

# An Evaluation of Cyanobacterial Diversity in Paddy and their Capability to Degrade Pesticides

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## **Abstract**

Paddy is one of the important cereal crops in the world that provides staple food for nearly more than half of the world's population. However 90 % of the world's rice is produced and consumed in the Asian region where only 6 countries (China, India, Indonesia, Bangladesh, Vietnam and Japan) comprising 80% of the world's production and consumption. The FAO estimated the world paddy production in 2017 to 754.6 million tonnes (500.8 million tonnes, milled basis); that is slightly more than in 2016 to 748.0 million tonnes (496.7 million tonnes, milled basis). The variation in soil physico-chemical properties across different selected sites may effects the soil quality and fertility of the paddy agro-ecosystem (Schoenholtz, 2000; Tale and Ingole, 2015; Kekane et al., 2015). Several physicochemical parameters like soil temperature, pH, electrical conductivity, total -N, -P and organic-C leads to quality and nutrient status of the paddy field soil of adjoining areas of Telangana. Results showed that all the five sampling sites i.e. have suitable pH, EC and also good amount of nutrients (N, P and C) which well supported the cyanobacterial growth in paddy agro-ecosystem. There are various factors that affect the distribution and abundance of cyanobacteria in the paddy field but pH is an important factor and it has been observed that an increase in pH along with decrease in light intensity and less nitrogen favored cyanobacterial growth. This information about physico-chemical parameters of paddy field soil and its correlation with the cyanobacterial diversity could be very useful for further study. A wide variation in soil physico-chemical characteristics across different selected sites could be due to variation in anthropogenic activities and consequently variation in occurrence of different cyanobacterial species.

**Key words:** Evaluation, Cyanobacterial Diversity, paddy, capability, pesticides.

## **1. Introduction:**

Paddy is one of the important cereal crops in the world that provides staple food for nearly more than half of the world's population. However 90 % of the world's rice is produced and consumed in the Asian region where only 6 countries (China, India, Indonesia, Bangladesh, Vietnam and Japan)

comprising 80% of the world's production and consumption. The FAO estimated the world paddy production in 2017 to 754.6 million tonnes (500.8 million tonnes, milled basis); that is slightly more than in 2016 to 748.0 million tonnes (496.7 million tonnes, milled basis). Although, rice production has been more than tripled since 1961 during the era of green revolution; but during last two decades, rice production was almost stagnant in spite of introduction of new technologies, hybrid rice varieties and improved irrigation facilities. However, in spite of change in food pattern, it is estimated that to over 800 million tonnes production of rice by 2030 and 525 million tons by 2050 (IRRI, 2000). In many Asian countries, rice fulfils more than 40 % of daily calorie intake of total calories from food consumption (Timmer and Dawe, 2007; Timmer et al., 2010). Although the percentage of consumption of rice decreased during several years but still rice is the main component of Asian diet. Some Asian countries also export the rice as primary agricultural products, which contribute to economy, but in terms of overall contribution in GDP; it contribute only 1-4 % in 2007.

But having highest harvested area, the yield still much lower than many other rice producing countries; it needs to be increased despite the limited options for expanding the area or irrigation coverage. Around 65% of the total population in India depends on rice and it accounts for 40% of their food production

### **Impact of pesticides on agro-ecosystem and environment**

The maximum portion of pesticides applied in paddy crop production lies unused in soil and contributes environmental contamination. Paddy growers often spray pesticides up to five to six times in one cropping season while only two applications may be sufficient. The pesticide also be taken up by non-target flora and fauna, leaches in to soil, and could be contaminate groundwater or potable water. These pesticides residues along with paddy flood water drained into irrigation canals; leads to further contamination in to rivers and lakes. The pesticides also creates a bigger problem i.e. biomagnifications, when they are accumulated in beneficial organisms like fish.

## **2. Review of literature:**

### **Cyanobacterial diversity in paddy fields**

The paddy fields considered as an ecosystem and provide diverse habitats for the variety of microorganisms. These habitats are microenvironments which exhibit different physico-chemical and biological properties. Having such heterogeneity of the habitats influences the structure and diversity of microbial communities in the paddy field ecosystem and facilitates various

microbiological processes; that are agronomically and biogeochemically important for paddy field ecosystem (Kimura, 2000). Cyanobacteria (Blue-Green-Algae, BGA) are one of the main microorganisms that naturally inhibit the paddy field ecosystem. They are a highly diverse group of prokaryotic microorganisms which performs oxygenic photosynthesis (Kulasooriya, 2011).

Some cyanobacteria also possess specialized cells called heterocyst which are capable to fix atmospheric nitrogen; so they can be used as natural bioinoculants. Kaushik (1994) reported that amount of nitrogen contributed by cyanobacteria to ricecrop varies from 20-30 kg/ha. It is also reported that cyanobacteria can improve soil characteristics by, modifying texture size and subsequent aeration (Ibrahim, 2007), increasing phosphorus content (Fuller and Rogers, 1952) and enhancing carbon content and water holding capacity (Richert et al., 2005).

Cyanobacteria naturally inhabit on the surface of paddy field soil as well as flood water. Anand and Hopper (1995); and Vijayan and Ray (2015), reported *Anabaena*, *Arthrospira*, *Cylindrospermum*, *Chroococcus*, *Nostoc*, *Lyngbya*, *Oscillatoria*, *Gleothecae*, *Gleocapsa*, *Microcystis*, *Merismopedia*, *Synechocystis*, *Leptolyngbya*, *Westilopsis*, *Syctonema*, *Phormidium*, *Trichodesmium*, *Haplosiphon* and *Fischerella* in paddy field of Kerala. In paddy fields of Nagaland, Singh et al. (1997) recorded maximum number of species of *Microcystis* and heterocystous genera as *Anabaena* and *Nostoc*. Some of the common cyanobacteria inhabiting paddy field are *Anabaena*, *Aulosira*, *Calothrix*, *Gleotrichia*, *Cylindrospermum*, *Nostoc*, *Fischerella*, *Syctonema*, *Tolypothrix* and *Wolleea* (Rai, 2001). Various workers have studied the cyanobacterial flora of paddy fields in our country (Ahmed et al., 1999; Tiwari et al., 2000; Nayak et al., 2001; Kaushik and Prasanna, 2002; Mishra and Pabbi, 2004; Choudhury and Kennedy, 2005; Rai, 2006; Nayak and Prasanna, 2007) and few attempts have also been carried to explore their diversity in the state of Orissa and reported cyanobacteria are: *Chroococcus*, *Fischerella*, *Nostoc*, *Lyngbya*, *Oscillatoria*, *Aphanocapsa*, *Microcystis*, *Microcoelus*, *Aulosira*, *Syctonema*, *Phormidium*, *Aphanocapsa*, *Microchaete*, *Dactylacocopsis*, *Symploca*, *Pseudoanabaena*, *Schizothrix* and *Hydrocoleum* (Bhakta et al., 2006; Dey and Bastia, 2008; Choudhary, 2009; Choudhary and Bimal, 2010; Dey et al., 2010). In Punjab region, *Anacystis*, *Anabaena*, *Cylindrospermum*, *Chroococcus*, *Fischerella*, *Nostoc*, *Lyngbya*, *Oscillatoria*, *Phormidium*, *Aphanocapsa*, *Synechocystis*, *Westilopsis*, *Microcystis*, *Plectonema*, *Dactylacocopsis*, *Synechococcus*, *Spirulina* and *Wolleea* were also reported. In Madhya Pradesh region, the cyanobacterial genus were reported are: *Anabaena*, *Aphanocapsa*, *Arthrospira*, *Cylindrospermum*, *Chroococcus*, *Fischerella*, *Nostoc*, *Lyngbya*, *Oscillatoria*, *Gleothecae*, *Gleocapsa*, *Microcystis*, *Westilopsis*, *Plectonema*, *Syctonema*, *Phormidium*, *Microchaete*, *Symploca*, *Stichosiphon*, *Trichodesmium*, *Haplosiphon*, *Stigonema* and *Fischerella*. Bhardwaj and Baruah (2013) reported

Anabaena, Aphanocapsa, Cyndrospermum, Chroococcus, Nostoc, Lyngbya, Oscillatoria, ApanotheceMicrocystis, Syctonema, Phormidium, Microchaete and Microcoelus in Telangana region. In southern region, Singh et al. (2016) reported Anabaena, Cyndrospermum, Chroococcus, Gleothece, Gleocapsa, Merismopedia, Nostoc, Lyngbya, Oscillatoria, ApanotheceMicrocystis, Arthrospira, AnabaenopsisTolypothrix, Rivularia, Calothrix and Microcoelus. Denoboyina and Shivakumar (2013) reported Anabaena, Cyndrospermum, Chroococcus, Gleocapsa, Nostoc, Lyngbya, Oscillatoria, Tolypothrix, Rivularia and Calothrix.

### **Cyanoremediation of pesticides**

Pesticides cause damaging effect on growth, photosynthesis, nitrogen fixation, biochemical composition and metabolic activity of paddy field cyanobacteria (Moustafa and Helling, 2002). Pesticide application is responsible for oxidative stress in cyanobacteria and also induced the production of reactive oxygen species (ROS), including superoxide radicals ( $O_2^-$ ), hydroxyl radicals (OH) and hydrogen peroxide ( $H_2O_2$ ). These reactive oxygen species (ROS) affect the cell membranes, proteins and nucleic acids; and could cause enzyme inactivation, protein denaturation, lipid peroxidation and DNA mutation and also damages the cellular components through oxidative (Imlay et al., 1998; Vandana et al., 2001). Maximum work related to effects

of pesticides on cyanobacteria is available with pesticides such as DDT, lindane, parathion, endosulfan, chlorpyrifos, glyphosate, molinate, monocrotophos and PCP. Singh (1973) isolated cyanobacteria i.e. Cyndrospermum sp., Aulosirafertilissima, and Plectonemaboyanurn from paddy fields; which were able to tolerate lindane (commercial perspiration) up to 80  $\mu\text{g}/\text{mL}$  conc. Singh (1973) also found that the growth of these cyanobacteria did not affected by the lindane concentrations up to 20  $\mu\text{g}/\text{mL}$ . It is also reported that Anabaena sp. PCC7120 and Nostocelliposporum, degraded A-HCH to a mixture of 1,2,3-and 1,2,4-trichlorobenzenes and forms an intermediate pentachlorocyclohexene (Kuritz and Wolk, 1995). Lee et al. (2003) investigated that Anabaena sp. strain PCC 7120 and Anabaena flos-aquae degraded the endosulfan into endodiol (main end product) and endosulfansulphate (trace amount). Although endodiol is a non-toxic metabolite to fish and other organisms, but endosulfan sulfate is much toxic like parent compound endosulfan and also persists much longer tolerance into soil environment in comparison to endosulfan (Kennedey et al., 2001). Megharaj et al. (1987, 1994), Orus and Marco (1991) and Subramanian et al. (1994) isolated Nostoc, Oscillatoria and Phormidium from methyl parathion enriched soil and grew in media supplemented with methyl parathion or other organophosphorus pesticides as a sole source of organic phosphorus and nitrate. They found that these cyanobacteria

were able to utilize phosphorus from the pesticide for their growth and development. Ibrahim et al. (2014) observed that *Anabaena oryzae*, *Nostocmuscorum* and *Spirulina platensis* were able to degrade and utilize malathion pesticide as a source of phosphorus. It is also found that under aerobic conditions *Anabaena* sp. PCC 7120 strain was able to reduce the nitro-group of methyl parathion to an amino group via a nitroso group intermediate (Barton et al., 2004).

Megharaj et al. (1994) said that cyanobacteria were able to oxidize the nitro group of para-nitrophenol accompanied by the release of nitrite into growth media. But enzymes involving in this process are unknown and further use of this released nitrite is likely to depend on the activity of nitrite reductase. Similar observations that nitrogen metabolism and phosphorus utilization from organophosphorus pesticides are interconnected (Subramanian et al., 1994); however, the researchers did not examine the possible effects of various sources of fixed nitrogen on biodegradation of organophosphorus pesticides (Kuritz, 1999). Lipok et al. (2007; 2009) investigated that *Spirulina* sp. was able to degrade glyphosate and stated that the rate of glyphosate utilization from the aqueous medium was independent of its initial concentration. Lipok et al. (2007) also suggested that the degradation mechanism of glyphosate by *Spirulina* sp. might be different from the mechanism shown by the other bacteria. It is also reported that *Anabaena* sp., *L. boryana*, *Microcystis aeruginosa* and *Nostoc punctiforme* are able to utilize the glyphosate as the only source of phosphorus (Forlani et al., 2008). Dyhrman et al. (2006) observed that marine cyanobacteria *Trichodesmium erythraeum* showed existence of phosphorus-dependent glyphosate transformation. Although a number of reports confirmed about phosphorus-dependent glyphosate transformation; but reports on the utilization of glyphosate as a source of nitrogen by cyanobacteria are not yet available in the literature. Ravi and Balakumar (1998) investigated the glyphosate degradation by *Anabaena variabilis* and also claimed that extracellular phosphatases are responsible in the hydrolysis of C-P bond of glyphosate; however, this claim has not been supported by the other researchers. In relation of this, it is stated that extracellular phosphatases have no significant role in glyphosate degradation (Forlani et al., 2008). Arunkumara et al. (2013) stated that cyanobacterial strains having the ability to use these phosphatases as a source of phosphorus could be applied for decontamination of pesticides. El-Nahhal et al. (2013) investigated the ability of cyanobacterial mats to degrade the pesticide acetochlor; and found that these cyanobacterial mats very efficient in degradation of acetochlor at high concentration. It is reported that *Phormidium valderianum* BDU 20041 strain is able to tolerate chlorpyrifos with oxido-reductase enzymatic activity to degrade the chlorpyrifos (Palanisami et al., 2009). Thengodkar and Sivakami (2000) investigated that *Spirulina platensis* are able to tolerate chlorpyrifos up to 80 ppm and

mineralize chlorpyrifos into TCP (3, 5, 6-trichloro-2-pyridinol) through the enzyme alkaline phosphatase (ALP). It is also reported that cyanobacterium *Synechocystis* sp. PUPCCC 64 strain was able to degrade chlorpyrifos into 3,5,6-trichloro-2-pyridinol (Singh et al., 2011).

### **3. Objectives**

1. Analyses of soil physico-chemical parameters and nutrients status of the selected paddy fields.
2. Isolation and identification of existing cyanobacterial diversity from paddy fields.
3. Screening of pesticide tolerant cyanobacterial strain(s).
4. Testing the efficacy of the selected cyanobacteria for pesticide degradation.
5. Analyses of the pesticide degraded end product by cyanobacteria.

### **4. Research Methodology**

Chlorpyrifos: General, Structure and mode of action Chlorpyrifos (O, O-diethyl O, 3, 5, 6-trichloro-2-pyridolphosphorothioate) is one of the most widely used organophosphorus insecticides (Lee et al., 2012). The properties of chlorpyrifos are given below. Chlorpyrifos is a broad spectrum insecticide, which is applied to kill a wide variety of insects. It was introduced in 1965 (Hayes and Laws, 1990). Primarily chlorpyrifos is used to kill mosquitoes in the immature, larval stage of development; but it is no longer registered for this use. Chlorpyrifos is effective in controlling a variety of insects, including cutworms, corn rootworms, cockroaches, grubs, flea beetles, flies, termites, fire ants, and lice (US EPA, 1986). It is commonly applied as an insecticide on cereals, cotton, field, fruit, nut and vegetable crops, and well as on lawns and ornamental plants (US EPA, 1984; Berg, 1986). It is also registered for direct use on sheep, turkey, for horse site treatment, for treatment of dog kennels, and for domestic dwellings, farm buildings, storage bins, and commercial establishments. It is available in emulsifiable concentrate, dust, flowable, pellet, spray, granular and wettable powder formulations. Chlorpyrifos kills insects upon ingestion by affecting the nervous system. It affects the nervous system by inhibiting the breakdown of acetylcholine (ACh), a neurotransmitter (Manahan, 1992). Chlorpyrifos binds to the active site of the cholinesterase (ChE) enzyme, which prevents breakdown of ACh in the synaptic cleft; resulted in the accumulation of ACh in the synaptic cleft causes overstimulation of the neuronal cells, which leads to neurotoxicity and eventually death (Ragnarsdottir, 2000; Singh and Walker, 2005). Chlorpyrifos also interferes with other enzymes, such as carboxylesterases and Aesterases; but functional role of these enzymes is not well understood, although they occur in many mammalian systems. The mechanism of

toxicity of chlorpyrifos resembles with other organophosphate insecticides such as malathion and parathion, thus, chlorpyrifos would not be effective against organophosphate-resistant insect populations.

Chlorpyrifos also able in cholinesterase inhibition in humans at high enough doses; result in the overstimulation the nervous system causing nausea, dizziness, confusion. But at very high exposures (e.g., accidents or major spills) it could be cause respiratory paralysis and death. So it should be primary concern for manufactures to aware about occupational exposure to chlorpyrifos. To prevent from this, current chlorpyrifos labels require information to wear additional personal protective equipment (chemical resistant gloves, coveralls, respirators) during workers handling and applying chlorpyrifos, and also restricting entry into treated fields for 24 hours up to five days.

## **5. Analysis**

### **Collection of soil samples**

Soil samples from each site were collected from the subsurface horizon (2–15 cm) at three different points and mixed thoroughly. The collected soil samples were brought to the laboratory in polythene bags, air dried and sieved before analyses. The soil texture of all the sites was sandy-clay in nature.

### **Physico-chemical analysis of soil samples**

The physicochemical properties of soil such as Temperature, pH and Electrical conductivity; and Nutrient status as Total-N, -P and Organic-C contents were measured as per standard procedures.

#### **Temperature**

The temperature of soil is directly linked to the temperature of the atmosphere because soil is an insulator for heat flowing between the solid earth and the atmosphere.

Procedure 1. Cleaned the surface of probe rod of thermometer. 2. Inserted the probe rod of digital thermometer in to soil up to 5-6 inches. Note the temperature immediately. 3. Washed the probe rod with distilled water. 4. Again inserted the probe rod in to the soil, and note the temperature. 5. At least 2-3 reading were taken per location. 3.2.3.2 Soil pH 3.2.3.2.1 Apparatus pH meter with a range of 0-14 pH, Pipette/dispenser, Beaker, Glass rod.

Reagents Buffer solutions (pH 4, 7 and 9) Deionized water

Procedure 1. Air dried soil for 1-4 days depending on the relative humidity and soil properties. Then grind the air-dried soil to pass 2 mm sieve and mix well. 2. Calibrated the pH meter, using buffers of pH 7.0, pH 4.0 and pH 9.2. 3. Weighed 10.0g of air dried soil sample into 50 or 100 ml beaker, add 20ml deionized water. 4. Allowed the soil to deionized water without stirring and then thoroughly stir for 10 second using a glass rod. 5. Stirred the suspension for 30 minutes and record the pH on the calibrated pH meter.

Electrical conductivity Electrical conductivity (EC) is expressed in units of milli-Siemens per meter (mS/m). Soil EC measurements may also be reported in units of deci-Siemens per meter (dS/m), which is equal to the reading in mS/m divided by 100.

.1 Apparatus Electrical conductivity meter, Beakers (25 ml), Erlenmeyer flasks (250 ml) and pipettes, Filter paper 2 Reagents 0.01M Potassium chloride solution: Dried potassium chloride at 600 C for two hours. Weighed 0.7456 g of it and dissolve in distilled water and make the volume to one litre. This solution gives an electrical conductivity of 1.412 mS/cm at 250C.

Aysal et al. (2004) reported the chlorpyrifos residues in food chain. During supervised trials on rice conforming to Good Agricultural Practise conducted in Columbia, the Philippines, Thailand, Vietnam, and India, maximum chlorpyrifos residue level of 0.5 mg/kg for rice, a supervised trials median residue of 0.12 mg/kg, and a highest residue level of 0.28 mg/kg were estimated. Chandra et al. (2010) reported chlorpyrifos residue in cauliflower in range of 0.024–0.07 mg/kg and brinjal in range of 0.018–0.021 mg/kg. Berg (1986) estimated LD50 for chlorpyrifos in rat body was 82–270 mg/ kg. These reports are evident that indiscriminate use of chlorpyrifos may cause serious human health problems. There is growing concern about the toxicological and environmental risks associated with chlorpyrifos residues. The persistent nature of the insecticide is a health hazard, and thus, there is a need to detoxify this moiety (Mukherjee et al., 2004).

### **Cyanoremediation of chlorpyrifos**

A number of researchers studied the effect of chlorpyrifos on cyanobacteria (Palanisami et al., 2009; Singh et al., 2013; Kumar et al., 2014). It is evident that insecticides altered the enzyme activities associated with antioxidant defence mechanism through inducing the production of singlet oxygen and other active oxygen species at various sites of photosynthetic electron transport chain and responsible for oxidative damage (Halliwell, 1987; Bagchi et al., 1995; Palanisami et al., 2009). In response to this, Cellular systems scavenge these active oxygen species by invoking antioxidative machinery such as superoxide dismutase, catalase, peroxidase, etc. (Patel and Chakrabarti, 1982;



Büyüksönmez et al., 1998; Palanisami et al., 2009). Kumar et al. (2014) investigates the effect of chlorpyrifos on the antioxidant level and changes in the fatty acid profile of *Chroococcusturgidus* NTMS12 and found increase in the activity of antioxidant enzymes proline, SOD and CAT. Under chlorpyrifos exposure conditions, *Chroococcusturgidus* NTMS12 showed in change its fatty acid profile by lowering the level of unsaturated fatty acids. It could be strong and effective defence mechanisms i.e. increased activity of cellular antioxidants and lowering the levels of cellular unsaturated fatty acids by cyanobacteria against the pesticide-induced stress. It is also reported that phosphatases play an important role in the biodegradation of chlorpyrifos (Madhuri and Rangaswamy, 2002; Thengodkar and Sivakami, 2010). Although few reports on the metabolization of pesticides by cyanobacteria are available (Subramanian et al., 1994; El-Bestawy et al., 2007; Lee et al., 2003), but detailed mechanism of chlorpyrifos degradation in cyanobacteria is not fully known from the paddy fields therefore, we selected cyanobacteria diversity from paddy soils and its efficacy to the tolerance and degradation of chlorpyrifos pesticide in laboratory with the objectives.

Morales et al. (2011) suggested that soil pH also influenced by the soil moisture content; leads change in the chemical equilibrium which further affects the form and effectiveness of soil nutrient elements present in the soil. In tropics, flooding commonly practiced for the paddy cultivation; it is observed that flooding facilitates the availability of phosphorus and potassium; but also reduces the availability of nitrogen, sulphur and zinc (Fan et al., 2008; Das et al., 2016). It is also observed that phosphorous availability initially enhanced by flooding in lowland rice soils and it is used by paddy to fulfil phosphorous requirement (Gupta et al., 2007). Reeves (1997) suggested that soil organic content could be a key soil physicochemical parameter to assess quality of soil and its maintenance is crucial for the productivity and long-term stability of paddy agroecosystem (Carter, 2002).

## **6. Conclusion:**

The variation in soil physico-chemical properties across different selected sites may effects the soil quality and fertility of the paddy agro-ecosystem (Schoenholtz, 2000; Tale and Ingole, 2015; Kekane et al., 2015). Several physicochemical parameters like soil temperature, pH, electrical conductivity, total -N, -P and organic-C leads to quality and nutrient status of the paddy field soil of adjoining areas of Telangana. Results showed that all the five sampling sites i.e. have suitable pH, EC and also good amount of nutrients (N, P and C) which well supported the cyanobacterial growth in paddy agro-ecosystem. There are various factors that affect the distribution and abundance of cyanobacteria in the paddy field but pH is an important factor and it has been observed that an increase in pH along with decrease in light intensity and less nitrogen favoured cyanobacterial

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