Chapter 5

Effect of Some Anions on the Mobility of Amino Acids in Soils by Thin Layer Chromatography
5.1 INTRODUCTION

Among the anionic forms of nutrients MoO\(_4^{2-}\), B\(_4\)O\(_7^{2-}\), C\(_2\)O\(_4^{2-}\), CO\(_3^{2-}\), HCO\(_3^-\) and H\(_2\)PO\(_4^-\) are important as these are utilized by plants in their anionic forms as such. Among these, MoO\(_4^{2-}\) and B\(_4\)O\(_7^{2-}\) are considered as micronutrients and play a vital role in the life process of plant development. Molybdenum is required for the reduction of absorbed nitrates into ammonia prior to formation of amino acids. It performs this function as a part of enzyme nitrate reductase. Molybdenum is also essential for nitrogen fixation carried out by nitrogen fixing bacteria in legumes. Responses of legumes to molybdenum application are mainly due to its need by the symbiotic bacteria. On the other hand, the role of boron is important in sugar transport within the plant. Boron has a role in cell division and is required for the production of certain amino acids. The application of boron in the form of solubor is preferred as it is highly soluble than sodium tetra borate. The calcium boron ratio in leaf tissues has been used to assess the boron status of crops. Boron deficiency is indicated by ratio >1200:1 for most crops. The total boron in soils varies from 7 to 80 ppm of which less than 5% boron of the total soil is available for plant use. It has also been revealed in literature that several organic compounds including carboxylic acids are produced in soils as results of chemical and/or biochemical [1] decomposition of organic matter remains in soil environment. These anionic species are found to play an important role in the translocation of plant nutrients and toxic heavy metals in soils. The CO\(_3^{2-}\), HCO\(_3^-\) ions are present in soils as these are released by their soluble salts. However, the phosphorus is the major nutrients and required in greater quantity. The most essential function of phosphorus in plants is energy storage and transfer. An adequate supply of phosphorus is required in an early stage of a plant development of its reproductive parts. Phosphatic fertilizers are applied to soils by farmers to get higher yields.

The significance of amino acids is well known in relation to soils and plants. Amino acids are found to exist in soils as a result of decomposition of proteins and soil organic matter [2, 3] and play a vital role in biochemical processes [4]. They are absorbed by clay to form clay amino acid complexes [5] and their products such as
indoles [6] act as growth promoting substances and hormones in plants. The role of these anions on the mobility of amino acids need to studied to know their significance in soil. This prompted to carry out such study by using soil thin layer chromatography, which has become an inexpensive tool for the mobility determinations in soils [7, 8].

5.2 EXPERIMENTAL

The soil (0-30 cm) sample was collected from agricultural farm of Aligarh Muslim University, situated in Aligarh district (U.P.) India. The Physio-chemical properties of the soil were determined by using analytical methods as described in chapter 2 and the values obtained are recorded in Table 5.1.

**Chemicals and Reagents**
All chemicals and reagents used were of Analytical Reagent grade.

**Stationary Phase**
Soil

**Mobile Phase**
Aqueous solution of (0.2 M) of \( \text{Na}_2\text{MoO}_4^{2-} \), \( \text{Na}_2\text{B}_4\text{O}_7^{2-} \), \( \text{Na}_2\text{C}_2\text{O}_4^{2-} \), \( \text{Na}_2\text{CO}_3^{2-} \) and (0.6 M) of \( \text{NaHCO}_3^- \), \( \text{NaH}_2\text{PO}_4^- \) salts with adjusted ionic strength (\( \mu = 0.6 \)) in 0.6 M \( \text{NaClO}_4^- \).

**Amino Acids Studied**
Alanine, serine, valine, leucine, histidine and glutamic acid.

**Test Solutions**
The test solutions (0.2 M) of all amino acids were prepared in double distilled water.

**Detector**
0.2% Alcoholic solution of ninhydrine (w/v).

**CHROMATOGRAPHY**

**Preparation of Soil TLC Plates**
For the preparation of soil TLC plates similar procedure was followed as described in chapter 3.

**Procedure**
An aqueous solution of 10 µL of each amino acid (0.2 M) was spotted at the base line on separate TLC plates with help of a micropipette. The plates were developed in closed chamber using distilled water (control) and various anionic solutions viz., (0.2 M) of \( \text{MoO}_4^{2-} \), \( \text{Ba}_4\text{O}_7^{3-} \), \( \text{C}_2\text{O}_4^{2-} \), \( \text{CO}_3^{2-} \); (0.6 M) of \( \text{HCO}_3^- \), \( \text{H}_2\text{PO}_4^- \) at constant ionic strength (\( \mu = 0.6 \)) as a mobile phase and allowed to develop up to a distance of 10 cm by ascending chromatographic technique. To prevent disintegration of soil in contact with developer, wetted strips of filter paper (about 2.5 cm wide) were wrapped around the bottom of plates before the development.

Developed plates were air-dried. The detection of amino acids on TLC plates was done by spraying 0.2% alcoholic solution of ninhydrin and heating in an oven at 70-80 °C for 10-15 min. Amino acids were detected as brick red colored spots, which were stable for several hr.

The \( R_F \) values in each case was calculated using the relation,

\[
R_F = 0.5 (R_L + R_T)
\]

Where,

\( R_L \) = Leading distance moved by amino acid.

\( R_T \) = Trailing distance moved by amino acid.

### 5.3 RESULTS AND DISCUSSION

An examination of the \( R_F \) values of the cyclic as well as aliphatic amino acids (Fig. 5.1) in natural soil (0 dose) follow the order: glutamic acid > histidine > valine > leucine > serine > alanine, that are in accordance with the same order of molecular weight (Table 5.2) except in the case of glutamic acid and leucine. The higher mobility of glutamic acid as compared to histidine may be due to the presence of two carboxylic groups indicating enhanced -I effect than imidazole ring present in histidine, thus making the glutamic acid less adsorptive due to the creation of negatively charge carboxylate ions and thus move at the faster rate. The higher mobility of valine than leucine may be due to its higher solubility in water. The mobility order: valine > leucine > alanine can be explained on the basis of the nature of alkyl groups capable of exerting +I effect making the nitrogen of -NH₂ group richer
in electron density posing unfavorable conditions for making the molecule to form zwitter ions. Thus making the molecule less attractive with the negatively charged sites of the soil colloids and move in the same order.

The effect of anionic species of the plant nutrients (Fig. 5.1) indicates that the initial lower concentrations of the anions used as mobile phase lead to the increase in the amino acids transport through soils. But at their higher concentrations the mobility is found to gradually decrease in most of the cases except in case of glutamic acid that followed a decreasing trend throughout the entire study. The increasing trend may be attributed to the blocking of the exchangeable/adsorptive sites by the preferential adsorption of sodium ions released from the respective salts of these anionic nutrients at their lower concentration as follows,

\[
\text{SOIL} \rightarrow M + Na - X \rightarrow \text{SOIL} \rightarrow Na + M^{n+} + X^{n-}
\]

Where M represent naturally occurring cations (Ca\(^{2+}\), Mg\(^{2+}\), K\(^{+}\), Na\(^{+}\) etc) at the exchangeable sites of the soil colloids; X denotes MoO\(_4\)^{2-}, B\(_4\)O\(_7\)^{2-}, C\(_2\)O\(_4\)^{2-}, CO\(_3\)^{2-}, HCO\(_3\)^{-} and H\(_2\)PO\(_4\)^{-}.

It is found that the exchangeable or adsorptive sites of the soil colloids get blocked. Thus facilitating the movement of amino acids to move at the faster rates. However, higher concentrations of these negatively charged anions might play a decisive role in the amino acids mobilization process due to their interaction with sodium saturated soils. This can be explained as: since the soil pH is 8.5 and becomes more alkaline with the increasing concentrations of the sodium salt of anionic species, as a result the adsorbed sodium ions may be converted into their hydrated forms that could possibly have exerted an attractive influence on these anions through hydrogen bonding as:

\[
\text{SOIL} \rightarrow Na-O-H + X^{n-} \rightarrow \text{SOIL} \rightarrow Na-O-H-X^{n-}
\]

Where \(n=1, 2\), the no. of negative charge

The adsorption of anions through hydrogen bonding/vander waals forces on specific sites is most likely to happen as each anionic species contains some active oxygen atoms capable of forming hydrogen bond in an alkaline soil medium. This
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contributes the net negative charge sites on the soil system. The electron density on these soil systems will vary and is expected to be governed by the nature and concentration of the anionic species. The overall interaction of the amino acids is expected to be linked as:

\[
\text{SOIL} \xrightarrow{\text{Na}^+ - \text{X}^- + \text{NH}_3^- - \text{CH}_2\text{COO}^-} \text{SOIL}
\]

Thus, the interaction of amino acids with negatively charged soil systems is expected to be governed by the ease of zwitter ion formation in the order:

\[
\text{NH}_3^- - \text{CH}_2\text{COO}^-, \text{NH}_2\text{CH}_2\text{COO}^-, \text{NH}_3\text{CH}_2\text{COO}^-
\]

Thus, the interaction of amino acids with negatively charged soil systems is expected to be governed by the ease of zwitter ion formation in the order:

\[
\text{H} - \text{C} - \text{C} - \text{OH}
\]

(Amino acid)

\[
\text{H} - \text{C} - \text{C} - \text{O}^-
\]

(Zwitter ion)

glutamic acid > histidine > serine containing \( \text{HOCH}_2\text{CH}_2\text{—} \) groups in decreasing order of -I effect. On the other hand, the mobility order for valine>leucine>alanine having respective groups: \( \text{CH}_3\text{—CH}—\text{CH}—\text{CH}—\text{CH}—\text{CH}_3—\) with decreasing order of +I effect has provided a basis of their interactive trend with soil colloid in the reverse order of their mobility.

However, the mobility of amino acids through soil with varying concentration of anionic species as their mobile phase depends upon the combined effect of the nature and concentrations of these ions as well as the nature of the amino acids through soil and thus, do not follow any specific trends as discussed above.

The above results are supporting the earlier work done and reported [9, 10].
REFERENCES


Table 5.1 Physico-chemical properties of soil

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
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<tr>
<td>PH (1:5, soils : water)</td>
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<tr>
<td>EC (1:5, soils : water) (dSm⁻¹)</td>
<td>0.86</td>
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<td>Organic matter (%)</td>
<td>0.45</td>
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<tr>
<td>Cation exchange capacity (CEC) (Meq 100 g⁻¹ soil)</td>
<td>16.1</td>
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<tr>
<td>Exchangeable cations (meq 100 g⁻¹ soil)</td>
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<tr>
<td>Na⁺</td>
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<tr>
<td>K⁺</td>
<td>0.5</td>
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<tr>
<td>Mg²⁺</td>
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<td>Exchangeable (mg kg⁻¹ soil)</td>
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<td>NH₄⁺ -N</td>
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<td>NO₃⁻ -N</td>
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<td>Phosphorus</td>
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Table 5.2 Structures and characteristics of some amino acids*

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Abbreviation</th>
<th>Molecular weight</th>
<th>Structure</th>
<th>Solubility (g 100 mL(^{-1}) water) at 25°C</th>
<th>Isoelectric point (pI)</th>
<th>pK(_a)</th>
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<tr>
<td>Alanine</td>
<td>Ala(A)</td>
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<td><img src="image" alt="Structure of Alanine" /></td>
<td>16.72</td>
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<td>Serine</td>
<td>Ser(S)</td>
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<td><img src="image" alt="Structure of Serine" /></td>
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<td>5.68</td>
<td>2.36</td>
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<tr>
<td>Valine</td>
<td>Val(v)</td>
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<td><img src="image" alt="Structure of Valine" /></td>
<td>7.09</td>
<td>5.96</td>
<td>2.32</td>
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<td>Leucine</td>
<td>Leu(L)</td>
<td>131</td>
<td><img src="image" alt="Structure of Leucine" /></td>
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<td>2.21</td>
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<td>Histidine</td>
<td>His(H)</td>
<td>155</td>
<td><img src="image" alt="Structure of Histidine" /></td>
<td>4.29</td>
<td>10.76</td>
<td>1.82</td>
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<tr>
<td>Glutamic acid</td>
<td>Glu(E)</td>
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<td><img src="image" alt="Structure of Glutamic acid" /></td>
<td>0.84</td>
<td>3.22</td>
<td>2.19</td>
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</tbody>
</table>

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Fig. 5.1 The Effect of some anions at constant ionic strength (μ=0.6) on the mobility (R_f) of amino acids in soil