pretreatment (10, 20 and 30 second(s)). The results revealed that samples exposed to various intensities of microwave showed significantly higher growth rates and biomass than that of non-irradiated controls. The result of PCR blasted with sequenced cyanobacteria in NCBI showed 97% homology to the 16S rRNA of Phormidium sp. The study revealed that various microwave intensities induce different physiological effects, depending on field strength and duration of exposure.

2.1.8 16S rDNA gene sequence analysis

Prokaryote genomes are small and compact. This applies to cyanobacteria as well, in which known genome sizes vary between 1.6 Mbp (Prochlorococcus marinus MIT 9301) to 9.01Mbp (Nostoc punctiforme PCC 73102) (http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi). Insufficient genetic information on cyanobacteria is available. By August 2008, 34
cyanobacterial genomes had been completely sequenced and many sequencing projects are currently in progress (http://bacteria.kazusa.or.jp/cyanobase/). Cyanobacteria possess a single circular chromosome and may have one or more plasmids (Kaneko and Tabata, 1997). The plasmids contain functional genes although the majority have unknown or hypothetical functions (Kaneko et al., 2003). In cyanobacteria the size of the genome reflects the number of genes. Oxygenic photosynthetic prokaryotes, cyanobacteria and prochlorophytes are genetically related on the basis of 16S rRNA sequences (Woese, 1987). It had been proposed that *Prochlorococcus* is closely related to the marine cluster *Synechococcus*, based on analyses using gene sequences from 16S rRNA and *rpoC1*, a subunit of DNA-dependent RNA polymerase (Urbach et al., 1998). These studies had been performed using 16S rDNA (small subunit) sequences. Actual genetic characterization, however, often rely on DNA sequencing, most commonly of the 16S rDNA gene (Comte et al., 2007).

Out of the 21 strains of *Leptolyngbya valderiana* screened, only four appeared to be positive for the microcystin synthetase gene by PCR analysis suggesting variability among strains. The variations may have resulted in response to environmental stress. For example, *L. valderiana* BDU 30501 is capable of utilizing ampicillin and phenol in nitrogen stress environments (Prabaharan et al., 1994). Likewise, degradation of lignin model dye Poly R-478 and organophosphorous pesticide by *L. valderiana* BDU 140441 and azo dyes (orange G) by *L. valderiana* BDU 20041 (Priya et al., 2006) signify the genetic differences leading to the adaptability of strains to various environmental conditions.

16S rRNA gene sequences of many isolates also revealed genetic differentiation on the order that is normally encountered between non-related species. These results indicated that several morpho-species in reality are composed of genetically divergent population or cryptic species as observed in other closely related taxa (Casamatta et al., 2003). However, 16S rDNA gene sequence analysis of these morphotypes exhibited distinction above genus level. Such designations remain provisional awaiting results from further characteristic studies involving large number of morphotypes and ecotypes (Komarek and Anagnostidis, 2005).

The distribution and diversity patterns of the cosmopolitan marine cyanobacterium, *Leptolyngbya valderiana* (Pseudanabaenaceae, Cyanobacteria) were studied by Jagadeesan et al. (2009). To assess the level of genetic diversity, morphotypes from different geographical
locations (Coast of south India and Andaman) were subjected to randomly amplified polymorphic DNA (RAPD) analysis and partial 16S rDNA sequence studies. Partial 16S rDNA sequence similarity values ranged from 91 to 99%. This genetic variability, within the marine strains of *Leptolyngbya valderiana* indicated the presence of cryptic species and studies that comprise ultrastructural morphology and additional molecular markers are essentially required to characterize and clarify the evolutionary patterns and biogeography.