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Effect of acidic soil on bacterial diversity in selected wheat fields of Gopalganj district in North Bihar

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Abstract : The present study was related to documentation of Bacterial diversity in selected sites with difference in soil acidity as pH is critical factor for shaping the microbial patterns in wheat field soils. The site 1 represented acidic soil, while site 2 was neutral due to upland location. Enumeration of heterotrophic bacteria and fungi were performed through heterotrophic plate count (HPC) with three replicates using conventional plating techniques from soils decimally diluted by sterile distilled water.

The bacterial phyla retrieved from site 1 were more diverse than those at site 2, and their bacterial compositions were quite different. Almost one third phyla at site 1 were protobacteria with 6 other phyla and in contrast soil at site 2 comprised of three main phyla as Actinobacteria, Bacteroidetes and Proteobacteria. Furthermore, only two species was common at both sites. The acidic soil of site 1 represented a non-optimal pH for bacterial growth and thus bacterial diversity, eveness and richness at this site were higher than those found in the site 2.

These results and the indices of population parameters examined in this study indicated that acidity might not play a main role for bacterial diversity in soil.

Keywords: Acidity, Bacterial diversity, Biodiversity indices.

INTRODUCTION

The soil environment composed of water and sediment are the most influencing complex for all microbial habitats. Amann et al. (2001) firstly quantified microbial diversity, however, geographical distribution yet to be partially investigated (Madigan et al. 2010). The major problem overlooked in used method of analysis. In past, culture media method was applied but recently it was realized that microbial diversities determined with use of cultivation-based methods due to the restriction of culture condition and media accounted for only 1% of total bacterial communities (Torsvik et al. 1990).

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In addition, recent studies have shown relations between microbes and environmental factors, such as geographical location, soil texture, land use (Hartman et al. 2008) Ph (Lauber et al. 2009), nutrients (Han et al. 2008), contaminants like oil (Liang et al. 2011), and heavy metals (Lee et al. 2008). The pH is regarded one of the most important factor in all of abovementioned in terms of shaping bio-geographical patterns (Lauber et al. 2009).

In different pH of soils, microbial community and its growth is also variable due to heavy metal deposition and anion availability. Furthermore, a major question arises in the case of pollution-free environment about factor that has the greater effect on microbial community dynamics. Therefore, the purpose of this study was to compare bacterial community structure in natural neutral soil obtained in a highland wheat fields with geochemical

parameters such as pH, concentration of ions and heavy metal concentration.

MATERIALS & METHODS

Soil samples were collected to determine the bacterial community structures of soils obtained from two selected sites with different pHs and ecological stability. The first sample was collected from diara region due to use of inorganic fertilizers, pesticides and anthropogenic activities in nearby Gandak River and site 2 of upland field with neutral pH. At each site, about 200 g of minimally disturbed soil with high litter contents was obtained from a depth of 10 cm at 5 to 10 randomly selected locations within an area of 100 m^2. Soil samples were homogenized and examined for microbial population.

The pH values of air dried soil were measured using a pH Meter after mixing them with distilled water in a ratio of 1:5 (w:v) for 30 min. Enumeration of heterotrophic bacteria and fungi using culture media. The heterotrophic plate count (HPC), were enumerated by three replicates using conventional plating techniques from soil samples decimally diluted with sterile distilled water. Bacteria and fungi were cultured by Nutrient Agar or Potato Dextrose Agar, respectively, and incubated at 30 °C for 48 h. Counting revealed 30 to 300 colonies per plate, and mean values were expressed in colony-forming unit (CFU) per gram of soil (dry weight).

The assessment of bacterial diversity was calculated Shannon-Weaver indices (H''), Simpson indices, evenness indices (E) developed by Pielou, and richness by Margalef. To unify the measurement criterion regarding diversity indices of Shannon-Weaver and Simpson, the reciprocal of the Simpson index was used because Shannon-Weaver index is a high number for high diversity, but Simpson index is a low number for high diversity.

RESULTS & OBSERVATIONS

The soil from site 1 had an acidic pH of 5.2, while that from site 2, which was located in upland area was almost neutral (pH 7.7).

The total concentration of anions at site 1 was greater than at site 2 (Table 1). In particular, the concentration of NO_3", which can be utilized immediately by microbes and plants, was much higher at site 1 (12.7 cmol /kg) than at site 2 (0.04 cmol /kg).

The CEC of 30.7 meq/100 g determined for soil from site 1 indicates relatively better conditions for microbial growth than at site 2 (13.0 meq/100 g). We observed bacteria belonging to the phylum Acidobacteria and the genus Prosthecobacter sp. (phylum Verrucomicrobia) were found to be more diverse in soils with small particles at site 1, whereas α-Proteobacteria dominated in large particle soils of site 2 (Table 2).

The study about occurrence of heavy metals revealed Chromium and Hg at both sites. Addressing the remaining, other metals in decreasing order, Zn was detected at the highest concentration at both sites, although its value at site 1 (47.26 mg/kg) was lower than at site 2 (58.43 mg/kg).Pb and Ni were present at site 1 at slightly higher concentrations (19.43 and 13.27 mg/kg vs. 13.04 and 11.44 mg/kg), which was unexpected. Cu had concentrations of 11.67 and 14.25 mg/kg at sites 1 and 2,respectively. So the concentrations of heavy metals, with the exception of Pb and Ni, were higher at site 2 than in soil from the site 1, as depicted in Fig. 1

In addition, contamination by Cd or Pb at concentrations of 5–55 mg/kg and 75–1660 mg/kg, respectively, reduced of bacterial numbers by up to 1 %. Accordingly, it would appear the relatively low concentration of heavy metals found at both sites was insufficient to have affected microbial growth. However, the slightly higher heavy metal levels at site 2 might have had a negative effect on the dynamics of the bacterial community.

Moreover, in accord with the abovementioned results concerning the geochemical property of soils, the number of heterotrophic bacteria/fungi was higher at site 1 than at site 2 (Table 2). In the site 1 soil sample, were more than ten times greater than that at site 2. Thus, the geochemical environment at site 1 seems to be more favorable for microbial growth than that at site 2.

Bacteria at both sites were affiliated with 12 phyla across the entire data set. There was 11 phyla at site 1 and 6 phyla of bacteria widely distributed. Three phyla, Proteobacteria (49.2 % at site 1 and 21.8 % at site 2), Actinobacteria (21.8 % site 1 and 29.8 % at site 2), and Cyanobacteria (9.8 % at site 1 and 17.5 % at site 2) dominated both sites. The next most abundant phylum, Planctomycetes (7.2 %), at site 1 was not observed at

site 2, whereas Bacteroidetes, which is regarded as a typical inhabitant of soil (Madigan et al. 2010), was much more abundant at site 2 (site 1; 0.5 % at site 1 and 24.3 % at site 2). Acidobacteria, known as bacteria occurring frequently in not only acidic soil but in all kinds of soil (Lauber et al. 2009) accounted only for a small proportion at both sites (2.7 % at site 1 and 1.1% at site 2, Table 3).

Table 1. Chemical properties of the analyzed soil.

Sample	pH	CEC (meg/100g)	Anion (cmol/kg DW) F	Cl	Nitrate	Sulphate	Total	Cation (cmol/kg DW) Ca	K	Mg	Na	Total
Site 1		30.8	.03	.52	1.26	1.19	3.0	10	.74	.98	.20	13.89
Site 2	7.8	13	.13	.47	.05	.98	1.63	12.1	.18	.62	.16	12.99

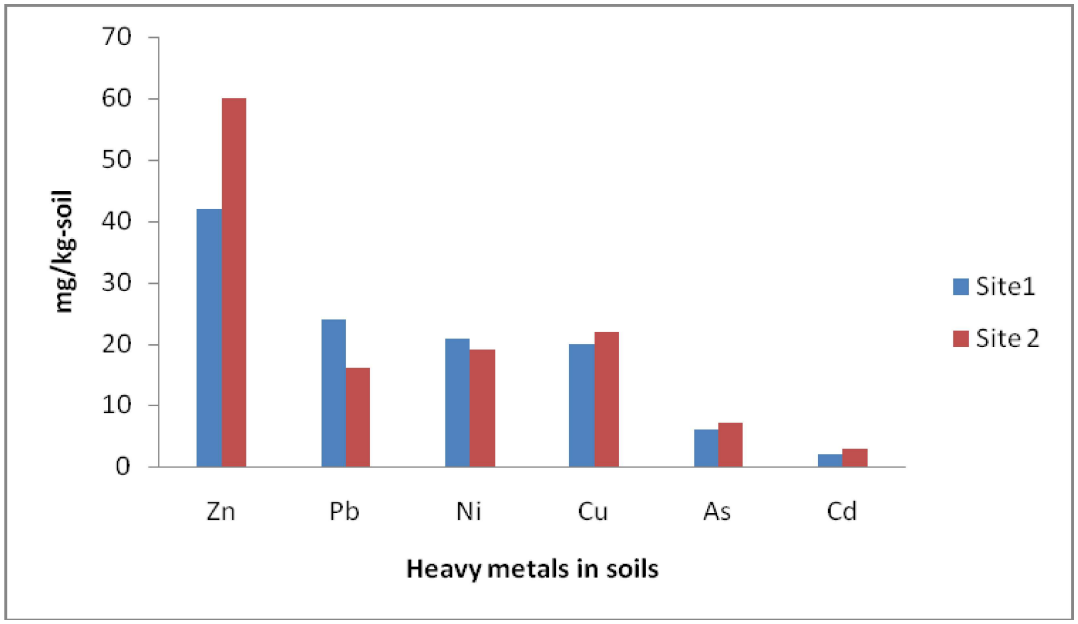

Figure 1. The concentrations of heavy metals in selected soils of site 1 and site 2.

Table 2. Microbiological characteristics evaluated by culturing bacteria and fungi.

Sample	HPC (bacteria CFU/g DW)	(Fungi CFU/g DW)
Site 1	9.2×10^{7}	3.6×10^{7}
Site 2	8.0×10^{7}	6.0×10^{7}

Table 3. Comparison of soil bacteria composition between two sites on the phylum level.

Phylum	Site 1 (%)	Site 2 (%)
Proteobacteria	49.2	21.8
Actinobacteria	21.8	29.8
Cyanobacteria	9.8	17.5
Bacteriodetes	0.5	24.3
Planctomycetes	7.3	0
Acidobacteria	2.6	1.1
Firmicutes	1.3	5.5
Chloroflexi	2.5	0
Verrucomicrobia	2.1	0
Elusimicrobia	1.2	0
Gemmatimonadates	0.3	0
Unidentified	1.4	0.1

Therefore, more detailed studies, including its functional capability, are needed to determine why these bacteria are present in given soil.

DISCUSSION

The optimal range (pH 6–8) required for microbial growth (Maier and Pepper 2009). The pH value of soil affects the solubility of chemicals by influencing ionization degrees (Maier and Pepper 2009). It should be added that the pH values at the two sites mentioned above are integrated results due to numerous interactions between cations and anions in the soil solution (Fierer and Jackson 2006). Just the large difference in pH values at the two sites implies that the geochemical environment of both sites differed. As has been reported by others (Lauber et al. 2009), we presumed that pH played a definite role on the diversities and compositions of bacterial community.

CEC, a measure of the capacity of soils and organic colloids to remove cations from solution, varies depending on the type of soil, and its value increases in line with the decomposition rate of organic matter by micro-organisms (Alexander 1977). According to Maier and Pepper (2009), the average of CEC of soils range from 15 to 20 meq/100 g, and that CEC values of <15 meq/100 g leads to low nutrient levels in soil because of a reduced capacity to retain cations and essential nutrients, such as NO_3 and PO_4. Particle size might change chemical properties by changing adsorption affinities (Maier and Pepper 2009). In addition, the availabilities of nutrients and organic matter also strongly influence bacterial abundances and diversities (Han et al. 2008).

It has been reported that heavy metals can not only inhibit microbial growth and activity but also shift bacterial populations from heavy metal nonresistant to resistant populations over time (Kelly et al. 1999; Roane and Pepper 2000). Kelly and coworkers (1999), in a laboratory investigation on the effects of a Zn smelter on microbes, added 6000 mg/kg of Zn to soil, and 15 days later found Zn level in soil had reduced to 4660 mg/kg, which was ascribed to adsorption on the surfaces both of soil and microbes (Lee et al. 2008), and that cultured bacteria (isolates) had reduced by 87 %. However, over the course of the experiment, it was found that the bacterial composition had changed from a nonresistant to a resistant population.

The numbers of heterotrophic bacteria (HPC) which is considered as an indicator of easily degradable organic compounds (Maier and Pepper 2009) were evidenced for degradable activity of microorganisms more at site 1.

CONCLUSIONS

In order to determine the effect of pH on bacterial diversity, we compared the bacterial communities of acidic soil (pH5.2) from site 1 with neutral upland soil at site 2. The pH may have an effect on bacterial diversity and thus the bacterial community in soil at site 1 was found to differ from that at site 2, and although the acidic soil of site 1 represented a non-optimal pH for bacterial growth,

the bacterial diversity, evenness, and richness at this site were higher than those found in the neutral pH soil at site 2. Accordingly, these results imply that pH might not be a critical factor for shaping bacterial diversity and its stability. However, this study was performed over a short period without regard of the effects of other environmental factors, such as precipitation, oxygen, and some other primary nutrients such as N and P. So nothing could be considered as conclusive proof. Therefore, we suggest more advanced detailed studies in a long term should be conducted to identify the environmental factors responsible for the establishment of particular bacterial community structure.

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