

## CYANOBACTERIA MODULATED CHANGES AND ITS IMPACT ON BIOREMEDIATION OF SALINE-ALKALINE SOILS

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### Abstract

Saline-alkaline (Usar) soils have high pH and undesirable salts on their surface. A halotolerant, heterocystous and nitrogen fixing cyanobacterium *Nostoc calcicola* Breb. BREB grow successfully on saline-alkaline soils of Eastern Uttar Pradesh. Soil pot experimentation has been conducted in laboratory condition to study the reclamation of saline-alkaline soils collected from investigated site. Both sterilized and natural soils were taken in earthen pots to observe the changes in soil properties inoculated with cyanobacteria and gypsum. In such treated soils significant decrease in pH, E<sub>Ce</sub> and Na<sup>+</sup> have been observed with cyanobacterial application. There also occurs a significant increase in organic carbon. Experimental reclamation of such soils has been found with cyanobacteria with or without gypsum. *N. calcicola* + gypsum seem to be a suitable combination for reclamation of saline-alkaline soils. This study highlights that a biological amendment with halotolerant cyanobacteria and gypsum in combination shows better option for bioremediation of saline-alkaline (Usar soils).

### Introduction

Increasing soil salinity, high soluble salts, sodicity and high exchangeable sodium all are serious land degradation issues worldwide (Vanessa *et al.* 2009). Saline-alkaline soils/salt affected soils (commonly known as Usar soils in India occupy more than 7 million hectare (Mha) land in India (NRSA 2003).

A scientific approach for practicing crop cultivation and to enhance crop productivity in saline/alkaline soil is the reclamation (amelioration) of soil salinity. The reclamation of such soils basically requires the replacement of exchangeable sodium by calcium and thereafter leaching of the exchanged sodium. Conventionally it is done by the approach of addition of gypsum (CaSO<sub>4</sub>·2H<sub>2</sub>O) or pyrite (FeSO<sub>4</sub>) followed by leaching of excess salts by flooding or extensive irrigation. Biological reclamation through cyanobacteria is another approach. Singh (1961) suggested that cyanobacteria could be used to reclaim alkaline soils because they grow successfully on saline/alkaline soils where most plants fail to grow. Pandey *et al.* (2005), Jaiswal *et al.* (2010) and Murtaza *et al.* (2011) have also suggested the role of cyanobacteria in reclamation of saline-alkaline soils. The objective of the present investigation was to explore the role of halotolerant cyanobacteria in bioremediation of saline-alkaline soils and to study their establishment and influence on soil properties.

### Materials and Methods

Soil samples were collected from investigated site (village Amra) is located 20 km away in south-west from Udai Pratap Autonomous College in the district Varanasi (25°20' N, 83°00' E) in the months of April i.e. driest season when very sparse patches of cyanobacterial population were there. The surface soil samples were taken from 0 - 15 cm depth from six different places of the

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area under investigation. Samples were taken in triplicate using soil sampler (auger). Collected soil samples were processed and used for estimation. Before and after inoculation of cyanobacterial species, soil pH and ECe were measured in a soil/water saturation extracts with pH meter and conductivity bridge, respectively following the methods of Richards (1954). Water soluble ions like-Ca<sup>2+</sup> (Versenate method), Na<sup>+</sup> (Flame photometrically), organic carbon and sodium adsorption ratio (SAR, mathematically) were also determined as described by Richards (1954). Two experimental sets were prepared, one with natural soil and other with sterilized soil under laboratory condition (30 ± 2°C and light intensity 25 µE/m<sup>2</sup>/sec).

Allen and Arnon (1955) sterilized medium having pH 9.5 has been used for maintaining the culture. Experimental soils (300 g) were kept in earthen pots (8 cm in height and 20 cm in diameter) and gypsum (powder) was mixed (200 mg/100 g of soil). Natural (unsterilized) as well as sterilized (1.05 kg /cm<sup>2</sup> pressure for 15 minute) soil sample were used for the experiment. After flooding with sterilized distilled water for 15 days, the cyanobacterial species like *Nostoc calcicola* Breb., *Calothrix* spp., *Anabeana* spp. (inoculum density of 20 µg protein/ml) were inoculated in pots. Four different sets of pot experimentation were done with both the sterilized soils. Pots of Set-1 were taken as control, Set - 2 were added with gypsum only, Set -3 comprised of gypsum + *N. calcicola* and Set - 4 was added with gypsum + mixture of cyanobacteria (*viz.*; *Calothrix* spp., *N. calcicola* BREB, *Anabeana* spp.) and observations were made for 3 months (May - July year needed).

Algal samples were scraped from upper 1 cm surface by using sterilized implements and the cyanobacteria present in the sample were identified with the help of Desikachary (1959) and Rippka *et al.* (1979). Enumeration of cyanobacterial species were done by Pandey *et al.* (1995). The analysis of variance was performed which indicated a significant effect of gypsum on cyanobacterial population. The effect of sterilization was also statistically significant at 5%.

### Results and Discussion

Table 1 shows the properties of the soil under observation before inoculation and Table 2 presents the succession of cyanobacterial genera both in natural and sterilized soils. In natural soils (unsterilized) all the cyanobacteria as reported in the Table 2 were observed but, in sterilized condition, only inoculated genera (*Calothrix* spp., *Nostoc calcicola*, *Anabeana* spp.) were reported.

**Table 1. Characteristics of the saline - alkaline soil before inoculation.**

pH	10.5
ECe (dS/m)	6.5
O.C. (%)	0.10
Ca <sup>2+</sup> (Me/l)	3.5
Na <sup>+</sup> (Me/l)	49.0

Fig. 1 (a - e) shows the alternation of soil properties by cyanobacterial inoculation in natural soil. All the treatments show decrement of pH, ECe and Na<sup>+</sup> but gypsum in combination with cyanobacteria shows higher decrement than gypsum alone. The same pattern of decrement was also found in case of sterilized soil as in natural soil (Fig. 2).

Application of gypsum and *N. calcicola* have shown lowering of pH and ECe than control pots. A considerable decrease in pH and ECe was also observed in the mixture of gypsum and *N. calcicola*. By comparing the data studied above, application of *N. calcicola* + gypsum seems to be superior over the gypsum + mixture of cyanobacterial form *viz.*, *Calothrix* spp., *N. calcicola* and *Anabaena* spp. for biological reclamation of saline-alkaline soil.

The pattern of change in ECe and organic ‘C’ after treatment in natural as well as sterilized soils has been shown in Figs 1 b, c and 2 b, c, respectively. In conditions (both natural and sterilized), gypsum and cyanobacteria resulted in enrichment of soil with organic carbon and reduction in ECe.

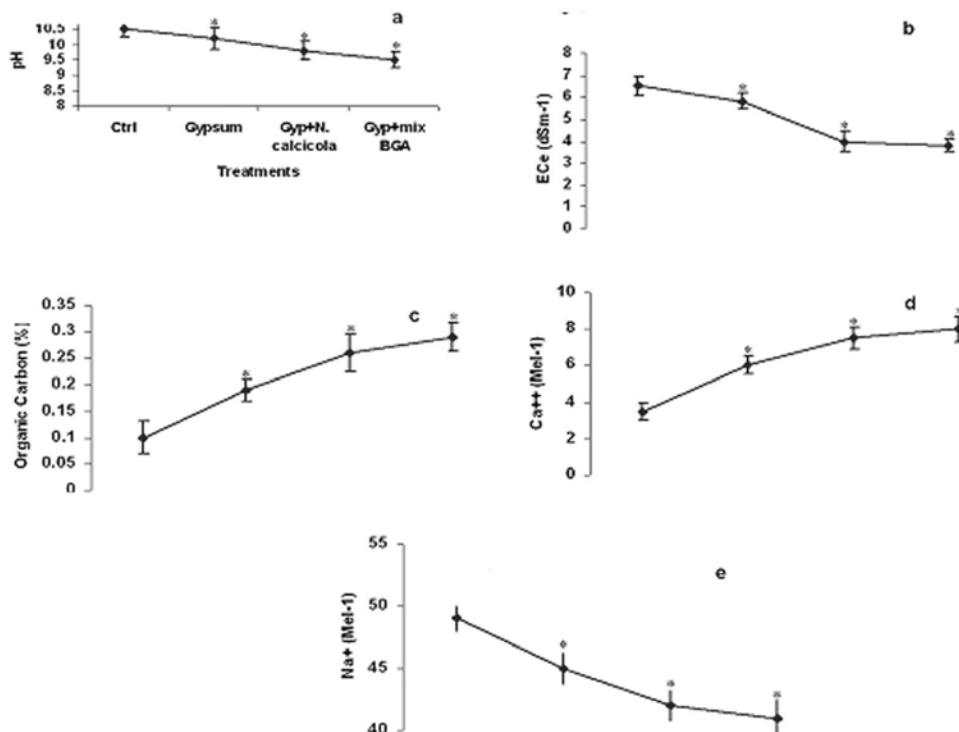


Fig. 1. Effect of cyanobacteria in altering soil properties in natural soil after 3 months of pot experiment.

\* Indicates significance from control.

**Table 2. Distribution pattern of cyanobacterial species in experimental pots.**

Forms	Natural soil				Sterilized soil			
	1	2	3	4	1	2	3	4
<i>Oscillatoria</i> sp.	A	A	A	A	-	-	-	-
<i>Microcoleus</i> sp.	A	A	A	A	-	-	-	-
<i>Phormidium</i> sp.	A	-	A	A	-	-	-	-
<i>Lyngbya</i> sp.	A	A	A	A	-	-	-	-
* <i>Calothrix</i> sp.	A	B	B	C	-	-	-	C
* <i>N. calcicola</i>	A	B	D	D	-	-	D	D
* <i>Anabaena</i> sp.	A	B	B	B	-	-	-	B
<i>Scytonema</i> sp.	A	A	B	B				

Pot 1 - control, 2 - gypsum, 3 - gypsum + *N.calcicola*, 4 - gypsum + mixture of cyanobacteria (*Calothrix* species, *Nostoc calcicola*, *Anabaena* species).

\* Inoculated species A - taxon present, B - common species, C - important, D - dominant.

Soil salinity is a pervasive problem especially in India and the reclamation of these soils is of massive practical importance to reinstate millions of acres of such barren lands once again under the plough (Jaiswal *et al.* 2010). It is clear from the Table 2 that heterocystous cyanobacteria (*Calothrix* sp., *Nostoc calcicola*, *Anabaena* sp.) were the more frequent inhabitants of the saline-alkaline soils than the non-heterocystous forms and since they have the property to add organic matter and nitrogen to the soil, they could be useful for starting plant succession in barren saline

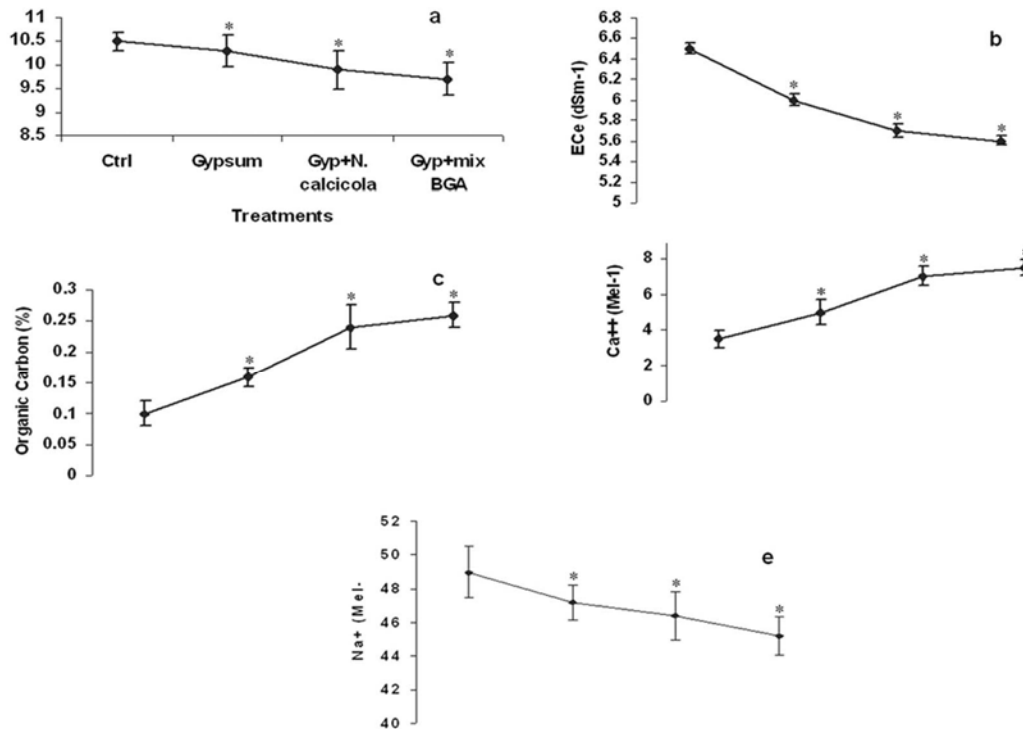


Fig. 2. Effect of cyanobacteria in altering soil properties in sterilized soil after 3 months of pot experiment.

\*Indicates significance from control.

soils (Ali and Sandhu 1972). Water leaching a pre-requisite for the reclamation of such soils accompanied by addition of organic matter and chemicals like gypsum or pyrites has also been recommended amendments (Singh 1961, Pandey *et al.* 2005). Due to the high pH of saline-alkaline soils, most likely as a result of  $\text{Na}_2\text{CO}_3$ , the addition of gypsum provides a source of  $\text{Ca}^{2+}$  which precipitates as  $\text{CaCO}_3$  and  $\text{Ca}(\text{HCO}_3)_2$ , resulting in a decrease in soil pH. In addition to decrease in soil pH, the addition of gypsum also leads to proton generation and thus a further reduction in pH occurs (Chorom and Rengasamy 1997). By the addition of gypsum in soil, there occurs an increment of  $\text{Ca}^{2+}$  and parallel decrement of  $\text{Na}^+$  (depicted in Figs 1 d, e and 2 d, e) which may be due to exchange of  $\text{Na}^+$  by  $\text{Ca}^{2+}$ . In reclamation of saline-alkaline soil, the conversion of  $\text{Na}^+$  clay into  $\text{Ca}^{2+}$  clay and leaching of excess  $\text{Na}^+$  occurs. Following the addition of gypsum and halotolerant cyanobacterial (*N. calcicola*) to sodic and saline soil, soil microbial biomass (SMB) and respiration rate increased despite adverse soil environmental conditions (Vanessa *et al.* 2009). The addition of gypsum decreases the pH ~ 10.5 - 9.5 in natural soil and 10.5 - 9.7 in sterilized soil. Following the incorporation of gypsum + *Nostoc calcicola* and gypsum

+ mixture cyanobacteria together, the E<sub>ce</sub> decreases (Figs 1, 2 b). The untreated saline soils (control) were highly alkaline and having high E<sub>ce</sub>. A significant improvement in soil properties has also been observed after cyanobacterial growth. Alkaline soils with high pH values and Na<sup>+</sup> content favour the growth of diazotrophic cyanobacteria with a consequent decrease in pH. The decrease in pH is also evident from our findings (Figs 1a and 2a) which are in line with the result of Singh (1961) who reported fall in soil pH from 9.2 to 7.5. Certain organic metabolites produced by cyanobacterial activities are also released in the soils which are responsible for maintaining the fertility of soil year after year (Ladha and Reddy 1995). The presence and succession of diazotrophic cyanobacteria in alkaline soil was reported by Singh (1961) and Singh *et al.* (2014). Frequent observation has been made for the organisms regarding cell characteristics, in that filament with, dead cells, spores and cells with granular body have been observed indicating last stage of their growth cycle. Keeping such views in mind the observation has been made for 3 months. Since gypsum itself is a source of calcium and presence of mucilage in such organisms are responsible for increase in calcium accumulation in soil.

Of the three inoculated genera *viz.*, *Anabaena* spp., *N. calcicola* and *Calothrix* spp., *N. calcicola* were found to be the most dominant forms in all the experimental earthen pots followed by *Calothrix* and *Anabaena* spp. (Table 2). *Nostoc* is known to be one of the most versatile diazotrophic cyanobacterial genera, observed in all types of environments, existing both in free living and symbiotic state. The members of this genus are capable of a variety of modes of C and N nutrition, which adapts them to diverse ecological habitats (Prasanna *et al.* 2008). *N. calcicola* adapts synthesis of organic osmoregulatory substances/compatible solutes and improvement in nitrogen assimilation and photosynthetic O<sub>2</sub> evolution which leads to its increased tolerance towards salinity stress (Borowitzka 1986). Moreover, compared to the algae, cyanobacteria belonging to the genus *Nostoc* offer another advantage: the improvement of total-N soil content, owing to the capability to fix atmospheric nitrogen (Obana *et al.* 2007). Jaiswal *et al.* (2010) also suggested *N. calcicola* as bioameliorating agent for saline-alkaline soil. Addition of *Nostoc calcicola* biomass to the saline/alkaline soils decreased the pH content and hence improved soil properties. The dominance of *N. calcicola* in saline/alkaline soils may be due to its salt tolerance, which suggests that *N. calcicola* could be a better phycotechnological approach for soil reclamation.

In conclusion, the role of cyanobacteria in bioremediation of saline-alkaline soil cannot be ignored. A combination of halotolerant cyanobacterium (*N. calcicola*) and gypsum is a considerable option for saline-alkaline/Usar soil reclamation and it should also be a matter of great concern in future experiments related to soil reclamation.

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