CHAPTER I

MOBILITY OF AMINO ACIDS THROUGH SOIL AMENDED WITH SOME PESTICIDES
Chapter I

INTRODUCTION

The significance of amino acids and pesticides is well known in relation to soils and plants. Amino acids are either directly added to (Rending, 1951; Sowden, 1956; Li et al., 1999) or found to exist in soil as a result of the decomposition of proteins and other organic matter (Waksman and Lomanitz, 1924; Waksman and Starkey, 1932; Scheffer et al., 1958; Summida et al., 1993; Appel et al., 1999). The amino acids play a vital role in the biochemical processes (Theng, 1974) and organic matter transformation in soil. Depending upon the pH of the soil environment, amino acids can exist as cations, zwitter ions or anions. This property enables them to be adsorbed or undergo exchange with metallic ions on soil and clay surfaces. By virtue of their nature, they form a variety of complexes (Talibuddin, 1955) with clays, lignins or soil organic matter and may undergo polymerization reaction. The products, such as indoles, act as growth promoting substances, hormones or auxins in plant (Waksman, 1952).
Pesticides, reaching soil either by direct application or run off from treated crop plants, get adsorbed on soil colloids, which have been intensively studied in recent years (Kaneko et al., 1978; Yanron, 1978; Camazano and Martin, 1987). It is found that pesticides treatment led to both qualitative and quantitative changes in the free amino acids composition of soils (Wainwright and Pugh, 1975; Mody and Munshi, 1990) and plants (Mehta et al., 1993). They also inhibit the synthesis of amino acids (Kishore and Shah, 1988; Shaner, 1989; Devine et al., 1993). It has been observed that pesticides contamination adversely effects protein and amino acid metabolism in aquatic animals (Reddy et al., 1991; Patil et al., 1992). Some studies have also been done to investigate the identification of amino acids by chromatography (Bremner, 1952; Stevenson, 1956), mobility of amino acids (Singhal et al., 1978) and pesticides (Khan and Khan, 1986) in soils and the effect of pesticides on soil microbial population growth (Martin, 1964; Parr, 1974; Greeves, et al., 1976; Alexender, 1977). But, there is a lack of literature about the effect of pesticides on the mobility of amino acids in soil medium.

In view of the significant relationship attached to amino acids, soils and pesticides, the present study has been conducted to investigate the mechanisms involved in the mobilization processes of certain amino acids through pesticides amended soil.
EXPERIMENTAL

For this investigation, Hirapur fine sandy loam soil (Aeric Halaquept) sample (0-30) was collected from Aligarh Muslim University Farm, situated in Aligarh district (U.P.), India. Physico-chemical properties of soil were determined as follows:

Determination of mechanical composition:

The mechanical composition of soil sample was determined by using international pipette method (Piper, 1950) as:

Apparatus and reagents required:

- 7, 200, 270 Mesh sieves (BSS).
- Graduated measuring cylinder.
- Constant temperature water bath.
- Pipette.
- Petridish.
- Electronic balance.
- 30% Hydrogen peroxide.
- 0.2N Hydrochloric acid.
- Sodium oxalate.

Procedure:

A 10 gm of dried, crushed and sieved through 7 mesh sieve (BSS), soil sample was dispersed in water after treating with 30% H₂O₂ and 0.2N
HCl using 50 ml sodium oxalate (8g/liter) as dispersing agent. The percentage of sand was calculated from the weight of the residues left behind on 200 mesh sieve (BSS). The suspension was then diluted to 500 ml and transferred to a graduated measuring cylinder, which was immersed in a constant temperature water bath at 25 ± 1°C throughout the course of pipetting. 10 ml of the sample was pipetted out carefully at specified intervals (4 mins. 15 sec. and 7 hrs. 5 mins.) of time from a depth of 10 cm and through 270 mesh sieve (BSS). The suspension, so obtained, was dried in a petridish and weighed and thus, the percentage of clay was calculated from weight of residues. The percentage of silt was calculated by subtracting the sum of percentage of all fractions (sand plus clay) from 100. The results are recorded in the table IV.

**Determination of pH:**

The pH of soil was determined with Elico pH meter (model LI 120) using glass and saturated calomel electrodes assembly. A 10 g soil was dispersed in 50 ml distilled water (1:5, soil: water) to prepare the suspension and was used to measure the pH of the soil. The result obtained is recorded in table IV.

**Determination of electrical conductivity:**

The electrical conductivity of the soil was measured by using Philips Conductivity Bridge with dip type cell at 30 ± 1°C. A 1: 5, soil: water ratio
was used to measure the electrical conductivity. The result obtained is given in table IV.

**Determination of organic matter:**

The organic matter of the soil was estimated by using Walkley and Black (1947) method:

**Regents required:**

- Aqueous solution of potassium dichromate (1N).
- Conc. sulphuric acid.
- Phosphoric acid (85%).
- Alcoholic solution of diphenylamine indicator.
- Aqueous solution of ferrous ammonium sulphate (N/2).

**Procedure:**

A 2 g soil sample, air-dried and passed through a 100 mesh sieve (BSS), was taken in 500 ml conical flask. 10 ml of 1 N potassium dichromate solution and 20 ml of concentrated sulphuric acid were added to it. The flask was shaken vigorously several times, allowed to stand for 30 minutes and thereafter 200 ml of distilled water, 10 ml of phosphoric acid and 1 ml of diphenylamine indicator were added to it. The excess of unreacted potassium dichromate was titrated against standard N/2 ferrous ammonium sulphate solution till the violet colour changed to purple and finally to green. Reagent blank determination was also carried out in the
same way. From the volume of dichromate solution used for oxidation, organic carbon was calculated by using the expression:

\[
\text{Organic carbon (\%)} = \frac{(\text{Blank titre} - \text{Actual titre}) \times 0.003 \times N \times 100}{\text{weight of dry soil in gm}}
\]

where,

N is the concentration of ferrous ammonium sulphate.

The value of organic carbon was converted to organic matter by multiplying with the factor 1.724. The result obtained for the percentage of organic matter is recorded in table IV.

**Determination of cation exchange capacity:**

Cation exchange capacity was determined by the Jackson (1958) method as:

**Reagents required:**

- 0.05N Hydrochloric acid.
- 1N Sodium acetate solution (pH 5).
- 1N Calcium chloride solution.
- EDTA solution.
- 1% Alcoholic solution of eriochrome black "T".
- Buffer solution of (pH 10).
- 2% Aqueous solution of sodium cyanide.
Procedure:

A 5 gm dried soil sample was taken in 100 ml conical flask. The soluble salts were washed out by treating the soil with 0.05N HCl and then washing with distilled water. It was further treated with 1N sodium acetate of pH 5.0 for 30 minutes with intermittent stirring in a 100 ml conical flask. The acidified sample was given five washings with 1N standard calcium chloride solution. The excess salts were removed by washing with 80% acetone until the excess $\text{CaCl}_2$ was removed, as indicated by a negative AgNO₃ test for chloride ion in the last washing. Finally calcium was exchanged from Ca - soil by sodium ions through five washings with a neutral 1N sodium acetate solution. The washing was collected and utilized in the determination of exchanged calcium ions by titrating it with a standard solution of EDTA, using 10 ml of the NH₄Cl-NH₄OH buffer of pH 10 and eriochrome black "T" indicator in presence of 1 ml of 2 percent NaCN solution as masking agent. A reagent blank was also determined simultaneously to avoid any error due to interfering ions. The blank reading was subtracted from the reading of calcium determination. From the volume of the EDTA solution used, the value of cation exchange capacity was calculated by using the following expression:

$$\text{Exchange capacity (meq. 100g}^{-1}\text{ soil) = } \frac{V \times N \times 100}{\text{Weight of the soil in gm}}$$

where,

$V$ is the volume of the EDTA solution and $N$ is the normality of EDTA solution. The result of the cation exchange capacity of soil is recorded in table IV.
Determination of exchangeable cations:

The exchangeable cations of soil were determined as follows:

**Apparatus and reagents required:**

- Buckner funnel.
- Systémicks flame photometer.
- Aqueous solution of ammonium acetate (1N).
- Nitric acid (6N).
- 30% Hydrogen per oxide.
- 6N Hydrochloric acid.
- Buffer solution (pH-10).
- Eriochrome black "T".
- Muréoxide indicator.
- 10% Potassium hydroxide.
- EDTA solution.
- Standard solution of Na and K.

**Procedure:**

A 50 g soil sample, air-dried, grounded and sieved through 100 mesh sieve (BSS), was taken into a 250 ml conical flask and then 100 ml 1N NH$_4$OAc solution was added to it. The contents of the flask were shaken for 20 minutes and allowed to stand overnight. The soil contents were then transferred into a Buckner funnel, in which moist whatman filter paper No. 42 was seated using a gentle pressure. The soil was leached
with an additional 400 ml of NH₄OAc. The filtrate, containing NH₄OAc extract of the soil, was evaporated to dryness on a steam plate. The dark coloured residue, containing organic matter, was treated with 2 ml of 30% H₂O₂ and 2 ml of 6N HNO₃ and heated to dryness on a steam plate. The dried organic matter free residue was then dissolved in 10 ml of 6N HCl and diluted with distilled water. It was filtered through Whatman filter paper No. 42 and the volume was made up to 100 ml. This solution was used for the determination of exchangeable Ca²⁺, Mg²⁺, Na⁺ and K⁺ in soil. Exchangeable calcium plus magnesium was estimated with 10 ml of above solution by EDTA titration, using a half test tube of buffer solution (pH-10) and 4-5 drops of eriochrome black “T” indicator. Calcium was also estimated separately using mureoxide indicator with 10% KOH as recommended by Jackson (1958). The volume of EDTA solution for magnesium was calculated by subtracting the volume for calcium from the volume of calcium plus magnesium used. The exchangeable sodium and potassium was estimated in the above solution using “Systronicks” flame photometer. The amount of exchangeable Ca²⁺, Mg²⁺, Na⁺, and K⁺ are recorded in table IV.

**Determination of mobility of amino acids by soil thin-layer chromatography:**

The mobility, in terms of Rᵢ value, of amino acids through soil was determined by soil thin-layer chromatography:
### Table IV

Physico-chemical properties of Hirapur fine sandy loam soil

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (1:5, soil: water)</td>
<td>8.5</td>
</tr>
<tr>
<td>Electrical conductivity (dS m⁻¹)</td>
<td>0.9000</td>
</tr>
<tr>
<td>Mechanical composition (%)</td>
<td></td>
</tr>
<tr>
<td>Sand</td>
<td>63</td>
</tr>
<tr>
<td>Silt</td>
<td>24</td>
</tr>
<tr>
<td>Clay</td>
<td>13</td>
</tr>
<tr>
<td>Soil organic matters (%)</td>
<td>0.41</td>
</tr>
<tr>
<td>Cation exchange capacity (meq. 100 g⁻¹ soil)</td>
<td>16.3</td>
</tr>
<tr>
<td>Exchangeable cations (Cmol (p+) kg⁻¹ soil)</td>
<td></td>
</tr>
<tr>
<td>Na⁺</td>
<td>1.0</td>
</tr>
<tr>
<td>K⁺</td>
<td>0.5</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>3.5</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>1.5</td>
</tr>
</tbody>
</table>
Apparatus and reagents required:

- TLC applicator with adjustable thickness.
- Uniform glass plates (20 x 4cm).
- Glass chamber (Jars of 25 x 10cm size) with covers.
- Micropipette.
- Glass sprayer.
- Oven.
- 100 mesh sieve (BSS).
- 0.01M Aqueous solutions of lysine, glycine, alanine, phenylalanine and valine.
- 0.2% Alcoholic solution of ninhydrin (w/v).
- Pesticides (parathionmethyl, dimethoate, carbendazim, chlorpyrifos, endosulfan and methyldemeton).

Procedure:

Preparation of plates:

For the measurement of mobility (Rf value), the soil sample was dried, grounded and passed through a 100 mesh sieve (BSS) to get uniform particle size. The soil was slurried in distilled water containing varying amount (0, 25, 50, 75 and 100 mg100g⁻¹ soil) of pesticides viz. parathionmethyl, dimethoate, carbendazim, chlorpyrifos, endosulfan and methyldemeton. The soil slurry was thoroughly mixed so as to get a homogenous mixture in each case and was then applied on glass plates
(20 x 4 cm) with the help of an applicator to have an uniform layer of 0.5 mm thickness. The coated plates were air dried at room temperature (30± 3°C). Two lines were scribed at 4 and 14 cm from base on all plates so as to allow a running distance of 10 cm for the study.

**Loading of amino acids and development of plates:**

A 15 µl amount of the respective 0.01 M amino acid solution was applied on the base line of the soil coated TLC plates as a single application with the help of a micropipette. Plates were then developed in glass chamber using distilled water as a developer up to a distance of 10 cm as indicated by the upper line on TLC plates by ascending chromatographic technique. To prevent disintegration of soil in contact with water, wetted stripes of filter paper (about 2.5 cm wide) were wrapped around the bottom of each plate before their development.

**Drying and detection of chromatograms:**

The developed plates were air-dried. Amino acids were detected by spraying a 0.2% alcoholic solution of ninhydrin (w/v) and keeping them in an oven at 70-80°C for 10-15 minutes till appearance pink-coloured spots. These spots were found to be stable for several days.

**Measurement of Rf values:**

\[ R_f \text{ value of amino acids} = \frac{\text{Frontal distance moved by the amino acid}}{10} \]

Results are recorded in fig. 6 and table V.
Concentration of pesticides (mg 100 g\(^{-1}\) soil)

Mobility of amino acids through soil amended with some pesticides.

→ Lysine  ← Glycine  ▲ Phenylalanine  ← Alanine  ← Valine

Fig. 6
# TABLE V

**MOBILITY OF AMINO ACIDS THROUGH SOIL AMENDED WITH SOME PESTICIDES.**

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Concentration of pesticides (mg 100g⁻¹ soil)</th>
<th>( R_f ) values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>00</td>
<td>25</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.20</td>
<td>0.14</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.54</td>
<td>0.64</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.70</td>
<td>0.80</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.77</td>
<td>0.69</td>
</tr>
<tr>
<td>Valine</td>
<td>0.95</td>
<td>0.86</td>
</tr>
</tbody>
</table>

**CARBENDAZIM**

| Lysine      | 0.20 | 0.15 | 0.12 | 0.10 | 0.08 | 0.16 | 0.13 | 0.11 | 0.09 |
| Glycine     | 0.54 | 0.60 | 0.65 | 0.62 | 0.60 | 0.60 | 0.64 | 0.64 | 0.62 |
| Phenylalanine | 0.70 | 0.78 | 0.81 | 0.85 | 0.89 | 0.74 | 0.79 | 0.83 | 0.87 |
| Alanine     | 0.77 | 0.71 | 0.67 | 0.64 | 0.60 | 0.73 | 0.70 | 0.68 | 0.64 |
| Valine      | 0.95 | 0.83 | 0.80 | 0.76 | 0.74 | 0.84 | 0.81 | 0.76 | 0.70 |

**CHLORPYRIFOS**

| Lysine      | 0.20 | 0.18 | 0.16 | 0.14 | 0.11 | 0.20 | 0.17 | 0.14 | 0.12 |
| Glycine     | 0.54 | 0.57 | 0.60 | 0.58 | 0.54 | 0.56 | 0.59 | 0.56 | 0.53 |
| Phenylalanine | 0.70 | 0.74 | 0.77 | 0.81 | 0.85 | 0.72 | 0.75 | 0.79 | 0.83 |
| Alanine     | 0.77 | 0.75 | 0.72 | 0.69 | 0.66 | 0.76 | 0.73 | 0.70 | 0.68 |
| Valine      | 0.95 | 0.88 | 0.82 | 0.77 | 0.73 | 0.91 | 0.88 | 0.84 | 0.79 |

**ENDOSULFAN**

| Lysine      | 0.20 | 0.18 | 0.16 | 0.14 | 0.11 | 0.20 | 0.17 | 0.14 | 0.12 |
| Glycine     | 0.54 | 0.57 | 0.60 | 0.58 | 0.54 | 0.56 | 0.59 | 0.56 | 0.53 |
| Phenylalanine | 0.70 | 0.74 | 0.77 | 0.81 | 0.85 | 0.72 | 0.75 | 0.79 | 0.83 |
| Alanine     | 0.77 | 0.75 | 0.72 | 0.69 | 0.66 | 0.76 | 0.73 | 0.70 | 0.68 |
| Valine      | 0.95 | 0.88 | 0.82 | 0.77 | 0.73 | 0.91 | 0.88 | 0.84 | 0.79 |

**DIMETHOATE**

| Lysine      | 0.20 | 0.14 | 0.11 | 0.08 | 0.04 | 0.14 | 0.12 | 0.10 | 0.06 |
| Glycine     | 0.54 | 0.64 | 0.67 | 0.70 | 0.64 | 0.62 | 0.64 | 0.67 | 0.60 |
| Phenylalanine | 0.70 | 0.80 | 0.85 | 0.89 | 0.93 | 0.80 | 0.84 | 0.88 | 0.91 |
| Alanine     | 0.77 | 0.69 | 0.65 | 0.62 | 0.58 | 0.70 | 0.67 | 0.63 | 0.60 |
| Valine      | 0.95 | 0.86 | 0.82 | 0.78 | 0.72 | 0.85 | 0.82 | 0.78 | 0.74 |

**METHYL PARATHION**

| Lysine      | 0.20 | 0.14 | 0.11 | 0.08 | 0.04 | 0.14 | 0.12 | 0.10 | 0.06 |
| Glycine     | 0.54 | 0.64 | 0.67 | 0.70 | 0.64 | 0.62 | 0.64 | 0.67 | 0.60 |
| Phenylalanine | 0.70 | 0.80 | 0.85 | 0.89 | 0.93 | 0.80 | 0.84 | 0.88 | 0.91 |
| Alanine     | 0.77 | 0.69 | 0.65 | 0.62 | 0.58 | 0.70 | 0.67 | 0.63 | 0.60 |
| Valine      | 0.95 | 0.86 | 0.82 | 0.78 | 0.72 | 0.85 | 0.82 | 0.78 | 0.74 |

**METHYL DEMETON**
RESULTS AND DISCUSSION:

The mobility of amino acids (Fig.6 Table V) in natural soil followed the order: valine > alanine > phenylalanine > glycine > lysine. This trend is in accordance with the increasing order of their molecular masses, except in case of phenylalanine and lysine. The lower mobility of phenylalanine as compared to valine, may be due to its lower solubility (Table VI) in water. On the other hand, the lowest mobility of lysine may be due to its higher adsorptive tendency over soil colloids, in which the chemical structure plays a decisive role in its chemical behaviour as:

\[
\begin{align*}
\text{Valine} & \quad \text{Soil pH 8.5} \quad \text{Lysine} \\
\end{align*}
\]

The presence of two protonated amino groups in neutral medium, out of which, one still remains in the cationic form even in the basic medium is most probably the cause of the more adsorptive behaviour of the molecule over negatively charged sites of the soil colloids with strong electrostatic force, resulting in its lowest mobility. Its adsorption on soil colloids can be illustrated as:

\[
\begin{align*}
\text{Soil colloids} + \text{Valine} & \quad \text{Soil colloids} \\
\end{align*}
\]
<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Molecular masses</th>
<th>Structures</th>
<th>Isoelectric points (pI)</th>
<th>Solubility (in g 100⁻¹ ml water)</th>
<th>Nature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>1.46.6</td>
<td><img src="image" alt="Lysine structure" /></td>
<td>9.59</td>
<td>Very soluble</td>
<td>Positively charged</td>
</tr>
<tr>
<td>Glycine</td>
<td>75.07</td>
<td><img src="image" alt="Glycine structure" /></td>
<td>5.97</td>
<td>24.99</td>
<td>Polar but uncharged</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>165.00</td>
<td><img src="image" alt="Phenylalanine structure" /></td>
<td>5.48</td>
<td>2.96</td>
<td>Nonpolar (hydrophobic)</td>
</tr>
<tr>
<td>Alanine</td>
<td>89.10</td>
<td><img src="image" alt="Alanine structure" /></td>
<td>6.00</td>
<td>16.51</td>
<td>Non polar (hydrophobic)</td>
</tr>
<tr>
<td>Valine</td>
<td>117.00</td>
<td><img src="image" alt="Valine structure" /></td>
<td>5.96</td>
<td>8.85</td>
<td>Non polar (hydrophobic)</td>
</tr>
</tbody>
</table>

The mobility order: valine > alanine > glycine can be explained on the basis of their increasing polarity order. The structure of valine, alanine, and glycine (Table VI) contain secondary, primary and no alkyl groups, respectively at their one end of the molecule, capable of inducing electrons (+I effect) towards the carbon attached with -NH$_3^+$ and -COO$^-$ groups in these amino acids. As a result, this part becomes rich in electron density but, poor in positively charged centre with diminishing cationic tendency in the increasing order of the number of methyl groups attached to these amino acids. The decreased cationic and increased anionic tendency may compel these molecules to follow the above mobility order in soil against the negatively charged sites over soil colloids.

The effect of pesticides on the mobility of amino acids (Fig.VI and Table.V) was found to have a decreasing trend in case of lysine, alanine and valine in the order: parathionmethyl > dimethoate > carbendazim > chlorpyrifos > endosulfan > methylidemeton whereas, increased mobility of phenylalanine has been observed in the reverse order throughout the entire range of pesticides (upto 100 mg 100g$^{-1}$ soil). On the other hand, the mobility of glycine increased at lower concentration and thereafter; it declined at higher concentration of pesticides. The varying mobility trend of amino acids in various pesticides systems can be understood by considering the chemical behaviour as supported by their structures:
The maximum reduction in the mobility of lysine, alanine and valine in parathionmethyl-soil system may be due to the presence of nitro (-NO₂) group which, is capable of forming the strong hydrogen bond with amino or protonated amino group, in amino acids at soil pH 8.5 that can be represented as:

The capacity of forming the hydrogen bonding is governed by the number of electronegative nitrogen or oxygen atoms in these pesticide molecules,
that are capable of retaining these amino acids in the order: parathionmethyl > dimethoate > carbendazim > chlorpyrifos > endosulfan > methlydemeton. The mobility of amino acids has been found to decrease in these systems with increasing doses of pesticides. However, in the case of phenylalanine it seems to be governed by its incapability for the formation of hydrogen bonding on increasing concentrations of pesticides, due to the presence of bulky phenyl group, that pose a steric hindrance resulting the increase of its mobility. On the other hand, the polarity of glycine enhances its mobility at lower concentrations (parathionmethyl and dimethoate upto 50 mg and carbendazim, chlorpyrifos, endosulfan and methlydemeton up to 75 mg 100g⁻¹ soil) due to its incapability in the formation of hydrogen bonding but, at higher pesticide concentrations the increased number of electronegative oxygen and nitrogen atoms of pesticides in soil system are capable of diminishing the mobility through hydrogen bonding.
References


