Assessment of Cyanobacterial diversity in paddy fields and their capability to degrade pesticides

SUMMARY OF
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Paddy crop is one of the important cereal crops of the world in terms of providing staple food for more than the 50 % of the world's populations. In Asia where 90 % of paddy is cultivated and consumed especially in countries like China, India, Indonesia, Bangladesh, Vietnam and Japan. Due to having major crop of Asian regions, paddy (rough rice) the basic staple for the majority of the population, including the region's million's of poor peoples. India is the second largest producer of the paddy where in north-east and south regions paddy is cultivated more than two or three times and imparts major portion in the daily diet in these regions.

Paddy is the crop which needs enough water to grow or mainly depends upon the rain for the good yields but due to global warming, there would be significant danger to the paddy production in terms of non-availability of good rainfall and arable land. Paddy crop is also affected by number of pests such as insects, diseases and weeds; which affects the paddy production in many folds in terms of loss in yields and quality. Although paddy growers uses variety of pesticides for the control of these but pesticides not only control the pests but also disrupt the paddy agro-ecosystem. Pesticides are responsible for the loss of beneficial organisms which play significant in the healthy ecosystem and pesticide residue which further creates problems in paddy ecosystem; somehow pollutes the nearby aquatic ecosystems.

Paddy agro-ecosystem has the many micro-habitats which supports the many types of communities of organisms such as microorganisms. Microbes play a beneficial role in maintaining healthy paddy agroecosystem by recycling of nutrients and soil fertility enhancement. The other beneficial microbes together with the photoautotrophic cyanobacteria or blue green algae (BGA) account about 60% of total biomass and have been considered as most successful and abundant photo-

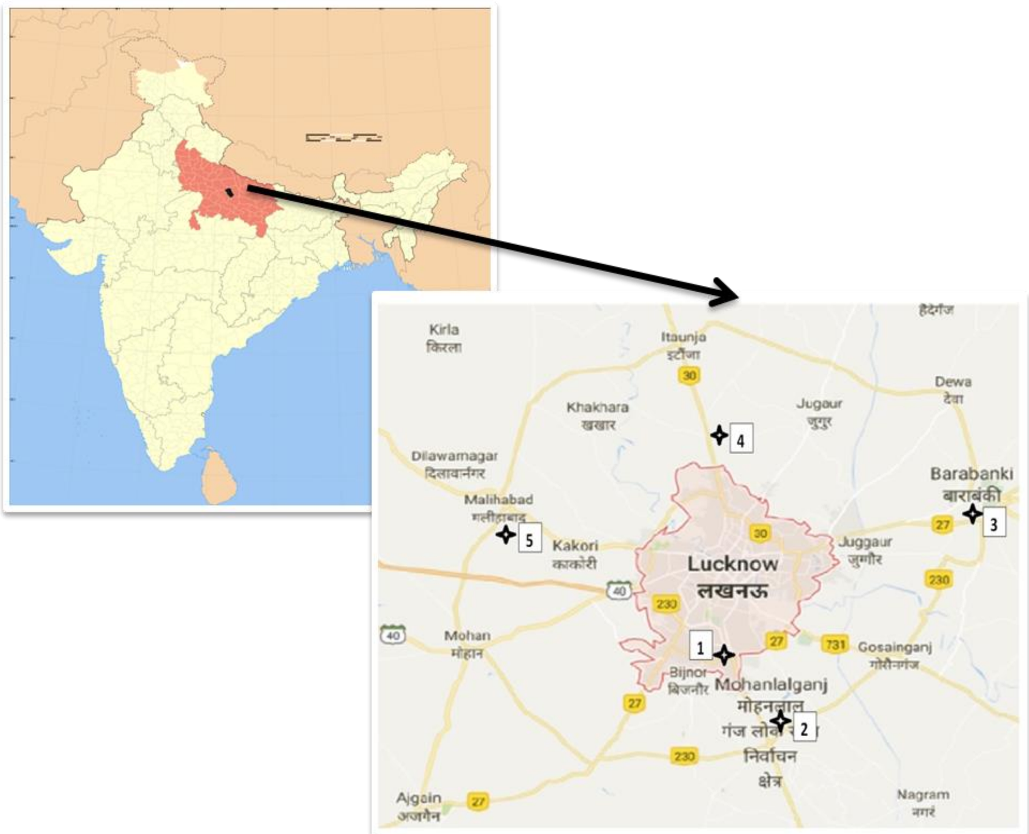
synthesizers on this Planet. The cyanobacteria have immense potential to contribute a very crucial role in agriculture and ecosystem services. A number of investigations have been conducted with aobjectives to improve our knowledge on the diversity, dynamics and roles of efficient soil microorganisms and their beneficial activities in the development of agricultural production. The advanced green-technologies such as bio-fertilizers consisting of cyanobacteria could be a powerful tool to remove soil contaminants, soil fertility restoration and enhanced agricultural production, rendering the paddy agro-systems more stress-tolerant. Furthermore, cyanobacteria can help to reduce the amount of energy input demands in the form of chemical fertilizers and mitigation of stress agro-ecosystems restoration. Now it well proved that the cyanobacteria are one of the important beneficial microorganisms of the paddy field agro-ecosystem. The cyanobacteria act as a bio-inoculants for the paddy crop, make available more nutrients and increase the quality of soil to the crops. Now days due to heavy dependence on pesticides to control pests in paddy crop, cyanobacteria also affected by the deleterious impacts of these pesticides. However, pesticides badly affected the cyanobacterial populations existed in the paddy fields; but some cyanobacterial genera were known to tolerate these pesticides in some extent and also able to degrade in lesser toxic intermediates.

So paddy field cyanobacteria could be promising entities to remediate pesticide contamination in paddy field agro-ecosystem can be implied in other areas related to remediation of various pollutants. The present research work describes the multidimensional role of cyanobacteria for reclamation and removal of pesticides of soil and sustainable paddy agriculture development. To investigate the ability of cyanobacteria to degrade the pesticides; there is firstly needs of the systematic study of cyanobacterial diversity in paddy fields and then to check paddy field cyanobacteria against pesticides.

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.

The sites selected for the study of the adjoining areas of Lucknow city, Uttar Pradesh were: 1) BBAU Campus; 2) Mohanlalganj; 3) Barabanki; 4) Baksi ka Talab; 5) Malhiabad.



Location of study sites

Soil samples were collected from the selected sites and the physico-chemical properties of soil such as temperature, pH, electrical conductivity (EC) and nutrient status such as total N, -P and organic-C contents were measured as per standard procedures. All the five sampling sites BBAU Campus, Mohanlalganj, Malihabad, Bakshi ka Talab and Barabanki showed suitable pH, EC and sufficient nutrients (N, P and C) which could be helpful for the good cyanobacterial growth in paddy agro-ecosystem (Kumar et al., 2018). This information about physico-chemical parameters of paddy field soil could be very useful to correlate with the cyanobacterial diversity.

Physico-chemical properties of soil samples collected from different selected sites of Lucknow district and adjoining area. (The values given are means of 3 independent experiments ±SE. N=15 (5 sites × 3 replicates)).

Parameters	**BBAU campus**	**Mohanlalganj**	**Barabanki**	**Bakshi ka Talab**	**Malhiabad**	**Significance level**
Temperature (^oC)	28.4 ± 0.08^a	28.5 ± 0.06^a	29.4 ± 0.04^b	30.1 ± 0.02^c	30.4 ± 0.04^c	$P < 0.001$
EC (ms cm^{-1})	0.25 ± 0.05^a	0.29 ± 0.03^b	0.25 ± 0.02^c	$0.12^b \pm 0.03$	$0.11^b \pm 0.05$	$P < 0.001$
Soil pH	8.4 ± 0.07^a	8.7 ± 0.01^a	7.5 ± 0.06^b	8.1 ± 0.01^c	7.9 ± 0.07^b	$P < 0.001$
Total-N (%)	0.17 ± 0.06^a	0.11 ± 0.08^b	0.12 ± 0.03^b	0.22 ± 0.02^c	0.14 ± 0.06^d	$P < 0.001$
Total-P (%)	0.7 ± 0.03^a	0.5 ± 0.04^b	0.6 ± 0.01^c	0.7 ± 0.07^a	0.6 ± 0.02^c	$P < 0.001$
Organic-C (%)	0.69 ± 0.07^a	0.43 ± 0.04^b	0.60 ± 0.09^c	0.81 ± 0.05^d	0.64 ± 0.07^a	$P < 0.001$

EC=electrical conductivity.Values of each row followed by the same letter(s) are not significantly different according to Duncan's multiple range test (P=0.05).

The cyanobacterial diversity in the paddy fields of BBAU Campus, Mohanlalganj, Malihabad, Bakshi ka Talab and Barabanki was found to follow no regular pattern. There were different diversity of cyanobacteria in the paddy fields of the adjoining areas of Lucknow city, Uttar Pradesh; it was 430 g^{-1} to infinite.

Cyanobacterial population at different study sites

SNo.	Study sites	MPN (g^{-1} dry soil)
1.	BBAU Campus	2100
2.	Mohanlalganj	430
3.	Barabanki	930
4.	Bakshi ka Talab	Indefinite
5.	Malhiabad	1500

The survey of paddy fields of adjoining areas of Lucknow city, Uttar Pradesh showed moderate cyanobacterial diversity. Further 10 cyanobacterial genera *Synechococcus* sp., *Gloeocapsa* sp., *Anabaena* sp., *Aphanocapsa* sp., *Chroococcus* sp., *Microcoleus* sp., *Gloeothece* sp., *Oscillatoria* sp., *Gleotrichia* sp., and *Aphanothece* sp. were isolated. The study has endorsed the ideal nature of paddy field for cyanobacterial growth.

Distribution of cyanobacteria in paddy field soil of selected sites of Lucknow city and adjoining area, Uttar Pradesh.

SNo.	Cyanobacteria	BBAU campus	Mohanlalganj	Barabanki	Bakshi ka Talab	Malhiabad
1.	*Synechococcus* sp.	-	-	+	+	+
2.	*Gloeocapsa* sp.	+	-	-	+	-
3.	*Anabaena* sp.	+	+	+	-	+
4.	*Aphanocapsa* sp.	-	+	-	+	-
5.	*Chroococcus* sp.	-	-	+	+	-
6.	*Microcoleus* sp.	-	-	-	+	-
7.	*Gloeothece* sp.	+	-	-	-	+
8.	*Oscillatoria* sp.	-	-	+	+	-
9.	*Gloeotrichia* sp.	-	-	-	+	-
10.	*Aphanothece* sp.	+	+	+	-	+

These isolated cyanobacteria were further treated with chlorpyrifos, a widely organophosphate insecticide which applied in agriculture for the control of various insects and as well as in public health for control of disease vectors like mosquitoes. It is also used for controlling termites in wood fences and furniture. The mode of action of chlorpyrifos is like inhibiting the acetylcholinesterase enzyme which controls the acetylcholine (a neurotransmitter). Due to this communication between the nerve cells disrupted, leads to the nervous system malfunction and finally causes death of the pest. All the 10 isolated cyanobacteria grown in BG-11 medium containing 5 ppm chlorpyrifos (a minimum dose that is double the recommended dose in paddy fields) for the 12-14 days for the screening of chlorpyrifos tolerant cyanobacteria. The growth of *Synechococcus* sp., *Aphanocapsa* sp., *Chroococcus* sp., *Microcoleus* sp. and *Oscillatoria* sp. showed the regular increase up to day 14 while the growth of *Gloeocapsa* sp., *Anabaena* sp., *Gloeothece* sp., *Gloeotrichia* sp. and *Aphanothece* sp. showed increase up to day 8 and further declined. After analysing the growth curve and inhibition % of all cyanobacteria, *Synechococcus* sp., *Aphanocapsa* sp., *Chroococcus* sp., *Microcoleus* sp. and *Oscillatoria* sp. proved to be chlorpyrifos tolerant cyanobacteria.

The chlorpyrifos tolerant cyanobacteria *Synechococcus* sp., *Aphanocapsa* sp., *Chroococcus* sp., *Microcoleus* sp. and *Oscillatoria* sp. were investigated to study the efficacy of these cyanobacteria to tolerate chlorpyrifos in greater amount. All cyanobacteria were treated further against 5, 7.5, 10, 15 and 20 ppm chlorpyrifos with control without no chlorpyrifos. At 5, 7.5, 10 ppm chlorpyrifos concentration, *Synechococcus* sp., *Aphanocapsa* sp., *Chroococcus* sp., *Microcoelus* sp. and *Oscillotoria* sp. showed similar growth pattern respectively; but at 15 and 20 ppm, *Aphanocapsa* sp. and *Microcoelus* sp. showed some slow growth in compare to *Synechococcus* sp., *Chroococcus* sp. and *Oscillotoria* sp.. In *Synechococcus* sp., *Chroococcus* sp. and *Oscillotoria* sp. which showed better growth at 15 and 20 ppm; *Oscillotoria* sp. showed maximum growth compared to *Synechococcus* sp. and

Chroococcus sp. So *Oscillotoria* sp. proved potential cyanobacteria that could be used for the further study of analysis of degradation of chlorpyrifos.

The 16S rRNA sequencing of *Oscillotoria* sp. further confirmed the cyanobacteria was *Oscillotoria* sp. which the showed 98-99 % similarity with *Oscillatoria* genera. The partial 16S rRNA gene sequences of was submitted to GenBank and an accession number MH 392711 was assigned with strain *Oscillatoria* sp. CYA8 CPF.

Chlorpyrifos stress caused degradation of photosynthetic pigments chlorophyll a, carotenoids phycobiliproteins (phycocyanin, allophycocyanin and phycoerythrin) in *Oscillatoria* sp. CYA8 CPF. To protect from the chlorpyrifos stress, *Oscillatoria* sp. increased the production of antioxidant enzymes SOD, POD and CAT, which played a significant role to overcome the oxidative stress caused by chlorpyrifos.

Photosynthetic pigments (mg L^{-1}) of *Oscillatoria* sp. CYA 8 CPF treated with different concentrations of chlorpyrifos at 5^{th} day.

Pigments (on 5^{th} day)	**0 ppm**	**5 ppm**	**10 ppm**	**ANOVA**
Chlorophyll a	0.24 ± 0.05^a	0.19 ± 0.05^b	0.14 ± 0.01^c	$P < 0.001$
Carotenoids	0.08 ± 0.01^a	0.06 ± 0.01^b	0.05 ± 0.0^c	$P < 0.001$
Phycocyanin	2.07 ± 0.05^a	0.92 ± 0.02^b	0.81 ± 0.06^c	$P < 0.001$
Allophycocyanin	3.62 ± 0.01^a	2.11 ± 0.05^b	1.95 ± 0.01^c	$P < 0.001$
Phycoerythrin	1.86 ± 0.05^a	1.15 ± 0.01^b	0.66 ± 0.04^c	$P < 0.001$

Values of each row followed by the same letter(s) are not significantly different according to Duncan's multiple range test (P=0.05).

There was in reduction in GSH content in *Oscillatoria* sp. CYA8 CPF which indicated that GSH (reduced form of glutathione) helps lowering the level of H_2O_2 through the conversion in to glutathione peroxidase. So the decreased level of GSH indicated that it might be helping in reduction of H_2O_2. While Proline content in *Oscillatoria* sp. increased which indicted the *Oscillatoria* sp. CYA8 CPF used proline accumulation to protect from the chlorpyrifos stress.

Oscillatoria sp. CYA8 CPF did not form any degradation product or nor it is released into the medium, but *Oscillatoria* sp. CYA8 CPF, intake good amount of chlorpyrifos which observed by a clear and good peak in GS-MS chromatogram of cell extract. The GC-MS spectra obtained showed that the chlorpyrifos might be degraded to some small metabolites which could not be identified using the available library database.

Finally it may be concluded that cyanobacteria have proven track record of restoring soil fertility they can be applied for refining and enhancing soil fertility of disturbed soil. However, there is still scope for further improvement in the cyanobacterial bio-technology and better exploitation of these microbes for better soil improvement process and paddy agricultural productivity. It is proposed that co-inoculations of paddy agricultural lands or degraded lands with selected pesticide tolerant cyanobacteria can be a suitable bio-formulation for improving the soil characteristics and soil detoxification. Nevertheless, there is dearth of information regarding the possible role of cyanobacteria in combination with other microbes in relation to their impact on removal of various pesticides from the paddy fields. Application of pesticide tolerant strains of cyanobacteria offer an ample scope in the field of sustainable paddy agriculture production and enriching soil fertility of degraded lands.