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Algal flora of the aridisols of Rohtak and salt-tolerance of the indigenous cyanobacteria

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Abstract: The algal flora in the salt-affected aridisols of Rohtak, a semi-arid region in Haryana was studied. The soil showed a gradient of salinity with electrical conductivity (EC) ranging from 0.4 to 21 dSm^{-1} in the rainy season and algal patches consisting of 20 species were recorded in the microquadrats. There were 9 species of cyanobacteria, 8 species of chlorophyceae and 3 species of bacillariophyceae. *Oscillatoria* was the most frequently found species followed by *Anabaena* and *Closterium*. The site with high alkalinity and low salinity (pH 8.9, EC 2.4 dSm^{-1}) showed the maximum number of algal species (12). In aridisols with higher EC level (8.9 to 21 dSm^{-1}) the algal species were restricted to only 4-6. The indigenous cyanobacterial strains from these soils were further studied for evaluating their range of halotolerance using artificially salinized Fogg's Culture medium. Four heterocystous species viz. *Anabaena* sp. *Nostoc commune, Cylindrospermum stangale* and *Hapalsiphon welwitschii*, and three non-heterocytous species, namely, *Spirulina, Oscillatoria* and *Chroococcus turgidus* tolerated salt stress upto EC 12 dSm^{-1}. Algal density in the soil of the study site was significantly less in dry season (5 x 10^2 g^{-1} soil) than in rainy season (12 x 10^3 g^{-1} soil). When exposed to artificial salt stress in culture medium algal growth in both the seasons was maximum at EC 8, followed by that at EC 12 dSm^{-1}.

Resumen: Se estudió la flora algal en los aridisoles afectados por sales de Rohtak, una región semiárida en Haryana. El suelo mostró un gradiente de salinidad, con una variación en las lecturas de conductividad eléctrica (CE) de 0.4 a 21 dSm^{-1} en la estación de lluvias, y parches de algas que consisten en 20 especies fueron registrados en los microcuadros. Hubo 9 especies de cianobacterias, 8 especies de Chlorophyceae y 3 especies de Bacillariophyceae. *Oscillatoria* fue la especie hallada con mayor frecuencia, seguida de *Anabaena* y *Closterium*. El sitio con alta alcalinidad y baja salinidad (pH 8.9, CE 2.4 dSm^{-1}) mostró el máximo número de especies de algas (12). En los aridisoles con niveles más altos de CE (8.9 a 21 dSm^{-1}) los números de especies de algas quedaron limitados a solo 4-6. Las cepas natives de cianobacterias de estos suelos fueron analizadas adicionalmente para evaluar su intervalo de halotolerancia usando el medio de cultiv de Fogg artificialmente salinizado. Cuatro especies heterocistosas, *Anabaena* sp., *Nostoc commune, Cylindrospermum stagnale* y *Hapalsiphon welwitschii*, y tres especies no heterocistosas, *Spirulina, Oscillatoria* y *Chroococcus turgidus* toleraron un estrés salino de hasta 12 dSm^{-1} de CE. La densidad de algas en el suelo del sitio de estudio fue significativamente menor en la estación seca (5 x 10^2 g^{-1} de suelo) que en la estación de lluvias (12 x 10^2 g^{-1} de suelo). Cuando se expuso al estrés salino artificial en medio de cultivo el crecimiento algal en ambas estaciones alcanzó su máximo a una CE de 8, seguida por el registrado a una CE de 12 dSm^{-1}.

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Resumo: A flora de algas nos aridosols salinos de Rhotak, uma região semiárida em Haryana foi estudada. O solo mostrou um gradiente de salinidade com uma condutividade eléctrica
(EC) variando entre os 0,4 e os 21 dSm^{-1} na estação chuvosa e manchas com algas tendo sido registadas nos micro quadrados de amostragem 20 espécies. Estavam representadas 9 espécies de cianobactérias, 8 espécies de chlorophyceae e 3 espécies de bacillariophyceae. A *Oscillatoria* foi a espécie mais representada a que se seguia a *Anabaena* e a *Closterium*. A estação com alcalinidade mais elevada e baixa salinidade (pH 8,9, EC 2,4 dSm^{-1}) mostrou a maior representação de espécies de algas (12). Nos aridosols com maiores níveis de EC (8,9 a 21 dSm^{-1}) as algas encontravam-se limitadas a 4-6 espécies. As linhas indígenas de cianobactérias destes solos foram seguidamente estudadas para avaliação do seu grau de tolerância ao sal através do recurso ao meio de cultura artificial salino de Fogg. Quatro espécies 'heterocystous' viz. *Anabaena* sp., *Nostoc commune, Cylindrospermum stagnale e Hapalsiphon welwitschii*, e três espécies não 'heterocystous', nomeadamente a *Spirulina, Oscillatoria* e *Chroococus turgidus* toleraram o stress do meio salgado até um valor de EC de 12 dSm^{-1}. A densidade das algas no solo da estação de estudo foi significativamente menor na estação seca (5 x 10^2 g^{-1} de solo) do que na estação chuvosa (12 x 10^3 g^{-1} de solo). Quando sujeita a um stress salino artificial num meio de cultura para algas, o crescimento das algas foi máximo em ambas as estações para valores de EC de 8 seguido pelo valor de EC de 12 dSm^{-1}.

Key words: Algal density, aridisols, cyanobacteria, halotolerance.

Introduction

The aridisols found in the arid and semi-arid tropics characterized by the accumulation of soluble salts or alkaline salts in the soil profile show restricted plant growth (Sinha *et al.* 1991). One of the recent approaches has been to reclaim and utilize such soils through the use of various biofertilizers like cyanobacteria (Kaushik & Ummat 1992; Kaushik *et al.* 1997). The role of cyanobacteria in soil improvement is due to their nitrogen fixing capability (Roger & Kulasooriya 1980) and their ameliorative effect on soils are well documented (Kaushik & Subhashini 1985). However, in the fields, artificial algalization has generally been tried with cultured strains of algae instead of indigenous strains while the latter due to their inherent capacity to tolerate salt conditions are likely to prove more useful as suggested by Roger *et al.* (1987).

With an objective to screen the halotolerant algal species from the salt-affected soils and with a view to exploring their potential as biofertilizers in saline and alkaline soils in future studies, the algal flora of the aridisols of Rohtak, a semi-arid region with wide range of salinity was studied and halotolerance of the indigenous cyanobacteria was studied at various salinity levels.

Materials and methods

Study site

The study site comprises an undisturbed stretch of waste-land located in Rohtak, northern India (28°55'N, 76°43'E) lying 219.8 m above mean sea level.

The climate of the region is semi-arid. During the study period there was a total rainfall of 406 mm during the rainy season. The soil is old alluvium, aridisol belonging to ellitic series having a sandy loam texture. The site shows numerous micro-reliefs and heterogeneous conditions, showing barren patches at certain areas and native plant growth dominated by *Salsola baryosma* on others. Presence of salt crusts was also observed at certain places of study site.

Soil characteristics

Soil Electrical Conductivity (EC) and pH were determined with 1:2 soil water suspension using an electrical conductivity bridge and a pH meter, respectively. Organic matter of the soils was estimated following the standard rapid titration method of Walkley & Black as described by Allen *et al.* (1986).

Study of soil algae

During the rainy season, algal patches were observed on the soil surface which were studied for algal composition using the quadrat method (Kumar & Singh 1982). The study area was demarcated into ten plots (3 x 3 m) based on variations in EC. Three microquadrats of 20 x 20 cm size were laid randomly in each of the ten plots. The algal patches occurring in the microquadrats were identified and frequency of each species was calculated using the formula F = Qo/Qt x 100; where F is frequency, Qo is the number of quadrats of occurrence and Qt is the total number of quadrats.

Screening of salt tolerant algae

Preparation of culture medium, dilution series of the soil inoculum and plating were done following Kaushik (1987). In order to screen the salt tolerant blue-green algae, Fogg's culture medium was used which was artificially salinized to different levels (EC 4, 8 and 12 dSm^{-1}). Salinity level upto 12 dSm^{-1} was selected because most of the crops cannot tolerate salinity beyond this level and thus soil algalization would be practically useful upto this level. Salinization was done by the addition of NaCl, Na_2SO_4, $MgCl_2$ and $CaSO_4$ in a proportion of 13:7:1:4 which is the normal ratio of these salts in the naturally saline soils of Haryana (Sinha *et al.* 1982). The non-salinized medium served as control. The pH of the medium was adjusted to 7.5. The autoclaved culture medium (non-salinized and salinized) was poured into sterilized Petri dishes using agar-agar as the solidifying agent. Each treatment was replicated thrice.

The soil inoculum was prepared by taking ten soil samples from the study site and pooling them. An inoculum of 10 g of soil from the pooled sample was transferred into 90 ml of sterilized distilled water, shaken thoroughly for an hour, and diluted to 10^{-2} and 10^{-3} concentration. The Petri dishes containing the culture medium with different EC levels were inoculated with 0.1 ml of the soil inoculum (10^{-3} dilution) after standardization.

The Petri dishes were incubated at 27 3°C under a continuous light intensity of 2000 lux in a B.O.D. incubator with fluorescent light fittings. Algal colonies developed at each salinity level were counted upto 21 days and identified.

The seasonal variations in site characteristics and variations in algal density due to EC levels were tested for significance using t-test following Sokal & Rohlf (1969).

Results

Soil characteristics

Soil of the study site was saline in nature and showed a gradient of soil EC varying from EC 0.4 to 21.0 dSm^{-1} in rainy season. The mean soil EC was much higher in dry season (EC 26 dSm^{-1}) than that in the rainy season (EC 6.75 dSm^{-1}) and these differences were found to be statistically significant ($P<0.01$). The pH of the study site was alkaline in both the rainy season (pH 8.4) and the dry season (pH 7.8). The differences were, however, not found to be statistically significant ($P<0.05$) when subjected to t-test. The soil organic matter was quite low in the study site, being 1.06% and 0.99% in the rainy and dry period, respectively (Table 1).

Algal composition

In the study site, 20 genera of algae were observed during the rainy season forming algal patches over the soil surface. A total of 9 cyanophycean genera were observed. The heterocystous blue-green algae included *Anabaena* sp., *Nostoc commune* and *Cylindrospermum stagnale*, whereas the non-heterocystous blue green algae included

Table 1. Soil characteristics of the study site in the rainy and dry seasons; the values are average of 10 samples.

Soil characteristics	Rainy season		Dry season		t-values comparing seasonal variation
	Range	Mean ±S.E.	Range	Mean ±S.E.	
$EC_{1:2}$ (dSm^{-1})	0.4 – 21.0	6.75 ± 1.87	18 – 32	2.3 ± 3.2	5.183*
$pH_{1:2}$	8.0 – 8.9	8.4 ± 0.10	7.5 – 9.2	7.8 ± 0.19	2.885^{NS}
Organic matter (%)	0.7 – 1.2	1.06 ± 0.16	0.8 – 1.1	0.99 ± 0.04	3.594^{NS}

*significant ($P<0.05$); NS = Not significant

Oscillatoria acuminata, Spirulina, Chroococcus turgidus, Gloeocapsa, Lyngbya and *Microcoleus* (Table 2). Eight genera of chlorophycean algae *Closterium, Pleurococcus, Chlorella, Zygnema, Cylindrocystis, Cosmarium, Chlorococcum* and *Oocystis* were observed in the study site. Among the *bacillariophyceae* 3 genera were observed, namely, *Pinnularia, Calonies* and *Diatoma*.

Out of all these genera, *Oscillatoria acuminata* showed the maximum frequency (63%) followed by *Anabaena*, with a frequency of 50%. The algae showing a frequency between 10 to 40% included *Spirulina, Chroococcus turgidus, Nostoc commune* and *Gloeocapsa* (blue-green algae); *Closterium, Pleurococcus, Chlorella, Cosmarium, Zygnema* and C*ylindrocystis* (green algae); and *Pinnularia* (bacillariophyceae). Algae having a frequency less than 10 included *Cylindrospermum, Microcoleus, Lyngbya, Chlorococcum, Oocystis, Calonies* and *Diatoma*.

The algal cover was not related to soil EC or pH as indicated by the cover area of algae in different plots. The plots IX with a high EC (12.3 dSm^{-1}) and pH 8.0 although had only four species but showed maximum cover of 1.00. The mean algal cover found on the soil surface was 0.15 cm^2 cm^{-2} ground area (Table 2).

Screening of salt tolerant blue-green algae

Colony formation by the indigenous algae of the study site in culture medium artificially salinized to EC 4, 8 and 12 dSm^{-1} is shown in Table 3 along with their natural occurrence at different EC levels in the study site. Out of a total of 9 genera of blue-green algae, *Nostoc commune* was found to tolerate salinity upto 8 dSm^{-1}, *Anabaena* upto EC 8 dSm^{-1} (rainy season) and EC 12 dSm^{-1} (dry season)

Table 2. Algal distribution in a waste-land of Rohtak along a gradient of soil salinity and varying pH.

Genera/Species		Sampling plots										Frequency* (%)
		I	II	III	IV	V	VI	VII	VIII	IX	X	
	EC (dSm^{-1})	0.4	2.3	2.4	3.0	3.1	3.2	5.7	7.8	12.3	21.0	
	pH	8.7	8.7	8.9	8.6	8.4	8.8	8.5	8.0	8.0	8.2	
Cyanophyceae												
Oscillatoria		+	+	+	+	+	+	+	+	-	+	63
Anabaena sp.		+	+	+	+	+	+	-	+	+	-	49
Spirulina sp.		+	-	+	+	+	+	+	+	-	+	33
Chroococcus turgidus		+	+	+	+	+	+	-	-	-	+	33
Nostoc commune		+	+	+	+	+	-	-	-	-	-	29
Gloeocapsa sp.		-	+	+	+	+	-	-	-	-	-	26
Cylindrospermum stagnale		-	-	+	-	-	+	-	-	+	-	6
Lyngbya		+	-	-	-	-	-	-	-	-	-	3
Microcoleus		-	-	-	-	-	-	+	-	-	-	3
Chlorophyceae												
Closterium		-	-	+	+	+	+	+	-	-	-	39
Pleurococcus		+	-	+	-	-	+	-	-	-	-	26
Chlorella		-	-	+	-	-	-	+	-	+	-	13
Zygnema		+	-	-	-	-	-	-	+	-	+	13
Cylindrocystis		-	-	-	-	-	+	+	-	-	-	13
Cosmarium		-	-	+	-	-	-	-	-	+	-	9
Chlorococum		-	-	+	-	-	-	+	-	-	-	6
Oocystis		-	-	-	-	-	-	-	-	-	+	3
Bacillariophyceae												
Pinnularia		+	-	-	-	+	-	-	-	-	-	9
Calonies		-	-	-	-	+	-	-	-	-	-	3
Diatoma		-	-	-	-	-	-	-	-	-	+	3
Total Species		9	5	12	7	9	8	8	5	4	6	
*Total Algal Cover (cm^2 cm^{-2} ground area)		0.13	0.09	0.06	0.07	0.05	0.12	0.03	0.09	1.0	0.03	

*Frequency and algal cover are based on observation of 30 microquadrats (20 x 20 cm size; 3 per plot)

Table 3. Growth of the indigenous blue-green algae from the aridisols in artificially salinized culture medium vs. their occurrence in saline conditions of natural site; EC in dSm^{-1}.

Algal species	Artificial saline culture								Natural site
	Rainy season inoculum				Dry season inoculum				
	Control	EC4	EC8	EC12	Control	EC4	EC8	EC12	EC Range
Heterocystous									
Anabaena sp.	+	+	+	-	-	+	-	+	0.1 – 12.3
Nostoc commune	+	+	+	-	+	+	+	-	0.2 – 3.1
Cylindrospermum stagnale	+	+	-	-	-	-	-	-	0.1 – 3.2
Hapalosiphon welwitschii	-	+	+	+	-	+	+	+	Not observed
Non-heterocystous									
Spirulina	-	-	+	+	-	-	-	-	0.4 – 21
Oscillatoria acuminata	+	+	+	+	-	-	-	-	0.1 – 21
Chroococcus tugidus	+	+	+	+	-	-	-	-	0.2 – 21
Microcoleus	-	-	-	-	-	-	-	-	5.7
Lyngbya	-	-	-	-	-	-	-	-	0.4
Gloeocapsa	-	-	-	-	-	-	-	-	0.2 – 123

and *Cylindrospermum stagnale* occurring only at EC 9 dSm^{-1} (rainy season). *Hapalosiphon welwitschii*, a branched heterocystous alga, which was not found in the surface patches of the natural site, readily formed colonies in culture medium at all the salinity levels, except in the control.

Among the non-heterocystous forms, *Chroococcus turgidus*, a unicellular blue-green alga tolerated a salinity upto EC 12 dSm^{-1} in rainy season but was absent in the dry season. *Spirulina* occurring at higher EC levels in the natural site formed colonies at EC 4 dSm^{-1} in culture medium, but was absent during the dry period. *Oscillatoria acuminata* occurring at all the salinity levels in culture medium during the rainy season was absent in dry period. *Gloeocapsa* and *Microcoleus* observed in the natural saline site failed to form colonies in the culture medium.

Growth of the salt-tolerant strains at various EC levels

Growth response of the cyanobacterial strains in artificially salinized culture media during dry and rainy season is shown in Fig. 1 (a and b). With the soil inoculum of the rainy season maximum algal growth occurred at EC 8 dSm^{-1}. A sharp increase in the algal density at EC 8 was observed 15 days onwards with maximum density of 61 x 10^3 g^{-1} soil on 21 days. Algal density at EC 12, EC 4 and control were 18 x 10^3 g^{-1} soil, 17 x 10^3 g^{-1} soil and 12 x 10^3 g^{-1} soil, respectively, at 21 days (Fig. 1a). Algal density at EC 8 dSm^{-1} was significantly higher ($P<0.01$) than at other EC levels, while all other differences in density were statistically not significant ($P>0.05$).

Algal growth from dry period soil inoculum resulted in a maximum density of 6 x 10^2 g^{-1} soil in the control. Density was highest at EC 8 (x 10^2 g^{-1} soil), followed by that at EC 12 (7 x 10^2 g^{-1} soil) whereas the density at EC 4 was 5 x 10^2 g^{-1} soil (Fig. 1b).

The differences in the density of algae at various salinity levels in the dry season were not statistically significant. However, the differences in algal density between the two seasons were significant at EC 4 and EC 8 ($p<0.05$) indicating thereby the significant influence of climatic conditions on the algal density.

Discussion

Although the spectrum of algal flora narrows down sharply in the arid and semi-arid regions, yet the present site having a wide range of soil EC (0.4 to 21 dSm^{-1}) and alkaline pH (8.0 to 8.9) shows several algal species forming surface crusts. There are some other reports on the occurrence of cyanobacteria in salt marshes (Hanne 1987), in saline soils (Sikander & Sander 1972) and in alkaline pH (Kroll 1990; Singh *et al.* 1995). All the cyanobacteria in the present site (except *Microcoleus* and *Lyngbya*) showed a wide range of tolerance as indicated by their natural occurrence at various levels of soil EC and pH. Likewise, the chlorophycean forms exhibited a wide range of tolerance as shown by their natural distribution, whereas bacillario-

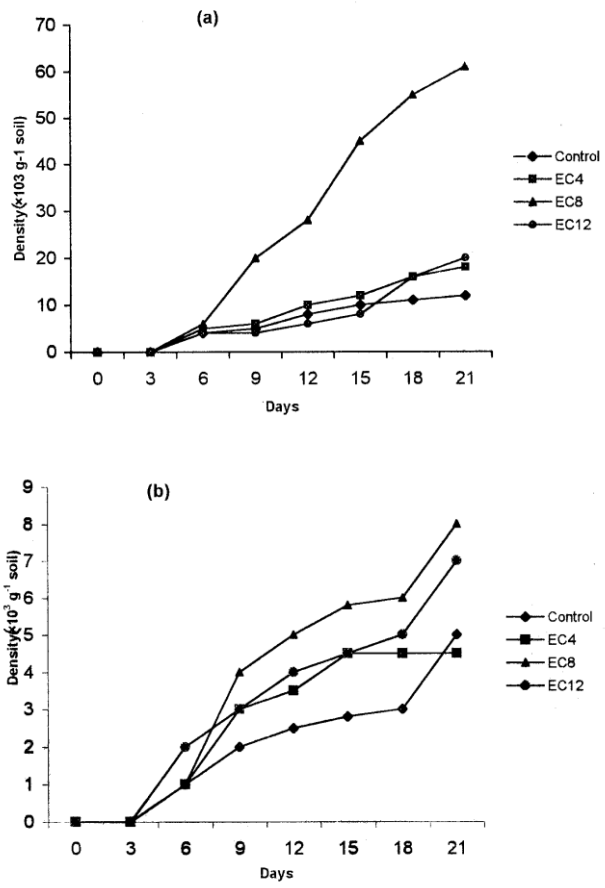

Fig. 1. Algal growth (density) in artificially salinized culture medium inoculated with pooled soil inoculum for the study site during (a) rainy season (b) dry season.

phycean algae were mostly absent form at the higher EC levels.

During screening of the salt-tolerant algae in artificially salinized culture medium, heterocystous cyanobacterium *Hapalosiphon welwitschii* was found to form colonies from the soil inoculum of dry season at all the EC levels indicating its high salt-tolerance. However, this algal species was not recorded in the algal patches formed on the soil during rainy season. This indicates that the alga exists in these soils in a less active form, may be due to some specific nutritional requirement and that is why on the availability of a nutrient rich medium it readily formed colonies even at high EC level. *Anabaena* sp. showed inconsistent response to increasing salinity of the medium, which may be due to the presence of more than one species of this genus in these soils having different degrees of salt-tolerance which needs to be explored. A wide range of salinity and alkalinity tolerance in *Anabaena* has earlier been reported (Sethi & Kaushik 1993). *Spirulina, Oscillatoria acuminata* and *Chroococcus turgidus* showed very high salt-tolerance in the soil inoculum of the rainy season, but not in the dry season, which may be due to the elimination of the algae from the surface layers of the aridisols due to scarcity of water. On the other hand, *Hapalosiphon welwitschii* formed colonies at high EC levels only in the dry season. This could probably be due to competitive superiority of this alga under conditions of water scarcity, which needs to be examined. Algae like *Microcoleus* and *Lyngbya* with a low frequency in the natural site also showed poor growth in the culture medium. Likewise, *Gloeocapsa*, which had a moderate frequency and salt tolerant behaviour in the natural site could not compete with other algal genera in the culture medium. *Nostoc commune* on the other hand, with a frequency of just 30 per cent, restricted to the non-saline plots in the natural site, exhibited salt tolerance upto EC 8 dSm^{-1} in both rainy and dry season in the culture medium. Thus, a diversity of salt tolerating potential was indicated in the algal genera of the aridisols. It was further indicated that while some genera show a marked halotolerant response only on the availability of optimal nutrient and growth conditions, as provided in the lab, others compete better under natural conditions. Greater algal density at EC 8 and 12 dSm^{-1} indicate that these algal genera have developed salt tolerating capacity, or rather become adapted to the presence of salts in the soil medium due to their exposures to the selection pressures in the aridisols for a long time. The role of selection trends in different ecological niches on different populations and their behaviour has been emphasized by Yablokov (1986) and Manchanda & Kaushik (1997). There is a need to exploit the diversity of salt tolerance and adaptive potential of the indigenous algal strains from the aridisols for their subsequent use as biofertilizers in problem soils.

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