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# Halotolerant bacterial diversity isolated from sodic soil samples of Allahabad, Uttar Pradesh

**Mohammad Hafeez, Rubina Lawrence, PW Ramteke, Suresh BG, Ram Bharose and Harison Masih**

## Abstract
The present research work was conducted to screen the diversity of salinity tolerant heterotrops, *Azotobacters* and phosphate solubilizing bacterial isolates to be used as bio-fertilizer. In present study, sodic soil samples were collected from different villages of Allahabad District was analyzed for their physicochemical properties and isolation of halotolerant bacterial diversity. Results of the study showed that out of 10 soil samples, 8 samples were sodic in nature (ESP ≥ 15 & pH ≥ 8.5) and 2 samples were saline sodic (EC ≥ 4.0, ESP ≥ 15 & pH ≥ 8.5). All samples are low in their available phosphate content and most are recorded low level of available nitrogen content. The maximum cfu/g soil was obtained in Village Panwari which was 74.0 cfu x$10^4$/g soil in Nutrient agar medium, 8.6 cfu x$10^4$/g soil in Ashbey agar medium and 63.0 and cfu x$10^4$/g soil in Pikovaskay agar medium and minimum cfu/g soil was recorded from Pawar, 36.0 cfu x$10^4$/g soil in Nutrient agar medium, 3.00, cfu x$10^4$/g soil in Ashbey agar medium and 29.0 cfu x$10^4$/g soil in Pikovaskaya agar medium. From the results of the study it is concluded that microorganisms are cosmopolitan in nature and can be isolated and applicable for their specific use in nature.

**Keywords:** *Azotobacter*, halotolerant, heterotrops, phosphate solubilizing bacterial, Sodic soil

## Introduction
In India, about 6.74 million ha saline-sodic soils are present. In which Indo-Gangetic Plain covers about 2 million ha of these soils, with a large area in Uttar Pradesh about 1.37 million ha (Rao *et al.*, 2014) [13]. Sodic soils have pH more than 8.5 and Exchangeable sodium percentage (ESP %) greater than 15, these soils has very less organic carbon and Microbial population. Soil degradation resulting from salinity and/or sodicity is a major environmental constrains with severe negative impacts on agricultural productivity and sustainability. The microbial community is fundamental component of ecosystems that play a crucial role in substantial agriculture. The soil is considered to be a complex environment and major reservoir of microbial genetic diversity (Ghazanfar *et al.* 2010) [6]. The biosphere is dominated by microorganisms that have much practical significance in agriculture (Sloan *et al.* 2006) [16].
Microbial diversity is defined as the total number of species (species richness) and relative abundance of these species (species evenness) in the environment. Due to their significance in genetic and biological diversity of microorganisms is an important area of scientific research (Ghazanfar and Azim 2009) [5]. The richness and evenness of bacterial communities effect selective pressures on shape diversity within communities. Microbial diversity is a general term used to include genetic diversity, i.e. the amount of different microbial species and distribution of genetic information within microbial species and ecological diversity, which showed variation in microbial community structure and complexity of interactions between microorganisms. The biological conversion of organic matter into organic acids and $CO_2$ by microorganisms in alkaline sodic soil containing $CaCO_3$ causes a reduction of soil pH and reduction in the exchangeable sodium percentage. Organic acids, aqueous, dissolved $CO_2$ carbonic acid react with $CaCO_3$ within the soil, freeing $Ca^{+2}$ ions. These $Ca^{+2}$ ions may then exchange with $Na^+$ ions absorbed to clay particles, allowing $Na^+$ ions to be leached down further to the soil profile thus decreasing the ESP% (Lehrsch *et al.*1993) [10].
(Mahmoud, *et al.*, 1978) [11] reported about salt tolerant strains of *Azotobacter*. (Hafeez, *et al.*, 2015) [7] reported about halotolerant *Azotobacter* spp. and its bio-formulations. The efficiency of $N_2$ fixing ability of many *Azotobacter chroococcum* isolates was generally better in non saline and slightly saline strains in comparison to saline strains. Suitable management practices for salt-affected soils are obviously different than in the normal soils. Therefore isolation of halotolerant bio-fertilizer strains from sodic soils that can sustain in these soils and their formulation applications can increase nutrient uptake by the plants in sodic soils.

So it is necessary to determine the ability of these bacteria to enhance plant productivity, their diversity, distribution and behavior in indigenous soil habitats because these organisms have a potential to be used as bio-inoculants for local soils.

## Materials and Methods

The present studies were conducted in the Research Laboratory of the Department of Industrial Microbiology, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India.

### Collection of Samples:

Sodic soil samples were collected from different field of Allahabad District. The collected samples were kept in polythene bags and brought to the laboratory for isolation. The samples were used to investigate their physiochemical properties and isolation of salinity tolerant bacterial isolates.

### Isolation of halotolerant bacterial isolates:

Halotolerant bacterial isolates were isolated from sodic soil on modified Nutrient agar media, Ashby's agar medium, and Pikovaskaya agar media using soil dilution pour plate method **(Khan, *et al.*, 2007) [8]**.

## Results and Discussion

### Properties of soil samples collected from different Villages of Allahabad, Uttar Pradesh

The soil samples collected for the isolation of desirable bacteria were analyzed for their different properties are presented in (Table -1). The data obtained showed that out of 10 soil samples analyzed 8 samples were sodic and 2 samples (Bundwan and Mubrakpur) were saline sodic in reaction. The maximum and minimum pH was found in Bundwan and Panwari Soil (9.96 and 8.67) (Fig. 1). In terms of exchangeable sodium percentage the maximum and minimum ESP also found in Bundwan and Panwari Soil (58.80 and 23.70) respectively (Fig. 2). The most of the soil samples (08) were low in available organic carbon and all samples were low in phosphate content. Out of 10 soil samples, 4 were high in available potassium content (> 250 Kg $ha^{-1}$). The properties of the soils are presented in (Table -1). Sodic soils are characterized by the (Sodium absorbtion ratio) SAR ≥ 13 or ESP ≥ 15 and high in sodium relative to calcium and magnesium. Exchangeable Sodium Percentage (ESP) is common measurement of sodium contents. Electrical conductivity (EC) is generally less than 4 dS/m and often less than 2 dS/m and Soil pH is greater than 8.5.

The dispersion of soil particles in sodic soils negatively affect the structure and porosity, which inhibits water movement into and through the soil by clogging pore space and can also cause severe crusting on the surface (Norton and Strom 2012) [12]. Similar study were conducted by (Arora *et al.* 2016) [1] with the saline soil from coastal Gujarat and sodic soil from Indo-Gangetic plains of Uttar Pradesh. The present results are agreements with the finding of (Bhadauria *et al.* (2010) [2] who reported that the chemical characteristics of the soil were in the range of (9.0 11.0) pH; E.C (1.5-7.6 $dSm^{-1}$); SAR (29.13-110.94 %) and ESP (29.4-60.9 %) in sodic soil of Ishwarpur, Village, Mainpuri District U.P, the results are also comparable with the findings of (Damodaran *et al.* 2013) [4] who reported that the pH and E.C ranges of saline sodic soil was 9.65 – 10.2 and 0.75 – 4.55 $dSm^{-1}$ in Raebareily district in Uttar Pradesh, India.

**Table 1:** Physico-chemical properties of soil samples collected from different Villages of Allahabad, Uttar Pradesh

| S. No. | Village | pH (1 : 2) | EC $dSm^{-1}$ (1 : 2) | ESP (%) | Org C (%) | Av. N (kg/ha) | Av. K (kg/ha) | Av. P (kg/ha) |
|---|---|---|---|---|---|---|---|---|
| **Block- Jasra** | | | | | | | | |
| 1. | Gauhania | 9.13 | 1.275 | 38.4 | 0.256 | 112.89 | 255.36 | 14.81 |
| 2. | Tikari | 8.87 | 0.739 | 28.8 | 0.335 | 137.98 | 194.88 | 10.51 |
| 3. | Bundwan | 9.96 | 5.700 | 58.8 | 0.217 | 75.26 | 123.20 | 12.10 |
| **Block- Kaudhiyara** | | | | | | | | |
| 4. | Panwari | 8.67 | 0.865 | 23.7 | 0.492 | 200.70 | 318.08 | 18.54 |
| 5. | Pawar | 9.38 | 1.647 | 49.0 | 0.355 | 144.25 | 232.96 | 17.37 |
| **Block- Phulpur** | | | | | | | | |
| 6. | Mubrakpur | 9.78 | 7.659 | 55.6 | 0.256 | 100.35 | 256.48 | 12.45 |
| 7. | Tardeeh | 9.22 | 0.455 | 28.7 | 0.591 | 200.70 | 161.28 | 18.87 |
| 8. | Parasinpur | 9.56 | 1.867 | 57.2 | 0.197 | 94.08 | 244.16 | 8.91 |
| 9. | Pooresood | 9.23 | 1.329 | 44.2 | 0.270 | 119.28 | 216.16 | 11.64 |
| **Block- Saidabad** | | | | | | | | |
| 10. | Yakoobpur | 9.38 | 1.753 | 48.7 | 0.512 | 156.8 | 257.60 | 9.88 |

**Note:** The soil samples are the mixed 10 different field soil samples from each Village. EC = Electrical conductivity, ESP = Exchangeable sodium percentage, Org C = Organic Carbon, Av N = Available Nitrogen, Av K = Available Potash, Av P = Available phosphoru.

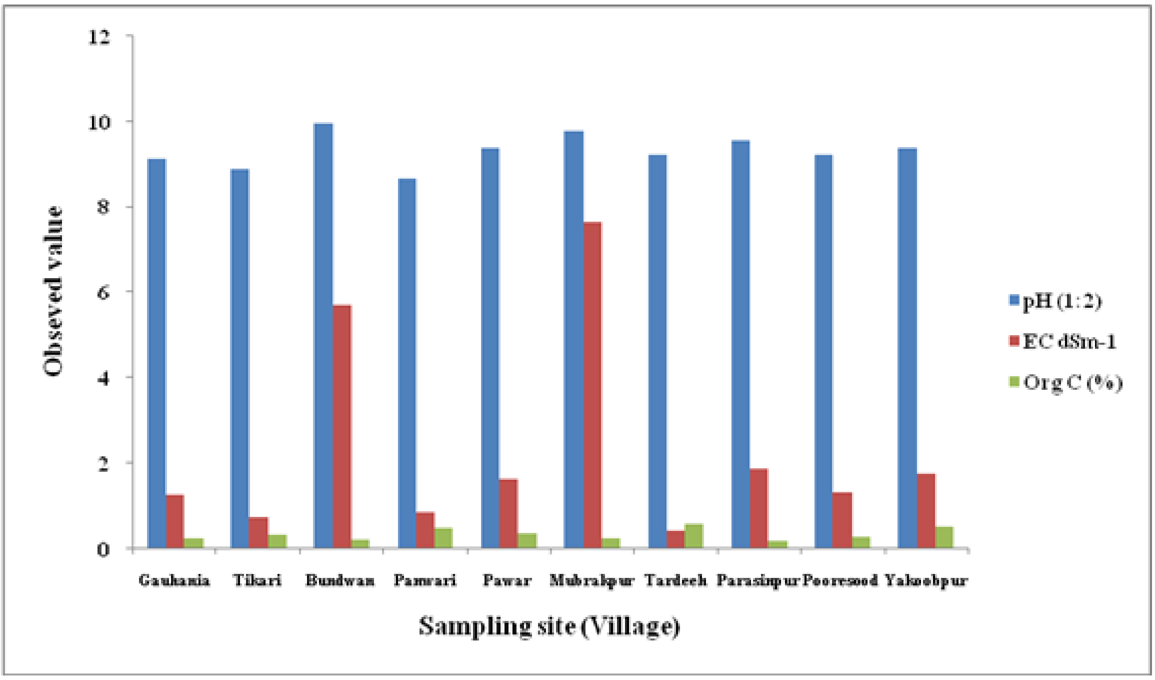


**Fig 1:** Physico-chemical properties of soil samples collected from different Villages of Allahabad, Uttar Pradesh

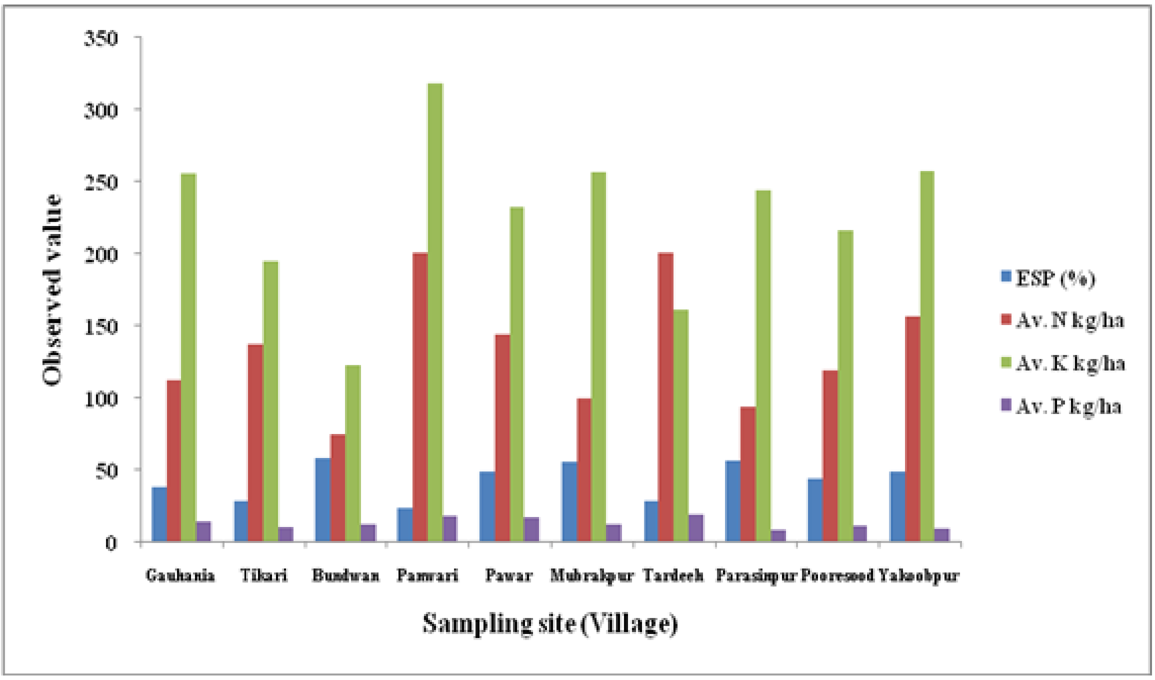


**Fig 2:** Chemical properties of soil samples collected from different Villages of Allahabad, Uttar Pradesh

**Isolation of halotolerant bacterial isolates**

The results depicted in (Table -2) showed that the soil samples from different Villages were examined for isolation of *Azotobacter* and phosphate solubiliziting bacteria. Out of 100 samples studied, 55 halotolerant bacterial isolates were isolated. Out of this 55 isolates 15 isolates were *Azotobacter* and 40 isolates as phosphate solubilising bacteria. In addition, for the calculation of total heterotrops were obtained. The maximum cfu-count were found in soil of Panwari Village, which was 74.0 cfu x$10^4$/g soil in Nutrient agar medium, 8.6 cfu x$10^4$/g soil in Ashbey agar medium and 63.0 and cfu x$10^4$/g soil in Pikovaskay agar medium. The minimum heterotrops were recorded in Village Pawar which was 36.0 cfu x$10^4$/g soil in Nutrient agar medium, 3.00, cfu x$10^4$/g soil in Ashbey agar medium and 29.0 cfu x$10^4$/g soil in Pikovaskaya agar medium (Fig. 3). The data was statistically analyzed by analysis of variance and found significant at 5% level of significance ($p$<0.05).

The results are correlated with the findings of (Kochar and Gera 2015) [9] who reported that the total bacterial counts of sodic soils on Jensen's, malate, Pikovaskaya's, King's B and soil extract agar media ranges from 3.44 - 6.23, 4.17 - 6.44, 3.77 - 5.45, 3.26 - 6.06 and 5.85 - 8.00 log cfu g-$^1$ soil, respectively. Moreover, it was also observed that salinity or sodicity levels of the soil samples affected the bacterial population. Damodaran *et al.* 2013 [4] reported that the population of bacteria ranged from 0.5 to 9.0 cfu gm-$^1$ soil in sodic soils. Saline and sodic soil effect about population and distribution in *Azotobacter* sp. sodic soil inhibit growth of *Azotobacter* sp. (Singleton *et al.* 1982) [15]. It has been reported that soil salinity plays an important role in the microbial selection process as environmental stress leads to reduce microbial diversity (Borneman *et al.* 1996) [3]. Isolation of these organisms from samples were clearly confirmed their cosmopolitan nature as reported by (Skinner and Bernfield 2005) [14]. On the basis, screening of the nitrogen fixing ability of *Azotobacter* and salt tolerance capacity of the isolates, potential 8 isolates of *Azotobacter* were selected for the further study.

**Table 2:** Effect of total viable count of sodic soil samples of different villages of Allahabad, Uttar Pradesh

| Sample no. | Sampling site (Village) | (CFU x$10^4$/g soil) | | |
|---|---|---|---|---|
| | | Total heterotrops | Ashbey medium for *Azotobacter* | Pikovaskaya medium for (PSB) |
| 1. | Gauhaniya | 60.7 | 5.1 | 48.5 |
| 2. | Tikari | 51.0 | 4.0 | 43.0 |
| 3. | Bundwan | 50.3 | 4.3 | 41.0 |
| 4. | Panwari | 74.0 | 8.6 | 63.0 |
| 5. | Pawar | 36.0 | 3.0 | 29.0 |
| 6. | Mubrakpur | 57.3 | 5.0 | 44.0 |
| 7. | Tardeeh | 65.0 | 6.6 | 55.0 |
| 8. | Parasinpur | 40.6 | 4.7 | 34.0 |
| 9. | Pooresood | 46.6 | 4.0 | 41.0 |
| 10. | Yakoobpur | 58.0 | 4.6 | 36.0 |
| | | Result | S.E. (±) | C.D. at 5% |
| | Due to Heterotrops | S | 2.605 | 5.184 |
| | Due to Sampling site | S | 1.682 | 3.346 |

**Note:** Total viable counts are the average values of composite soil samples of each Village

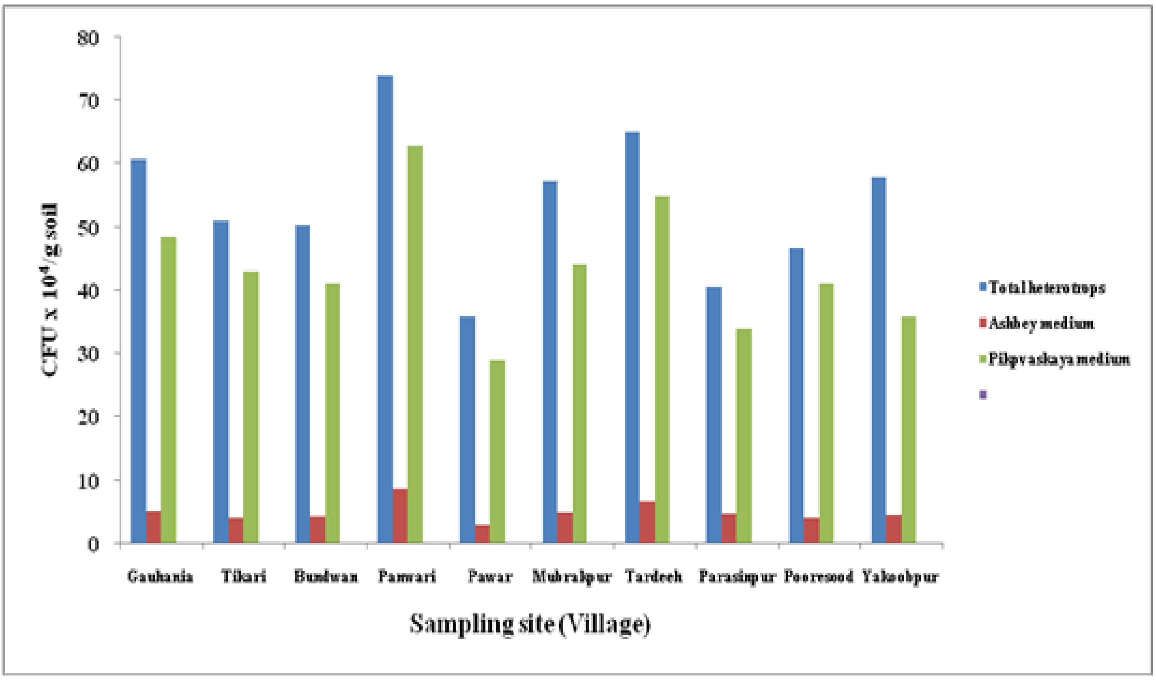


**Fig 3:** Incidence of total viable count of sodic soil samples of different villages of Allahabad, Uttar Pradesh


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