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Distribution and correlation of Soil bacterial population and chemical properties in salt affected landuses of Kaithal and Fatehabad district of Haryana

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Abstract

This study attempts to examine the relationship between soil bacterial population and physico-chemical properties of soil in Kaithal and Fatehabad district of Haryana. Soil pH in the study ranged from 7.7 to 10.32 in the selected sites. EC in the study ranged from 200 to 1780 µS/m at 25 °C. Organic carbon ranged from 0.10 to 1.32% in the present study. Majority of soil samples showed medium status of available nitrogen which ranged from 0.0005 to 0.02%. Soil phosphorus is most available for plant use at pH values of 6 to 7. Potassium content ranged from 0.001 to 0.05%. The present study revealed that bacteria isolated from bottom segment showed higher growth rate in anaerobic condition. A decreasing trend of total microbial population and organic carbon content of soil were found with increase in depth. The maximum number of bacterial colonies was observed at the depth of 0-30 cm. Gram's staining Revealed that 0-30 cm depth was occupied by majority of Gram negative bacteria in Haryana. 30-60 cm depth was analyzed, there was a slight but significant difference in the bacterial population and majority were Gram positive. Simultaneously there was less variation with respect to colony morphology and pigment production as compared to the depth of 0-30 cm.

Keywords: Soil bacterial population, soil phosphorus, organic carbon, Kaithal, Fatehabad

Introduction

Increasing soil salinity and alkalinity are serious worldwide land degradation issues, and may even increase rapidly in the future (Wong *et al*., 2009) [33] Salt-affected soils occupy an estimated 952.2 million ha of land in the world that constitutes to nearly 7% of the total land area and nearly 33% of the area of potential arable land. In India problem of salt affected soils is predominantly in Indo gangetic plains, arid and semiarid regions and account for 6.727 million ha i.e. 2.1% of geographical area of the country. Alluvial alkali soils: These soils are located mainly in the semi-arid tracts of Indo-Gangetic Plains of Punjab, Haryana, and Uttar Pradesh. These soils have developed under impeded drainage due to nearly impermeable clayey subsoils usually underlain by hard-indurated caliche (calcium carbonate concretions) pan with a high fluctuating ground water table (Bhargava, 1977) [5]. A soil is classified as sodic if exchangeable Na accounts for more of 15% of the CEC (Exchange Na percentage –ESP– >15). However, crops sensitive to Na toxicity are affected at ESP >7 (e.g. peach, citrus, strawberry). Classification of saline soils and the assessment of the negative effects of salinity on crops are based on the electrical conductivity (EC) of the saturation extract of the soil. If the EC of the soil is higher than 4 dS/m it is defined as saline. The degree of acidity or alkalinity of a soil is a very relevant property affecting many other physicochemical and biological properties. Some of the soil fertility features affected by soil pH include: (a) Availability of mineral elements to plants in the soil. At high pH, the solubility of many metals and trace elements is decreased, including essential nutrients for plants such as Fe, Mn, Cu or Zn. Deficiency of Fe, known as iron chlorosis, is frequent in basic soils (typically in calcareous ones). Extreme pH values decrease microbial activity in soils, which affects many soil processes (for instance, soil organic matter decomposition, nitrification, and biological N_2 fixation under acidic conditions. In soil the microorganisms play a vital role, though, these constitute less than 0.5% (w/w) of the soil mass, but play a key role in soil properties and processes participating in oxidation, nitrification, ammonification, nitrogen fixation, and other processes which lead to decomposition of soil organic matter and transformation of nutrients (Amato and Ladd, 1994) [1].

Though, differences in salinity tolerance among microbes result in changes in community structure compared to non-saline soils (Gros *et al*., 2003) [9]. Future research could investigate the effect and properties of organic materials such as decomposability and nutrient content on microbial tolerance to osmotic stress. Consequently, drastic changes in physical and chemical attributes in soils may upset the delicate plant-microbe relationship. Sodium affected soils have smaller average size pore spaces which can inhibit water and gas flow in the root zone of plants. High levels of sodium ions present can affect the ability of the plant to uptake other necessary ions and diminishes the rate of nitrate absorption (Ogle and St. John, 2010) [37]. Together, the physical and chemical properties of soil exert control over the composition and diversity of soil bacterial communities in agricultural and dry ecosystems (Zhang *et al*., 2019) [36]. Due to the heavy reliance of plants on soil microorganisms, an evaluation of soil microbes and soil health is necessary to determine how the soil microorganisms are being affected by salts and how this could be affecting the plants.

Material and methods

Study site description

For the present investigation, the soil samples were collected from the salt affected soil of Haryana. Two district of Haryana kaithal and Fatehbad were selected for the study. Kaithal district (2317 sq.km) lies between 29°31′27.43″N 76°09′02.99″E, 30°13′07.45″N 76°47′59. 44″E, under the old alluvial plain covering agro-ecological zone (Yamuna alluvial plain, hot and semiarid region with length of growing period of 90–120 days). It consists of two administrative sub-divisions *viz,* Kaithal & Guhla and six blocks *viz,* Kaithal, Pundri, Rajaund, Guhla, Kalayat and Siwan. The climate varies from arid to semiarid. The average rainfall of the district is 500–600 mm. The net cultivable area is 2.02 lakh ha, the area under forest is 3000 ha and barren and uncultivable land covers 2000 ha. Fatehabad district is located in an alluvial plain of Indo-Gangetic basin in the western part of Haryana. The geographical area of the district is 2520 sq. km., which is 5.4 per cent of the state share. Bhakra and Western Yamuna are the two major canals which irrigate most part of the district. The climate of the district is of tropical type with intensively hot summer (470C in June) and cool winter (20C in December and January). The average rainfall of the district is 312 mm. The sub-soil water of the district is overall brackish or saline. The quality of water varies from place to place.

Fatehabad district is located in an alluvial plain of Indo-Gangetic basin in the western part of Haryana. The geographical area of the district is 2520 sq. km., which is 5.4 per cent of the state share. Bhakra and Western Yamuna are the two major canals which irrigate most part of the district. The climate of the district is of tropical type with intensively hot summer (470 C in June) and cool winter (20 C in December and January). The average rainfall of the district is 312 mm. The sub-soil water of the district is overall brackish or saline.

Soil sampling was done in the month of July before onset of monsoon in these areas. Soil samples were collected from predetermined depths *i.e.* 0-30 and 30-60 cm, basically from plantation, agriculture, agroforestry landuses, samples were transferred to a polythene bag and the bag was tightly closed with proper labeling. In the laboratory, samples were divided into two parts one for microbiological parameters which were stored in deep fridge at 4^{o}C temperature and one for physico-chemical parameters the sample for physico-chemical parameters were air dried, grinded and sieves through 2 mm mesh sieve.

Physical and chemical analysis of soil

Soil pH was determined by using calomel electrode by 1:2.5 soil water ratios. Soil organic carbon (SOC) was determined by (Walkley and Black Method, 1934) [43]. Soil texture analysed by Hydrometer method (Bouyoucous, 1962) [44]. Bulk density of every sample was estimated by standard core method (Wilde *et al*., 1964) [38]. Soil available nitrogen was analyzed Subbiah and Asija (1956) [45]. Potassium by (Hanway and Heidel, 1952) [39], Determination of available phosphorus is by the Olsen method (Olsen, *et al*., 1954) [40]. Micronutrients like Zinc, copper, iron, magnesium, were estimated with the help of AAS (Lindsay and Norvell, 1978) [41].

Bacteriological analysis

For the isolation of bacteria, serial dilution method given by Johnson and Curl 1972 [42] was followed using Nutrient Agar medium. Nutrient agar was poured in each sterilized petridishes. For each dilution two petridishes were used. 1 mL of 10^{-5} dilution was spread on the petridish having nutrient agar media. Then the petridishes were incubated at 37 ^{o}C for 48 hours in inverted position. After 48 hours the different colonies were counted with the help of colony counter. The number of bacteria per gram soil was calculated by using the following formula- Three replicates were maintained for each sample. Inoculated Petri plates were then incubated upside down at 30 ± 1 °C for 24 hours in a BOD incubator. The number of bacterial colonies was counted and the Colony Forming Unit (CFU) was calculated based on dry weight basis.

Statistical analysis

Data were summarized as mean ± SD (standard deviation).Pearson correlation analysis was done to assess associations between the variables. A two tailed values less than (p<0.05) was considered statistically significant. Analysis was performed using SPSS software (version 16.0).

Results and Discussion

Bacterial population

The population of aerobic halophilic microbes was studied to determine their distribution with the availability of different physicochemical parameters with increasing depth in two district of Haryana. The present study revealed that bacteria isolated from bottom segment showed higher growth rate in anaerobic condition. A decreasing trend of total microbial population and organic carbon content of soil were found with increase in depth (Fig.1). The maximum number of bacterial colonies was observed at the depth of 0-30 cm, there is more diversity in bacterial population on the basis of colony morphology and pigmentation as shown in figure 1. Csonka, 1989 studied that Proline, glycine and betaine are the main organic osmolytes and potassium cations are the most common inorganic solutes used as osmolytes accumulated by salinity tolerant microbes.

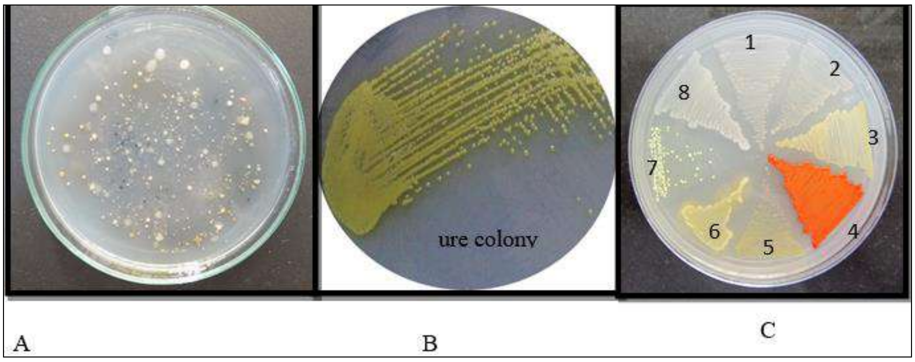
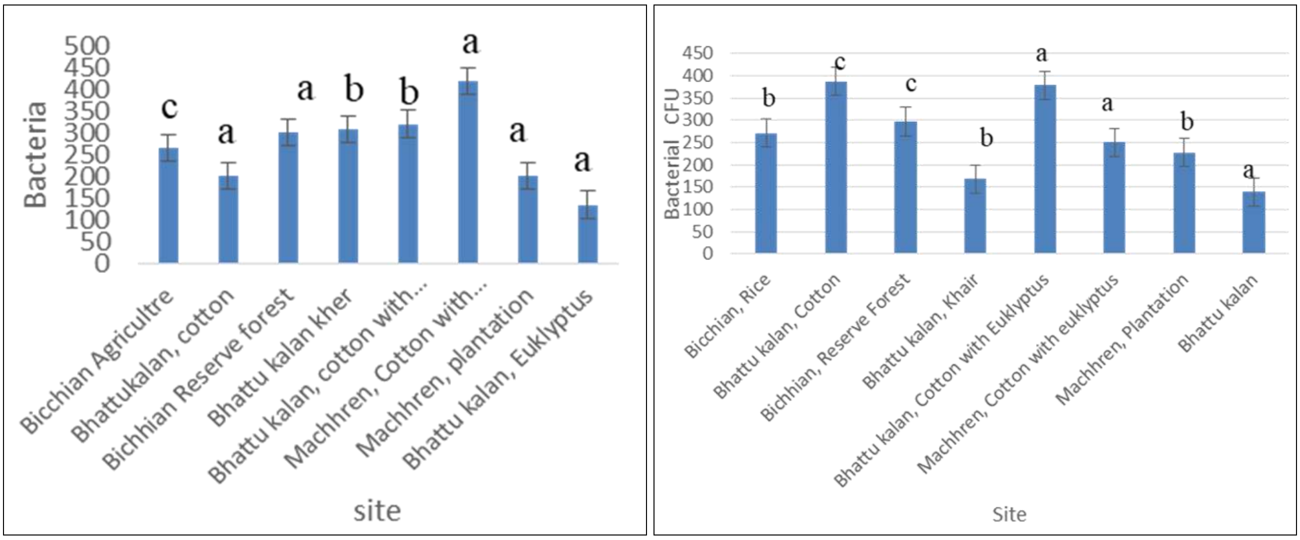


Fig 1: Isolation and purification of bacteria from the soil of Haryana (Fatehbad): 1 ml of 1g soil (depth 0-30 cm) spreaded on NAM plate, B, C a single colony of bacteria is streaked on NAM plate. The numbering 1-8 indicated bacteria with different type of colony morphology and pigmentation.

Gram's staining Revealed that 0-30 cm depth was occupied by majority of Gram negative bacteria in Haryana. There was a significant difference in the bacterial population as 030 cm soil depth was having higher number of bacterial population (fig: 1) and majority were Gram positive. Simultaneously there was less variation with respect to colony morphology and pigment production as compared to the depth of 0-30 cm.



A B

Fig 2: Soil bacteria population under salt affected soils (A)at 0-30 cm depth (B) at 30-60 cm depth

Chemical properties of soil

Soil chemical property is one of the important aspects of soil fertility that determines the health of soil. Majority of the soil samples studied were in the range of moderately alkaline to strongly alkaline pH, which may be due to the presence of more exchangeable sodium, calcium and magnesium on the soil exchangeable complex contributing to alkaline nature. Soil $pH_{(1:2)}$ in the study ranged from 7.7 to 10.32 in the selected sites. Soil electrical conductivity $EC_{(1:2)}$ is a measure of the amount of salts in soil (salinity of soil). It is an important indicator of soil health and it affects crop yields, crop suitability, plant nutrient availability, and activity of soil microorganisms which influence key soil processes including the emission of greenhouse gases such as nitrogen oxides, methane, and carbon dioxide (Smith & Doran, 1996) [28]. EC in the study ranged from 200 to 1780 µS/m at 25 °C. Low organic carbon may be due to the factors like high temperature and poor management of soil as reported by Chalwade *et al*. (2006) [8]. Organic carbon ranged from 0.10 to 1.32% in the present study. The low organic carbon content of these soils could due to rapid decomposition of organic matter in semiarid climatic condition. Majority of the soils studied fall in the category of lower organic matter indicating poor soil fertility status. Majority of soil samples showed medium status of available nitrogen which ranged from 0.0005 to 0.02%. The decrease of nitrogen availability might be due to the low organic matter in the surface soils and also the semi-arid climatic condition which have favored rapid oxidation and lesser accumulation of organic matter which resulted in low nitrogen content, Bandopadhyay *et al*. (2004) [4]. Shakir *et al* 2006 [46] reported that negative but highly significant correlation coefficients of exchangeable sodium percentage and total dissolved salt times exchangeable sodium percentage with particles density are same (-0.98**) in different soil texture. Phosphorus (P) is essential for plant growth and it stimulates growth of young plants, giving them a good and vigorous start. Soil phosphorus is most available for plant use at pH values of 6 to 7. P Kumar *et al* 2018 [25] reported 30.86 kg ha^{-1} to 43.27 kg ha^{-1} phosphorus in salt affected soils of Vani Vilas command area of Hiriyur taluk. Phosphorus in the above study ranged from 0.0004 to 0.01%. However, a low to medium range of soils available P under study area may be mostly affected by past fertilization, pH, organic matter content, texture and various soil management and agronomic practices Verma, Patel, Toor, and Sharma (2005) [31]. Potassium content ranged from 0.001 to 0.05% it is important for early growth stimulation, increasing protein production, improves the efficiency of water and improves resistance to diseases and insects.

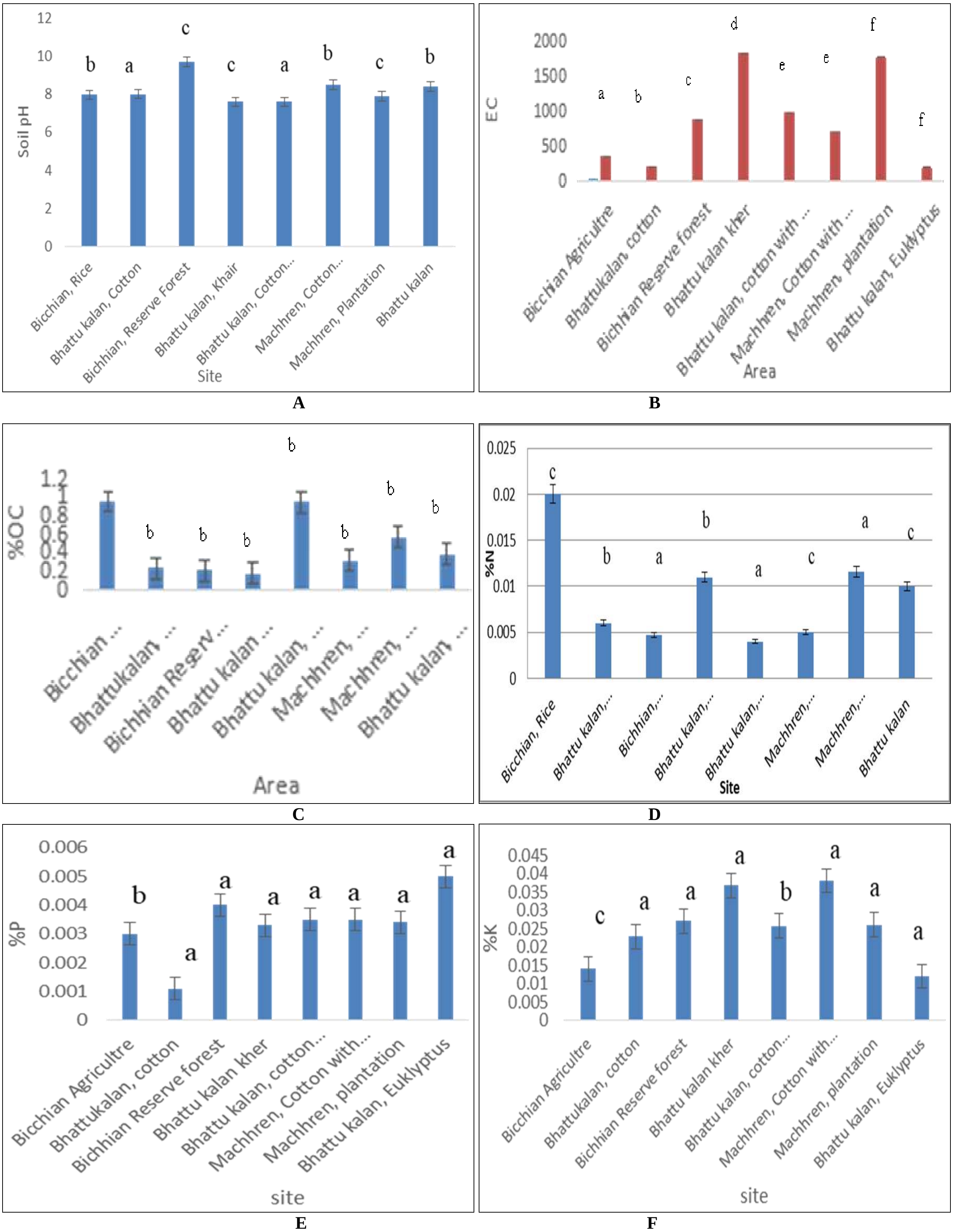


Fig 3: Soil chemical properties under salt affected soils (A, B, C, D, E, F) at 0-30 cm depth

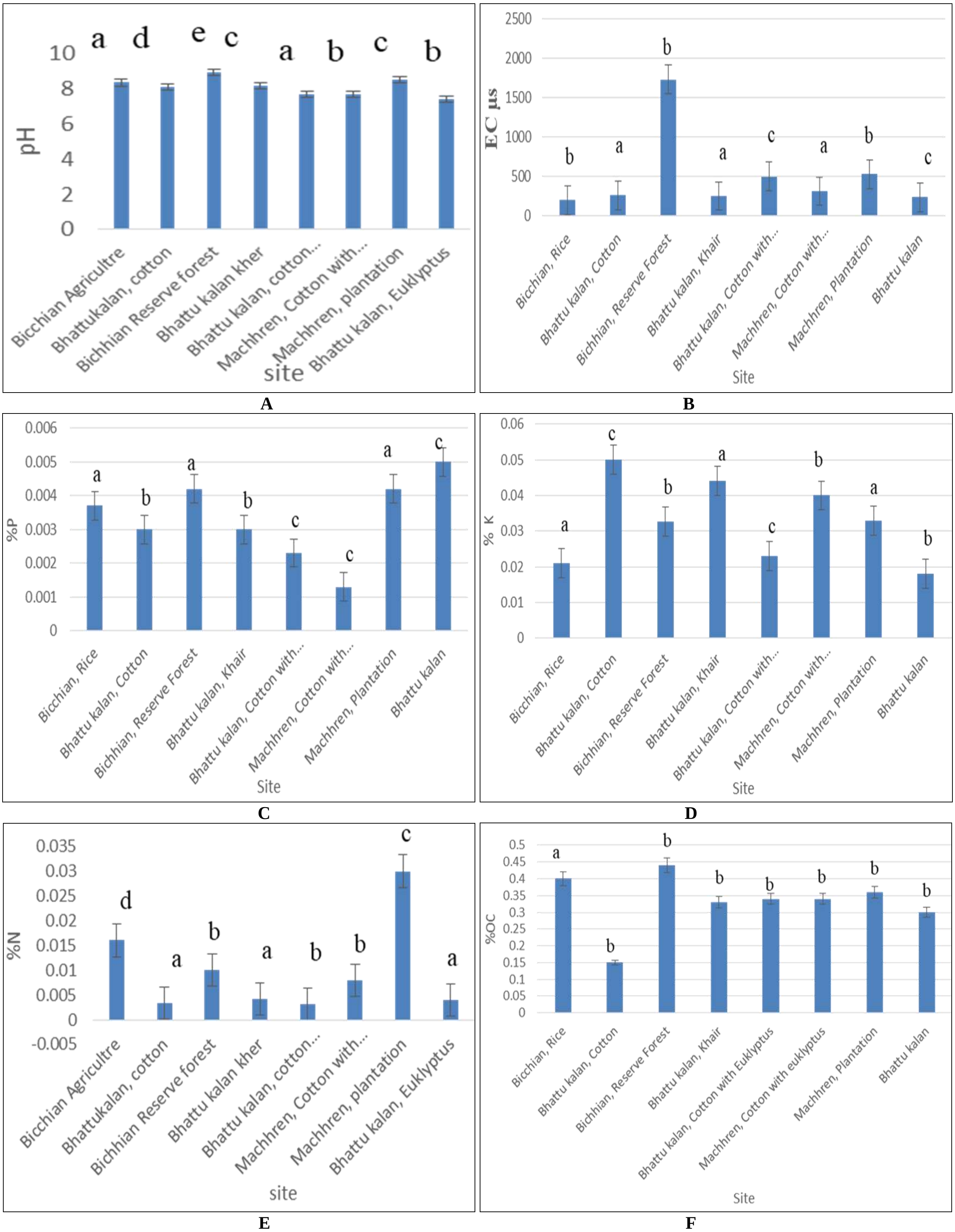


Fig 4: Soil chemical properties under salt affected soils (A, B, C, D, E, F) at 30-60 cm depth

Correlations among soil properties

Correlation coefficient (r) values of bacterial CFU and the various physico-chemical properties at 0-30 cm and at 30-60 cm soil depths in salt affected soils of Kaithal and Fatehabad district of Haryana during the study period are depicted in table1 and table 2 respectively. Soil EC and Soil pH were significantly correlated ($r=0.779^{**}$) at 0-30 cm depth. Soil bacteria population negatively correlated with soil pH (r = -0.220), (r= -0.250) at both the depths respectively. Organic carbon negatively correlated with soil pH (r=-0.360), (r=-0.096) at both the depths respectively. Available Nitrogen negatively correlated with soil pH(r=-0.182) at 30-60 depth. Available Phosphorus negatively correlated with soil pH (r=-0.140) at 30-60 depth. Available potassium negatively correlated with soil pH (r=-0.027), (r=-0.220) at both the depths respectively. Soil EC was having negative correlation with bacterial population (r=-0.026), Organic carbon(r= -0.180), available Potassium (r=-0.015) Available Nitrogen (r=-0.015) at 0-30 depth and positive correlation with, Liu *et al.* 2021 [15] studied the composition of the soil bacterial community relative to soil chemical properties in reclaimed soil after long-term monocultural cotton production in arid and extremely arid areas. They found that available K, N, and P, total N, and pH are all key factors in shaping soil bacterial communities. Available Phosphorus (r=0.170) whereas at 30-60 cm depth Soil EC negatively correlated with all the parameters except with bacterial population (r= 0.052).Soil bacterial population negatively correlated with organic carbon (r=-0.102) available Nitrogen (r=-0.021) available phosphorus (r=-0.426) and significantly correlated with available potassium (r=0.504*) at 0-30 cm depth whereas at 30-60 cm depth there was negative correlation with organic carbon only (r=-0.009).Organic carbon positively correlated with Available nitrogen, available phosphorus, available potassium at both the depth. Available nitrogen negatively correlated (r=-0.085) with phosphorus at 30-60 cm depth. Available phosphorus positively correlated with potassium at both the depth. Soil bacterial diversity is critical to the functioning and long-term sustainability of soil ecosystems (Liu *et al.*, 2014) [14]. We found that, across arid and extremely arid environments, available N and K content strongly influence the diversity of soil bacterial communities in fields. A possible explanation is that nitrogen is an important resource for bacterial growth, and thus high nitrogen content may promote soil bacterial biomass and diversity.

Table 1: Correlations among soil properties under different land uses of Haryana at 0-30 cm depth

Correlations among soil properties at 0-30 cm depth under different landuses of Haryana						
	pH	**EC**	**Bacteria**	**OC**	**N**	**P**
EC	0.779**					
Bacteria	-0.220	-0.026				
OC	-0.360	-0.180	-0.102			
N	0.028	-0.015	-0.021	0.270		
P	0.154	0.170	-0.426	0.080	0.201	
K	-0.027	-0.015	0.504*	0.582	0.230	0.360

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 2: Correlations among soil properties under different land uses of Haryana at 30-60 cm depth

Correlations among soil properties at 30-60 cm depth under different landuses of Haryana						
	pH	**EC**	**Bacteria**	**OC**	**N**	**P**
EC	0.140					
Bacteria	-0.250	0.052				
OC	-0.096	-0.184	-0.009			
N	-0.182	-0.026	0.095	0.380		
P	-0.140	-0.106	0.440*	0.035	-0.085	
K	-0.220	0.082	0.230	0.027	0.027	0.320

*. Correlation is significant at the 0.05 level (2-tailed).

Conclusion
Soil salinity is a world-wide threat to agricultural, forestry and ecosystems because it reduces plant growth and microbial functioning. The present study revealed that bacteria isolated from bottom segment showed higher growth rate in anaerobic condition. A decreasing trend of total microbial population and organic carbon content of soil were found with increase in depth. This study provides evidence for the importance of soil type and its interactions with biological and chemical parameters. The extremophiles, in particular thermophiles and alkalophiles, have been a subject of extensive investigation. Identification of areas with extreme environments including, the isolation of extremophiles, and investigations on the associated microbial wealth for biotechnological applications are of great importance, both for basic and applied research.

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