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RESEARCH ARTICLE

Seasonal Distribution Pattern Analysis of Cyanobacteria Isolated from Saline/Alkaline Soils.

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Abstract

‘Usar’ soils that occur widely in certain parts of U.P. and Bihar exhibit extreme ecological conditions in characteristically having a high pH, high degree of salinity and low moisture content during dry weather. Soil salinity is one of the most serious factors limiting the productivity of crops including staple diet in many countries. Cyanobacteria, the cosmopolitan, photosynthetic prokaryotes have been reported to grow extensively on ‘Usar’ soils. These are capable of thriving in condition which is considered to inhabitable tolerating desiccation, high temperature, extreme pH and high salinity illustrating their capacity to acclimatize to extreme environments. Therefore a study was conducted from July 2012-June 2013 to find out the influence of soil properties on cyanobacterial population of saline-alkaline soils. We selected six experimental sites for our study. On the basis of our results we could reach to the conclusion that the growth of cyanobacteria was slow in highly saline soil during summer due to high pH, only a few genera like *Nostoc* and *Calothrix* could survive during highly saline condition. High amount of soluble cations and anions adversely affects inhibit the growth of cyanobacteria especially high Na^+ content during summer reduces the population of cyanobacteria. The heterocystous strains were found to be more resistant to salinity like *Nostoc calcicola*, *N. commune*, *Calothrix bressvissima* while non-heterocystous strains like *Microcoleus* and *Lyngbya* favored less saline condition and began to appear after few rain showers when slight decrease in pH was observed. Although some of the non-heterocystous strains like *Phormidium* and *Oscillatoria* persisted at high salinity during summer but in very low quantity.

Introduction

Land degradation is the major problem being faced by man today. Out of the total geographical area of 329 m.ha., 175m.ha. is considered as affected. Salt affected soils cover a big chunk of total Indian cultivable land. About 10m.ha. have been suffering from salinity and alkalinity problems alkaline soils, 4.50 Mha saline soils 5.50 Mha in India. Extensive occurrence of alkaline soils has been reported from the Indo-Gangentic plains, a flood plain of river Ganges, in Northern India (Sharma et. al. 2004). The area badly affected by this problem in U.P. is about 1.2 million hectares. Eastern U.P. district like Gazipur, Azamgarh, Jaunpur and Varanasi are amongst worst affected areas having big stretches of sodic soils. Total Geographical Area (TGA) in Varanasi is 1381 square Km and total wasteland is 59.27 square km. Out of these, 14.2 square Km is slightly salinity/alkalinity affected, 8.00 square Km moderately and 0.18 square Km highly salinity-alkalinity affected. So, percentage of TGA in Varanasi is 4.29% only by NRSA, 2003. These problematic salt affected soils are by and large, barren in the Gangetic alluvial plain of U.P. Soil toxicity generally refers to the salinity and/or alkalinity of soil. Both saline and alkaline soils are commonly called as ‘Usar’. Saline soils are the soil that have developed due to the influence of sodium salt (mainly NaCl or Na_2SO_4) where as alkaline soils have developed mainly due to the influence of Na_2CO_3 and $NaHCO_3$.These salt

affected soils are not suitable for crop production and affect plant growth although they have high agricultural potential. Such soils are unproductive and hard due to the presence of undesirable salts on the surface.
The study of saline soil ecosystem is a vital part of the study of biodiversity of cyanobacterial communities. A seasonal change in soil chemical properties has been documented by Sarvankumar et al., 2008. The pH is particularly considered an important factor in influencing cyanobacterial distribution and abundance in soil because it directly or indirectly influences a wide range of chemical, biological and physico-chemical soil reactions and properties. Seasonal changes in salinity or alkalinity significantly influence the diversity of cyanobacteria in soil. These organisms initially appear on land after the first shower of mansoon and prefer neutral to slightly alkaline condition. The diversity and population dynamics of cyanobacteria in 'Usar' soils has been studied by several groups or researchers. (Singh 1961; Pandey et al. 2005). Cyanobacteria have been found to differ considerably in their ability to resist salt stress.

The distribution of N_2- fixing cyanobacteria has been investigated almost in every soil ecosystem and increment or reduction of the cyanobacterial population may continuously affect the nitrogen status of the soil (Somporn et al. 2003). Therefore, this investigation was aimed to observe the changes in the diversity of the indigenous BGA assemblage in the 'Usar' fields with seasonal variations in soil properties.

Material and Methods

Study Area

The study was performed for one year at selected sites in Varanasi city from July 2012 to June 2013. The climate of the region is tropical monsoonal. The year is divisible into a hot and dry summer, a humid rainy season and a cold winter season. The ambient mean temperature was lowest in December (9.9 to 26.1 °C) and highest in May-June (27.8 to 40.9 °C). The rainy months remained warm and wet, with humidity reaching close to saturation (Kumar et al 2015). The day length is recorded as longest in June (about 14 hours) and shortest in December (about 10 hours). Wind direction shifts predominantly westerly and south - westerly in October through April and easterly and north - westerly in remaining months. Six study area were selected and one sites for collection of sample were selected on the bases of plot distribution curve and test for normality to exclude outliers sites in respect to a single variable, salinity, at each study area.

Collection of Soil Sample and analysis

Soil samples were collected from predefined sites in summer, rainy and winter season during July 2012-June 2013. A composite soil sampling was performed at each site. Soil samples were collected 5-10 cm bellow the surface after digging a pit of 4"x 4" inch and packed into poly bag and delivered to the laboratory on the same day to avoid unpredictable changes and interference in characteristic. The collected samples were dried at shaded place at room temperature, grinned and sieved for removal of debris and stones. The sieved soil was used for analysis.

The soil samples were analyzed with respect to their electrical conductivity (EC) and pH ranges using soil/water suspension in (1:2.5w/v) with microprocessor pH-EC-TDS meter model 1615. Soluble cations were determined by EDTA method and Flame photometrically method respectively (Chopra & Kanwar 1991; Tandon 1993). The soluble anions were determined by volumetric method (Chopra & Kanwar 1991).

Moist culture of soil algae were prepared by spreading a layer of soil (about 1 cm. thick) and moistened with sterilized distilled water periodically in Petri dish covered with a sheet of glass both previously sterilized (John 1942).

In about a fortnight after incubation, the visible growth of algae appear in the culture, one of the replicates was disturbed for microscopic examination while other were left undisturbed for further observation. For making unialgal culture, a few drops of the culture of the algal flora were transferred to soil agar plates with the help of fine pipettes. After 12-15 days, Petri dishes were observed for algal colonies. The colonies were then transferred into various nutrient medium for their isolation and identification.

Statistical Techniques

The sodium absorption ratio were calculated by following formula

$$\mathbf{SAR = Na^{+} / \sqrt{(Ca^{2+} + Mg^{2+})/2}}$$

Result and Discussion

Seasonal variation in saline/alkaline soil

Seasonal variation among the physic-chemical properties of saline/alkaline soil was studies and is represented in table 1. pH, EC, SAR, soluble cations (Ca^{2+}, Mg^{2+}, Na^{2+} and K^{+}) and anions (Cl^{-}, CO_3^{-}, HCO_3^{-} and SO_4^{2-}) variable is undertaken for study to describe the current status of alkaline/saline soil which collected from predefined study sites. Veerapatti (Site 1), Rameshwaram (Site 2), Chaubeypur (Site 3), Harahua (Site 4), Kachhwa (Site 5) and Ayar (Site 6) study sites were considered for study.

The pH ranges 0.57, 0.45 and 0.38 were recorded in summer, rainy and winter season, respectively. The highest pH 9.9 was recorded at site 6 during the summer where as the minimum pH (8.1) was recorded at site 4 during the rainy season.

The EC ranges 0.57, 0.96 and 1.32 were recorded in summer, rainy, and winter season, respectively. The highest EC (5.94) was recorded at site 2 during the summer where as the minimum EC (3.6) was recorded at site 6 during the rainy season.

Content of water soluble cations namely Ca^{2+}, Mg^{2+}, Na^{2+} and K^{+} in Mel^{-1} also varied at all six experimental sites. Among them Na^{2+} was found to be dominating cation followed by Ca^{2+}, Mg^{2+} and K^{+}. Water soluble anions viz Cl^{-}, CO_3^{-}, HCO_3^{-} and SO_4^{2-} in Mel^{-1} were also found in different amount at all six experimental sites. Cl^{-} was the dominating among followed by HCO_3^{-}, CO_3^{-}, and SO_4^{2-} .Sodium absorption ratio (SAR) also varied at all six experimental sites.

Cyanobacterial population diversity

The present study shows the seasonal variation in the cyanobacterial population in respect to salinity and is represented in table 2. The apparent growth of algae in the 'Usar' soils was confined to the rainy season when the soils were moist. Mixed population of cyanobacterial strains were seen which was comprised of filamentous heterocystous, filamentous non heterocystous and some coccoid forms. Rainy season showed the maximum variety of cyanobacteria in comparison to winter and summer.

Cyanobacteria reached to the maximal biomass in the rainy season. Following the first rain shower the cyanobacterial community was dominated by *Microcoleus*, *Aphanothece* sp., *Aphanocapsa* sp. and *Calothrix* sp. After about a month *Lyngbya*, *Cylindrospermum*, *Nostoc* sp. was appeared as small patches on the soil surface.

At the end of rainy season *Anabaena orientalis* and *Nostoc calcicola* started to appear while *Microcoleus* and *Calothrix* still persisted in lesser quantity. Onset of winter *Aulosira* was observed on the soil surface and was found to intermingle with *Phormidium* and *Oscillatoria*. In summer poor algal growth was observed. Cyanobacterial patches were completely absent on soil surface due to high saline condition. Under laboratory condition only a few genera like *Nostoc calcicola* survive and grow during summer. The cyanobacterial growth was greatly influenced by some a biotic factors like light, temp., water availability salinity etc. *Nostoc* and *Calothrix* appeared as higher salinity tolerate strain, although *Phormidium* and *Oscillatoria* persisted during summer but in quantity Nostoc calcicola was dominant in all six sites showing in table (1).

Salt affected soils adversely affect plant because of the total concentration of salts (salinity) in the soil solution and because of concentration of specific ions especially sodium. Increase in Na^{+} concentration in soils can cause dispersion of soil clay particles and organic matter resulting in surface crusting reduced infiltration and lower hydraulic conductivity (Yang et al. 2011; Qadir et al. 2006). Such soils are quite unproductively and are very difficult to manage. Halotolerant cyanobacteria can tolerate these conditions but the growth and diversity on such soils is constrained by the specific toxicities of Na^{+}, OH^{-} and HCO_3^{-} as well as by the very poor soil physical condition and slow permeability to water.

Seasonal variation in the soil properties has been observed. Soil properties like pH, EC and soluble cations and anions were found to be high in summer samples contained more salts because of intense evaporation that brought the salts back to the surface. Increasing SAR and EC (salt concentration) in these soils are attributed to addition of salts and sodium by irrigation water and dissociation of salts already present in these soils (Gangegunte et al. 2005). High SAR is also responsible for limitation of Ca^{2+} andMg^{2+} due to Na^{+} induced displacement (Kopittke & Menzies 2005). Soil reaction or pH is one of the most commonly measured soil parameter because it directly or indirectly influences a wide range of chemical, biological and physico-chemical soil processes. Soil properties greatly influence the cyanobacterial diversity. Values of pH and Ec were found to fluctuate throughout the year at all the sites. Hence the variation in the cyanobacterial diversity was also observed along with the soil properties and this was in accordance with the results (Pathak et al. 2014). The pH values were lower in rainy season than in winter and summer. Salt contents were found to be low in rainy season perhaps because soils under investigation subjected to leaching during rainy season. So excess soluble salts were removed and pH lowered down. Water logging in alkali soil is known to reduce the soil pH and hence this may also have been responsible for reducing the pH during rainy season.

Salinity inhibits general protein synthesis especially stress protein. So, the growth of cyanobacteria was slow in highly saline soil during summer due to high pH and exchangeable Na^+. High concentration of NaCl adversely affects different metabolic processes of cyanobacteria including inhibition of growth, photosynthesis, adverse effects of NaCl on enzyme activities and fluidity of membrane was also seen by Srivastava et. al., 2005.

Fluctuation in salinity during rainy, winter and summer season provide growth opportunity for a variety of algal species with varying halotolerance. The heterocystous strains were found to be more resistant for saline soil while non- heterocystous favoured less saline condition. Some non- heterocystous strains like *Microcoleus* and *Lyngbya* began to appear after few rain showers when slight decrease in pH was observed.

The present results agree with previous that blue-green algae are quite tolerant of high alkalinity. Alkaline soil with high pH and Na^+ content favour the growth of diazotrophic cyanobacteria with a consequent decreases in pH by Singh, 1961. The property of being able to grow in such adverse habitats thus adding organic matter and nitrogen to the soil is a useful one for starting plant succession in barren soils.

The soil salinity (Ec) and alkalinity (pH and Na^+) are the main factors which determine the diversity and dominance of cyanobacteria in 'Usar' soils (Singh et. al. 2014). Beside this temp., light, moisture content of soil is also key factors which determine the cyanobacterial diversity.

Table 1 Physico-chemical properties of saline/alkaline soil of study site-2013

Sites	**Season**	**p^H**	**EC**	**Ca^{2+}**	**Mg^{2+}**	**Na^+**	**K^+**	**Total**	**Cl^-**	**CO_3^{2-}**	**HCO_3^{2-}**	**SO_4^{2-}**	**Total**	**SAR**
			dSm^{-1}	**MeL^{-1}**	**MeL^{-1}**	**MeL^{-1}**	**MeL^{-1}**		**MeL^{-1}**	**MeL^{-1}**	**MeL^{-1}**	**MeL^{-1}**		
1	Summer	9.58	5.13	2.1	1.31	50.88	1.11	55.4	36.42	3.47	10.29	1.31	51.49	35.76
	Rainy	8.5	3.75	3.1	2.9	37.75	0.63	44.38	32.7	2.3	9.21	0.41	44.62	22.89
	Winter	8.78	4.54	2.8	2.9	43.9	1.1	50.7	39.58	3.79	11.21	1.21	55.79	27.68
2	Summer	9.33	5.94	2.25	1.8	50.28	1.28	55.61	45.66	3.44	10.25	1.61	60.41	34.42
	Rainy	8.35	4.92	2.9	2.6	42.8	0.63	48.93	40.29	3.52	8.5	1.15	53.46	26.43
	Winter	8.8	5.43	2.2	1.9	48.45	1.05	53.6	43.58	4.00	11.5	0.8	59.88	33.61
3	Summer	9.63	5.68	2.2	1.3	50.38	1.18	55.06	17.03	19.53	23.53	1.08	61.17	34.61
	Rainy	8.55	3.95	3.1	2.9	37.18	0.5	43.68	12.33	15.58	17.23	1.05	45.19	22.56
	Winter	8.98	4.95	2.5	2.1	47.5	0.95	53.05	14.75	17.88	21.3	0.8	54.73	31.09
4	Summer	9.42	4.98	2.26	1.9	48.2	1.28	53.6	39.43	3.8	11.87	1.51	56.61	33.01
	Rainy	8.1	3.9	3.15	2.98	33.9	0.43	40.46	32.21	3.5	8.21	0.98	54.94	20.59
	Winter	8.6	4.21	2.8	2.4	38.1	1.2	44.5	35.53	4.21	9.16	1.91	50.81	23.96
5	Summer	9.72	5.2	2.1	2.48	50.95	1.38	59.91	42.45	3.91	11.47	0.78	58.61	36.39
	Rainy	8.5	3.8	2.9	2.1	35.1	0.58	40.68	34.12	3.31	8.15	0.86	46.44	21.63
	Winter	8.9	4.91	2.6	2.45	44.9	1.15	51.1	38.24	4.51	12.2	0.83	55.78	29.07
6	Summer	9.9	5.32	2.12	1.98	52.68	1.28	50.06	17.28	19.28	24.81	1.89	63.26	37.17
	Rainy	8.46	3.6	2.96	2.1	38.1	0.6	43.8	14.15	15.51	17.45	1.26	48.37	23.21
	Winter	8.72	4.5	2.9	2.65	40.95	0.98	47.48	14.78	16.88	19.3	0.92	51.88	25.37

Table 2 Seasonal variation in cyanobacterial population in saline/alkaline soil -2013

Species	**Site**						
	Season	**1**	**2**	**3**	**4**	**5**	**6**
Microcoleus chthnoplates	Summer	28	32	30	30	36	38
	Rainy	58	55	52	61	58	62
	Winter	19	15	12	20	18	18
Microcoleus vaginates	Summer	32	30	27	35	35	35
	Rainy	53	57	54	56	62	57
	Winter	15	17	14	18	21	19
Scytonema sp.	Summer	32	35	32	36	40	48
	Rainy	20	12	9	22	18	15
	Winter	0	0	0	5	2	0
calothrix marchica	Summer	66	63	60	70	68	68
	Rainy	28	36	32	32	42	36
	Winter	0	0	0	5	4	0
Lyngbya major	Summer	0	0	0	5	11	0
	Rainy	19	20	17	23	22	15
	Winter	0	0	0	5	9	0
Lyngbya rubida	Summer	0	0	0	2	12	0
	Rainy	43	38	35	48	43	42
	Winter	0	0	0	4	6	0
Tolypothrix sp.	Summer	15	15	12	19	20	18
	Rainy	0	0	0	9	15	0
	Winter	0	0	0	4	4	0
Nostoc calcicola	Summer	68	67	64	73	73	72
	Rainy	89	80	77	94	92	86
	Winter	43	40	37	49	48	45
Nostoc punctiformae	Summer	54	52	49	58	60	56
	Rainy	74	71	68	78	79	82
	Winter	39	35	32	42	45	38
Nostoc commune	Summer	43	40	38	48	55	45
	Rainy	57	53	52	62	66	65
	Winter	34	30	26	40	40	30
Anabaena orientalis	Summer	0	0	0	6	8	0
	Rainy	35	32	29	40	42	32
	Winter	20	18	15	23	22	18
Aulosira firtilissima	Summer	0	0	0	6	4	0
	Rainy	30	28	24	36	38	30
	Sinter	21	19	16	25	19	15
Phormidium anamola	Summer	19	15	13	22	15	10
	Rainy	0	0	0	4	13	0
	Winter	17	17	11	18	20	15
Oscillatoria subbrevis	Summer	10	11	10	15	15	15
	Rainy	13	13	7	18	20	12
	Rinter	15	13	11	18	18	18
Plectonema radiosum	Summer	11	9	5	16	22	13
	Rainy	0	0	0	8	0	0
	Winter	13	11	8	14	12	8

Conclusion:-

It is observed that heterocystous strains like *N. calcicola* and *Calothrix* sp. appeared to be the most frequent blue-green algae in 'Usar' soils and so they may be useful as a first colonizer of such soils. Nostoc is known to be one of the most versatile diazotrophic cyanobacteria genera, observed in all types of environments, existing both in free living and symbiotic state. Such halotolerant N_2-fixing cyanobacterial strains can be used to improve properties of salt affected soils due to their survival under stress condition.

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