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Amino Acid Distribution Patterns in Soils*

by

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Introduction: It has been almost definitely established by soil scientists that 37—50 per cent of the nitrogen of the soil is of proteinaceous nature. Though considerable work has been done along these lines in other countries no work has been done so far on the nature of soil nitrogen in the soils of South India with special reference to amino acid distribution patterns. The object of the present investigation, reported in this section, is to detect the protein components i. e., amino acids of soil hydrolysates and identification of the amino acids present in the typical soil types of the Madras and Kerala States of South India.

Materials and Methods: The soils taken up for the present study were (1) Black (2) Alluvial (3) Brown (4) Red (5) High altitude latosol of Nanjanad (6) Medium altitude latosol of Kallar and (7) Plains latosol of Pattambi.

The method as adopted by Bremner (1950) was followed for the detection of amino acids. One hundred gm of soil were hydrolysed by boiling under reflux in a 750 ml conical flask for 24 hours with 6 N HCl. The ratio of acid to soil was 4 ml of 6 N HCl. for every gram of air dried soil. Since the black soil was slightly calcareous the calcium carbonate from that soil was removed by leaching with cold 0·1 N HCl. before the actual hydrolysis. The hydrolysis mixtures were filtered through a Buchner funnel using Whatman No. 50 filter paper and the residues washed thoroughly with hot water. The filtrate was concentrated on a water bath several times to remove HCl. The residues were dissolved in water and the solution brought to pH 7·0 by addition of sodium hydroxide. The precipitate formed on neutralization was removed by filtration to eliminate salts, washed with hot water and the filtrate concentrated to small volume and desalted in an electric desalter (Reco model) employing 2 N sulphuric acid as the electrolyte and sheep's bladder as the semipermeable membrane. The brown precipitate formed during desalting was removed by filtration and the filtrate concentrated on a water bath and the residue dried in a vacuum desiccator. When it was almost dry it was dissolved in a small quantity of alcohol and this was used for spotting in chromatographic examination

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Partition chromatography: Whatman No. 1 filter paper of the size 22" x 18" was used for the development of two dimensional chromatogram in a Chromatocab. The spotting was done with a micro-pipette care being taken to see that the diameter of the spot did not exceed one cm. The spot was dried with the heat developed by a 100 watts electric bulb. The first run was given through using n-butanol-acetic-acid—water mixture in the ratio of 4:1:5. The lower layer was discarded and only the upper layer was used. The first run was conducted for a period of 12 hours. At the end of 12 hours the paper was taken out from the Chromatocab, the solvent boundary marked with graphite pencil and the paper air dried.

When completely dry, it was turned through right angles and the second run was conducted through with aqueous phenol containing potassium dihydrogen phosphate and sodium citrate as buffers. The second run was allowed for a period of 12 hours in an atmosphre saturated with phenol inside the Chromatacab. Two separate Chromatacabs were used for the butanol and phenol run, i. e., for the first and second solvent respectively. After the second run with phenol, the solvent boundary was marked and the paper dried in air. When completely dry, the paper was sprayed with 0·1 per cent (W/V) ninhydrin in butanol from an all-glass spray bottle. Immediately after spraying with ninhydrin the paper was dried in an oven at 65° C for the development of colour. The spots which were pinkish in colour were marked and the identification was done with reference to standard chromatograms run already with pure components. The identification was mainly based on the Rf values of the pure amino acids. The results of the study with the two-dimensional chromatograms are given in figs 1 to 4.

Results: The 6 N hydrochloric acid hydrolysate of black soil contains eight amino acids, namely, glycine, alanine, aspartic acid, glutamic acid, cystine, histidine, phenylalanine and tyrosine (Table 1). The hydrolysate of alluvial soil is found to contain glycine, alanine, serine, aspartic acid, asparagine, glutamic acid, cystine, arginine, phenylalanine, tryptophan and hydroxy-proline. Brown soil contains eleven amino acids. They are glycine, alanine, valine, aspartic acid, asparagine, arginine, cystine, tryptophan, proline and hydroxy-proline. Red soil contains phenylalanine in addition to the amino acids contained in the brown soil. The high altitude latosol contains glycine, alanine, glutamic acid, cystine, methionine, histidine, phenylalanine, tyrosine, and tryptophan. Medium altitude soil of Kallar, which can also be brought under the group of latosols, contains twelve amino acids same as of the red soil. The amino acids found in the plains latosol of Pattambi are glycine, alanine, glutamic acid, cystine, methionine, histidine, phenylalanine and tyrosine.

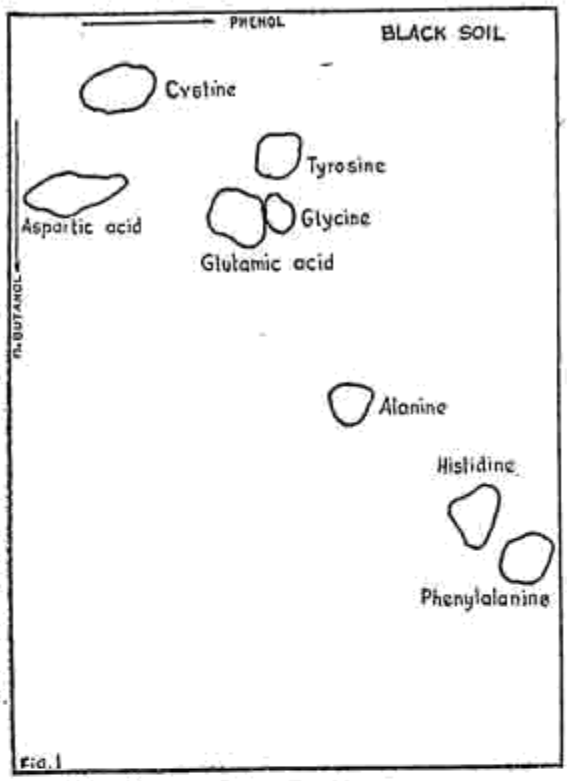

Fig. 1

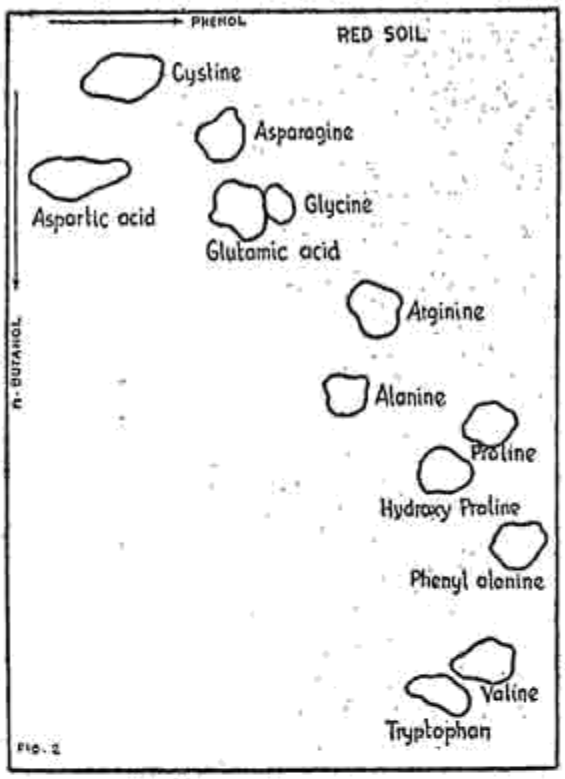

Fig. 2

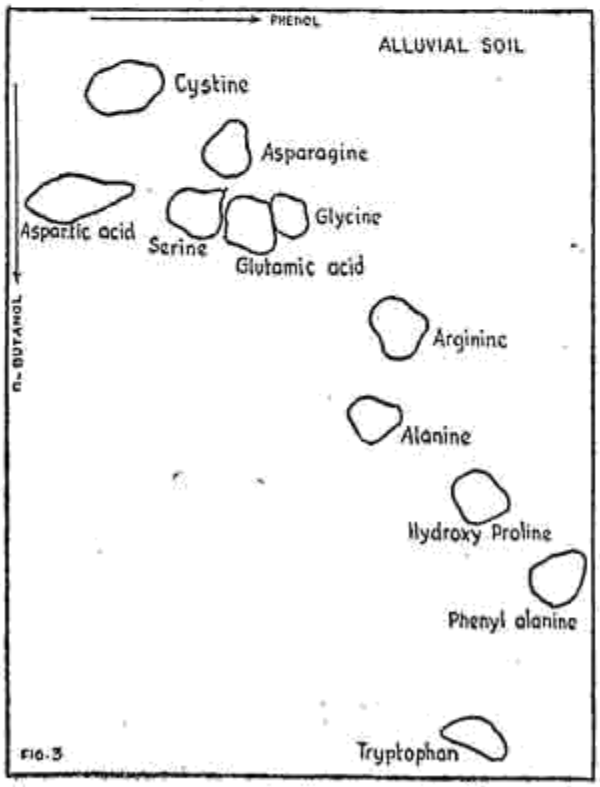

Fig. 3

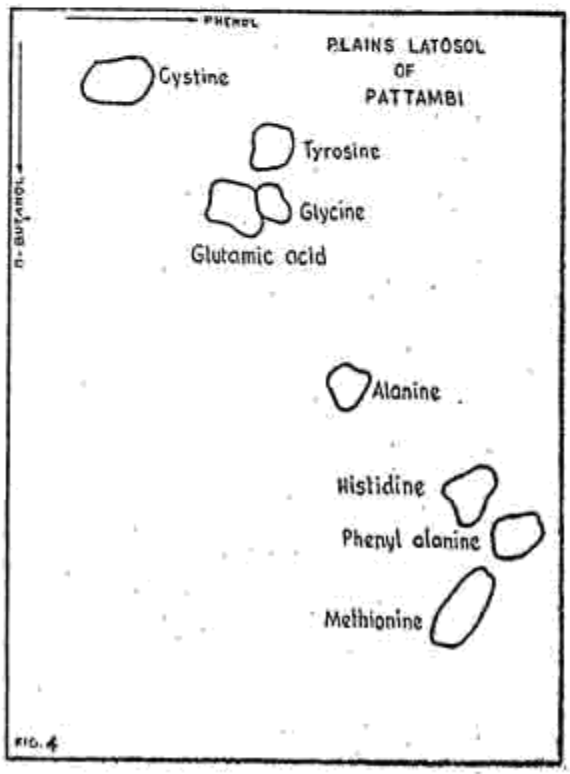

Fig. 4

TABLE 1. *Amino acid Distribution Patterns in Soils*

Amino acids	Black	Red	Brown	Alluvial	High altitude latosol of Nanjanad	Medium altitude latosol of Kallar	Plains latosol of Pattambi
Glycine	+	+	+	+	+	+	+
Alanine	+	+	+	+	+	—	+
Valine	—	+	+	—	—	+	—
Serine	—	—	—	+	—	+	—
Aspartic acid	+	+	+	+	—	+	—
Asparagine	—	+	+	+	+	+	—
Glutamic acid	+	+	+	+	+	+	+
Cystine	+	+	+	+	+	+	+
Methionine	—	—	—	—	+	—	+
Arginine	—	+	+	+	—	+	—
Histidine	+	—	—	—	+	—	+
Proline	—	+	+	—	—	+	—
Hydroxyproline	—	+	+	+	—	+	—
Phenylalanine	+	+	—	+	+	+	+
Tyrosine	+	—	—	—	+	—	+
Tryptophan	—	+	+	+	+	+	—
Total number identified	8	12	11	11	9	12	8

\+ Sign denotes the presence of amino acid.

— Sign denotes the absence of amino acid.

Discussion: In the seven types of soils examined it is found that all the soils contain a good number of amino acids. This result is in agreement with several workers, namely Kojima (1947), Bremner (1949, 1950), Dodd *et al.* (1953), Simonart and Peeters (1954) Young and Mortensen (1958) and Putnam and Schmidt (1959). The amino acids present are presumably the derivatives of the protein present in the soil. It is found that in general the higher the organic matter content of the soil the greater is the number of amino acids occurring in the soil. The amino acids detected in the present study may be divided into different groups as follows:

1. *Aliphatic amino acids:*
   - (a) Monoaminomonocarboxylic acids: Glycine, alanine, valine and serine.
   - (b) Monoaminodicarboxylic acids and their amides: Aspartic acid, asparagine and glutamic acid.
   - (c) Sulphur containing amino acids: Cystine and methionine.
   - (d) Basic amino acids: Arginine and histidine.
2. *Aromatic amino acids:* Phenylalanine and tyrosine.
3. *Heterocyclic amino acids:* Tryptophan, proline and hydroxyproline.

Cystine, glycine and glutamic acid are commonly present in all the soils. Valine is present only in red and brown soils, and medium altitude latosol of Kallar. Serine is recorded in alluvial and medium altitude latosol of Kallar only. Aspartic acid is found in all the soils except in the high altitude and plains latosols. Asparagine is present in black, brown and alluvial soils and medium altitude latosol of Kallar and is absent in red soil, high altitude latosol of Nanjanad and plains latosol of Pattambi. Methionine is present only in high altitude latosol of Nanjanad and plains latosol of Pattambi. Arginine is found in all soils except in black soil and plains latosol of Pattambi. Histidine and tyrosine are present only in black soil, high altitude latosol of Nanjanad and plains latosol of Pattambi. Phenylalanine occurs in all soils except brown soil and plains latosol of Pattambi. Tryptophan is present in red and alluvial soils and high altitude latosol of Nanjanad and medium altitude latosol of Kallar and absent in others. Proline is absent in all soils except in red and brown soils and medium altitude latosol of Kallar. Hydroxy-proline is detected in red, brown and alluvial soils and medium altitude latosol of Kallar only. Alluvial soil is very closely similar to brown soil and medium altitude latosol of Kallar. High altitude latosol of Nanjanad and plains latosol of Pattambi are similar to each other. Bremner (1949) reported that he could not detect tryptophan in both the acid and alkaline hydrolysates of the soils examined by him. But tryptophan has been detected in four soils out of the seven soils examined in the present investigation.

Baumann (1887) indicated that Russian black soils contained amino and amido compounds. The black soils studied in the present investigation is found to contain about eight amino acids. Kojima (1947) found serine, valine, threonine, aspartic acid, glutamic acid, hydroxy glutamic acid, hydroxylysine, leucine and isoleucine in the hydrolysate of a muck soil from New York. In the present investigation five amino acids out of the ones mentioned above are obtained in the medium altitude latosol of Kallar which is high in the organic matter content and hence comparable to the muck soil mentioned above.

By an examination of the extract of forest soils Simonart and Peeters (1954) observed that alanine, serine, aspartic acid and glutamic acid occurred in all samples studied. In the present study it is observed that glycine, glutamic acid and cystine are commonly occurring in all the seven soils studied. It is significant that in the present study the simple aliphatic amino acids glycine and alanine and the sulphur containing amino acid cystine are found to be common components of all soils studied. Young and Mortensen (1958) reported the presence of glycine, alanine, valine,

serine, threonine, aspartic acid, glutamic acid, lysine, leucine and isoleucine. In the present study excepting threonine and lysine all the other amino acids are found to occur in the soils.

Summary: Seven soils of the Madras and Kerala States of South India coming under six different soil groups have been examined for the distribution pattern of amino acids in their acid hydrolysate using paper chromatography. Sixteen amino acids of the aliphatic and aromatic groups of amino acids, some sulphur containing amino acids and a few heterocyclic amino acids have been identified. The maximum number of amino acids is found in the soils containing higher amounts of organic matter.

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