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Cyanobacterial Composition under Sewage-Irrigated and Tubewell-Irrigated Agroecosystems in Rohtak City, Haryana, India

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Abstract

Soil cyanobacterial communities can be used as an indicator to provide signal of changes, particularly in the soil quality. Cyanobacteria are highly sensitive to heavy metals and pesticide residues in the soil and their use can provide information on the accumulation of such chemicals and their possible detrimental effects on crop plants. In the present investigation, cyanobacterial communities of sewage irrigated-soils (SIS) and tubewell-irrigated soils (TIS) were investigated. The SIS and TIS were sampled, located along the sewage drain of Rohtak city, Haryana, India. The SIS were found to be rich in inorganic nitrogen and phosphorus, with high heavy metal content. Soil samples were analyzed using enrichment culture techniques in BG-11 medium with and without nitrates. Fifty one and eighty one cyanobacterial isolates were enumerated from the SIS and TIS samples, respectively using the dilution method on solid media. The analysis revealed low species diversity in SIS as compared to TIS samples. The result of the investigation suggested that cyanobacteria tended more to reflect influence of sewage water through changes in community structure. The heavy metal residues and other toxic chemicals in the soil may be the factor responsible for less cyanobacterial diversity as the correlation analysis reveals negative correlation of heavy metal content of the soil with cyanobacterial abundance. It becomes vital that sewage irrigated soils must be maintained and the management should be directed towards ecological restoration.

Keywords: Cyanobacteria, Sewage-irrigated soil, Tube-well irrigated soil, Abundance

Introduction

Soil ecosystems serve as the most important non-aqueous habitats for cyanobacteria (Zenova *et al.,* 1995). Cyanobacteria are a diverse group of prokaryotes able to live under a wide range of environmental conditions (Stal and Krumbein, 1985;

Whitton and Potts, 2000). The activities of cyanobacteria contribute to soil formation, stability of mature soils (Metting, 1981) and they carry out an important role in nutrient re-cycling. These prokaryotic, photosynthetic micro-organisms are a potential source of combined nitrogen because they are capable of fixing atmospheric nitrogen (Goyal, 1997). They also facilitate the survival, growth and establishment of plants in extreme habitats. Thus, cyanobacteria play a key role in ecosystem functioning.

Cyanobacteria are one of the main components of the microbiota in fields (Ladha and Reddy, 2003) and play an important role in the maintenance and build-up of soil fertility (Roger and Ladha 1992; Kaushik and Prasanna 2002; Peoples *et al.,* 1995). Cyanobacterial growth is affected by a variety of physical and chemical factors, including temperature, pH and nutrient concentration. Any change in these conditions and any introduction of toxicants into the system are accompanied by an alteration in the composition of preceding cyanobacterial flora. Today, the increasing population especially in the metropolitan cities has rapidly expanded the discharge of partially-treated or untreated sewage to the water bodies thus magnifying the nutrient load and generation of wastewater. Therefore, sewage water has been commonly employed as a source of irrigation. Application of sewage water improves the physico-chemical properties and nutrient status of the soil and increases crop production as it supplies N, P, K and also valuable micronutrients than the crop requires (Panicker, 1995). On the other hand, the use of sewage water in agriculture is associated with health risks because of the presence of pathogenic micro-organisms (Toze, 2006), metallic contaminants like Cu, Ni, Cd, Cr, Zn (Misra and Mani, 1991) and polychlorinated substances (Fytili and Zabaniotou, 2008).

Brookes *et al.* (1986) evaluated the effects of metal-rich sewage solids on cyanobacteria development and N_2-fixation in soil and found cyanobacterial development was restricted on sewage-amended soils relative to manure-amended soils. Mårtensson and Witter (1990) evaluated the effects of long-term metal-rich sewage applications on the activity of diazotrophs and specifically cyanobacteria, finding that although diazotrophic N_2-fixation rates were similar in sewage-amended and manure amended soils and reference soils, following glucose addition, fixation rates were significantly lower in sewage-amended soils than reference soils (manure-amended soils had significantly higher rates than both). Also, cyanobacterial growth was delayed in sewage-amended soils relative to manure-amended and reference soils. Leigh (2010) found no evidence of an inhibitory effect of sewage application on diazotrophs, even though it is well-established that addition of labile N (as contained in biosolids) inhibits N_2-fixation. It may be possible that viable cyanobacteria in the biosolids accounted for the temporarily higher level of N_2-fixing activity, as there were blue green microbial mats on the surfaces of biosolids and biosolids-amended soils first observed approximately 14 days post-amendment.

The presence of metallic contaminants, PCBs and other toxic waste in sewage water has become a pervasive threat to the soil ecosystems due to increasing use of sewage water for irrigation. These contaminants can have toxic effects on many different types of organisms and affect biological processes at various levels of organization viz.

cellular, population, community and ecosystem. Toxic effects of heavy metals upon soil micro-organisms and microbially-mediated processes have been reviewed by Duxbury (1985) and Doelman (1986).

Studies concerning the use of bio-indicators have suggested biodiversity as a basic indicator of landscape quality and a fundamental tool for assessing the impact and success of the remediation process. The main purpose of this study was to: (i) analyze the cyanobacterial communities of sewage irrigate soils of Rohtak city, Haryana, India, (ii) analyze the relationships between cyanobacterial diversity and environmental variables (surrounding land use, habitat condition) by comparing their relative abundance in SIS and TIS, and (iii) to identify the community indicators useful to evaluate the effect of human impact. This may also act as a valuable tool for assessing the ecological quality of these ecosystems.

Materials and Methods

Area of investigation

The study was conducted in Rohtak city which is located between 76°25′ and 76°94′ East longitudes and 28°35′ and 28°80′ North latitude, lying at 219.84 meters above sea level. The climate in this area is classified as continental. The annual rainfall of Rohtak is 455mm, most of which is received during the last week of June to September contributed by the south-west monsoon.

Sampling

To assess the reaction of soil cyanobacterial communities to agricultural practices, the soil samples were collected from depth of 0-10 cm from ten sewage-irrigated fields located along the sewage drain of Rohtak city, Haryana, India. Before chemical analysis, they were air dried, powdered and sieved through a 2 mm sieve. Stones and plant materials were removed manually. A portion of soil was analyzed for edaphic parameters as per the methods given in USDA Handbook No. 60 (US Salinity Lab Staff, 1954). The heavy metals were analysed by the method of Lindsay and Norvell (1978). The remaining air dried soil samples were used to estimate cyanobacterial composition.

Soil cyanobacteria

Cyanobacteria were cultured on BG-11 medium with or without combined nitrogen. The chemical composition of BG-11 is given in table-1 (Stanier *et al.,* 1971). The media was solidified with 1.5% agar. Media was sterilized for 15 minutes in an autoclave and then transferred into sterile plastic Petri dishes.

Dilution on solid media was chosen for cyanobacterial counts. 10 g of soil was transferred to 90 ml of sterile water and shaken for half an hour. Then serial 4 fold dilutions of homogenate liquid were prepared. Each petridish containing solidified BG-11 media was inoculated with 1ml of dilute soil suspension, 3 replication of each dilution was used (Lukesova, 1993). These were then incubated for 3 weeks at 27±3°C under continuous illumination using white fluorescent light (3000 lux).

Table 1 Chemical composition of BG-11 medium

Ingredients	**Quantity(g/l)**
K_2HPO_4	0.04
$MgSO_4.7H_2O$	0.075
$CaCl_2.2H_20$	0.036
Citric acid	0.006
Ferric ammonium citrate	0.006
EDTA	0.001
Na_2CO_3	0.02
Trace metal mix	1ml/L
H_3BO_4	2.86
$MnCl_2.4H_2O$	1.81
$ZnSO_4.7H2O$	0.222
$Na_2MoO_4.2H_2O$	0.390
$CuSO_4.5H_2O$	0.079
$Co(NO_3)_2.6H_2O$	0.0494

The number of cyanobacteria was estimated by counting the cyanobacterial colonies that developed on agar plates of the most suitable dilution. In order to identify the cyanobacteria, strains were isolated into unialgal cultures by repeated streaking. All cyanobacterial species were identified by direct microscopic examination of wet mounts with the help of keys given by Desikachary (1959).

Statistical analysis

Data reported concerning physico-chemical parameters of the soil samples are the mean± standard deviation of samples. A correlation analysis was performed using Microsoft Excel package and analysed for their significance using Pearson's tables.

Result and Discussion

The edaphic features of sewage-irrigated soil and tubewell-irrigated soil of Rohtak city were analysed by the authors (Rana *et al.*, 2010a) and are given in table 2. Edaphic characteristics of sample collected from study areas indicated that the SISs were weakly alkaline with a pH value of 7.84, 0.38 units less than that of TISs. The soil pH influences the solubility and nutrient availability and also acts as prime factor affecting potential metal availability (Merrington *et al.,* 2003). Soil algae composition depends largely on pH of soil as cyanobacteria are unable to survive in acidic conditions (Brock, 1973) except a few. The analysis of sewage soil samples reveals high chloride, bicarbonate, calcium and magnesium content as compared to TISs. Organic carbon and phosphorus content of sewage soil was quite high due to irrigation with sewage water which adds a significant amount of organic matter to soil. The analysis of SIS samples showed that the contents of DTPA-extractable micronutrients ie. Zn, Cu, Fe and heavy metals Ni, Cd, Pb has increased appreciably due to long term application of sewage water. The degradation of sludge organic matter may be a significant factor in release of heavy metals in sewage sludge amended soils (McBride, 1995). The heavy metal content of TIS was considerably less as compared to SIS.

Table 2 Physico-chemical analysis of sewage-irrigated and tubewell-irrigated soil

Parameters	Tubewell irrigated soil. Mean values ± S.D.	Sewage water irrigated soil. Mean values ± S.D.
pH (1:2)	8.15 ± 0.1718	7.84 ± 0.0989
E.C. (ds/m)[1:2]	0.975 ± 0.0181	1.65 ± 0.0302
Carbonates	Nil	Nil
Bicarbonates(mg/l)	197.57 ± 6.32	246.14 ± 12.54
Chlorides (mg/l)	275.2 ± 4.15	388.5 ± 12.99
Ca + Mg (mg/l)	215.14 ± 14.14	253 ± 6.42
Organic carbon (%age)	0.707 ± 0.0335	0.882 ± 0.0372
Potassium (mg/l)	8.13 ± 0.283	20.14 ± 3.225
Sodium (mg/l)	11.27 ± 1.217	13.69 ± 1.512
Phosphorus (Kg/hec.)	14.15 ± 0.704	20.98 ± 2.50
Fe (mg/l)	2.482 ± 0.828	11.24 ± 1.487
Mn (mg/l)	0.462 ± 0.0610	2.04 ± 1.05
Ni (mg/l)	0.311 ± 0.0644	4.28 ± 1.418
Cd (mg/l)	N.D.	0.61 ± 0.050
Zn (mg/l)	2.538 ± 0.877	10.62 ± 1.33
Cu (mg/l)	0.652 ± 0.2498	3.82 ± 0.587
Pb (mg/l)	1.06 ± 0.304	3.59 ± 0.370

Microbial biomass and species diversity are strongly determined by the environmental factors. The greater adaptability of cyanobacteria to environmental variations and trophic independence from carbon and nitrogen enable them to be ubiquitous. Their role in the soil ecosystem is manifold, the most important role being the fixation of nitrogen and carbon, besides promoting release of nutrients. Enrichment of such soils with the indigenous cyanobacterial isolates may help in ameliorating the land and making them suitable for application as biofertilizers (Nayak and Prasanna, 2007). The cyanobacterial composition of SIS and TIS of Rohtak city is given in table-3. Cyanophytes are among the dominant forms as they are more adaptable to disturbed extreme environments or changes in nutritional status and other environmental changes (Reuter and Muller, 1993). The dominance of cyanobacteria in late summer due to depletion of organic nutrients in lakes is well known.

Among soil properties, pH is a very important factor in growth, establishment and diversity of cyanobacteria, which have generally been reported to prefer neutral to slightly alkaline pH for optimum growth (Singh, 1961; Kaushik, 1994). The relative abundance of cyanobacteria in rice soils and biofertilizer inocula from different countries revealed that significant correlation could be made with respect to pH of soils (Roger *et al*., 1987). In the present investigation, soil samples were collected from SISs and TISs, differing in their EC , pH and evaluated for cyanobacterial abundance. A total of 51 cyanobacterial isolates were recorded from SISs and 83 cyanobacterial isolates were recorded from TISs. Highest percentage abundance of heterocystous forms was observed at pH 8.1, of TISs. In terms of non-heterocystous forms, sewage soil samples with pH 7.8 recorded high abundance.

Table 3 Major genera under sewage-irrigated and tubewell-irrigated soil ecosystem

Genus	Total number of strains (Sewage irrigated soil)	Relative abundance (%)	Total number of strains (Tubewell irrigated soil)	Relative abundance (%)
Oscillatoria	12	100	5	22
Phormidium	8	100	3	22
Lyngbya	13	100	1	11
Hapalosiphon	-	-	8	56
Westiellopsis	5	56	12	78
Cylindrospermum	-	-	11	100
Calothrix	5	56	17	100
Nostoc	3	22	14	100
Anabaena	3	22	7	89
Spirulina	1	11	3	56
Tolypothrix	1	11	2	11

Cyanobacteria belonging to 9 genera were isolated from SISs which included 3 non-heterocystous forms: *Oscillatoria Phormidium, Lyngbya* and 6 heterocystous forms: *Westiellopsis, Calothrix, Nostoc, Anabaena, Spirulina* and *Tolypothrix.* However, filamentous non-hetrocystous isolates were abundantly found, showing the highest relative abundance of 100% in samples from SISs as compared to samples from TISs. During 20 years of monitoring in Lake Kasumigaura, the dominant phytoplankton has drastically changed, from *Microcystis* to filamentous cyanobacteria including *Oscillatoria, Phormidium*. The change was concurrent with the increase of TN: TP ratio of lake water. Since the optimum N:P ratio of *Oscillatoria* is higher than that of *Microcystis* (Tilman, 1982), change of dominant phytoplankton was due to change of N and P ion balance in the lake. Species belonging to 11 genera namely *Oscillatoria, Phormidium, Lyngbya, Hapalosiphon, Westiellopsis, Cylindrospermum, Calothrix, Nostoc, Anabaena, Spirulina and Tolypothrix* were recorded in TISs of Rohtak city, Haryana, India. The number of isolates belonging to genera *Cylindrospermum, Calothrix, Nostoc* showed relative abundance of 100% while lowest values were recorded for *Lyngbya* and *Tolypothrix.*

The community structure of algae may be modified by the use of sewage water which contains toxic substances and high metal content. Excessive use of untreated sewage water may lead to destruction of the algal soil components. Besides this, pesticides are also being used in agricultural fields. Pesticides are known to affect algae in vitro and *in- vivo*, with algal species varying considerably in their sensitivity to various pesticide concentration (Megharaj *et al.,*1999; Mostafa and Helling, 2002). Pre-emergent herbicides would influence the range of genera and the number of algal cells present at any given time (Mc Cann and Cullimore, 1979) and cyanobacteria in particular (Metting and Rayburn, 1979).

Table 4 Correlation coefficient values(r) between cyanobacterial abundance (measured as MPN/g soil) and pH, EC, heavy metal content of soil samples studied

Parameters	Correlation coefficient values(r).
pH	0.733*
E.C.	-0.909**
Fe	-0.643*
Mn	-0.273
Ni	-0.341
Cd	-0.289
Zn	-0.517
Cu	-0.619
Pb	-0.47

*Significant at 5% level r>0.632, (n=10),
** Significant at 1% level r>0.765, (n=10).

Soil pH is an important factor which determines the biodiversity and dominance of cyanobacteria on a given soil surface. Fogg (1956) reported that, under natural conditions, most of the cyanobacteria grow in neutral to alkaline conditions and sometimes the growth of diazotrophic cyanobacteria in rice fields is limited by low pH (Whitton and Potts, 2000). Correlation analysis between the abundance of cyanobacterial population and pH, EC and heavy metal content of the soil sampled is shown in Table 4.

The cyanobacterial abundance was found to be positively correlated with pH and exhibited significant negative correlation with EC of soil samples studied. The heavy metal content of the soil was found to show negative correlation with cyanobacterial abundance. However, the correlation was not statistically significant except in case of iron. The cyanobacterial species isolated from sewage irrigated soil were shown to be tolerant to heavy metal concentrations due to the prolonged exposure to such environment (Rana *et al.,* 2010b).

The present study showed the presence of various cyanobacterial species in the sewage-irrigated soil and tubewell-irrigated soil. Stabilisation of disturbed ecosystem like sewage irrigated soil is dependent upon successful establishment of the most effective and dominant cyanobacterial community. These species may also act as bioindicators reflecting the degrading impact on agroecosystems. Successful vegetation would depend on maintaining these essential algal species and developing methods to re-inoculate these species in disturbed soil ecosystem to enhance their fertility and to maintain soil structure.

Authors' contributions: **Dr. Lalita Rana** (Assistant Professor) was responsible for experimental, project design and wrote the manuscript. **Dr. Sunil Chhikara** (Assistant Professor) made conceptual contributions, Statistical analysis, calculations and also corresponding author.

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