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Soil salinity differentially influences soil physicochemical properties and nutrient availability in rhizospheric soils of grasses growing in hyper-saline-arid regions

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Abstract

Soil salinity is increasing overtime in agricultural soils as well as in natural ecosystems particularly in arid and semi-arid regions due to low rainfall and high temperature. In this study, eighteen sites in Punjab from the Cholistan to Thal deserts, and Potohar Platues were selected to investigate the effect of soil salinity with increasing soil depths on soil physicochemical properties and nutrient availability. Soil texture of these soils varied from loamy sand and sandy loam of saline hyper-arid habitats to clay loam of saline semi-arid habitats. Soil saturation percentage, moisture contents, and soil organic matter generally decreased with increasing soil depth. Topsoil ECe ranged from 58 dS m^{-1} in Kallar Kahar (KKr) to 31 dS m^{-1} in Noorpur Thal (NpT) habitat while for deep soil (25 cm depth), ECe ranged from 50 dS m^{-1} in KKr to 21 dS m^{-1} of Khanewall (Knw) habitat. Soil Na^+ and Mg^{2+} contents increased with increasing ECe levels of different habitats but K^+ decreased in most of the cases. Soil Ca^{2+} decreased both with increasing soil salinity as well as with soil depths except for moderately dry Chak # 87A-Feroza (87A), Chak # 70-Feroza (H70), and Hasilpur (HsP) habitats. Most of the salinity causing cations i.e. Na^+, Ca^{2+}, and Mg^{2+} were concentrated in topsoil thereby causing higher ECe levels in surface soils. In comparison, NO_3^-, PO_4^{3-} and NH_3 generally reduced with increasing depth and by increasing intensity of salinity. It was concluded that soil salinity severally distorted soil physical properties and affected nutrient distribution particularly those of anions in different soil horizons that explains the growth restrictions imposed by high soil salinities on native vegetation of hyper saline arid regions.

Keywords: Salinity; soil health; nutrient availability; vegetation; rangelands

Introduction

Soil salinity is the most imperative ecological restriction that causes extensive losses in crop yield in agricultural fields and destroys vegetation of natural plant communities all over the world *(*Alam *et al*., 2015). Vegetation growing in saline areas shows stunted growth and causes formation of barren stretches of land due to poor plant development or death of vegetation indicating a direct consequence of high concentration of salts in the soil (Shrivastava, 2015). Other impacts of salt stress includes changes in soil properties, decreased uptake of water and imbalance of ions and mineral availability to the plant (Vinocur and Altman, 2005; Ali *et al.*, 2013). Such ionic imbalance in the lithosphere severely affects growth and survivorship of plants (Brucet *et al*., 2009). According to statistics, there is about 9.54×10^8 km^2 of saline soil worldwide forming 7% of the land surface and 5% of the cultivated land (Praxedes *et al*., 2010).

In general, plant growth is adversely affected by high salinity where plants grow slowly and, therefore, vegetation structure is altered altogether in saline communities. Salinity induced problems to plant growth and survival are observed when high amounts of salts accumulate in the root zone that negatively affects plant growth (Bharti, 2014). Excess salts in the root zone hinder plant roots to absorb water from surrounding soil. This lowers the amount of water available to the plant, regardless of the amount of water present in the root zone (Rani, 2019). Some of the earliest indicator plant species of saline soils include many salt tolerant grasses found growing in saline patches of semiarid and arid areas.

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Such hyper saline soils support only the growth of salt tolerant plants including many halophytic grasses where glycophytes cannot survive or show irregular growth patterns with poor plant vigor (Flowers, 2010). Studying salt tolerant plants growing in native habitats can be a key to unlock the relationship of soil high salinities with altered nutrient availability in saline communities (Wang, 2006).

Nutrient disturbances under salinity stress causes reduced plant growth by affecting the availability, transport, and partitioning of essential nutrients. Extreme salinity may cause nutrient deficiencies or imbalances, due to the competition of Na^+ and Cl^- with nutrients such as K^+, Ca^{2+}, and NO_3^- (Hu, 2005). In saline soils, high Na^+ concentration negatively affects K^+ uptake and accumulation within plant cells and organs, however, salt tolerance in plants is correlated with selectivity for K^+ uptake over Na^+ (Paksoy, 2010). Another critical factor in saline soils is soil depth where many nutrients and salts show differential accumulation in different depths. For example, potassium and available phosphorus tend to predominate near the surface, especially in clayey soils, whereas magnesium and calcium are more commonly distributed in the lower horizons (Fierer, 2003). Thus in hyper saline soils, nutrient availability might be altered altogether with increase in soil depth that limits root growth to surface layers particularly in shallow rooted plants such as grasses (Hameed *et al.*, 2008).

The most immediate effect of salinity induced disturbance in nutrient recycling and availability on natural communities can be observed as altered diversity and distribution of plants especially those of halophytic grasses (Kamal *et al.*, 2021). Depending upon the tolerance limits and degree of the plant to withstand salinity levels, certain grasses such as *Ochthochloa compressa, Lasiurus scindicus, Panicum antidotale, Cymbopogon jwarancusa, Leptochloa fusca,* and, *Aeluropus lagopoides* are frequently found growing in these hyper saline arid environments (Ahmad *et al.*, 2004). These communities and other halophytic vegetation grow in patches rather than forming dense or evenly distributed cover. Such random distribution of plants indicates the limitation imposed by high soil salinities on growth and survival of natural vegetation and is directly linked to direct effects of salinity stress (Kathiresan and Bingham, 2001).

Naturally, vegetation is growing in saline environments from ages and, therefore, provides an excellent opportunity to understand how these plant species are capable to survive and sustain growth in such a harsh environment. It was hypothesized that soil physicochemical properties and nutrient availabilities might vary as a result of a differential accumulation of salts (mainly Na^+) at different soil profiles. The objectives of the present study were (i) to investigate the effects of soil salinity on soil physicochemical properties in saline hyper arid habitats, (ii) to study the influence of soil salinity on availability of essential nutrients in various soils and (iii) how soil salinity differentially affects nutrient availability with increasing soil depths. Addressing these research questions will provide a key to understand the mechanisms of salinity tolerance in the salt tolerant grasses and augment efforts to rehabilitate the proposed study regions in future studies.

Materials and Methods

Study sites

Eighteen sites were selected from five ecozones including Cholistan desert, Pakka Anna, Sahianwala, Kallar Kahar, and Thal desert of Punjab to study the influence of soil salinity on soil physicochemical properties and nutrient availability in the root zone of natural vegetation especially grasses. Cholistan desert is located in south-east of Punjab between 27 to 29 degrees North and 69 to 73 degree East and contains many saline patches in the form of salt-flats. The soil of the Cholistan desert is dark gray-brown to blackish, somewhat saline with pH ranging from 8.0-9.0 (Arshad and Akbar *et al*., 2002). Pakka Anna**,** a hyper-saline land is situated in the vicinity of Faisalabad, Punjab. Its geographical coordinates are 31°14'0" North, 72°48'0" East. The soil is sandy-loam with a pH range of 7.5 to 8.5 (Akram *et al*., 2016). Sahianwala, another saline arid area, is situated in Faisalabad, Punjab and its geographical coordinates are 31°38' North, 73°14' East. Kallar Kahar is situated in Jhelum, Punjab, and its geographical coordinates are 32° 47' 0" North, 72° 42' 0" East. It is a major part of Potohar and consists of hyper-saline areas with rocky plateau. The plants present in these regions face extremely harsh climates due to high salinity, drought, and high temperature (Arshad *et al*., 2008). Thal desert is situated in Punjab, Pakistan between the Jhelum and Sindh rivers near the Potohar Plateau. It is a desert with little vegetation mostly thorny bushes. The whole land is arid and alluvial with sand dunes covering 50 to 60% of the area with rare lowland salt pans (Kamal *et al.*, 2021). The habitat details are presented in Table 1 while a map of collection sites is given in Figure 1.

Collection of soil samples

Soil samples were collected with a soil auger at different soil depths (0-25 cm) from rooting zones of grasses growing in different regions of Punjab as listed in Table 1. The soil column was split into three depths *i.e.* 0-5 cm, 6-15 cm, and 16-25 cm. Samples were arranged, labeled, and listed. A 200 g of fresh soil sample was weighed and dried in oven at 110 ± 5 °C (230 ± 9 °F) overnight. After

Table 1: Habitat details of the six grasses collected from various eco-zones of Punjab, Pakistan

Site No.	Species	Abb.	Site Name	Abb.	Habitat Type*	Soil Texture
01	*Ochthochloa compressa*	*Oc*	Noorpur Thal	NpT	Desert	Sandy Loam
02			Khanpur	KnP	Saline Arid	Sandy Loam
03			Kallar Kahar	KKr	Saline Semi-Arid	Clay Loam
04			Khushab	Ksb	Semi Semi-Arid	Clay Loam
05	*Lasiurus scindicus*	*Ls*	87A-Feroza	87A	Desert	Loamy Sand
06			Hasilpur	HsP	Desert	Loamy Sand
07			Perrowal	Pwl	Arid	Loamy Sand
08	*Panicum antidotale*	*Pa*	Khanewall	Kwl	Arid	Loamy Sand
09			Paka Anna	PkA	Saline Semi-arid	Clay Loam
10			Noorpur Thal	NpT	Desert	Loamy Sand
11	*Cymbopogon jwarancusa*	*Cj*	Yazman	Yaz	Desert	Loamy Sand
12			Khanpur	KnP	Desert	Loamy Sand
13			Chak#70-Feroza	H70	Semi-arid	Clay Loam
14	*Leptochloa fusca*	*Lf*	Sahianwala	Shw	Saline Semi-arid	Clay Loam
15			87A-Feroza	87A	Desert	Sandy Loam
16			Noorpur Thal	NpT	Desert	Loamy Sand
17	*Aeluropus lagopoides*	*Al*	Khushab	Ksb	Saline Semi-arid	Loamy Sand
18			Kallar Kahar	KKr	Saline Semi-Arid	Clay Loam

*The habitat types reported in this manuscript are the major ecological zone on a broader scale considering all soil, geographic and climatic attributes. Out of the 5 habitats [Cholistan desert (Cho), Pakka Anna (PkA), Sahianwala (Shw), Kallar Kahar (KKr), and Noorpur Thal desert (NpT)], 18 sites with saline patches and/or salt flats were selected for sample collection as shown in Figure 1.

removing from oven, the samples were cooled to room temperature, re-weighed and used to calculate soil moisture content as follows:

$$\%\ moisture\ contents = \frac{wet\ soil\ (g) - dry\ soil\ (g)}{dry\ soil\ (g)}\ x\ 100$$

The remaining soil samples were air-dried and ground by pestle and mortar to break soil aggregates and then sieved through a 2 mm sieve. Then soil was thoroughly mixed and stored in a paper bag till further analysis.

Determination of soil physicochemical properties

Soil texture: The percentage of sand (0.05 - 2.0 mm), silt (0.002 - 0.05 mm) and clay (< 0.002 mm) was estimated by hydrometer method (Bouyoucos, 1962; Day, 1965; FAO, 1974). Air-dried and sieved soil sample (<2.0mm) was weighed and put in 1000 mL beaker. Then, 60 mL of dispersing solution was added to the beaker and placed on reciprocating horizontal shaker for 24 hours. The suspension was transferred to the sedimentation cylinder and deionized water was added to bring the final volume to 1 L. The suspension was allowed to equilibrate at room temperature for two hours, stirred well and a hydrometer reading was taken on a blank solution as "R_b".

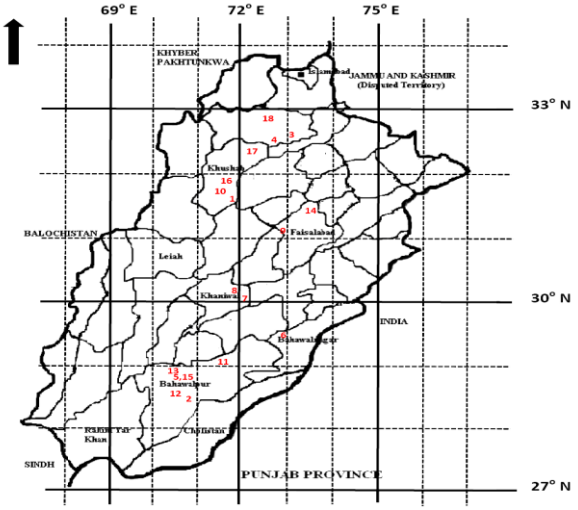

Figure 1: Map of Punjab, Pakistan showing the collection sites of six grasses. The numbers shown in map corresponds to the sample collection sites listed in Table 1

Determination of combined silt and clay contents: The plunger was inserted to thoroughly mixe contents, dislodging sediment from the bottom of the cylinder. Then 2 drops of amyl alcohol were added to the surface. The hydrometer reading R_{sc} was taken 40 seconds after mixing. The collective percentage of silt and clay was determined by the following formula:

$$\% \text{ [Silt + Clay] (w/w)} = \frac{\text{Rsc} - \text{Rb x 100}}{\text{Oven dry soil (g)}}$$

Separation of silt and clay contents: The above mentioned process was repeated and the suspension was left undisturbed for four hours. After which time, the hydrometer was inserted, and another hydrometer reading (R_c) was taken. The separate percentages of clay and silt were calculated as follows:

$$\% \text{ Clay (w/w)} = \frac{\text{Rc} - \text{Rb x 100}}{\text{Oven dry soil (g)}}$$

% Silt (w/w) = [% silt + Clay (w/w)] - [% clay (w/w)]

Determination of sand content: After determining R_c, the suspension was passed through a 50 mm sieve. The suspension was washed till water passing through sieve become clear. The leftover sand was transferred to 50 mL pre-weighed beaker. The sample was dried overnight at 105 ^oC, cooled in a desiccator, and re-weighed. The percentage of sand was calculated as follows:

$$\% \text{ sand (w/w)} = \frac{\text{Sand weight x 100}}{\text{Oven dry soil (g)}}$$

Sand weight = [Beaker + Sand (g)] - [Beaker (g)]

After measuring sand, silt, and clay percentages, the textural class was assigned by using USDA textural triangle (USDA, 1987).

Organic matter: Organic matter of the soil was determined by Walkley-Black (1934) method. A 2g of soil sample was transfer to a 500 mL flask and 10 mL of 1 N $K_2Cr_2O_7$ was added. Then 200 mL conc. H_2SO_4 was added by a dispenser and gently mixed. The solution was allowed to stand for 30 min. and 200 mL deionized water was added to make a clear suspension. Then, 10 mL of 85% H_3PO_4 and 0.2 g NaF was added. Following this, 10 drops of ferroin indicator solution was added just before the titration and titrated against 0.5 N Fe^{2+} to wine red endpoint. Percent carbon was calculated by using following formula:

$$\% \text{ C} = \frac{(\text{B}-\text{S}) \text{ x N of Fe2+}}{\text{g of soil}} \text{ x } \frac{12}{4000} \text{ x } 100$$

Where: B = mL of Fe^{2+} solution used to titrate blank; S = mL of Fe^{2+} solution used to titrate sample, 12/4000 = milliequivalent weight of C in g.

Percent organic matter was calculated by using formula:

$$\% \text{ organic matter} = \% \text{ C x } \frac{100}{58} = \% \text{ C x } 1.72$$

Preparation of a saturated paste: The soil saturated paste was prepared following the standard procedure of Rhoades (1982). A 200 g soil sample was weighed, air-dried and sieved into a porcelain dish. Then, a measured quantity of deionized water was slowly added and mixed with a spatula to form a paste. The paste was allowed to stand for an hour and saturation was rechecked by adding more water. The soil paste was left for half an hour and then filtered with a vacuum filtration system having Buchner funnel fitted with Whatman No. 42 filter paper. The filtrate was collected and used for the measurements of EC, pH and ionic contents (Na^+, K^+, Ca^{2+} and Mg^{2+}) following Rhoades *et al.* (1989)

Determination of saturation percent: Saturation percentage was calculated by following formula:

$$\text{SP} = \frac{\text{Amount of water added in ml x 100}}{\text{Mass of the air dried soil (g) x [(100 - Pw)/100]}}$$

Where P_w is water contents of the soil

EC measurements: The methodology given in USDA Handbook 60 (Richard, 1954) was followed for EC measurement of saturation paste extract using ECe meter (WTW series, USA) at standard temperature of 25 ^oC. Before taking readings, the EC meter was calibrated using a 0.01 N KCl solution. All readings were expressed as deciSiemens per meter (dS m^{-1}).

pH measurement: The soil pH of the above saturated paste was determined following the procedure of McKeague (1978) and McLean (1983) by using pH meter (Ino LAB pH/Cond 720). The pH meter was calibrated using solutions of known pH of 4.0 and 7.0 before taking all readings.

Na^+, K^+ and Ca^{2+} contents: The concentration of Na^+, K^+ and Ca^{2+} in soil extract was analysed by a flame photometer (PFP-7, Jenway, England). A graded series of standards ranging from 10 to 100 mg kg^{-1} was prepared and the standard curves were drawn. The OD values of the mineral elements generated by the flame photometer were compared with the standard curves. The actual quantities were computed and expressed as mg g^{-1} of soil dry weight.

Mg^{2+} contents: The Mg^{2+} content of the soil extract was determined by atomic absorption spectrophotometer (AAnalyst 300, Perkin Elmer, Germany) pre-calibrated with known standards. The final concentration was expressed as mg g^{-1} of soil dry weight.

PO_4^{3-} contents: Soil PO_4^{3-} was appraised spectrophotometrically by the method of Yoshida *et al*. (1976). Air-dried and sieved soil sample (5 g) was added to 100 mL $NaHCO_3$ extraction solution and shaken well for 30 minutes. The mixture was filtered by Whatman filter paper No 40. Soil extract (35 mL) was taken in a 50 mL flask and 10 mL vanadate solution was added to it. The final volume was made to 50 mL by adding distilled water. After 10 minutes, the reading was taken at 405 nm by using a spectrophotometer (UV-1100). The PO_4^{3-} content was estimated by following equation:

$$PO_4^{3-}\ mg/100g = \frac{O.D.at\ 405nm\ x\ factor}{10} \times \frac{100}{Soil\ dry\ weight\ at\ 105\ ^{\circ}C}$$

NO_3^- and NH_3 content: Soil NO_3^- and NH_3 were appraised spectrophotometrically by following method of Kowalenko and Lowe (1973). The estimation was performed by converting it into ammonium sulphate and titration against H_2SO_4 using appropriate indicator. A soil sample of 5 g taken in digestion flask was digested following Wolf (1982). Then, digestion mixture (5 g) and 20 mL conc. H_2SO_4 was added to it. The flasks were placed on electric heater and temperature was gradually increased from 350 to 380 ºC. Digestion was continued till the solution became clear and volume of the acid digested sample was maintained to 50 mL. The sample was then transferred to 250 mL volumetric flask. A 20 mL volume of H_3BO_3 was added along with few drops of indicator. A portion of diluted sample (25 mL) was run through the micro-kjeldahl distillation unit and released ammonia was trapped in boric acid solution collected in a conical flask. Finally, it was titrated against H_2SO_4 till the endpoint (green color) was achieved. The total N content was estimated by following formula and used to calculate NO_3^- and NH_3 contents:

$$N\%\ in\ soil = \frac{(sample\ titration - blank)\ x\ normality\ x\ 14\ x\ dilution}{sample\ weight}$$

Cl^- content: Soluble chloride content was measured by silver nitrate titration method after Richards (1954). Soil saturation extract (5mL) was taken in a flask with the help of pipette and four drops of potassium chromate solution were added. The mixture was titrated against silver nitrate solution until permanent reddish-brown color appeared. A blank sample without soil was also run to subtract the blank reading from all samples. The chloride content was estimated as follows:

$$Cl^-\ (meq\ L^{-1}) = \frac{(V - B)\ x\ N\ x\ R\ x\ 1000}{Wt.}$$

Where, V = Volume of 0.01 N $AgNO_3$ titrated for the sample; B = Blank titration volume; R = Ratio between total volume of the extract and extract volume used for titration; N = Normality of the $AgNO_3$; and Wt. = Weight of air dried soil

Statistical analysis

All sampling was done in three replicates. For graph preparation and other data analysis, all sites for each grass were arranged in order of their soil salinity levels. The LSD values (5%) were calculated and used to place letters of significance separately for habitat (expressed as capital letters) and soil depths (expressed as small letters) on graphs. The influence of different habitats on soil physicochemical properties were determined by redundancy analysis (RDA) performed using CANOCO for windows (v 4.5). The response curves (RC) were also drawn with the same software to determine the response of soil nutrients at various depths against the increasing soil salinity gradients by keeping soil depths on axis 1 (as indicated by the direction of arrow in each response curve).

Results

Soil physicochemical properties

The saturation percent (SP) of root zone soil differed significantly among habitats as well as with increasing soil depths. The maximum SP was recorded in topsoil (0-5 cm) of most of habitats, which decreased significantly with increasing soil depths. However, this was not true for all three habitats (Ksb, 87A, and HsP) of *Ls*, one habitat (Yaz) of *Cj*, and another habitat (H-70) of *Lf* where it increased significantly with soil depth. The maximum saturation percentage of soil collected from rooting zone of *Oc* observed at KKr was significantly higher than other two habitats *i.e.* KnP and NpT. In *Pa*, the maximum SP was observed in soil collected from Knw. The SP of *Cj* habitats differed non-significantly amongst NpT and KnP while significantly lower soil SP was recorded for the Yaz habitat (Figure 2).

The moisture contents (MC) differed significantly among habitats as well as with increasing soil depths. Generally, the maximum MC was recorded in the topsoil (0-5 cm) of most of the habitats that decreased significantly with increasing soil depths. However, this was not true for KnP of *Oc*, Knw of *Pa*, and 87-A of *Lf* where it increased significantly with soil depth. The maximum MC of soil collected from rooting zone of *Oc* as observed at NpT was significantly higher from other two habitats *i.e.* KnP and KKr. The Ksb habitat of *Ls* showed the maximum soil MC. In *Pa*, the maximum soil MC was observed in the soil collected from PkA. The MC of *Cj* habitats differed significantly among NpT, Yaz and KnP while significantly lower soil MC was recorded for the soil collected from Yaz

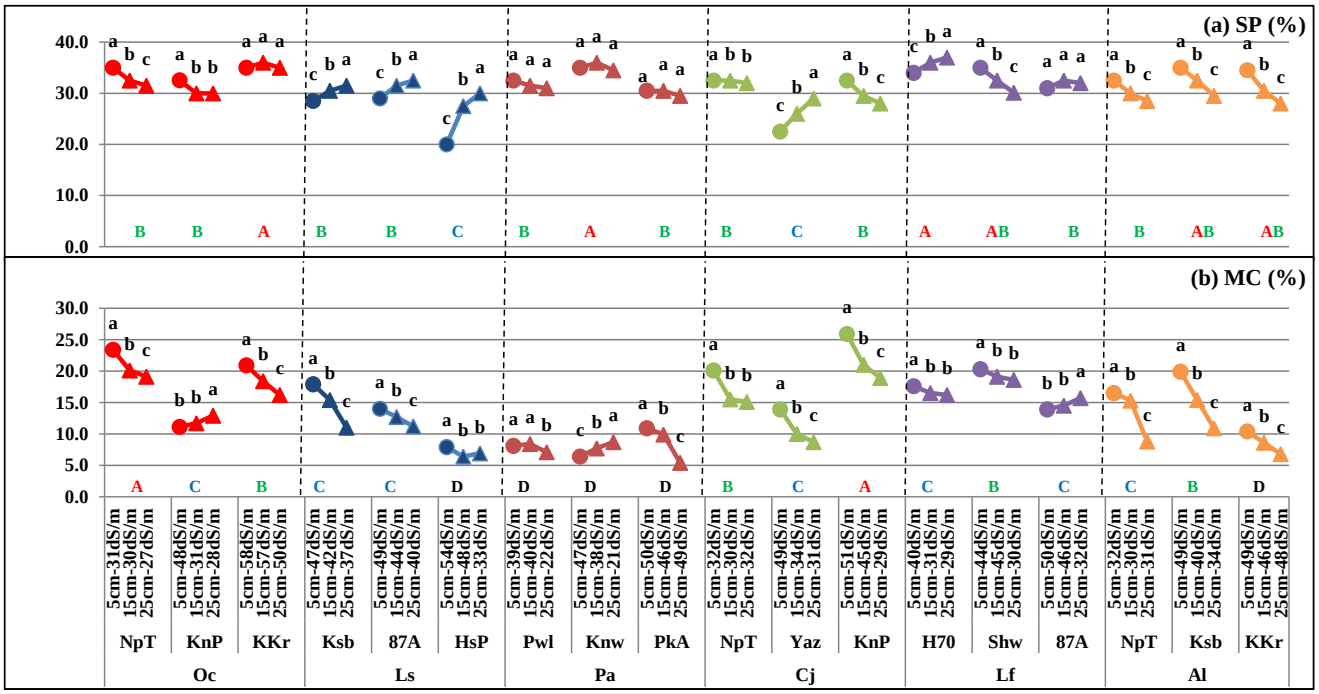

Figure 2: Mean values for SP and MC (%) of the soil samples collected from various saline habitats of the Punjab province. All sites for given species are arranged in order of increasing salinity gradients and ECe values rounded to nearest zero. Capital letters (A, B, C) represent significance of means calculated for all habitats. The small letters (a, b, c) represent significance of means at various soil depths for one habitat type. Abbreviations are given at start of manuscript. Pointers ( ) indicate trend (increase or decrease) from 5cm topsoil ( ) to 15 and 25 cm (( ) soil depths

habitat. The maximum MC of *Lf* rooting zone soil was noted at Shw. The soil collected from rooting zone of *Al* also differed significantly from all three habitats (Figure 2).

Statistically non-significant differences among habitat types or within species were observed. Nevertheless, the maximum pH value observed in KKr of *Oc* significantly differed from all other sites. There were also statistically non-significant differences with increasing soil depths in all habitats except 87A of *Ls* and Yaz of *Cj* where soil showed a significant decrease with soil depth. Maximum pH noted for the soil of KKr habitat of *Oc* differed significantly from other two sites (KnP and NpT). Similarly, soil of HsP habitat for *Ls* showed higher pH that also differed significantly from other two sites *i.e.* Ksb and 87A of *Ls* (Figure 3).

The analysis of the organic matter for the rooting zone soil generally showed non-significant differences for some habitat types. The maximum organic matter was noted in KnP habitat of *Oc*, while for *Al*, the minimum OM existed in Ksb habitat. Generally, OM differed non-significantly with varying soil depths but it is not true for the rooting zone soil of *Cj* collected from NpT and KnP habitats, where soil OM significantly increased with the increasing soil depth. The soil of *Lf* from Shw habitat, and, soil of *Al* from NpT, Ksb, and KKr habitats showed a statistically significant decrease in OM with increasing soil depths. The soil of KKr habitat of *Oc* showed the minimum OM that differed significantly from NpT and KnP. Shw habitat of the *Lf* showed significantly decreased soil OM as compared to H70 and 87A. NpT habitat of *Al* has higher OM contents that differed significantly from Ksb and KKr habitats (Figure 3).

Soil analysis of different sites showed remarkable differences in the soil ECe values as well as highly significant statistical variation with increasing soil depths was noted. The general trend revealed a decrease in soil ECe with increasing soil depth from the surface. Maximum soil ECe (58.50 dS m^{-1}) was noted in KKr habitat of *Oc* and the minimum ECe (21.10 dS m^{-1}) was observed in Knw habitat of Pa. The soil ECe values also differed significantly among different ecotypes. The rhizospheric soil of KKr ecotype of *Oc* showed the maximum ECe (58.50 dS m^{-1}) that differed

significantly from rhizospheric soil of KnP and NpT ecotypes. The ECe values of rhizospheric soil from Ksb, 87A, and HsP habitats of *Ls* showed a statistically significant decrease with increasing soil depths. Similar variation was noted in Pwl, Knw, and PkA habitats of *Pa* that differed significantly from each other. NpT rhizospheric soil of *Cj* showed remarkably lower ECe (32 dS m^{-1}) as compared to Yaz and KnP. The rooting zone soil of 87A of *Lf* ecotype exhibited significantly higher ECe than Shw and H70 habitats. KKr and Ksb soil of *Al* species showed higher ECe value than the NpT habitat (Figure 4).

mg L^{-1}). The soil Na^+ values also differed significantly among the rhizospheric soil collected from various habitats. The rhizospheric soil *Oc* of KKr habitats showed the maximum Na^+ concentration that differed significantly from KnP and NpT. Ksb, 87A, and HsP habitats of *Ls* showed a statistically significant decrease with increasing soil depths. NpT of *Cj* showed remarkably lower Na^+ concentration (1067.00 mg L^{-1}) as compared to Yaz and KnP habitats. 87A habitat of *Lf* exhibited statistically significant higher Na^+ concentration differing from Shw and H70 habitats (Figure 4).

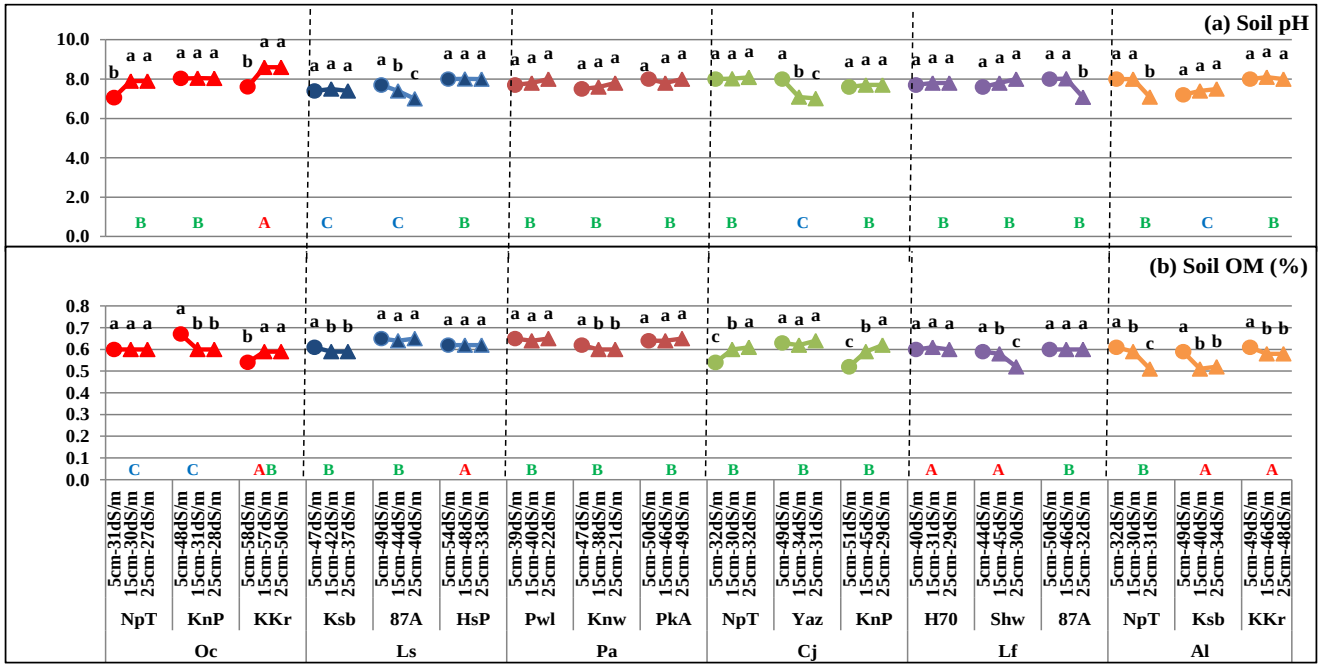

Figure 3: Mean values for pH and OM (%) of the soil samples collected from various saline habitats of the Punjab province. All sites for given species are arranged in order of increasing salinity gradients and ECe values rounded to nearest zero. Capital letters (A, B, C) represent significance of means calculated for all habitats. The small letters (a, b, c) represent significance of means at various soil depths for one habitat type. Abbreviations are given at start of manuscript. Pointers ( ) indicate trend (increase or decrease) from 5cm topsoil ( ) to 15 and 25 cm ( ) soil depths

Nutrient availability

The soil Na^+ concentration mostly corresponded to the soil ECe values of their respective habitats. Generally, the highest Na^+ concentration observed in the topsoil (0-5 cm) decreased with increasing soil depths. However, it was not true for PkA habitat of *Pa*, NpT habitat of *Cj* and NpT, Ksb habitats of *Al* that showed the same Na^+ concentration at all soil depths. The maximum Na^+ concentration was noted in KKr soil of *Oc* (2842.10 mg L^{-1}) and the minimum in Ksb habitats soil of *Ls* (681.3 mg L^{-1}), and, NpT of *Al* (751.00

Generally, K^+ concentration increased from the surface with increasing soil depths. Though the rhizospheric soil of KKr habitat of *Oc* showed the maximum K^+ concentration, it was statistically non-significant among three habitats (NpT, KnP, and KKr). The rooting zone soil of *Ls* collected from Ksb habitat showed significantly lower K^+ content. The Knw and PkA habitats of *Pa* showed statistically significant differences in K^+ concentration as compared to the soil of Pwl that exhibited very low K^+ concentration. The maximum K^+ concentration observed in KnP habitat of

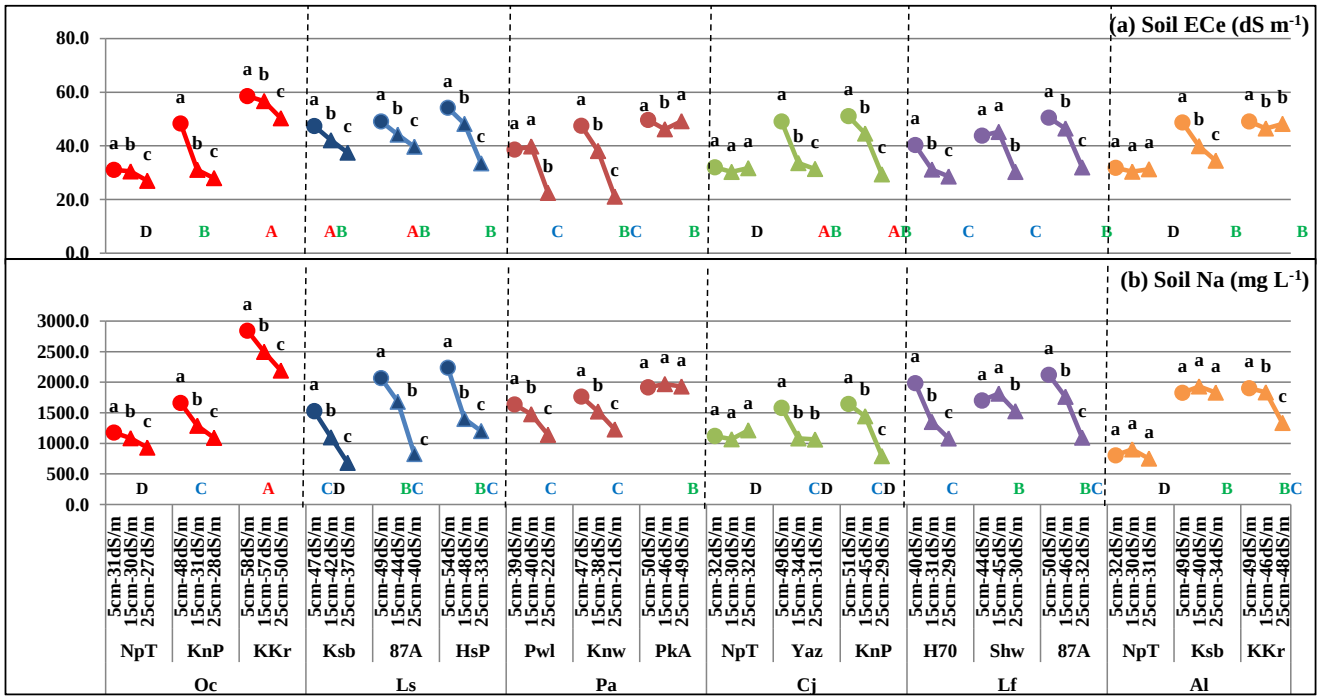

Figure 4: Mean values for ECe and pH of the soil samples collected from various saline habitats of the Punjab province. All sites for given species are arranged in order of increasing salinity gradients and ECe values rounded to nearest zero. Capital letters (A, B, C) represent significance of means calculated for all habitats. The small letters (a, b, c) represent significance of means at various soil depths for one habitat type. Abbreviations are given at start of manuscript. Pointers ( ) indicate trend (increase or decrease) from 5cm topsoil ( ) to 15 and 25 cm (( ) soil depths

Cj differed significantly from NpT and Yaz habitats (Figure 5).

The Ca^{2+} concentration showed significant variation among all sites. Generally, Ca^{2+} concentration decreased with increasing depths. However, it is not true for Ca^{2+} concentration in soils collected from 87A, HsP habitat of *Ls* and H70, Shw, and 87A habitats of *Lf* where soil Ca^{2+} concentrations increased significantly with increasing depths. The maximum Ca^{2+} concentration recorded in soil of NpT *Oc* habitat differed significantly from KnP and KKr. The Ca^{2+} concentration of Ksb habitat of *Ls* showed significant variation from 87A and HsP habitats. The maximum Ca^{2+} content observed in *Cj* soil of NpT habitat slightly varied with Yaz and KnP. All three sites of *Lf* (H70, Shw, and 87A) and *Al* (NpT, Ksb, and KKr) showed a highly significant decrease with increasing soil depths (Figure 5).

A general decreasing trend in soil Mg^{2+} concentration with increasing soil salinity at various soil depths of all habitats was noted except for sites 87A, HsP of *Ls*, and H70 of *Lf*. The highest Mg^{2+} concentration noted in habitat KKr of *Oc* differed significantly from two other sites *i.e.* NpT and KnP. Two habitats (87A and HsP) of *Ls* differed significantly from Ksb habitat. The Pwl habitat of *Pa* exhibited lowest Mg^{2+} concentration in soil that differed significantly from Knw and PkA. Yaz and KnP sites of *Cj* had higher Mg^{2+} concentration in soil as compared to NpT. Statistically highly significant differences were noted for all three sites (H70, Shw, and 87A) of *Lf* (Figure 6).

In general, the phosphorus (as PO_4^{3-}) content showed a decreasing trend. Maximum PO_4^{3-} content was noted in topsoil, which, gradually decreased in lower depths. The maximum PO_4^{3-} concentration noted in soil of KnP habitat of *Oc* differed significantly from the two other sites (NpT, KKr). The two habitats *i.e.* 87A and HsP of *Ls* showed a significant decrease from top to deeper soils. The PO_4^{3-} concentration in rooting zone of *Pa* collected from Pwl habitat showed a significant decrease with increasing depth. All three sites of *Cj* (NpT, Yaz, and KnP) exhibited statistically significant differences among habitats with a gradual decrease in PO_4^{3-} concentration from surface soil to deeper layers. The 87A habitat of *Lf* showed higher PO_4^{3-} that differed from other two sites (H70 and Shw). Sites NpT

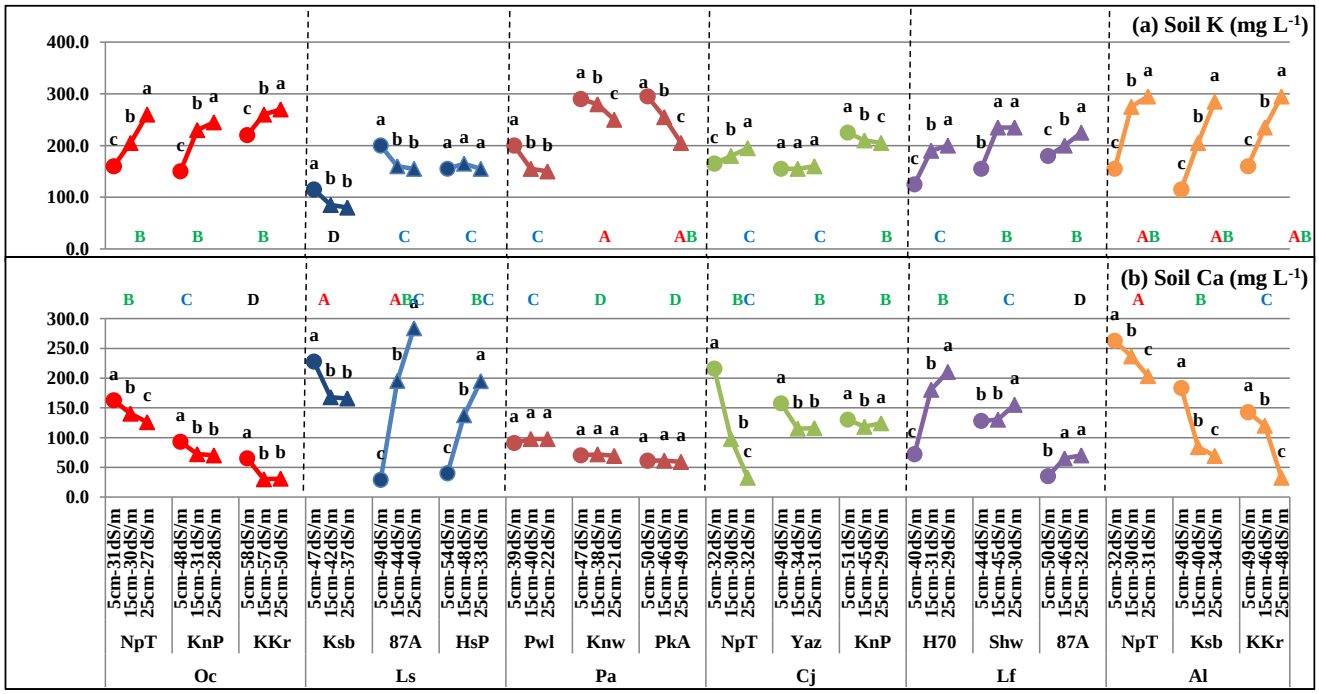

Figure 5: Mean values for K and Ca of the soil samples collected from various saline habitats of the Punjab province. All sites for given species are arranged in order of increasing salinity gradients and ECe values rounded to nearest zero. Capital letters (A, B, C) represent significance of means calculated for all habitats. The small letter (a, b, c) represent significance of means at various soil depths for one habitat type. Abbreviations are given at start of manuscript. Pointers ( ) indicate trend (increase or decrease) from 5cm topsoil ( ) to 15 and 25 cm (( ) soil depths

and Ksb of *Al* exhibited a decreasing trend with increasing soil depth (Figure 6).

Soil nitrate showed significant variation among habitats with a general decreasing trend with increasing soil depths. The soil of KnP *(Oc*), PkA *(Pa)*, NpT and Yaz (*Cj*), and, Shw and 87A (*Lf*) habitats represented an increasing trend with increasing soil depths. The maximum NO_3^- concentration noted in topsoil of KKr *(Oc*) site differed significantly from KnP and NpT habitats. The rooting zone soil of *Ls* collected from Ksb habitat showed highly significant differences with other two sites *i.e.* 87A and HsP. The *Pa* topsoil from KnP habitat showed a much higher value of NO_3^-. The Knw and PkA habitats of *Pa* showed a decreasing trend in NO_3^- concentration with increasing soil salinity. The maximum NO_3^- concentration noted in a deeper zone of NpT of *Cj* species differed non-significant with Yaz while differed significantly from KnP habitats. All three habitats of *Lf* (H70, Shw, and 87A) and *Al* (NpT, Ksb, and KKr) showed significant differences between habitats with increasing soil salinity (Figure 7).

The NH_3 concentration exhibited significant differences among habitats as well as with varying soil depths. Generally, an increasing trend was noted with increasing soil depths. However, *Oc* of KnP, *Ls* from Ksb, 87A, and HsP, *Pa from* PkA, and *Cj* from KnP exhibited a decrease with soil depths. *Pa* from Pwl, *Cj* from NpT, *Lf* from H70 and *Al* from NpT did not show any significant variation in NH_3 content at various soil depths. The NH_3 contents also varied significantly at different study sites. The NpT site showed significantly higher NH_3 concentration in the rhizospheric soil of *Oc* that differed significantly from KnP and KKr sites. The maximum NH_3 concentration noted at Ksb site for *Ls* differed statistically from other sites 87A and HsP. In comparison, NH_3 content in *Pa* collected from Knw, and PkA sites also differed significantly from Pwl site. The NH_3 content of 87A site for *Lf* was significantly higher from H70 and Shw sites. The maximum NH_3 concentration was noted in Ksb site that differed statistically from KKr and NpT sites of *Al* ecotypes (Figure 7).

A non-significant change in soil Cl^- concentration was noted with increasing depths. However, it was not true for KnP habitat of *Oc*, habitats 87A and HsP of *Ls*, habitats

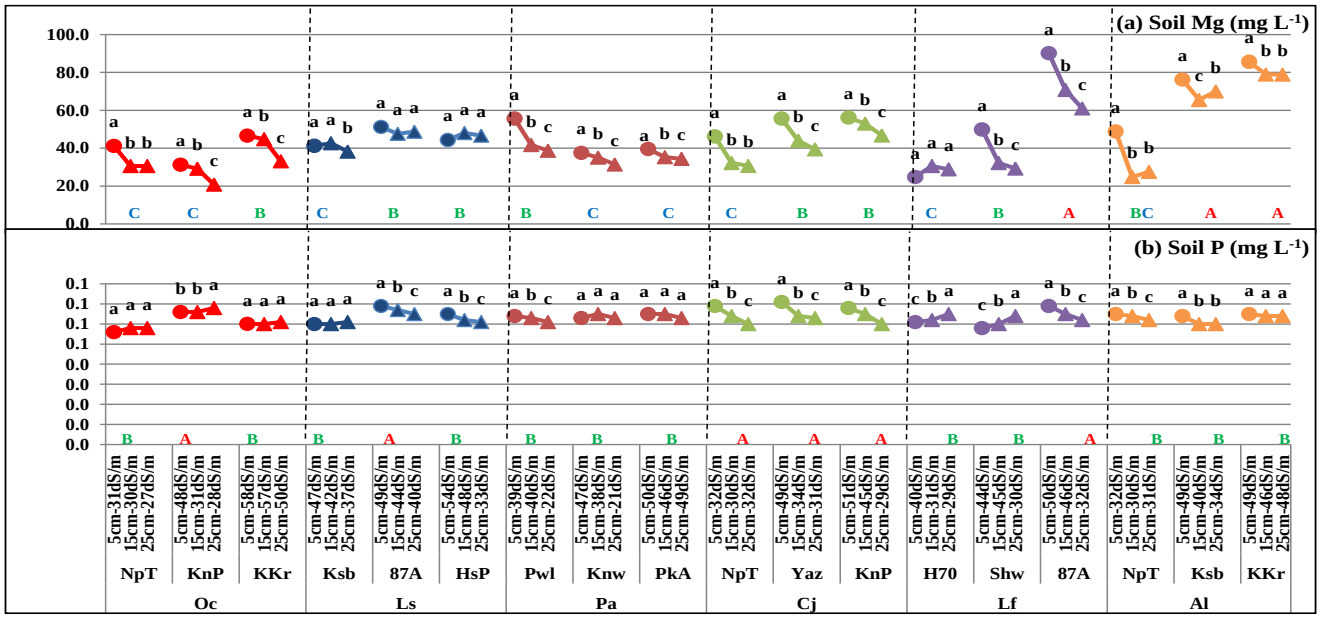

Figure 6: Mean values for Mg^{2+} and PO_4^{3-} of the soil samples collected from various saline habitats of the Punjab province. All sites for given species are arranged in order of increasing salinity gradients and ECe values rounded to nearest zero. Capital letters (A, B, C) represent significance of means calculated for all habitats. The small letter (a, b, c) represent significance of means at various soil depths for one habitat type. Abbreviations are given at start of manuscript. Pointers ( ) indicate trend (increase or decrease) from 5cm topsoil ( ) to 15 and 25 cm (( ) soil depths

NpT and KnP of *Cj*, and, habitat 87A of *Lf* that showed a statistically significant decreasing trend from surface to deep soil horizons. In comparison, Cl^- content significantly increased with increasing soil depth in NpT habitat of *Oc*, Pwl and PkA habitats of Pa, KKr habitat of *Al*. Among sites, Cl^- concentration differed significantly from each other in all habitats. The maximum Cl^- concentration noted in KKr habitat of *Oc* differed significantly from NpT and KnP habitats. Similarly, HsP site of *Ls* exhibited maximum Cl^- concentration that differed significantly from other two habitats 87A and Ksb. The higher Cl^- concentration was noted in KnP habitat of *Cj* that also differed significantly from NpT and Yaz habitats. The soil of highly saline habitat KKr of *Al* showed maximum Cl^- concentration that differed significantly from moderately saline habitats NpT to the least saline habitat Ksb habitats (Figure 8).

RDA biplot of rhizospheric soil's physicochemical properties

Biplot of soil physicochemical redundancy analysis (RDA) at 5cm depth showed a strong influence of Na^+, ECe, SP, pH, MC, NO_3^-, PO_4^{3-}, NH_3, and OM on distribution of *Ls* (87A and HsP). The *Al* ecotypes from KKr was mostly affected by soil K^+ contents, *Oc* from NpT and KnP by soil Cl^-, and *Al* from NpT by soil Ca^{2+}. All other species did not show the influence of any specific soil physicochemical properties at 5cm soil depth since these ecotypes plotted either at the center or at distance from the soil-physicochemical under study (Figure 9a).

The RDA biplot at 15 cm soil depth showed that many soil properties like Na^+, ECe, pH, SP, MC, OM, Mg^{2+}, PO_4^{3-}, NO_3^- and NH_3 had a more or less variable influence on distribution of ecotypes of *Al* (Ksb and KKr), *Pa* (Pwl and PkA) *Ls* (87A), *Lf* (87A) and *Oc* (KKr). The soil Cl^- and K^+ content had a significant influence on *Oc* (NpT) and soil Ca^{2+} on *Al* (NpT) ecotypes. Ecotypes of other grasses like *Ls* (Ksb and HsP), *Lf* (H70, Shw) *Cj* (Yaz, KnP, and NpT), *Pa* (Knw), and *Oc* (KnP) were plotted in the center of the RDA biplot showing almost equal influence of all soil properties on these ecotypes (Figure 9b).

At 25 cm soil depth, the physical (SP, MC, OM) and chemical properties (Na^+, ECe, pH, PO_4^{3-}, Mg^{2+}, NO_3^-, and NH_3) had a significant correlation with rhizospheric soil of *Pa* (PkA), *Al* (Ksb) and *Cj* (NpT) ecotypes. *Cj* (KnP), *Oc* (NpT), and *Al* (NpT) ecotypes were associated with soils with higher Cl^- and K^+ content. Soil Ca^{2+} had a significantly higher link with *Ls* (Ksb and 87A) and *Lf* (H70) ecotypes. All other ecotypes of different grasses *i.e. Ls* (HsP), *Pa* (Pwl and Knw), *Cj* (Yaz), *Al* (KKr) *Oc*

(KKr and KnP), and *Lf* (Shw and 87A) showed no clear link with any soil physicochemical attributes analyzed in this study (Figure 9c).

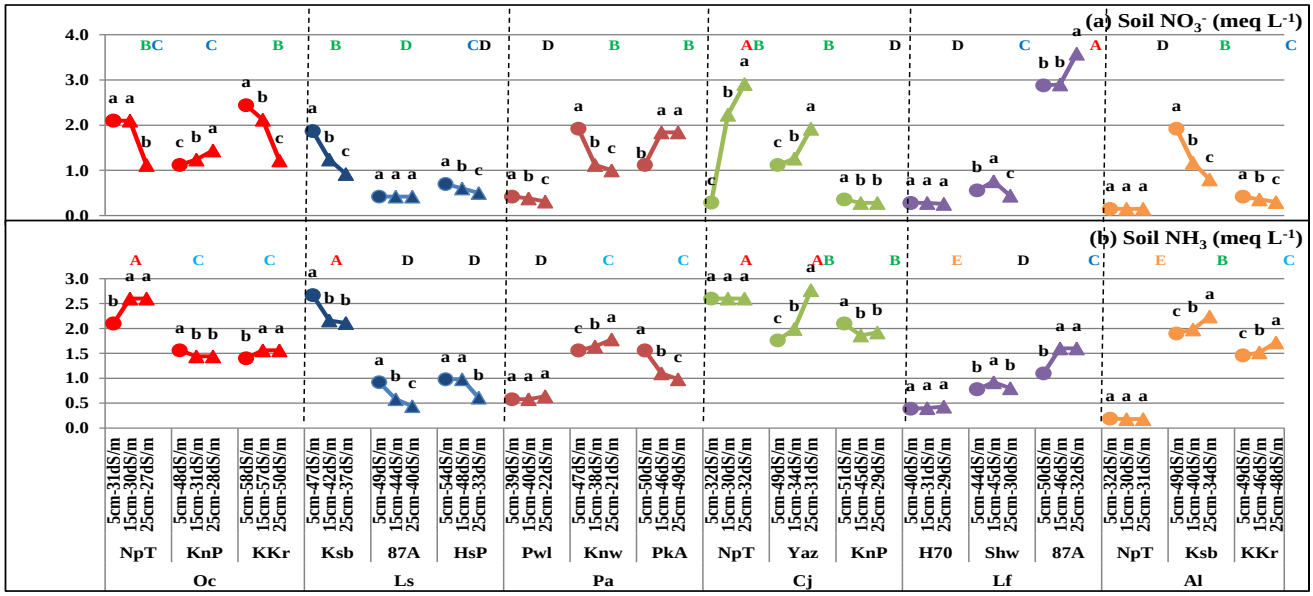

Figure 7: Mean values for NO_3^- and NH_3 of the soil samples collected from various saline habitats of the Punjab province. All sites for given species are arranged in order of increasing salinity gradients and ECe values rounded to nearest zero. Capital letters (A, B, C) represent significance of means calculated for all habitats. The small letter (a, b, c) represent significance of means at various soil depths for one habitat type. Abbreviations are given at start of manuscript. Pointers ( ) indicate trend (increase or decrease) from 5cm topsoil ( ) to 15 and 25 cm (( ) soil depths

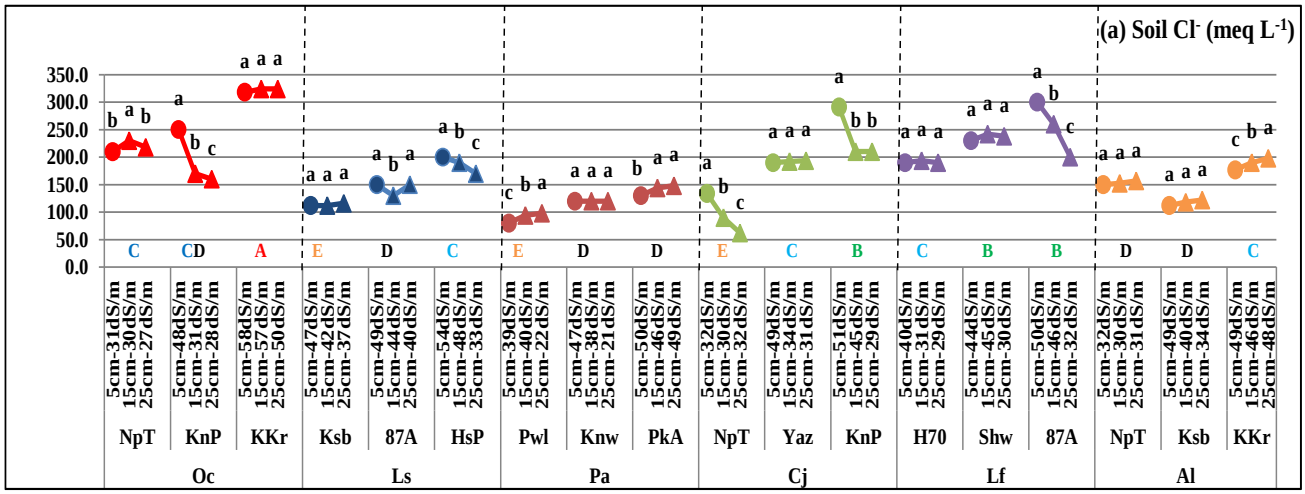

Figure 8: Mean values for Cl of the soil samples collected from various saline habitats of the Punjab province. All sites for given species are arranged in order of increasing salinity gradients and ECe values rounded to nearest zero. Capital letters (A, B, C) represent significance of means calculated for all. The small letter (a, b, c) represent significance of means at various soil depths for one habitat type. Abbreviations are given at start of manuscript. Pointers ( ) indicate trend (increase or decrease) from 5cm topsoil ( ) to 15 and 25 cm (( ) soil depths

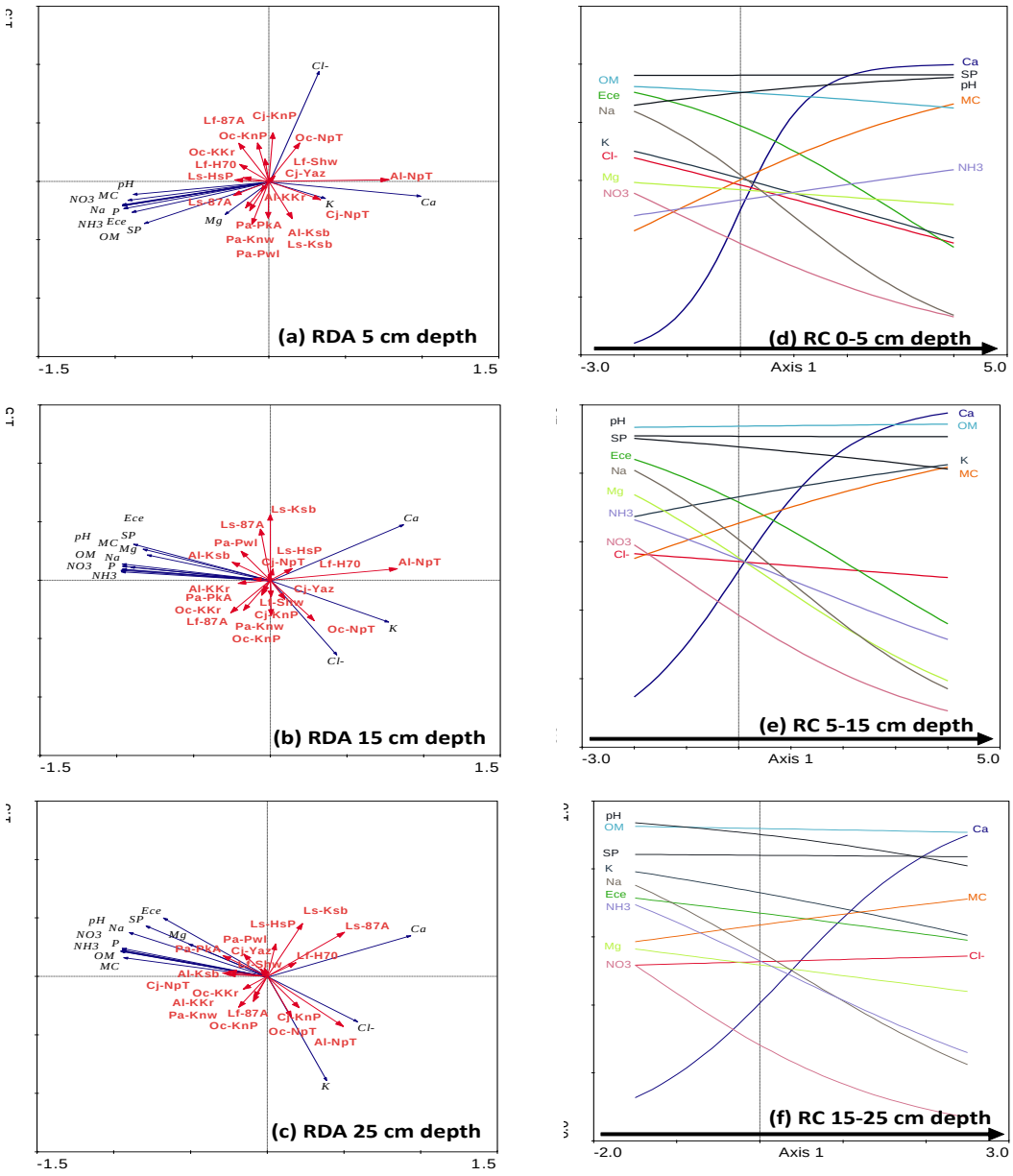

Figure 9: Redundancy analysis (RDA) and response curves (RC) for various soil-physicochemical attributes of six grass populations collected from habitats of varying soil salinity across Punjab. Direction of arrow in RCs indicates response of soil attributes along increasing soil depth from 0 to 25 cm (Abbreviations are given at start of manuscript)

Response curves (RC) for root biochemical attributes at various soil depths

The distribution of soil nutrients along with increasing soil depth showed differential response. In general, soil pH, organic matter, and saturation percent were evenly distributed from topsoil (0-5cm) to deep soil layers *i.e.* up to 25 cm as seen by zero slopes of these attributes in RC graphs. Soil MC, Ca^{2+} K^{+} and NH_3 showed a positive slope indicating leaching towards increasing depth while ECe, Na^{+}, K^{+}, Cl^{-}, Mg^{2+} and NO_3^{-} showed a negative slope with increase in soil depth indication tendency of these ions to accumulate in surface soil in all studied saline-arid habitats (Figure 9 d, e, f).

Discussion

Some plants show adverse effects of salt stress on growth and physiology by the presence of relatively low levels of salts in soils (salt sensitive plants) while others can survive at high soil salinity (salt-tolerant plants) or even some plants flourish under hyper saline conditions (halophytes) (Hameed *et al*, 2008; Kamal *et al*., 2021). Adaptability mechanisms in halophytes are directly linked to the soil physicochemical properties and composition of mineral nutrients and salt concentration in soil solutions (Hameed *et al*., 2008; Arabbeigi, 2014). In present study, soils collected from 18 sites of 5 different habitats were investigated to explore variation in soil composition and mineral nutrients that could have a direct influence on vegetation structure and distribution of native fauna in these regions.

The present study revealed a variable response of soil physicochemical properties and nutrient availability to soil salinity of different habitats. In the present study, rhizospheric soils of different grass ecotypes (*Ochthochloa compressa, Lasiurus scindicus, Panicum antidotale, Cymbopogon jwarancusa, Leptochloa fusca,* and *Aeluropus lagopoides*) were collected from habitats with varying soil salinity ranging from 30 dS m^{-1} to 55 dS m^{-1}. The SP of some habitats NpT, KnP, Pwl, Knw, KKr, and Shw decreased while increased with increasing rhizospheric soil depth of other habitats Ksb, 87A, HsP, Yaz, and H70. This was linked to differential composition of soil particles (sand, silt, and clay) in different soil profiles where the former group of desert environments had sandy soil, and, the preceding group of arid environments had mostly clay-loam soils (Kamal *et al.*, 2021). MC in all habitats invariably decreased with increasing depth that was a sign of increasing dryness of all saline-arid habitats. This can be a reason that the studied desert habitats were mostly inhibited by shallow rooted grasses and other small plants (El-Keblawy, 2016). Among other soil attributes, soil pH did not vary significantly among habitats but slightly increased with increasing depths. OM was evenly distributed (or slightly increased in some cases) within all soil layers studied except in extreme desert habitats (KnP, NpT, Yaz) where it sharply decreased. These findings indicated a strong inhibition of organic matter decomposition in lower soil layers as a direct consequence of increasing dryness with increasing soil depths (Emiru, 2013). Another reason can be reduced leaching of OM to lower layers in dry environments due to extremely low rainfall where OM tends to accumulate in upper soil layers (Emiru, 2013).

Soil Na^+ and Mg^{2+} contents increased with increasing ECe levels of different habitats but K^+ decreased with some exceptions. Soil Ca^{2+} contents strongly decreased both with increasing soil salinity as well as with soil depths except for moderately dry 87A, HsP, and H70 habitats. Considering the soil depths, most salinity causing cations *i.e.* Na^+, Ca^{2+} and Mg^{2+} were concentrated in upper soil layers thereby causing higher ECe levels in surface soils. Such high concentration in upper soil layers was because of higher evapotranspiration rates accompanied by low rainfalls in these saline-arid environments (Manzoor, 2017). Soil K^+ showed an increasing pattern in some saline hyper-arid habitats (NpT, KnP, KsB, Kkr, H70, 87A, Shw) while slightly decreased in some other moderately-saline habitats (Pwl, Knw, HsP). In comparison, NO_3^-, PO_4^{3-} and NH_3 were generally reduced by increasing intensity of salinity, with exceptions of NpT, Yaz and KnP habitats (Fortmeier and Schubert, 1995). It has been reported earlier that salinity causes a significant reduction in anions like NO_3^-, PO_4^{3-}, SO_4^{2-} contents in soil solutions (Nasr *et al.*, 1977). Our findings clearly indicate that high soil salinity disturbed soil nutrients availability thereby causing nutritional disorders in plants by disrupting availability, absorption, and transport (Nublat *et al.*, 2001; Ashraf, 2004; Munns and Tester, 2008). High Na^+ contents in soil solutions also caused a reduction in other nutrients due to a change in permeability of root cell membrane (Greenway and Munns, 1980). External osmotic potential of the saline media reduces the germination and seedling growth of various species by preventing water uptake or due to the toxic effects of Na^+ and other cations on growth of natural vegetation (Murrillo-Amador *et al*., 2002).

The RDA analysis indicated that the distribution of *Pa* (Knw, PkA), *Ls* (HsP) and *Al* (Kkr) ecotypes were strongly influenced by soil ECe, Na^+, PO_4^{3-}, NH_3, NO_3^-, OM, pH, SP, and MC. The soil Cl^- content had a strong influence on distribution of *Cj* of KnP and *Oc* of NpT. *Al* (NpT) was mainly influenced by soil Ca^{2+} content. The remaining ecotypes of different species showed a centered response indicating identical influence of soil properties on their distribution in various saline environments. Since these populations are growing in these hyper saline environments over long time periods, they must have evolved specific adaptation at physiological and anatomical level to cope high salinity (Hameed, *et al*., 2008; Hameed, *et al*., 2010). The response curves for different soil attributes plotted against increasing soil depth showed that most of soil attributes (ECe, Na^+, Mg^{2+}, NH_3, Cl^-, and NO_3^-) showed a negative slope (decrease) with increase in soil depth indicating their accumulation in surface soils. Some soil physicochemical attributes *i.e.* MC and Ca^{2+} and occasionally K^+ and NH_3 exhibited a positive slope with increase in soil depth that clearly indicated their leaching to deep soil layers thereby reducing their availability and uptake by plants. These results indicated that ECe, Na^+,

Mg^{2+}, and Cl^- were the key soil attributes interfering with nutrient availability and limiting growth and survival of these populations in saline environments (Qadir, *et al*., 2006). Some soil physicochemical attributes like SP, OM, and pH did not show any significant change (zero-slope) with increasing soil depth indicating no significant change with soil depth (Emiru, 2013).

Conclusion

The studied habitats exhibited hyper-saline soils accompanied by extreme aridity. The highest topsoil salinity level was recorded as 58 dS m^{-1} for KKr habitat while at least 31 dS m^{-1} was noted in dry NpT desert habitat. In KKr habitat the soil ECe dropped to 50 dS m^{-1} at 25 cm soil depth marking this site as one of the most saline study sites. Generally, some soil physicochemical attributes like SP, OM, and pH did not show any significant change with increasing soil depth. Na^+, Mg^{2+}, and Ca^{2+} were the major cations concentrated in surface soils making the surface soils more saline than deeper soils. Soil K^+, NO_3^-, PO_4^{3-} and NH_3 showed increased leaching tendency to deep soil layers thereby reducing their availability and uptake by plants. To conclude, soil ECe, Na^+, Ca^{2+} Mg^{2+}, and Cl^- were the key soil attributes interfering with nutrient availability and limiting growth and survival of vegetation growing in saline environments.

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