Land use Effect on the occurrence and distribution of Azotobacter in Hamadan soils, Iran

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Abstract
Azotobacter is a nitrogen-fixing bacterium, found in soils world-wide, with many features relevant to energy consumption and carbon sequestration. For assessment the effects of agricultural practices and land use on the occurrence and distribution of Azotobacter, the present investigation was made in the pastures, deciduous and coniferous woodlands, and dry and irrigated (with sewage and river waters) farmlands of Hamadan in northwestern of Iran. Sampling was carried out at depth of 0-30 cm with maximum of plant cover diversity in may, 2003. According to heterogeneity of lands, sampling plan was completely randomized with unequal numbers of repetitions. Some soil physical, chemical and biological properties were investigated. Data statistically analyzed for standard deviation (s), and F-test to assess the land use effect on each soil property. Means were calculated and Duncan’s new multiple range test was made to assess the soil management systems. The highest population of Azotobacter was found in soil sampled from sewage water irrigated farmland. Among soils, dry farmlands and deciduous woodland soils had the lowest fertility. The lowest population of Azotobacter was found in soil sampled from deciduous woodland. The occurrence of Azotobacter correlated positively with soil organic matter (SOM), electrical conductivity, total nitrogen, available phosphorous, available potassium, C/N ratio, and substrate-induced respiration (SIR) and negatively with soil carbonates.

Key Words: Azotobacter, management practices, soil properties.

Introduction
Soil microorganisms promote plant growth in many ways. Azotobacter can affect plant growth directly, either by the nitrogen it fixes (Zapater et al. 1982 and Zaid, 1992), or through growth promoting substances, indol-3-acetic acid, gibberellins and cytokinins (Barea and Brown, 1974; Pareek et al. 1996 and Zahir et al. 1997), or indirectly by change in the microflora of the rhizosphere (Barea and Brown, 1974).

Preliminary study of occurrence of Azotobacter chroococcum in soils have shown that its population is lower than 10⁵ in 1 g of dry soil (Subba Rao, 1993). Occurrence of Azotobacter is strongly depended on soil properties and agricultural practices. Azotobacter was abundant in soils near neutrality, and decreased in accordance with increasing acidity, being absent at pH values of 5.80 and below (Harris, 1973). Soil organic matter is an important factor affecting soil biological activities. The stimulating influences of humic and fulvic acid on the growth and efficiency of nitrogen fixation of Azotobacter have been reported by Gaur and Mathur, (1966) and Bhardwaj and Gaur, (1970). Mishustin and Shilnikova, (1971) reported that application of nitrogen and phosphorus fertilizers have significant negative and positive effects on Azotobacter population respectively. Hartley and Schlesinger (2002) used the acetylene reduction assay to analyze soil nitrogenase activity at the Jornada Long-Term Ecological Research site (northern Chihuahuan Desert, New Mexico, U.S.A.). Their findings indicated that labile carbon and
inorganic N may exert a stronger control on nitrogenase activity than phosphorus or micronutrient levels.

The objectives of this study were (i) to determine the effects of management practices on the occurrence and distribution of Azotobacter in soil, and (ii) to investigate the relationships of Azotobacter populations with some biological and physico-chemical properties of soil in different uses in Hamadan, northwestern of Iran.

**Materials and Methods**

The present investigation was made in dry and irrigated farmlands, pastures, deciduous and coniferous forests of Hamadan, in northwestern of Iran. In May, 2003, sampling was carried out at depth of 0-30 cm from root zone of plants with maximum diversity. According to heterogeneity of the experimental sites, sampling plan was completely randomized with unequal numbers of repetitions (≥3).

Soil samples were analyzed for clay, silt, sand, equivalent CaCO₃ (CCE), pH, electrical conductivity (EC), Cation-exchange capacity (CEC), organic carbon (OC), total nitrogen (TN), Olsen available phosphorus, available K, basal respiration, and substrate induced respiration (SIR) according to methods of soil analysis parts 1 and 2: published by SSSA (Klute, 1986; Page, et al, 1982), and applied methods in soil biology and biochemistry (Kassem and Nannipieri, 1995). Azotobacter populations were estimated by plate count method. Soil suspension and dilutions were prepared. Two media were used for study of Azotobacter population in soil samples. The first one was Ashby’s mannitol agar. For inhibition of the growth of gram positive bacteria and actinomycetes it was modified by addition of 1 ml crystal violet solution (0.5 % in ethanol). The second media for Azotobacter enumeration was LG medium. Colony forming unites on the solid media were numbered after a week of incubation at 27 °C (Kassem and Nannipieri, 1995; Subba Rao, 2001).

Data statistically analyzed for standard deviation (s), and F-test to assess the land use effect on Azotobacter populations. Means were calculated and Duncan’s new multiple range test was made to assess the soil management systems. Pearson linear correlations were performed to ascertain whether the Azotobacter populations were correlated with soil physical and chemical properties. So, the relationship between Azotobacter populations and the other soil properties were analyzed by correlation analysis.

**Results**

Azotobacter populations in soils sampled from farmlands irrigated with wastewater and river water numbered on Ashby’s medium were $10.06 \times 10^6$ and $6.13 \times 10^6$ g⁻¹ soil respectively. They are significantly higher than that numbered in the other soils (FIG.1). Likewise, Azotobacter population in soil sampled from farmlands irrigated with wastewater numbered on LG medium was $30.65 \times 10^6$ g⁻¹ soil, having a significant difference with the other soils. Azotobacter population in soils of the coniferous forest was the lowest one. It was also considerably low in soils sampled from the ranges and dry farmlands. Estimate for Azotobacter population on LG medium was $11.66 \times 10^6$ g⁻¹ in soils sampled from farmlands irrigated with river water. It was $10.65 \times 10^6$ g⁻¹ in soils from deciduous forest. These estimates are significantly different from the population estimated for the coniferous forest ($3.75 \times 10^6$ g⁻¹ soil).

Estimates for Azotobacter population by LG medium with compare to Ashby’s medium were higher. It is related to the differences between their constituents. Carbon and energy sources used
in the both media are different, and crystal violet used in modified Ashby’s medium may be more toxic than bromothymol blue used in LG medium.

Land use and management practices can change soil properties, controlling soil microbial population and activities. Table 1 shows the relationship between the Azotobacter population and some of the other soil properties. In these calcareous soils the correlation between Azotobacter population and soil carbonates was negative and significant. The correlation between Azotobacter population and soil salinity was positive in these non-saline soil.

The correlation coefficients between Azotobacter population and soil organic carbon, total nitrogen, available P and K were positive and strong. Strikingly basal respiration exhibited a significant negative correlation with Azotobacter numbered on LG medium. Soil basal respiration highly depends upon both the soil organic matter and microbial populations. The investigated soil had low organic matter so organic carbon limitation on basal respiration is more than limitation of microbial populations. There were significant positive correlation coefficients between substrate induced respiration and soil Azotobacter populations numbered on Ashby’s and LG medium. The associations and positive correlation coefficients between SOM, microbial biomass, and fine particles are well documented in vegetated soils (Gupta and Germida, 1988; Schnitzer and Kodama, 1992; Hassink et al., 1993; Kiem and Kandeler, 1997; Ley, et al., 2001). Maximum respiration response upon addition of substrate (SIR) is proportional to the size of the living microbial biomass (Anderson and Domsch, 1978).

Azotobacter populations numbered on Ashby’s and LG medium showed strong positive correlations with soil C/N ratio.

Discussion
The results indicated that there are significant differences between the biological parameters of soils, used and managed differently. Coniferous forest with low level of fertility showed a lower biological activity namely Azotobacter population. Allelopathic compounds such as tannins and aromatics may be toxic for bacteria and especially Azotobacter species (Inderjit and Weston, 2001). Irrigated farmlands especially those irrigated with raw municipal wastewater compared to coniferous forest had the highest microbiological activities.

This study supports earlier findings that organic fertilization rapidly benefits soil microbial biomass and activity, but provide few indications that the irrigation with wastewater affect soil microbial biomass, community structure, or activity. Although it is beyond the scope of this study to address possible effects of use of the wastewater in irrigation, it appears that there are strong and positive relationships between soil fertility and some microbiological indices.

Acknowledgments
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References


Figure 1. Effects of management practices on the Azotobacter population in different land. 1) Coniferous forest, 2) Deciduous forest, 3) Ranges, 4) Dry farmlands, 5) Farmlands irrigated with river water and 6) Farmlands irrigated with untreated municipal wastewater. (Values for each medium followed by different letters are significantly different at the 0.05 probability level).

Table 1. Pearson correlation coefficients of the soil Azotobacter populations and some soil physico-chemical properties.

<table>
<thead>
<tr>
<th></th>
<th>Azotobacter in Ashby’s medium</th>
<th>Azotobacter in LG medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand</td>
<td>0.170248</td>
<td>0.141409</td>
</tr>
<tr>
<td>Silt</td>
<td>-0.2475</td>
<td>-0.31134 *</td>
</tr>
<tr>
<td>Clay</td>
<td>-0.00683</td>
<td>0.118442</td>
</tr>
<tr>
<td>CEC</td>
<td>0.02781</td>
<td>-0.07043</td>
</tr>
<tr>
<td>CCE</td>
<td>-0.23201 *</td>
<td>-0.27356 **</td>
</tr>
<tr>
<td>EC</td>
<td>0.40437 **</td>
<td>0.35574 **</td>
</tr>
<tr>
<td>.pH</td>
<td>-0.07953</td>
<td>-0.12382</td>
</tr>
<tr>
<td>OC</td>
<td>0.43995 **</td>
<td>0.46560 **</td>
</tr>
<tr>
<td>Total N</td>
<td>0.45153 **</td>
<td>0.49216 **</td>
</tr>
<tr>
<td>Available P</td>
<td>0.59433 **</td>
<td>0.35217 **</td>
</tr>
<tr>
<td>Available K</td>
<td>0.52382 **</td>
<td>0.31009 **</td>
</tr>
<tr>
<td>C/N ratio</td>
<td>0.233015 *</td>
<td>0.211862 *</td>
</tr>
<tr>
<td>Basal respiration</td>
<td>-0.01963</td>
<td>-0.20405 *</td>
</tr>
<tr>
<td>SIR</td>
<td>0.49180 **</td>
<td>0.4142 **</td>
</tr>
<tr>
<td>Azotobacter in Ashby’s m.</td>
<td>1</td>
<td>0.47497 **</td>
</tr>
</tbody>
</table>

** Correlation is significant at the 0.01 level.
* Correlation is significant at the 0.05 level.