Microstructural and Adsorption Behavior of Non-polar Amino acids in Soil Amended with Polyethylene Glycol

NISHA TEWATIA[1]*, SHAGUFTA JABIN[1] and LOVENEESH KUMAR[2]

[1]Department of Applied Sciences (Chemistry), School of Engineering and Technology,
Manav Rachna International Institute of Research & Studies, Faridabad, Haryana, India.
[2]Department of Chemistry, University of Delhi, New Delhi, India.
*Corresponding author E-mail: nisha.tewatia13@gmail.com

ABSTRACT

Amino acids aid in the growth and development of plants. Soil microbes outcompete plants for amino acids because they are great source of carbon and nitrogen for microbes. To overcome this problem, new agricultural practices are required. Due to minimal integration and ecological environmental preservation, polyethylene glycol is used as a soil-stabilizer to study its stabilization effect on soil. Six soil samples (0%-5% Polyethylene Glycol) were prepared to study the effect of polymer on soil-amino acid interaction. Five non-polar amino acids, viz., glycine, alanine, 2-aminobutyric acid, leucine and methionine, on soil were studied with soil thin layer chromatography. Scanning electron microscopy and X-ray diffraction studies successfully marked the presence of Polyethylene Glycol in the soil. Mobility of amino acid is positively correlated with the concentration of Polyethylene Glycol in the soil. The effect of polymer on soil-amino acid interaction could be applied as a new reference for polymer-improved soil engineering.

Keywords: Amino acid, Scanning electron microscopy, Soil stabilization, Soil thin layer chromatographic technique, Water-soluble polymer, X-ray diffraction.

INTRODUCTION

The necessity for food has prompted worldwide extensive research in the field of agriculture because of the world population's fast expansion. To feed the expanding world population, crop production must be raised[1]. Amino acids, one of the nitrogen sources that aid in the plant's development, are an integral part of soil[2]. Amino acids play significant roles in plants, including those of si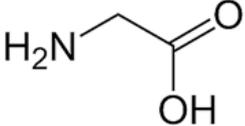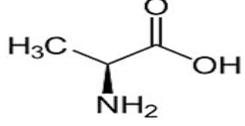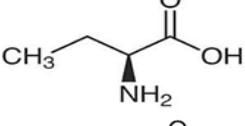g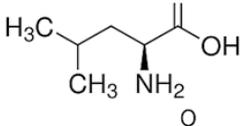naling molecules, regulators of root and shoot architecture, schedulers of flowering, and helpers in stress defense[3,4]. They are typically regarded as components of protein synthesis and as secondary metabolite substrates. According to a previous study, 8% of the plant's total nitrogen absorption was from amino acids only[5]. Organic nitrogen (ON), which includes amino acids, peptides, and proteins, is used by plant roots in addition to inorganic nitrogen (IN), which is present in the forms of ammonium (NH_4^+) and nitrate (NO_3^-) ions[6,7]. According to published research, soils contain roughly 40%

of the total nitrogen as amino acids[5]. Soluble ON dissolved in soil solution are pools of ON in soil that may be accessible to plant roots[8]. The main supply of nitrogen for mineralization is called soil organic nitrogen (SON).[20–30] kg per ha of SON is found in arable agricultural soils with amino acids constituting the greatest pool. It has been concluded from previous research that plant roots use amino acids as they are great sources of nitrogen and carbon for them and agricultural soils often have a higher capacity for amino acid adsorption and hence only IN would be available to plants[9]. It has been observed that annual nitrogen uptake in plants namely arctic, alpine and boreal forest ecosystems cannot be explained by the accessibility of inorganic nitrogen (IN) (net nitrogen mineralization rates) only because their major source of nitrogen is ON[9]. This demonstrated the significance of additional nitrogen availability for plants. The latent significance of ON in plant nutrition has also been established for specific species in various environments. As per literature, free amino acids present in soil failed to calculate the effect of adsorbed amino acids on the nutritional contribution of plants[10]. Plants and soil-microbes

quickly absorb amino acids therefore the free amino acid pool is limited and unstable in the soil. According to several studies, amino acids have half-lives in soils that range from 1.7 to 28.7 hours[11]. Furthermore, due to the amino acid-soil particle's strong interactions, amino acids find a challenge to move through the soil and its availability for plant uptake[9]. The pace at which nutrients diffuse through the soil has a significant impact on plant-microbe competition and nutrient availability. The ability of amino acids to bind to the soil matrix has a significant impact on how quickly they diffuse through soils. It has also been suggested that several intrinsic characteristics of amino acids, such as their molecular weight, charge characteristics and nitrogen content may influence how they behave in soil. The molecular weight of amino acids ranges widely, from glycine's molecular weight of 75 to tryptophan's molecular weight of 204. Several studies have shown that charged amino acids are strongly sorbed to soil solids; whereas neutral amino acids are not. Therefore, five different non-polar and neutral amino acids used in this study namely glycine, alanine, 2-aminobutyric acid, leucine and methionine (Table 1).

Table 1: Various amino acids used in this study and their characteristics

Sr. No	Amino acid	Molecular weight (g/mol)	Solubility (g/100 mL in H_2O at 25ºC)	Nature	Structure
1	Glycine	75.07	24.9	Non-polar and uncharged	
2	Alanine	89.09	16.7	Non-polar and uncharged	
3	2-aminobutyric acid	103.12	21.0	Non polarand uncharged	
4	Leucine	131.17	0.99	Non polarand uncharged	
5	Methionine	149.21	3.38	Non polarand uncharged	
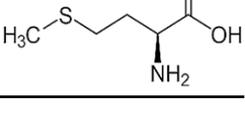
Due to adsorption mechanisms and soil microbial biomass consumption, a significant amount of these amino acids seem to be unavailable to plants, which ultimately affects the plant growth and crop yield. To overcome this problem, it is becoming more and more common to utilize appropriate soil stabilizers to enhance soil properties. Soil stabilizers can be categorized into five main groups based on their primary components: lime cement stabilizers[12,13], slag silicate stabilizers[14,15,16,17], ionic stabilizers[18,19,20], bio-enzyme stabilizers[21,22] and polymer stabilizers[23,24,25,26,27]. Because of benefits like minimal integration, a steady curing effect, and ecological environmental preservation, soil stabilization using a water-soluble polymer (WSP) has reportedly become a hotbed of study as soil stabilization materials.

The polymer-based soil treatment, which uses water soluble polymer (WSP), disposed off as a waste from industries such as paper, water, food processing, mineral processing, textile, pharmaceuticals, surface coating, petroleum production and oil and natural gas recovery, etc. has been broadly considered for integration as an environmentally sustainable soil improvement technique[28,29]. WSPs influence soil grains by interacting with the soil particles. Polymers have capability to affect the amino acid adsorption through the soil bed which ultimately affects the plant's nitrogen uptake. In clay environments, polymers may cause direct ionic interaction with clay particle surfaces and results into changing the clay fabric and causing the soil particles to be arranged geometrically[30]. In earlier studies, the use of microscopic techniques such as energy-dispersive X-ray spectroscopy has been commonly used in examining the interactions between soil and polymers[31]. To bridge this knowledge gap, the study was aimed to examine and analyze the effects of PEG on soil from Faridabad district of Haryana by employing XRD as well as SEM techniques. Among a variety of organic WSP, PEG was selected in this study owing to its wide usage in various applications[32,33], for promising results in effecting adsorption of amino acid and other organic content on soil surface.

Polyethylene glycol is classified as a synthetic and nonionic WSP. Macrogols are another name for PEGs. This polyether is made up of the repeated ethylene glycol units $[-(CH_2CH_2O)]$. PEG has the same number assigned by the Chemical Abstracts Society (CAS) as that of polyethylene oxide, though they differ in their molecular weights. Any hydroxyl initiator combined with ethylene oxide can be anionically polymerized to create PEG. Hydroxyl groups can be obtained from ethylene glycol, water or any diols. They can also be produced by ring-opening polymerization from epoxyethane. Linear PEG has two functional groups only, i.e., ether and alcohol. The chemical structure of PEG is shown in Figure 1.
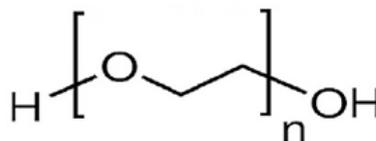

Fig. 1. Chemical structure of PEG

The objective of the present study is to assess the effect of polyethylene glycol (PEG) on soil-amino acid interaction by investigating the mobility of amino acid through soil bed by using soil thin layer chromatography (Soil-TLC) technique. Soil-TLC is one of the most versatile techniques used to study the mobility of amino acids on soil beds in earlier times also[34]. It is regarded as an effective analytical method for tracking and identifying the adsorption behavior of various amino acids through soil treated with PEG at varying concentrations as a stationary phase. The study and the extent of interaction between soil and polymer was done with the help of microstructural analysis such as scanning electron microscopy (SEM) and X-ray diffraction study (XRD). Furthermore, limited research could be found on the microstructural study of polymer-stabilized soils and its inherent relationship with agricultural qualities[35]. The study is thought to be very helpful in facilitating the design of remediation for an amino acid deficiency for plant uptake as they are already adsorbed by soil microbial mass more in case of unstabilized soil as compared to the soil stabilized with the help of polymer. Identifying the significant knowledge gaps and the kinds of study required close to them is another goal of this study.

MATERIAL AND METHODS

Materials

Soil sample was taken from the area of Aravali sites in Faridabad district of Haryana, India (28.44°N, 77.28°E). Five uncharged and non-polar amino acids viz. glycine, alanine, 2-aminobutyric

acid, leucine and methionine along with a visualizing agent, ninhydrin, have been purchased from CDH (India). Polyethylene glycol (PEG) has been purchased from Sigma Aldrich. Micropipette, used in soil-TLC has been bought from Abdos Labtech in India. Chromatographic glass jars and plates were purchased from a laboratory bought from Sisco, India.

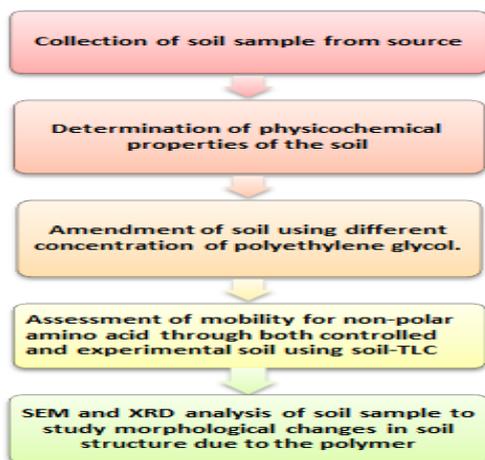The study has been carried out by adopting the following scheme/methodology (Figure 2).

Fig. 2. Methodology adopted during the study

Preparation of sample and solution for analysis

Before being crushed in a mortar, the sample was desiccated at ambient temperature in desiccators. It was then put through a sieve with a 150 μL size in order to preserve consistency in particle size. Soil suspensions of 1:5 (soil: water) ratio were prepared using distilled water. 0.1 M solution of amino acid was also prepared using distilled water. Five different solutions of PEG of varied concentration from 1% to 5% (w/v) were prepared in the distilled water. PEG stock solution (5%) was prepared through dissolving 5 g PEG into 100 mL distilled water. To get concentrations of 1% to 5%, aliquots from the stock solution were collected and diluted with ultrapure water. By dissolving 0.2 g of ninhydrin in 100 mL of ethanol, 0.2% ninhydrin was obtained. The chemical reagent, ninhydrin is a well known visualizing agent used for the detection of ammonia, primary/ secondary amines and amino acids in the analyte. The substance produces Ruhemann's purple, a dark purple compound, when it combines with the amino acid component present in the soil (Figure 3).
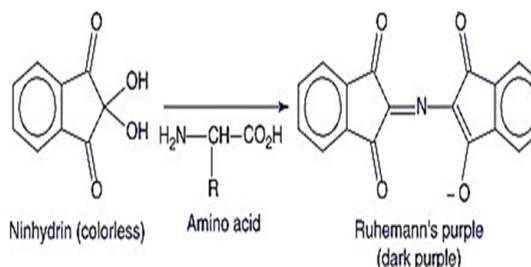

Fig. 3. A reaction between ninhydrin and an amino acid, leading to the formation of a dark purple compound known as Ruhemann's purple

Physicochemical properties of soil

The internationally recognized method of the "International pipette" was used to analyze the mechanical composition of soil[36]. A pH meter (Elico model L1-10T) was used to determine the pH of the soil. A conductivity cell (Hanna HI 8314, USA) was used to calculate the soil's electrical conductivity. Calculations have been made to determine the moisture percentage and moisture content (of both dry and wet) of soil, also the Jackson approach was used to evaluate the exchangeable cations[37]. The results have been tabulated in Table 2.

Table 2: Physico-chemical properties of soil

Parameter	Value
Mechanical composition (%)	
a) Sand	81
b) Silt	06
c) Clay	13
Electrical conductivity (mS)	0.38
pH (1:5, soil:water)	8.14
Determination of moisture content	
a) Moisture content	10.60
b) Moisture percentage (wet soil)	22.00
c) Moisture percentage (oven dried)	27.58
Exchangeable cations (CmolKg^{-1} soil)	
a) Na$^+$	0.72
b) K$^+$	0.92
c) Ca^{2+}	6.50
d) Mg^{2+}	2.20

Preparation of chromatographic glass plates and Soil-TLC

To act as an absorbent and to find out the Rf values of five amino acids, the prepared soil sample was used as discussed earlier. Following that, it was put through an appropriate filter to guarantee uniform particle size. The soil slurry was prepared using distilled water. Six distinct glass plates (dimensions of 200x35x0.25 mm) were used to apply the resulting homogeneous slurry to obtain various results for the comparative study from these

six PEG solutions with various concentrations. The coated plates were placed in six appropriately sized glass chromatography jars and left to air dry before being studied further. After that, 15 µL of amino acid is applied onto six dried soil-coated plates at 2 cm using a micropipette. Different PEG solutions (0%; 1%, 2%, 3%, 4% and 5% PEG) were used as a developing agent during the procedure. On each of the glass plates, soil-TLC is allowed to flow until the developing agent has traveled 10 cm. For the remaining four amino acids employed in the study, the same process has been adopted.

Microstructural analysis
Scanning Electron Microscopy

Using a Jeol (Japan) JSM 6510Lv SEM apparatus operating at a 15 kV accelerating voltage, materials were imaged using SEM. Using a typical SEM, a 1 cm to 5 µ area can be scanned with a 20 x–30,000 x magnification and 50–100 nm spatial resolution. In SEM, a concentrated beam of electrons interacts with the specimen and creates images of the adsorbent's surface that reveal the sample's topography and chemical composition[38].

X-Ray Diffraction (XRD)

A Rigaku D/Max-2500 X-ray diffractometer was used to conduct the examination of X-ray diffraction. A Cu-Kα X-ray tube (Cu-K = 1.540538) was used, with a current of 20 mA and an input voltage of 40 kV. The two-theta (2) value of the soil samples was measured and ranged from 5 to 75 degrees. During the study, a total six diffractograms (each for 0%-5% PEG solution) were obtained to shed some light on soil-polymer interactions.

Detection of chromatograms and Rf value

To identify the amino acids on the glass plates, 0.2% ninhydrin solution was sprinkled on them after the plates had been allowed to air dry at room temperature for two to three hours. Until the plates developed long-lasting purple spots, they were all kept in an oven at a temperature of 70ºC to 80ºC. Following a few hours of spot stabilization, the R_f value of the amino acids in each case was calculated.

RESULT AND DISCUSSION

The molecular weight for glycine, alanine, 2-aminobutyric acid, leucine and methionine is 75.07 g/mol, 89.09 g/mol, 103.12 g/mol,

131.17 g/mol and 149.21 gm/mol respectively. Fig. 4(a) shows R_f values of glycine, alanine, 2-aminobutyric acid, leucine and methionine through pure soil (0% PEG) as a stationary phase as 0.52, 0.81, 0.82, 0.86 and 0.85 respectively, which indicates that R_f values increase with increase in molecular weight of amino acid except the anomalous behavior shown by methionine. In case of soil amended with 1% PEG solution, the values obtained for glycine, alanine, 2-aminobutyric acid, leucine and methionine are 0.54, 0.82, 0.83, 0.92 and 0.77 respectively, as shown in Fig. 4(b). These two cases clearly indicate that R_f value has a positive correlation with size and molecular weight of amino acids, with an exceptional behavior of methionine. A similar trend was again observed with the other concentrations of PEG, as shown in Fig. 4(c) to 4(f). The size of amino acid affects its diffusion rate through the amended soil. The mobility of amino acids through soil bed increases with increase in size and molecular weight of amino acids. One of the key elements influencing adsorption to soil solids is molecular weight, with heavier amino acids exhibiting poorer adsorption due to increased steric hindrance. The exceptionally reduced R_f value in case of methionine may be attributed due to the presence of extra element sulfur (S) which is not present in the other molecular structures of the amino acids studied here. It has been found in one of the studies that methionine is an essential amino acid which is known to be found in low quantities in crops, plants and vegetables[39]. So it has been studied extensively by soil scientists how to increase its content in the plants.

Another remarkable observation was made in this experiment that the R_f value of amino acid in soil phase increases with the increased concentration of PEG solution as well, which focuses on the fact that mobility of amino acid increases in the soil bed mixed with high dosages of the polymer in soil. It may be due to the strong interaction between polymer and clay particles of the soil. The strong interaction between the two, results in the unavailability of the adsorption sites onto the soil layer for amino acids to adsorb by soil microbes but it makes the polymer available for plants as a source of nitrogen. The findings indicate that the highest level of mobility was seen in the case of leucine in pure soil as well as in amended soil samples. R_f values of leucine were

found to be 0.86 and 0.98 in cases of minimum and maximum concentration of polymer in the soil respectively. However, R_f values are found to be 0.52 and 0.75 in case of glycine with both minimum and maximum concentration of polymer in the soil respectively as shown in Fig. 4(a-f). Owing to the soil's strong glycine adsorption capability, R_f values for glycine in all cases have been found to be low. This can be attributed to its low molecular mass and lesser steric hindrance to get adsorbed on soil bed among all the used amino acids. Adsorbent and adsorbate are in equilibrium as the adsorption process progresses. The chemisorption phenomena unite the adsorbent and adsorbate here. Size of amino acid affects its diffusion rate through the amended soil. The mobility of amino acid through soil phase increases with increase in size and molecular weight of amino acid as

reported above. Furthermore, it is interesting to note that, in comparison to soil supplemented with varying concentrations of PEG, the R_f values of all amino acids were found to be lowest in pure soil as stationary phase. This is likely due to weaker mobilities and higher adsorption of amino acids through the pure soil bed. But, the presence of the PEG in soil makes these adsorption sites less available for the adsorption of amino acid and ultimately enhances the mobility of amino acid through the polymer amended soil bed. It may be inferred that the relationship between amino acids and the solid phase of the soil, which is ultimately impacted by the presence of polymer in the soil, has a major impact on the distribution and bioavailability of amino acids. R_f value of amino acid in soil phase increases with increase in concentration of PEG solution.
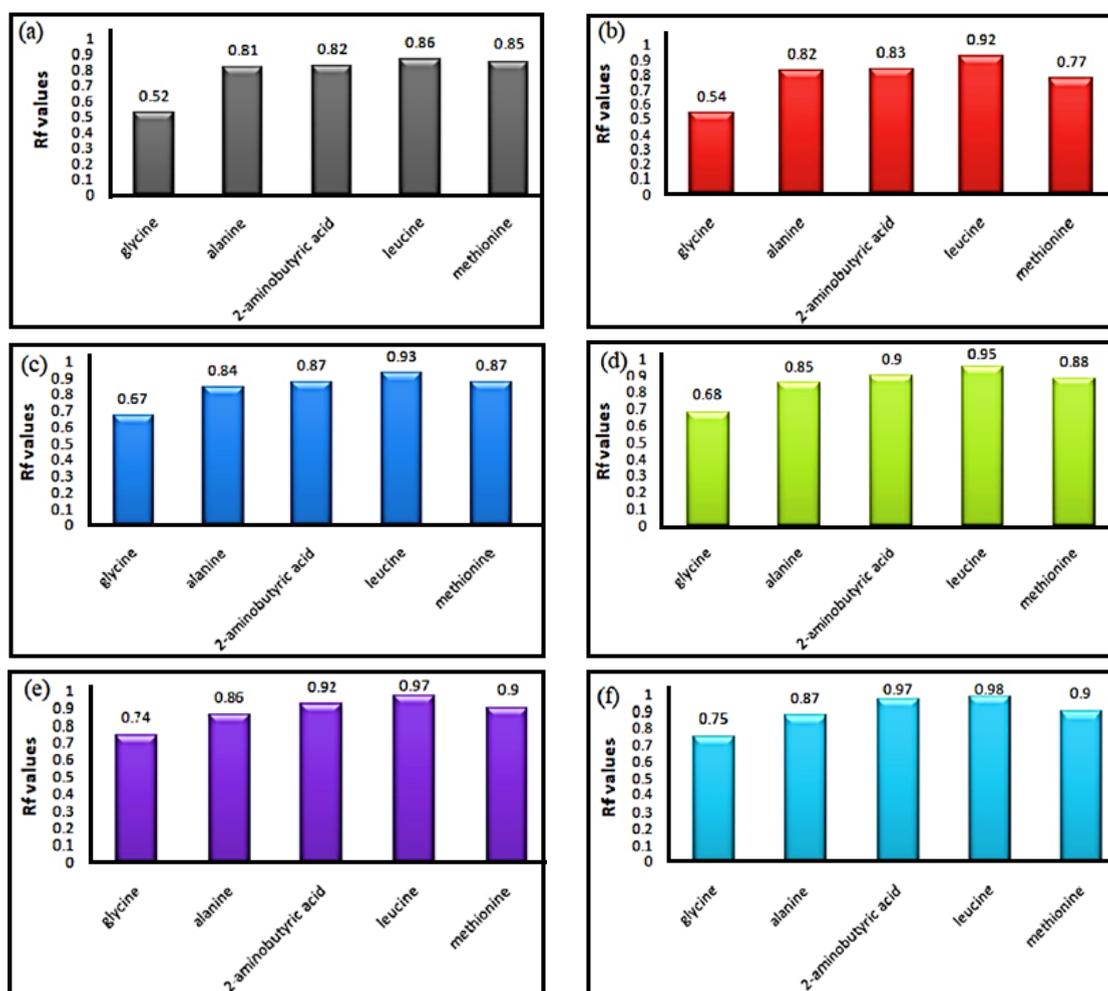

Fig. 4. R_f values of different amino acids in case of soil with (a) 0% PEG (b) 1% PEG (c) 2% PEG (d) 3% PEG (e) 4% PEG (f) 5% PEG

Total six SEM images of the soil samples (each for 0%-5% PEG solution) have been obtained to assess how well the interaction between soil and polymer exists. Mobility of amino acids increases due to physicochemical linkages and membrane structure formation on soil beds. Increased PEG concentration improves the ability of molecules to interact with aggregates and increases the number of long-chain macromolecules that cover the aggregate surfaces. As a result, the surface of the soil aggregate will form an enhanced membrane structure which will increase the soil's cohesiveness. Therefore, mobility of amino acid through the soil increases with the increase in the PEG concentration. Fig. 5(a) shows SEM image of controlled soil (0% PEG) at x5000 magnification scale. On the surface of pristine soil, several uninhabited places in the shape of holes or channels can be seen. Nevertheless, it has been shown that when the polymer is added to the soil in varying amounts, some of the polymer penetrates the soil's interior and fills up the holes between the majorities of the particles, strengthening the forces that hold the particles together[38]. This is clearly shown in Fig. 5(b)-5(f), where the polymer has occupied the spaces between the particles after meeting the soil, which was not present earlier. The ability of molecules to associate with soil aggregates is improved with an increase in PEG concentration, and the surfaces of the aggregates are covered by more long-chain macromolecules as shown by SEM images.
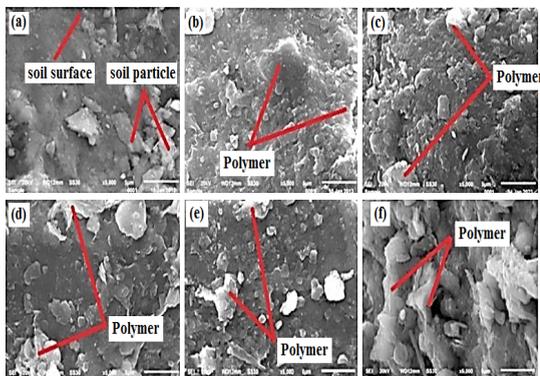

Fig. 5. SEM images of soil samples (a) 0% PEG (b) 1% PEG (c) 2% PEG (d) 3% PEG (e) 4% PEG (f) 5% PEG

XRD obtained for controlled soil (pure soil with 0% PEG) contains the prominent peaks of calcium silicate (CaSiO$_3$) at 2 values 26.56° and 36.50°, aluminum silicate (Al$_2$SiO$_5$) at 2θ values 39.66°, and 60.16°, magnesium silicate (MgSiO$_3$) at 2θ value 42.51°, and quartz (SiO$_2$) at 2θ values 20.85°, 27.93°, 45.79°, 60.01° and 68.08° as shown

in Fig. 6(a)[40]. Eventually, when the diffractogram for pure soil is stacked with the other five diffractograms, each for the soil mixed with 1%-5% PEG solution, two main observations were made: (1) four additional peaks were observed at 2θ values 14.62°, 19.00°, 22.50° and 29.78° as shown in Fig. 6(b), (2) the intensity of the observed peaks is increasing with the concentration of the polymer (Figure 6(c)).
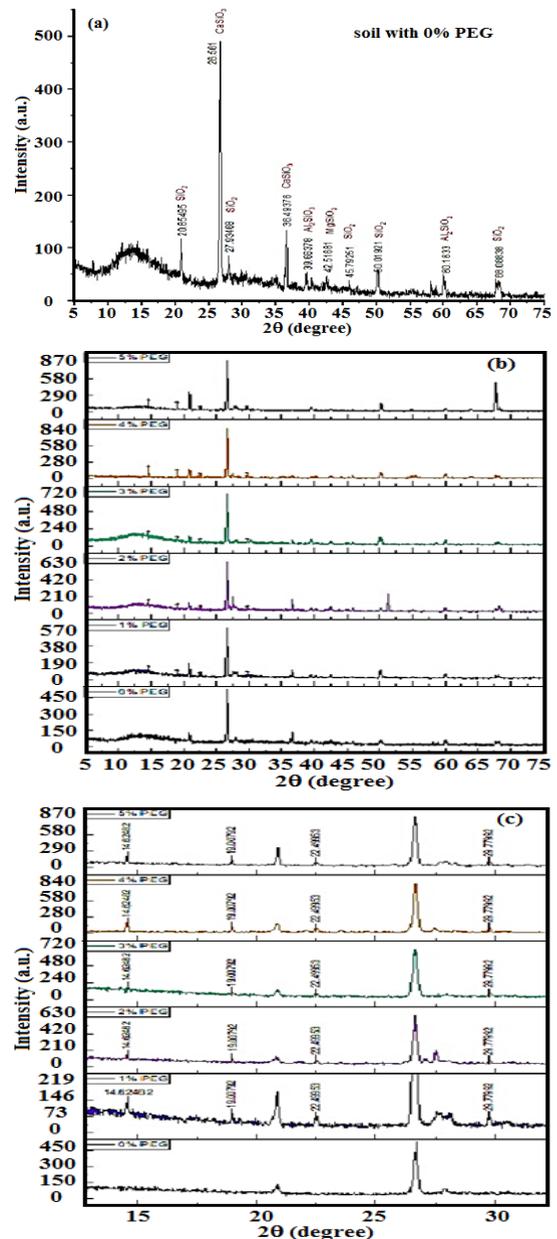

Fig. 6. XRD images of soil samples (a) 0% PEG (b) stacked XRD for 0%-5% PEG with the additional peaks of PEG highlighted (c) stacked XRD for 0%-5% PEG with the intensities mentioned for the peaks of PEG

From these observations, it can be easily concluded that the emergence of four additional peaks are due to organic moiety, PEG, in the soil, which were earlier not present in the case of controlled soil analysis but appeared only for the soil amended with 1%-5% polymer. The emergence of four additional peaks in each of the 1%-5% PEG-soil mixture during XRD analysis is clearly indicating the presence of an organic moiety (PEG) in the soil sample[41]. Moreover, the increase in intensity of the peaks with the concentration of the polymer added to the soil, clearly signifying towards the more

arranged structures of the soil due to the polymer (Table 3). The intensity of XRD peaks depicts the information related to arrangements of atoms in a crystallite. In this case also, the increased peak intensities is clearly inferring towards the more arranged and crystalline structures of soil due to the presence of PEG. Peak intensities are basically due to crystallographic arrangements, the thermal vibration of atoms and relative site of lattice atoms within the elementary cell[42]. XRD analysis is successful in justifying the interaction of polymer with soil.

Table 3: XRD Peak intensities of PEG-soil Mixtures for various 2θ in case of different PEG concentrations

PEG concentration (%)	Peak Intensity (a.u.)			
	2θ = 14.62°	2θ = 19.00°	2θ = 22.50°	2θ = 29.78°
1	150	99	75	75
2	165	110	88	79
3	199	125	95	95
4	205	150	97	99
5	210	165	100	102

CONCLUSION

Due to today's demand for high food production, it is a necessity of the era to enhance the availability of amino acids for plants. Soil-polymer interaction directly affects the plant uptake of nitrogen and nutrient consumption. By controlling the adsorption of polymers by soil-microbes, the plant uptake of amino acids can also be controlled. Soil thin layer chromatography (soil-TLC) is used in the laboratory to study the extent of adsorption for five different uncharged and non-polar amino acids, viz., glycine, alanine, 2-aminobutyric acid, leucine and methionine, on both pure soil and soil treated with PEG qualitatively. In the present study, glycine, having the lowest molecular weight, among all the five amino acids, tends to move slowly through the soil bed. On the other hand, leucine is the fastest among all. Although, Methionine has the highest molecular weight, but surprisingly, an irregular trend is observed during its study. The anomalous behavior of methionine can be attributed to the presence of sulfur (S) atom which might be interacting with the soil particles in a different way. Still more studies in future are required to make this clearer. SEM images prove how well the soil-amino acid interaction occurs in every case where soil is mixed with different concentrations of PEG[43]. PEG being a nonionic polymer shows

interaction with soil particles through Vander Waal forces of interactions. Polymers get adsorbed broadly on clay surfaces by uncoiling and spreading themselves over the surfaces. Increased PEG concentration improves the ability of molecules to interact with soil aggregates which increases the amount of long-chain macromolecules that cover the aggregate surfaces. As a result, the surface of the soil aggregate will form an enhanced membrane structure, which will increase the soil's cohesiveness. Therefore, mobility of amino acid through the soil increases with the increase in the PEG concentration. The results from XRD also conclude the presence of the polymer on the soil and clearly indicate about the more arranged structures of the soil. Based on the results obtained during this study, an attempt has been made to comprehend the soil's microstructural features, which are impacted by PEG and affect the soil's overall agricultural qualities. The effect of polymer on soil-amino acid interaction could be applied as a new reference for polymer-improved soil engineering, especially for the plant uptake for nitrogen in future studies.

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Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

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