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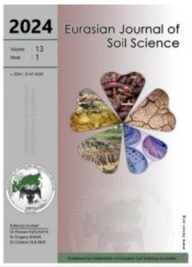

Characterization of humic acids from soil of Delhi regions and their impact on plant growth

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Abstract

Humus materials are considered complex organic substances generated through a chain of chemical reactions and responsible for many processes in soil that ultimately govern soil health. The structural and functional characteristics of humus depend upon the location, quality, and microbial abundance of the soils. However, the differential characteristics of soil organic matter, seasonal changes, parent rock, plant cover, microbial abundance, and anthropogenic activities majorly affect it. The present study has aimed toward the extraction of humus from five different locations in the Delhi region of India and their characteristics were investigated through elemental analysis, Fourier Transform infrared (FT-IR) spectroscopy, and UV spectroscopy. The results showed that there was a higher degree of unsaturation detected in the Forest soil sample. The results of FT-IR showed the presence of characteristic peaks of humus in the samples however the intensity of bands was weak in sample disposable site soil sample and clayey soil sample due to the variation in soil physicochemical properties. The study also aimed to assess the growth of *Oryza sativa* (rice) plants observed in the hydroponics system. The significant finding was observed with the forest soil sample in 1000 mgL^{-1} and treatment in which the growth was minimum in clayey soil of 1500 mgL^{-1}. Our investigation infers the diverse nature of humus in different soils and its implications for plant growth, highlighting the importance of understanding soil organic matter for sustainable agriculture and soil health management.

Keywords: Humus, humic acid, fulvic acid, organic matter, soil health.

Introduction

Soil organic matter is the key component of soil that affects the physicochemical properties of soil. The organic matter is made up of non-living heterogeneous material derived from microbial and chemical degradation of the organic pool in the soil (Lal, 2020). These processes are termed as humification process which generates humus which is further resistant to degradation. Humus substances are mainly associated with soil nutrient flux, carbon restoration, pollutants movements, soil aggregation behaviour, and soil fertility (Körschens, 2002). Humus is mainly composed of fulvic acids, humic acids (HAs), and humin materials. It features a network of single strands connecting clusters of humic material and minerals, forming a carbonaceous structure (Jackson, 1993). They exist as groups of peptide amphiphiles, carbohydrates, N-heterocyclics, and alkyl-aromatics. Although some polysaccharides, proteins, lipids nucleic acids were also found in the humus (Enev et al., 2014). The clay fractions predominantly consist of phenols, lignin, lipids, and fatty acids. The primary functional groups within the HAs molecule include carboxylic, phenolic, and alcoholic, along with minor groups like hydroxy, methoxy, thiol, etc. The Humic substances (HS) represent around 80% of organic matter. The structures and compositions of humus largely depend on the quality of organic matter, soil properties, and microbial activity, involving several polymerization, degradation, and transformation

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processes leading to the generation of humus materials in soils (Wei et al., 2019). The composition of marine humus is generally composed of aliphatic compounds while the presence of certain carboxylic acids and phenolics in humus was due to input of lignin, and tannins from terrestrial materials (Hayes et al., 2017). Humus derived from coal exhibits significant variations in mineral components (aluminosilicate), the number of functional groups, and water content (Volkov et al., 2021).

Several theories were earlier presented for the determination of humus structures (Ndzelu et al., 2020, Chen et al., 2020). According to Stevenson (1994)'s study, they are composed of heterogeneous organic macromolecules similar to the original material. There is significant concern in controlling the commercial potential of humic acids due to their ability to enhance plant growth (Thakur et al., 2023). Few studies have reported that HAs are composed of hydrophobic materials stabilized by neutral pH and hydrophobic forces while fulvic acids are mainly formed from hydrophilic compounds derived at any pH (Kolchanova et al., 2021). However, detailed structural characterization of humus materials has been discussed less, and thus a critical extraction procedure is essential for the derivation of proper structures.

For the determination of qualitative and quantitative features of humus, fractionation of organic matter is preferred as it can generate more valuable information about the humus produced. Previously, various extraction techniques like the use of chelating agents, cationic exchange resins, organic solvents, aqueous saline solutions, and alkaline solvents were employed. Due to the establishment of multiple procedures, there was a lack of proper determination of humus composition and humus characteristics. Hence, the International Humic Substances Society (IHSS) has implemented an extraction method involving the treatment of samples with a potent base (typically 0.1 or 0.5 mol L^{-1} NaOH) within a nitrogen-rich environment (Li et al., 2022). Despite numerous chemical alterations in the structural compositions, this methodology successfully yielded an optimal composition and provided representative compositions similar to those found in the original material.

Characterizing the HAs is vital for understanding their structural composition and applications in agriculture, environmental science, and biotechnology. Analytical techniques like Elemental Analyzer, UV spectrophotometry, and FTIR spectroscopy reveal molecular characteristics and interactions, providing insights into the organic nature of HAs. UV spectrophotometry examines ultraviolet light absorption, identifying functional groups and the aromatic nature of HAs. FTIR spectroscopy helps by identifying the specific functional groups. These methods collectively offer a comprehensive understanding of HA's chemical composition, facilitating its use in diverse applications, from soil improvement to wastewater treatment.

The growth-promoting activity of plants in response to varying concentrations of HAs extracted from soil is a subject of keen scientific interest in agriculture. The Has are evident to influence plant growth through multiple mechanisms. When applied to plants at different concentrations, HA can enhance root development, nutrient uptake, and overall plant vigour (Adani et al., 1998) . Studies have shown that lower concentrations of HAs can stimulate root growth and improve nutrient absorption by facilitating ion exchange processes in the root zone. Conversely, higher concentrations of HAs might exert different effects, potentially leading to improved photosynthetic efficiency, increased biomass, and enhanced resistance to environmental stresses (Castro et al., 2022). However, the response of plants to varying HAs concentrations is complex and can be species-specific, as different plants possess varying capacities to respond to these organic compounds (Stuijfzand et al., 1999) . As such, investigations into the growth-promoting potential of HAs at different concentrations contribute to our understanding of soil components and plant development, extending the pursuit for sustainable agricultural practices.

In the present study, we aimed towards the determination of spectral and chemical characteristics of HAs obtained from soil of five different locations. Characterization of the extracted HA was performed through FT-IR, UV-Vis spectrophotometry, and an elemental analyzer. This study was also aimed at the assessment of the growth-promoting potential of the extracted HAs from different locations. For this purpose, *Oryza sativa* was used as model plants using a reliable hydroponic rice seedling culture system to examine the various plant physiology parameters.

Material and Methods

Chemicals and Reagents

All the chemicals used in the extraction and characterization stages were of high purity and analytical research grade. Reagents and chemicals used in the study include hydrochloric acid (HCl), sodium hydroxide (NaOH), distilled water, potassium bromide (KBr), sodium pyrophosphate ($Na_4P_2O_7$), sodium hypochlorite (NaClO)and deuterium oxide (Merck). Hoagland solution macronutrients- magnesium sulphate heptahydrate ($MgSO4·7H_2O$), calcium nitrate ($Ca(NO_3)_2$), potassium dihydrogen phosphate (KH_2PO_4), potassium nitrate

(KNO_3), ethylenediaminetetraacetic acid iron (III) sodium salt ($C_{10}H_{12}FeN_2NaO_8{\cdot}H_2O$) and micronutrients- boric acid (H_3BO_3), manganese chloride tetrahydrate ($MnCl_2{\cdot}4H_2O$), zinc sulphate heptahydrate ($ZnSO_4{\cdot}7H_2O$), sodium molybdate dihydrogen monoxide ($Na_2MoO_4{\cdot}H_2O$), copper sulphate pentahydrate ($CuSO_4.5H_2O$), the Technical-grade HA was obtained from Sigma-Aldrich. *Oryza sativa* seeds were purchased from Indian Agriculture Research Institute (IARI).

Sampling Site

Soil samples were collected from 0-20 cm depths at five diverse locations within the Delhi-NCR region, India. The collected samples represented various soil types, including forest soil, agricultural field soil, riverbank soil from the Yamuna, landfill soil near Ghazipur landfill site, and clayey soil from a pond in Gautam Buddha Nagar, Uttar Pradesh, India. Specifically, the soil from the forest area near Gwal Pahadi, Gurugram, Haryana, situated in the Aravalli Hills, was classified as 'undisturbed' due to its minimal human impact and was located 17 km from Gurugram, India. The agricultural field soil was collected from Bulandshahr District, Uttar Pradesh, India, while riverbank soil was obtained from the Yamuna bank at Kalindi Kunj, Delhi, India. The soil near the Ghazipur landfill site of Ghaziabad, Uttar Pradesh, and the clayey soil near a pond in Bulandshahr District, Uttar Pradesh, India were also part of the study. The collected samples were designated as S1, S2, S3, S4, and S5 labels respectively (Figure 1). These locations were chosen based on significant differences in pedogenetic factors, parent material, landform, land use, and management practices (Table 1). The selection process also carefully considered the molecular-level diversity within the soil samples (Senesi and Loffredo, 1999; Lehmann and Kleber, 2015). Multiple sub-samples were gathered, air-dried, and sifted through a 2.0 mm mesh for subsequent chemical analysis, following the methodology as explained by Xiao et al. (2021).

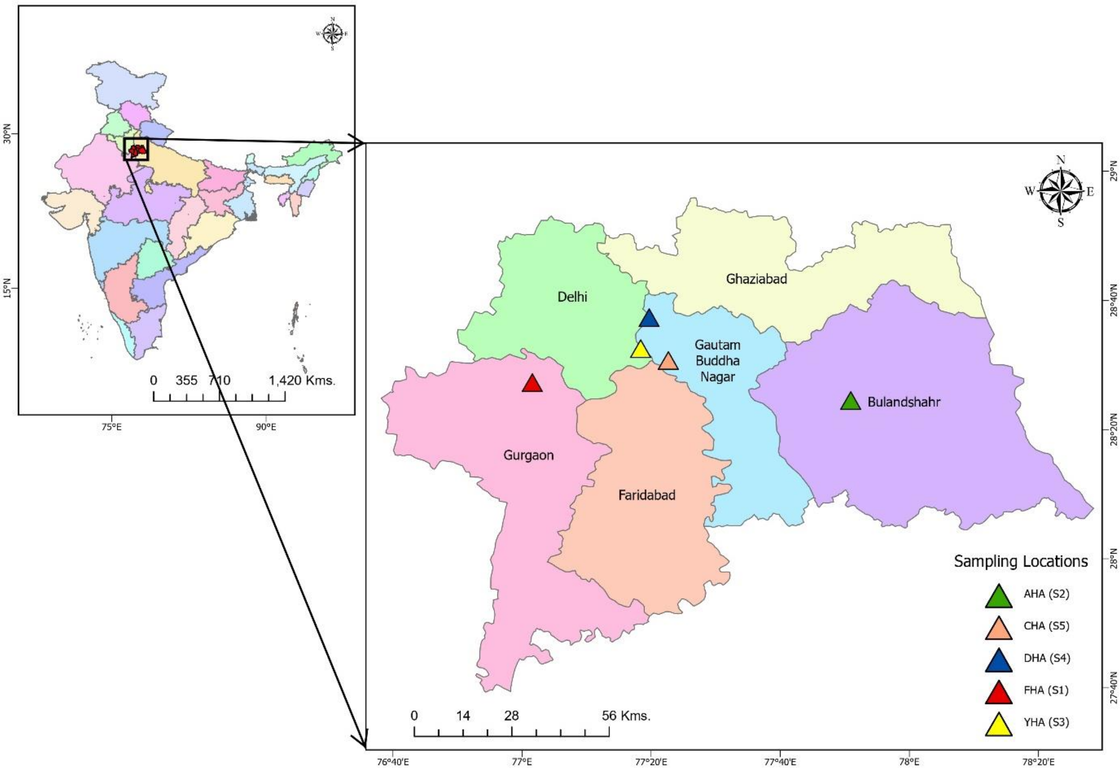


Figure 1. Soil sampling location on the map of Delhi NCR, India

All experiments were conducted at a room temperature of 25±1°C and humidity 50%.

Table 1. Soil sampling locations and their geo-coordinates in and around Delhi National Capital Region (NCR) of India

S. No.	Sampling Site	Location	Latitude	Longitude
1	S1 (Forest HA-FHA)	Gwal Pahadi, Gurgaon, Haryana, India	28.457523	77.026344
2	S2 (Agriculture HA-AHA)	Bulandshahr District, Uttar Pradesh, India	28.411331	77.848434
3	S3 (Yamuna HA-YHA)	Yamuna bank at Kalindi Kunj, Delhi, India	28.545267	77.306092
4	S4 (Disposal HA-DHA)	Ghazipur landfill site of Ghaziabad, Uttar Pradesh, India	28.625242	77.327989
5	S5 (Clay HA-CHA)	Gautam Buddha Nagar, Uttar Pradesh, India	28.514580	77.377594

Extraction and Purification of HA

The extraction of HAs in the soil was done corresponding to the standard method of the International Humic Substance Society (IHSS) (IHHS, 2024). The extraction was performed using 0.1 M HCl, and subsequently extracting during the night with 0.1 M NaOH under an atmosphere of N_2. The pH was set to 2.0 by adding 6.0

M HCl. Then, the remnants were collected and further extracted again. The contents existed centrifuged at 5,000 RCF for 10 minutes before being redissolved in a solution containing 0.1 M KOH under a N_2 atmosphere The clustered colloidal particles were then collected by subjecting it to centrifugation at 40,000 RCF for 15 minutes after this solution was adjusted to 0.3 M using KCl. The precipitated HAs were retrieved through centrifugation after the supernatant was centrifugally acidified by adjusting the pH to 2.0 using 6.0 M HCl. After being treated twice for 24 hours with 0.5% HF + HCl, the HAs were centrifuged at 5,000 RCF. The material that precipitated was dialyzed and subsequent freeze-drying following a rinse with 200 ml of 0.01 M HCl. The treated samples were then obtained through centrifugation at 5,000 RCF (Figure 2).

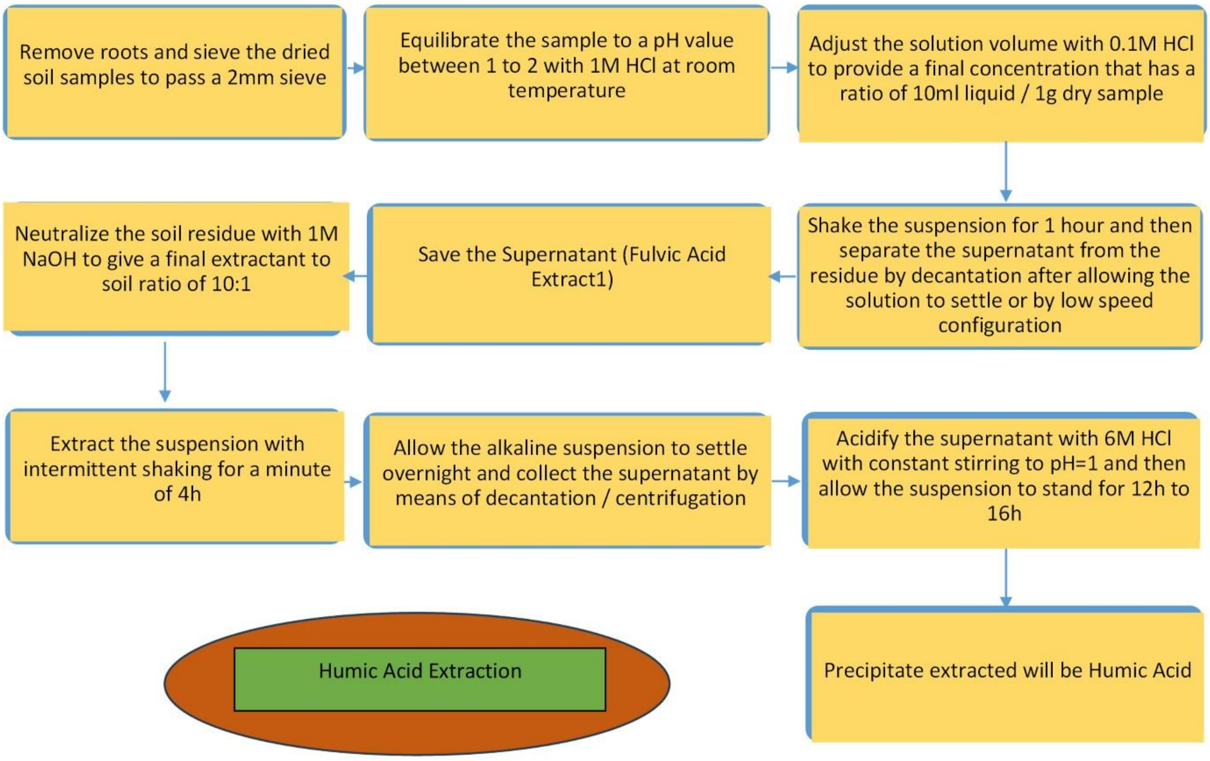


Figure 2. Flow chart illustrating the methodology of Humic Acid extraction steps.

Characterization of Humic Samples

Elemental Analysis

After extraction and purification, the obtained HAs fractions, elemental compositions of the HAs were determined by the elemental analyser (CHNS-O EA 3000). The percentage of O content was determined by subtracting the percentages of C, H, N, and S after making corrections for moisture and ash content in the data. The following formula was used for the calculations: $\%O = [100 - (\%C + \%H + \%N + \%S)$

UV-Visible Spectrophotometry

UV-VIS spectroscopy was done using AJX-1600 spectrophotometer where the spectra were taken corresponding to the methods described by Datta et al. (2022) using a wavelength range of 200 to 800nm, at 25°C, with a 1 cm optical path in a solution created by diluting 20mg of HAs in 1 L of $NaHCO_3$ (0.05 $molL^{-1}$), with a pH 8.0. To calculate the relationship coefficient E4 /E6, the absorbance at 465 nm was divided by that obtained at 665 nm (Datta et al. 2022) .

A separate stock solution of HA (10 mgL^{-1}) was prepared using $Na_4P_2O_7$ alkali solvent. Using this solution, multiple standards working solutions with HA concentrations ranging from 0.002% to 0.012% were also prepared in solvent. The absorbance of HA in the UV-VIS spectral range was measured in the mixture of $Na_4P_2O_7$ alkali solvent. To establish the calibration series, each standard working solution was transferred into a spectrophotometric cell, and its absorbance was recorded at two wavelengths, 465 and 665 nm.

Fourier Transform Infrared Spectroscopy (FT-IR)

The spectral analysis was performed using a Bruker Vertex 70 FTIR spectrophotometer equipped with a Platinum attenuated total reflection (ATR) module. To achieve this, the lyophilized and purified samples were converted into tablets and compressed together with KBr. Subsequently, FT-IR spectra ranging from 400 to 4000 cm^{-1} were generated. A resolution of 4 cm^{-1} was utilized, and 10 scans were performed to generate the set of 10 spectra. Origin software (version 5.0, 2007) was used to assess these spectra.

Plant Materials and Growth Conditions

Rice seeds (*Oryza sativa* L.) were procured from Indian Agriculture Research Institute (IARI), New Delhi, India. Before use, seeds of consistent size underwent surface sterilization using solution of NaClO at a concentration of 10% (v/v) was applied for a period of 10 minutes. Following this, they were thoroughly rinsed with distilled water and immersed for a duration of 4 hours. Subsequently, the healthy and seeds of consistent size were placed in 150 mm petri dishes, covered with filter paper of Whatman no. 1, and moistened with Hoagland's solution at half-strength with a pH of 6.5 as described by Arditti and Dunn (1969). The seeds were further allowed for germination in the absence of light at a temperature of 28 ± 2°C and for 4 days. The ensuing young seedlings were grown under a photon flux density (PFD) of 150 μmol photons m^2 s^{-1} and kept within a relative humidity range of 50–60%. This environment followed a day/night cycle lasting 12 hours each at a consistent temperature of 28 ± 2°C for a duration of 8 days inside a growth chamber. Following this period, seedlings of consistent size were selected and relocated into half-strength Hoagland's solution at half-strength for a 7-day acclimatization period. Subsequently, the leaves were carefully stored at −86 °C until further analyses. After the 7-day treatment period, root and shoot samples were gathered from both treated and untreated (control) seedlings, and different parameters were measured for analysis.

Determination of Growth Parameters and Photosynthetic Pigment

The parameters related to growth, such as fresh and dry weight, along with root and shoot length, were assessed following the random harvest of both untreated (control) and treated rice seedlings. From each sample, 10 seedlings were selected at random, and then divided into root and shoot portions for subsequent measurement of their respective length and fresh weight. To ascertain the dry weight, the root and shoot segments were enclosed in butter paper and underwent oven drying at a temperature range of 65-75°C for 48 hours, following which their weights were measured. To assess total chlorophyll and carotenoids, 25 mg of fresh leaves were extracted in 5 ml of 80% (v/v) acetone for each sample. The quantification of chlorophyll and carotenoids was conducted following the procedures outlined by Arnon (1949) and Ikan (1969), respectively. There were total of 16 treatments viz, T1 (control) to T16, and three concentrations were taken, 500 mg L^{-1}, 1000 mg L^{-1}, and 1500 mgL^{-1} as per the description of the treatments is given in the table 2.

Table 2. Description of treatments

Treatment Code	Doses (mg L^{-1})	Treatment Details
T1	Not Applicable	Control (Nutrient Broth)
T2	500	
T3	1000	FHA (Forest HA)
T4	1500	
T5	500	
T6	1000	AHA (Agriculture HA)
T7	1500	
T8	500	
T9	1000	YHA (Yamuna HA)
T10	1500	
T11	500	
T12	1000	DHA (Disposal HA)
T13	1500	
T14	500	
T15	1000	CHA (Clayey HA)
T16	1500	

Results and Discussion

Elemental Analysis

The elemental composition is the most fundamental trait of the organic compound of any sample. The results of the elemental analysis, along with the atomic ratios (C/H and C/N) and the ash content of the samples are provided in Figure 3. The results were obtained after corrected the moisture and remaining ash contents were assessed through thermogravimetric analysis. The content of %C was highest in S1 followed by S2, S3, S4, and S5. However, the %H was maximum in S2. %N was followed the different trend S2>S1>S3>S4>S5. %O and %S was highest in S1.

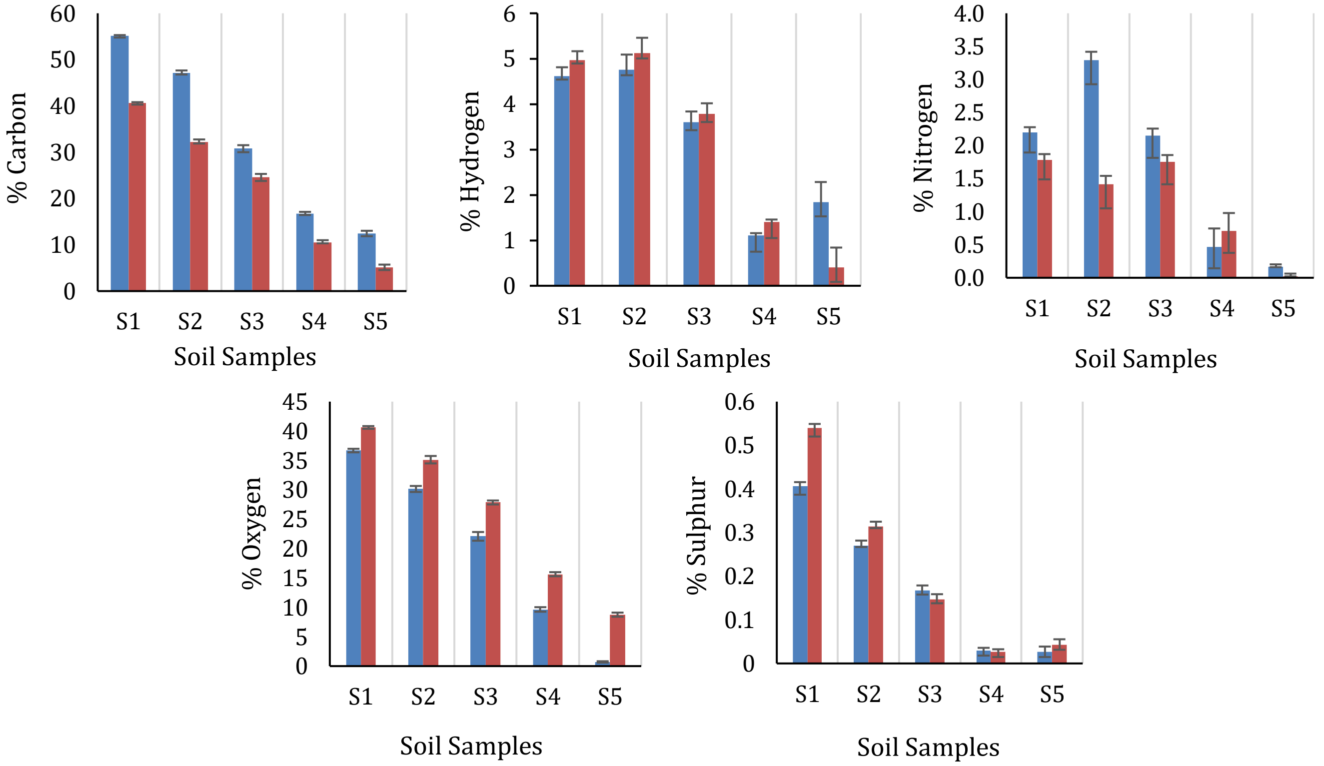
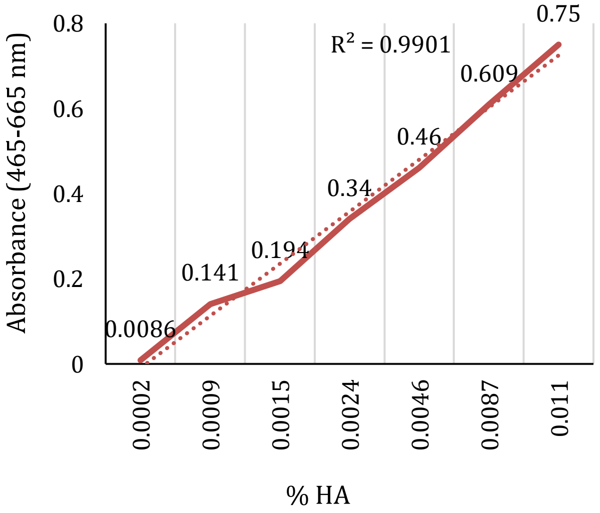


Figure 3. Elemental analysis of the samples for percent content of Carbon, hydrogen Nitrogen, oxygen and sulphur

UV-Vis Spectroscopy

The UV absorption serves as an effective indicator of the unsaturated carbon content in samples, presenting a rapid, simple, and highly sensitive approach for molecular characterization (Figure 4). The spectra of the samples were showing higher absorbance (200-300 nm) for sample S2, due to the presence of more chromophores of double bonds (C=C, C=O). These results were corroborating the above section with higher aromatic structures (higher H/C) in sample S1. The percentage HA was maximum in S1 that is 0.089% followed by S2, S3, S4 and S5 (Figure 5).



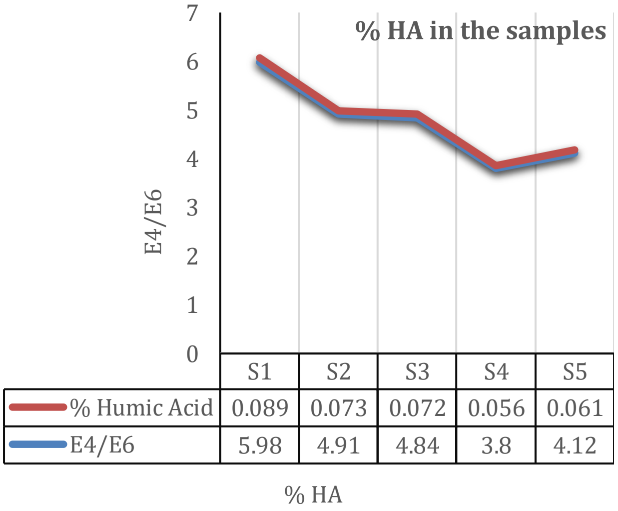


Figure 4. Calibration curve of the standard Humic Acid prepared at the absorbance of 465-665 nm

Figure 5. Percentage of Humic Acid in the soil samples selected for the studies

FT-IR Analysis

To identify the conformational alterations and content fluctuations of functional groups of HAs from various locations, FTIR spectra were collected. The spectroscopic data about tested samples has been provided in Figure 6. The spectrum was divided into two bandwidth regions for a wider understanding of bands. All the samples were showing almost similar absorption patterns due to the basic skeleton structures of the HAs. However, few differences in the intensity and band shifting were found owing to their origin sites. The spectra existing 3500-3000 cm⁻¹ were showing the bands of stretching of O–H, minor stretching of N–H, and displaying hydrogen-bonded –OH groups (Cocozza et al., 2003). The intensity of these bands was prominent in S1, S2 and S3 however it gets decreased in S4 and S5 due to the presence of less matured humus substances. Since in the S4 major organic reactions owing to humus formation were absent therefore it is showing immature humus substances. The groups that appeared between 2900-2300 cm^{-1} were major due to involving asymmetric and symmetric stretching of C–H in CH_2 groups. These groups were absent in S4 and S5, and very prominent intensity was detected in S2 due to a chemical reaction that occurred at that site. The absorption bands at 1740 were corresponding to the stretching of C=O in COOH and other carbonyl groups. The bands occurring between 1600-1690 cm^{-1} were arising from vibrations in the aromatic C=C skeleton, stretching of C=O in amide groups (designated as amide I band), and C=O in quinone and/or hydrogen-bonded conjugated ketones. Additionally, the presence of a peak at 1550 cm^{-1} was associated with N–H deformation and C=N stretching (referred to as amide II band), as well as stretching of aromatic C=C. There were several absorption bands detected between 1200-1400 cm^{-1} were denoting the presence of asymmetric bending of C–H in CH3 groups, deformation of O–H, and stretching of C–O in phenolic OH, bending of C–H in CH_2 and CH_3 groups, anti-symmetric stretching of COO–, and stretching of C–O in aryl esters. The extra peak observed at 1130 cm^{-1}, associated with the skeletal vibration of C–O–C stretching, could possibly be attributed to the presence of cellulose residues in the sample (Ertani et al., 2011). Many peaks between 500-1000 cm^{-1} were denoting the existence of lignin residues in the samples, as reported by Rodríguez-Lucena et al. (2009). There was a remarkable shift in the intensity was detected at each peak. In sample S1 the peaks were more prominent followed by S2, S3, S4 and S5.

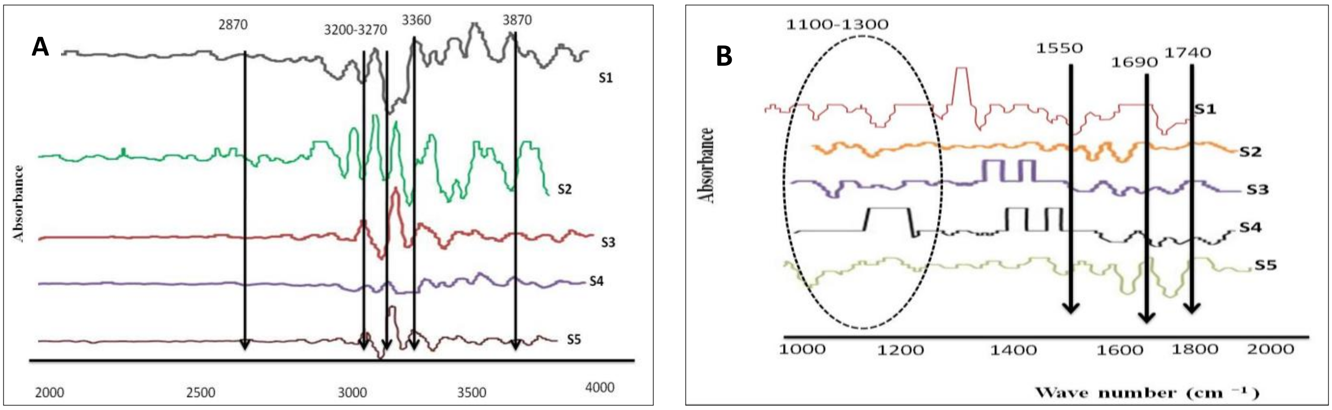


Figure 6: FT-IR spectra of the Humic Acids samples extracted from soil of the five different locations across Delhi, NCR, India (A) illustrate the diverse functional groups present in the range 2000 to 4000 cm^{-1},and (B) illustrate the diverse functional groups present in the range 1000 to 2000 cm^{-1}

A higher percentage of C, and H in S1and S2 were suggesting a lower degree of humification than that of S3, S4, and S5. The H/C, N/C, values were almost similar for S2 and S3 however they were highest for S2, suggesting a higher degree of unsaturation. The higher values in S2 were denoting the presence of less substituted aromatic rings due to shorter transformation reactions.

Hydroponics Setup with Rice Using Various Concentrations of HA

Treatments significantly affected the fresh mass, length, protein content, chlorophyll content and carotenoids content of the rice seedlings. All the treatments gave significantly higher values. The best treatment for all the parameters was T3 in which the value of the shoot fresh mass was 0.79 g/seedling, and the fresh root mass was 0.325 g/seedling. The value of maximum total protein content is 19.1 mg g^{-1} FW, maximum chlorophyll content is 29 mg g^{-1} FW and the maximum carotenoids is 551 mg g^{-1} FW. The least values of all the parameters were observed in T11.

Discussion

The elemental composition serves as a fundamental aspect that unveils the organic makeup of each sample. Carbon, nitrogen, hydrogen, oxygen, and sulfur work together in organic compounds, showing how important the elemental composition is in understanding how systems function. Our study assess the elemental composition's core role in describing the organic composition of examined samples. This comprehensive analysis focusses on essential ratio of element (C/H and C/N) alongside ash content. The adjustments for water content and remaining ash content, executed through analysis using thermogravimetric, contribute to these outcomes. Aligned with Amir et al. (2004), composting induced substantial nitrogen content (%N) reduction and a higher carbon-to-nitrogen (C/N) ratio. Parallel to their findings, our study indicates increased oxygen content (%O) and a slightly elevated oxygen-to-carbon (O/C) ratio post-composting, collectively suggesting compositional transformations. It's important that hydrogen (%H) and carbon (%C) content experienced limited variation during this process, aligning with Amir et al.'s observations. The distribution of carbon content (%C) across sampling stations resonates with Tadini et al. (2022), revealing higher %C in S1, followed by S2, S3, S4, and S5. Their study demonstrated declining soil carbon content (%C) with depth across integrated agricultural systems, paralleling our depth-related trend.

Table 3. Impact of different treatments on seedling length and fresh biomass, total chlorophyll, carotenoid, and protein content in rice seedlings

Treatments	Fresh mass (g/ seedling)		Length (cm/seedling)		Total Protein (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
	Shoot	Root	Shoot	Root			
T1(Control)	0.68±.0.006	0.291±0.008	16.1±0.47	8.58±0.35	15.8 ± 0.19	25 ± 1.76	463.0 ± 5.19
T2	0.52±.0.008	0.212±0.032	13.1±0.66	6.40±0.70	12.8 ± 0.15	21 ± 1.43	383.2 ± 4.29
T3	0.79±.0.029	0.325±0.010	18.3±0.45	9.36±0.90	19.1 ± 0.23	29 ± 1.99	551.0 ± 6.17
T4	0.60±.0.004	0.253±0.023	14.2±0.62	7.40±0.59	14.4 ± 0.17	23 ± 1.61	427.5 ± 4.79
T5	0.48±.0.004	0.183±0.007	12.1±0.53	5.90±0.34	12.3 ± 0.15	20 ± 1.38	378.0 ± 4.23
T6	0.62±0.014	0.244±0.009	14.9±0.38	7.65±0.12	14.6 ± 0.18	22 ± 1.54	412.0 ± 4.61
T7	0.51±0.012	0.195±0.012	12.8±0.81	6.33±0.28	12.1 ± 0.15	20 ± 1.41	373.1 ± 4.18
T8	0.62±0.007	0.263±0.006	14.4±0.78	7.93±0.44	14.2 ± 0.17	23 ± 1.57	384.5 ± 4.31
T9	0.68±.0.006	0.165±0.008	16.1±0.47	8.58±0.35	15.8 ± 0.19	25 ± 1.76	463.0 ± 5.19
T10	0.49±.0.029	0.321±0.010	17.3±0.44	8.36±0.80	15.1 ± 0.23	29 ± 1.99	561.0 ± 6.81
T11	0.20±.0.008	0.126±0.032	11.1±0.66	5.40±0.70	11.8 ± 0.15	19 ± 1.43	356.2 ± 4.29
T12	0.30±.0.002	0.242±0.023	12.6±0.61	5.70±0.54	13.4 ± 0.17	25 ± 1.31	438.5 ± 4.58
T13	0.38±.0.004	0.173±0.007	13.1±0.53	6.90±0.34	14.3 ± 0.14	22 ± 1.34	385.8 ± 3.68
T14	0.42±0.014	0.144±0.009	16.9±0.38	6.95±0.17	13.6 ± 0.18	21 ± 1.36	321.0 ± 3.25
T15	0.48±0.012	0.205±0.012	11.8±0.81	6.45±0.28	12.1 ± 0.11	21 ± 1.48	328.8 ± 3.85
T16	0.31±0.007	0.011±0.006	11.2±0.78	5.63±0.44	14.2 ± 0.17	23 ± 1.57	329.5 ± 4.02

Mirroring Polyakov et al. (2019), our %N analysis reveals a distinctive trend among sampling stations: S2>S1>S3>S4>S5. Their focus on HAs underscores the relationship between nitrogen (%N) and hydrogen (%H) content within HAs, aligned with our observations. Additionally, the heightened oxygen (%O) and sulphur (%S) content in S1 resonates with Polyakov et al. (2019)'s emphasis on HAs' elemental composition. Despite differing sample types, our study's elevated oxygen and sulphur content in S1 echoes similar organic composition trends.

Our study demonstrates that UV absorption serves as quickly and highly responsive for characterizing the molecular composition of samples, particularly in terms of unsaturated carbon content. The UV spectra exhibited elevated absorbance within the 200-300 nm range for sample S2, indicating a higher presence of chromophores associated with double bonds (C=C, C=O). These findings align with the observation of greater aromatic structures (evidenced by a higher H/C ratio) in sample S1. The analysis revealed that sample S1 had the highest percentage of HA at 0.089%, followed by decreasing percentages in the order of S2, S3, S4, and S5. Our research underscores the utility of UV absorption analysis as a valuable tool for assessing the unsaturated carbon content in samples and provides valuable insights into the relative HA content among the tested samples.

In a series of studies on the characterization of HS, UV-Vis spectroscopy has emerged as a valuable analytical tool. Enev et al. (2014) compared HS from various sources, including soil, compost, and lignite, and employed UV-Vis spectroscopy to assess their structural differences. They found that the existence of O-containing functional groups followed a distinct order across these materials. Additionally, the study by Chen et al. (2002) focused on natural organic matter (NOM) fractions and reference soil HA (SHA) and utilized UV-Vis spectroscopy to reveal variations in the excess of aromatic C=C and methoxyl (–OCH_3) functional groups. Furthermore, Chen et al. (1977) emphasized the significance of the E4/E6 ratio, a key UV-Vis parameter, for characterizing humic and fulvic acids, highlighting its relationship with particle size, pH, and various chemical properties. Pedra et al. (2008) explored the effects of organic amendments on soil HAs using UV-Vis spectroscopy, particularly noting alterations in the E4/E6 ratio and the aliphatic and aromatic characteristics of soil HAs. Altogether, these studies underscore the versatility and applicability of UV-Vis spectroscopy in elucidating the structural characteristics of HS from various sources.

The FT-IR analysis conducted on various samples of HAs from diverse locations has revealed critical insights into their compositional and structural characteristics. These findings can be discussed in conjunction with relevant studies, focuses on the complexity of HAs in different environmental contexts. In the spectral zone of 3500-3000 cm^{-1}, bands associated with O-H stretching, minor N-H stretching, and the presence of hydrogen-bonded -OH groups were observed, consistent with previous studies (Woelki et al., 1997). The intensity of these bands was notably higher in samples S1, S2, and S3, suggesting a greater presence of matured HS, while S4 and S5 exhibited lower intensities, indicating the presence of less matured humic compounds.

The bands in the 2900-2300 cm^{-1} range corresponded to asymmetric and symmetric C-H stretching of CH_2 groups. This aligns with the absence of these bands in S4 and S5, where unique chemical reactions may be occurring, as reported by Tu et al. (1993). Absorption bands at 1740 cm^{-1} were attributed to the C=O stretching of COOH and other carbonyl groups, consistent with the presence of these functional groups (Woelki et al., 1997). The range of 1600-1690 cm^{-1} indicated aromatic C=C skeletal vibrations and C=O stretching of amide groups (amide I band), consistent with observations in previous studies (Tu et al., 1993). The peak at 1550 cm^{-1} was linked to N-H deformation and C=N stretching (amide II band) as well as aromatic C=C stretching, corroborating findings by Senesi et al. (1999).

Additional absorption bands between 1200-1400 cm^{-1} pointed to various functional groups, including C-H asymmetric bending in CH_3 groups, O-H deformation, C-O stretching of phenolic OH, C-H bending of CH_2 and CH_3 groups, anti-symmetric stretching of COO-, and C-O stretching of aryl esters aligning with the results observed by Senesi et al. (1999). The presence of cellulose residues in the sample was suggested by an additional band at 1130 cm^{-1} (C-O-C stretching skeletal vibration), similar to findings by Senesi et al. (1999). The detection of lignin residues aligns with the presence of similar aromatic components observed in previous studies (Senesi et al., 1999).Variations in the intensity of these spectral features were observed among the different samples. S1 exhibited the most prominent peaks, followed by S2, S3, S4, and S5. This variance suggests differences in the degree of humification and the degree of unsaturation, as reported by previous research (Tu et al., 1993; Senesi et al., 1999). The FT-IR analysis of HAs presented in this study parallels and extends the findings of previous research. The characterization of functional groups and spectral features provides valuable insights into the diverse composition and structural characteristics of HAs across various environmental sectors. These findings contribute to an enhanced comprehension of the role of HAs in organic matter decomposition and nutrient cycling (Tu et al., 1993; Woelki et al., 1997; Senesi et al., 1999).

In our study, we have found that the various treatments significantly influenced the growth and biochemical composition of rice seedlings. Specifically, all treatments yielded significantly higher values compared to the control group, indicating their positive impact on the seedlings. Treatment T3 stood out as the most effective, with the highest shoot and root fresh mass, maximum total protein content, chlorophyll content, and carotenoid content. In contrast, treatment T11 consistently exhibited the lowest values across all parameters. These findings emphasize the substantial influence of the treatments on rice seedling growth and biochemical composition, with T3 demonstrating the most favourable outcomes.

Our research aligns with previous studies on HS and their role in promoting plant growth and enhancing biochemical composition. Nardi et al. (2021) highlighted the importance of understanding the connection between the chemical structure of humic substances (HS) and their impact on biological activity is crucial. They highlighted the significance of specific functional groups in HS that could elicit favorable physiological responses in plants including hormone-like signalling pathways. This aligns with our findings where treatment T3 significantly increased protein content, chlorophyll content, and carotenoid content in rice seedlings, indicating its positive impact on biochemical composition. Our results are also consistent with the study by Vaccaro et al. (2015), which examined the effects of HS on maize seedlings. They found that HS positively influenced nitrate metabolism, leading to increased soluble protein and amino acid synthesis, as well as the activity and transcription of enzymes involved in nitrogen assimilation. Similarly, in our study, treatment T3 significantly increased protein content, suggesting improved biochemical composition in rice seedlings.

The study by García et al. (2014) investigated the effects of HA under water stress conditions on rice plants. They observed that peroxidase activity was maintained by HA, reduced lipid peroxidation, and regulated abscisic acid levels, indicating its protective effects on plants. In our study, treatment T3 consistently outperformed other treatments across various parameters, suggesting its potential to promote rice seedling growth and biochemical enhancement, which is in line with the protective effects demonstrated by HA in the study.

This study discusses about the elemental composition, UV absorption and FTIR analysis of humic acids (HAs). The study explores compositional changes during composting and highlights the utility of UV-Vis spectroscopy and FTIR analysis in characterizing HAs. It also connects these findings with some previous reported literature. Additionally, the research investigates the impact of various treatments on rice seedling growth and biochemical composition. These results align with previous studies emphasizing the positive effects of humic substances on plant growth and biochemical composition.

Conclusion

Humic acids play a significant role in the fertility of soil and plant health. In present study, assessment of diverse characteristics of HAs from various locations of Delhi NCR has been done. Through elemental analysis, we determined that the samples exhibited variations in C, H, N_2, O_2 and S content, with sample S1 showing the highest C and S levels. UV-Vis spectroscopy highlighted differences in the unsaturated carbon content among samples, with sample S2 displaying the highest absorbance in the 200-300 nm range, indicative of more double bonds. Moreover, our FT-IR analysis focuses on the structural and compositional disparities among HA samples, with S1 and S2 exhibiting more intense bands, suggesting a lower degree of humification. The hydroponics experiments revealed significant effects of different treatments of HAs on rice seedlings' growth and biochemical characteristics. Treatment T3 has been found to be the most effective by promoting significant growth in root and shoot length, increased root and shoot fresh mass increased protein, chlorophyll, and carotenoid content in rice seedlings. On the contrary, treatment T11 consistently showed the least helpful to the seedling health across all parameters. The findings contribute to a understanding of the HAs and their role in organic matter composition in the various soil. This study also emphasizes the potential for optimizing promoting and utilization of HAs to enhance sustainable crop growth by ensuring soil and plant health for sustainable agriculture practices.

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