SOIL STUDIES

RESEARCH PAPER

Spectroscopic, thermic and metal binding properties of commercial humic acid and leonardite

Ahsen Ertem[1]* iD, Emre Karmaz[1] iD, Kadri Avağ[1] iD, R. Murat Peker[1] iD

[1] Soil, Fertilizer and Water Resources Central Research Institute, 06172 Ankara, Türkiye

Abstract

Humic acids are formed from a combination of variable chemical groups found in natural sources containing partially aromatic groups in an amorphous structure. These substances have been shown to significantly impact plant biomass, affecting nutrient distribution through direct interaction with the plant and nutrient supply and regulation through indirect interaction. The lack of a regular and repeating molecular structure in humic substances makes them challenging to determine. Humic substances used in agriculture commercially are humic and fulvic acid or combinations of both in different proportions. In our research, we used spectroscopic and thermal methods to understand the structures of commercial samples. We conducted FT-IR (Fourier Transform Infra-Red) spectroscopy to analyze the structure's bonds, thermal analysis to comprehend its thermal properties, and NMR (Nuclear Magnetic Resonance) analysis to obtain information about the carbon content. By combining, comparing, and interpreting all these methods, we can predict commercial samples' spectroscopic and thermal properties during the production stage. This can serve as a foundation for researchers studying or utilizing humic acid.

Introduction

Humic substances have been used in agriculture for a long time due to their beneficial effects on plant nutrition. Humic acid is composed of a large and complex structure consisting of carbon, hydrogen, nitrogen, and oxygen. These substances, which are the source of humic acid, are amorphous, partially aromatic, and naturally occurring. Humic substances are categorized into humin, humic acid, and fulvic acid (Chen and Avnimeleek, 1986). The main benefits of humic substances are that they affect the distribution of nutrients by direct interaction and provide and regulate nutrients through their indirect effects (Schnitzer and Khan, 1978). Humic acids are macro-organic molecules containing polymeric phenolic compounds that can form complexes with metal cations (Schnitzer and Khan, 1978).

Humic substances have large supramolecular structures, which can cause their properties to vary depending on their source. Therefore, characterization and content determination are of great importance. Studies on this subject have continued increasingly in recent years. Like this "For instance, five coal samples from China were oxidized with hydrogen peroxide to obtain humic acids (HAs). These HAs were characterized by physical and chemical methods, showing differences in yield, ash content, aromaticity,

molecular weight, and functional groups (Shuangdui et al., 2021). In the study aimed at researching the molecular characterization of organic matter in the soil, the fractions of humic matter, humic substances, fulvic acids, humic acids, and humins were investigated and broad spectrum spectroscopic (UV-VIS and VIS - near IR) analyses were performed. In addition, electrochemicals (zeta potential, particle size diameter, and polydispersity index were examined. All data were brought together to investigate the relevant differences in behavior, formation, and composition, and it was determined that humic substances differ in terms of behavior, formation, composition, and sorption properties (Ukalska-Jaruga A. et al., 2021).

Additional studies have employed spectroscopic methods (FT-IR/ATR and CP/MAS 13C-NMR - Carbon 13 Nuclear Magnetic Resonance) and elemental composition analysis to evaluate the thermal decomposition and quality of humic acids from various sources, revealing differences in the degree of humification. Moreover, spectroscopic methods (FT-IR/ATR and CP/MAS ^{13}C -NMR) and elemental composition analysis were used to determine the effect of humic acids extracted from peat and lignite on the thermal decomposition of HA and to evaluate the quality of humic acid in this article, which examined the thermal decomposition of ammonium nitrate and humic acid mixtures Analysis of the spectra revealed differences in the degree of humification of humic acids extracted from various raw materials (Nieweś et al., 2023).

Sodium humate samples were isolated from soil, compost, and South Moravian lignite, and FT-IR, fluorescence spectroscopy, and ^{13}C- NMR analyses were performed on the samples. The density of chemical groups decreases in the order of compost, soil, lignohumate, and lignite. It is observed that sodium humate samples isolated from soil, compost, and lignite are more polycondensed (Enev et al., 2014)

Humic substances have many beneficial properties. In addition to agriculture, it is also used in medicine and animal husbandry. Humic acid has been found to reduce damage caused by the retention of heavy metals and pesticides when taken orally in animals. In recent years, the use of humic acids in medicine has been investigated in the solution of very common problems such as asthma, bronchitis, flu, stomach disorders, kidney stone disorders, hemorrhoids, skin cancer, stopping bleeding, anemia, excessive sleep, and oversleeping. Sleepiness accumulation, pollution and residue problems caused by chemical fertilizers and regulators do not apply to humic acids.

Certain humic acid derivatives, once purified from humate substances, are found to possess similar molecular structures and chemical characteristics. However, a study conducted with humic acids extracted from different types of soils revealed significant variations. Humic acid obtained from andisol soils was found to be rich in carboxyl and carbonyl groups with a high C ratio, while being poor in hydroxyl, and low in H and N. Conversely, humic acid obtained from entisol soils exhibited low carboxyl and carbonyl groups. Furthermore, aquatic humic acids were found to have higher H and N values compared to terrestrial humic acids (Yonebayashi and Hattori, 1988). Additionally, humic acid extracted from lignite humate contains more nitrogen, carbohydrates, and aromatic compounds than those obtained from non-lignite sources. Moreover, humic acid extracted from domestic wastes contains more carbon, exhibits a more aliphatic structure, contains fewer oxygen groups, and is more heterogeneous than those extracted from peat and leonardite (Aşık, 2008).

In Türkiye, the use of humic acid is increasing, but its application often overlooks source variations and soil-plant characteristics. The Ministry of Agriculture and Forestry regulates commercial humic acid products, classifying them as organic according to the Ministry's 2018 directive (Anonymous, 2018). To use humic acid resources more efficiently and healthily, their structures need to be characterized in detail.

The study conducted thorough final characterization analyses on humic acid and leonardite products, commonly used in the market, uncover their molecular properties. This research marks the first time that the spectroscopic and thermal properties of various commercial humic acid products have been collectively examined at a national level.

Material and Method

Humic acid and Leonardite samples

The samples analyzed in this study were selected from commonly used products in the market to ensure compliance with relevant regulations. Among the samples, three were identified as leonardite, one as liquid potassium humate, and four as solid potassium humate.

Characterization of Materials

^{13}C-NMR analyses targeted within the scope of the project were carried out at the Middle East Technical University Central Laboratories. FT-IR and Thermogravimetric analyses (TGA) were performed at Bilkent University National Nanotechnology Center. Elemental analyses of the samples were performed with the ICP-OES device at the Soil, Fertilizer, and Water Resources Central Research Institute. In addition, total humic acid + fulvic acid percentage, organic matter percentage, total nitrogen percentage, amount of Na and S, moisture, pH, and EC parameters were determined in the laboratories of the Central Research Institute of Soil, Fertilizer and Water Resources.

The moisture content of the samples was determined by subjecting them to 70°C after initial wet weighing. pH and EC values were measured by diluting

the samples 1/10 using potentiometric methods. Organic matter analysis was conducted for 5 hours at 550°C using a modified AOAC method. Total carbon, nitrogen, and sulfur analyses were performed in duplicate using the TruSpec-Leco brand elemental analysis. Total potassium, water-soluble potassium, and total phosphorus analyses were carried out following the Kacar &Kutuk -2009 method. Additionally, total sodium analyses were performed using a Jenway brand flame photometer. The total phosphorus analysis was conducted using the coloring method with a Libra-Biochrom brand UV-visible spectrophotometer.

All measurements were conducted twice, and the average value was considered as the result. Analysis of calcium, magnesium, iron, copper, manganese, zinc, cadmium, lead, cobalt, chromium, and nickel additives involved three repeated readings using the Varian 720-ES ICP-OES device. The Merck brand ICP multi-element standard solution IV served as the reference standard. Statistical evaluation of the results was deemed unnecessary.

Elemental Analysis

The device used in elemental analysis can simultaneously detect carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) in the structure of inorganic and organic substances in solid, liquid, or gas samples. With the elemental analyzer, microanalysis of all homogeneous organic compounds such as petrochemical industry (oils and derivatives), industrial chemistry (polymer), environment (soil, sediment, water), and drug and protein analysis can be performed. The sample was prepared by precise weighing between 0.10 - 0.20 g and analyzed with an instrumental analyzer (Leco TruSpec NHC-S). Because of these analyses, the percentages of carbon, hydrogen, and nitrogen, which form the main skeleton of the molecules of our samples, were determined.

FT-IR Analyze

Fourier transform infrared (FT-IR) spectroscopy is a method frequently used in basic sciences, health sciences, and engineering by detecting the bonds in the structure of molecules. This method is based on the absorption of infrared rays falling on intramolecular bonds by the vibration and rotational movements of the bonds. However, this phenomenon is observed only in polar molecules with dipole moments. IR spectra can be obtained by pelleting solid samples with potassium bromide (KBr) or analyzing them directly with an ATR unit with IR spectrometry. Direct IR spectra of liquid and gas samples can be obtained with ATR (Attenuated Total Reflection unit). The IR spectrometer, an indispensable device for both academic and industrial studies, can be applied in almost every field. It is often used to determine organic and inorganic molecules (Yildiz et al., 1997). In this study, an FT-IR/Thermo device with a diamond-tipped

ATR unit was used at Bilkent University National Nanotechnology Research Center (UNAM) laboratories. As sample number 9, Acros Organics brand powder humic acid salt produced as a laboratory chemical with a purity of 50-60 % was used.

^{13}C- NMR Analyze

NMR is the most powerful technique for determining the structures of organic compounds. It can be used to examine a wide variety of nuclei: ^{1}H, ^{13}C, ^{15}N, ^{19}F, and ^{31}P are examples. All atoms with an odd atomic number and/or mass number have nuclear spin. The nucleus (proton, ^{1}H, etc.), which rotates around itself (rotational motion) and has an electric charge, creates its magnetic field. When spinning protons are placed in an external magnetic field, they rotate under the influence of this magnetic field. The magnetic fields of these rotating protons tend to be either in the same direction as the external field or in the opposite direction to the external field.

When the energy of a photon with the right amount of energy is absorbed, the direction of its magnetic field changes. The energy difference between the two states is corrected by the strength of the magnetic field (strength of the magnet). If all protons absorbed the same amount of energy in the same magnetic field, not much information would be obtained. Protons protect them from the influence of the external magnetic field. Spinning electrons reduce the effect of the external field by creating an excited magnetic field in the opposite direction to the external magnetic field. Protons in molecules depend on chemical environmental conditions.

They are preserved in varying amounts. The signal number indicates how many different types of protons there are. The position of the signals (chemical shift) indicates how protected the proton is. The intensity of the signals tells you how many protons there are. The division of signals shows how many protons are in neighboring atoms (Erdik, 1998).

TG/DTG/DTA Analysis

Thermal methods are based on the study of the dynamic relationship between temperature and some properties of the system, such as mass, reaction rate, or volume. There are about twelve methods of thermal analysis; the methods we used in the project are as follows; Thermo Gravimetric Analysis (TGA/DTG) and Differential Thermal Analysis (DTA).

Thermo Gravimetric analysis (TGA)

In Thermo Gravimetric Analysis, the mass (weight) of the sample is continuously monitored as the temperature is heated from ambient conditions to temperatures reaching 1200°C. The mass-temperature graph is called a "thermogram" and is used in qualitative/quantitative determinations. The thermogravimetric analyzer consists of a precision analytical balance, an oven, an oven temperature

controller and programmer, and a recorder. The logger plots the mass of the sample versus the temperature. In an inert atmosphere, auxiliary systems are also needed to provide this when necessary. With TGA, the purity, degradation behavior, and chemical kinetics of the sample are examined.

Differential thermal methods are used to determine the composition of natural or artificial substances. The thermal behavior of inorganic compounds such as silicates, ferrites, clay, oxides, ceramics, and glasses can be monitored by differential thermal measurements. The monitoring processes consist of melting, dissolution, hydrogen removal, oxidation, reduction, adsorption, degradation, and solid-state reactions. The most important applications are drawing phase diagrams and investigating phase transitions.

Thermal analyses were performed in Bilkent University National Nanotechnology Research Center (UNAM) laboratories. Analyzes were carried out with TA Instruments Q500 device in a dry air environment at a heating rate of 10°C min^{-1}, using sintered α-Al_2O_3 as a reference, in a platinum crucible at a temperature range of 20-900°C and using 5-10 mg of sample. Sample number 9 was used as the standard. It is an Acros Organics brand powdered humic acid salt, produced as a standard laboratory chemical with 50-60% purity.

Differential Thermal Analyze (DTA)

In Differential Thermal Analysis, the heat absorbed or emitted by the system is observed by measuring the temperature difference between a chemical system (sample) and an inert reference compound (can be aluminum, silicon carbide, or glass particles). In operation, the temperatures of the system and reference are increased at a constant rate. The temperature difference between the sample and the reference is monitored as a function of temperature (Yıldız et al., 1997).

The sample is prepared for analysis by placing it in an inert micro crucible with precision weighing in the thermal analyzer. Because of the analysis, thermograms, differential thermal graphs, and differential thermal analysis graphs are interpreted.

ICP-OES Analyze

Metal contents of samples prepared by licking in a microwave oven will be determined by ICP-OES. The ICP-OES instrument is an analytical technique that analyzes many different elements in metal, sediment, soil rock, industrial waste, anode sludge, aluminum and magnesium anode, coal ash, ore, and pre-treated beneficiation products. ICP stands for Inductively Coupled Plasma and is an optical emission spectrometer. This technique involves the excitation of the sample with argon plasma reaching temperatures of 10.000 K by electromagnetic induction and identification of the excited elements according to the specific wavelengths they emit. Plasma is obtained by electromagnetically exciting argon gas in induction windings with a radio frequency (RF) generator. This happens when the hot plasma ionizes the incoming gas and the process continues continuously. The fundamental principle of inductively coupled plasma spectroscopy is to measure the emission emitted by the excitation of atoms and ions in the plasma, an electrically conductive gaseous medium containing a high concentration of equivalent amounts of cations and electrons.

Results and Discussion

Analyzes

Physical and chemical analyses

Analyzes were carried out on eight commercial samples 3 of the samples are products originating from leonardite, 1 is liquid potassium humate and 4 are solid potassium humate. Study findings show that products originating from leonardite exhibit high organic matter and humic + fulvic acid content. Additionally, the analysis revealed that the liquid humic acid was particularly rich in potassium. Although the analysis results of the selected samples vary, the results of leonardite-derived products and potassium humate-labeled products are similar in most parameters. When the organic matter amounts were examined, high values such as 50.63% and 54.2% in the first 2 products and 95.93% in the third product were found in the leonardite-derived products. It was determined that the liquid sample had the lowest organic matter content at 4.05%. Potassium humate samples have 35.8-34.41-66.86-44.51% organic matter, respectively. Leonardite-derived products have much higher moisture content than humate-derived products, between 20.90-48.94% moisture values and 3.54-13.13% moisture values. While humate-derived products have more basic pH values of 9.97-10.34, leonardite-derived products have more acidic values of 7.58-7.63-8.04. Total nitrogen values were determined slightly higher in humate-based products. While it is 1.03% in leonardite products, it is 1.44% in Humate-based products. When the parameters of total potassium, water-soluble potassium, and total phosphorus are compared, it is seen that the values of humate-derived products are 15-20 times higher than the others.

The interaction of a molecule with metal elements that have electropositive properties, such as plant nutrients, is measured by the electronegativity characteristics of that molecule. Electronegativity is the tendency of an atom in a compound to attract bond electrons. If the molecule contains more than one covalent bond, the bond dipole moments have a vector result. Polar molecules with a net dipole moment interact electrostatically with each other and with other molecules. The vector direction and magnitude

of the net dipole moment are determining factors in these interactions.

The humic + fulvic acid ratio, which is the most important analysis parameter of the samples, is 72.23-64.10-50.14%, respectively, in leonardite-origin products. The rate in the liquid sample was determined as 13.97%. For humate derivative products, it is 67.97-68.38-71.89-64.16%, respectively. When the ratios of the elements contained in the samples are examined, it can be said that calcium, magnesium, chromium, lead,

and sulfur are more in leonardite-sourced products, while sodium and zinc elements are more in humate-sourced products. Since the amounts of other elements vary from sample to sample, no correlation can be established between sample sources. While cadmium and cobalt remained below the detection limit in all samples, manganese, nickel, lead, and copper elements could not be measured in some samples when they remained below the detection limit (Table 1-2).

Table 1. Analysis of selected commercial humic acids (1)

Analyzes	Analysis method	Leonardit-1	Leonardit-2	Leonardit-3	Liquid Humic Acid
Organic matter (%)	AOAC 967.03-04-05	50,63	54,22	95,93	4,05
Humidity (%)	AOAC 1995	48,94	37,90	20,90	--
pH	1/10 Potansiyometry	7,58	7,63	8,04	12,1
EC (mS cm^{-1})	1/10	2,06	1,96	3,05	26,00
Total nitrogen (N) (%)	TS EN 15478	1,089	1,080	0,92	0,31
Total carbon (C) (%)	Elemental analysis device	34,05	34,15	22,10	6,33
Total potassium (K) (%)	Kacar and Kutuk, 2009	0,04	0,06	0,19	3,9
Water-soluble Potassium (K) (%)	Kacar and Kutuk, 2009	0,05	0,05	0,09	2,28
Total (H+F) acid (%)	TSE 5869	72,23	64,10	50,14	13,97
Total phosphorus (P) (%)	Kacar and Kutuk, 2009	0,44	0,46	0,20	0,002
Total calcium (Ca) (mg L^{-1})	ICP-OES	93140	79470	45330	15466
Total magnesium (Mg) (mg L^{-1})	ICP-OES	3777	4940	18443	1411
Total iron (Fe) (mg L^{-1})	ICP-OES	5871	126830	37040	396,80
Total manganese (Mn) (mg L^{-1})	ICP-OES	32,89	45,08	277,10	1,78
Total copper (Cu) (mg L^{-1})	ICP-OES	6,68	129,30	53,00	UDL
Total zinc (Zn) (mg L^{-1})	ICP-OES	1,44	14,57	UDL	0,62
Total cadmium (Cd) (mg L^{-1})	ICP-OES	UDL	UDL	UDL	UDL
Total lead (Pb) (mg L^{-1})	ICP-OES	UDL	UDL	UDL	UDL
Total chromium (Cr) (mg L^{-1})	ICP-OES	57,81	67,00	95,80	14,2
Total cobalt (Co) (mg L^{-1})	ICP-OES	UDL	UDL	UDL	UDL
Total nickel (Ni) (mg L^{-1})	ICP-OES	7,65	35,97	324,00	4,56
Total sulfur (S) (%)	Elemental analysis device	2,55	4,32	6,07	1,11
Total sodium (Na) (%)	Flame photometry	0,04	0,02	0,13	0,07

UDL: Under Detection Limit, Cu<0,015 ppm, Pb <0,09 ppm, Cd<0,03 ppm, Co<0,01 ppm, Zn<0,01 ppm

Table 2. Analysis of selected commercial humic acids (2)

Analyzes	Analysis method	Humat-1	Humat-2	Humat-3	Humat-4
Organic matter (%)	AOAC 967.03-04-05	35,89	34,41	66,86	44,51
Humidity (%)	AOAC 1995	7,82	7,98	13,13	3,54
pH	1/10 Potansiyometry	10,16	10,34	9,97	10,32
EC (mS cm^{-1})	1/10	16,50	15,44	11,91	21,8
Total nitrogen (N) (%)	TS EN 15478	1,37	1,38	1,53	1,47
Total carbon (C) (%)	Elemental analysis device	46,02	43,90	46,55	41,05
Total potassium (K) (%)	Kacar and Kutuk, 2009	6,05	6,82	5,62	13,26
Water-soluble Potassium (K) (%)	Kacar and Kutuk,2009	11,93	9,36	2,81	9,59
Total (H+F) acid (%)	TSE 5869	67,97	68,38	71,89	64,16
Total phosphorus (P) (%)	Kacar and Kutuk, 2009	3,24	3,41	3,97	0,61
Total calcium (Ca) (mg L^{-1})	ICP-OES	8517,00	7511,00	9124,00	13766,00
Total magnesium (Mg) (mg L^{-1})	ICP-OES	1093,90	1280,40	1358,404	4687,00
Total iron (Fe) (mg L^{-1})	ICP-OES	17139,00	13201,00	16415,00	5274,00
Total manganese (Mn) (mg L^{-1})	ICP-OES	UDL	0,22	40,00	128,89
Total copper (Cu) (mg L^{-1})	ICP-OES	46,60	43,97	14,85	22,92
Total zinc (Zn) (mg L^{-1})	ICP-OES	23,00	272,00	74,34	49,92
Total cadmium (Cd) (mg L^{-1})	ICP-OES	UDL	UDL	UDL	UDL
Total lead (Pb) (mg L^{-1})	ICP-OES	5,84	11,65	6,40	0,56
Total chromium (Cr) (mg L^{-1})	ICP-OES	12,88	11,48	9,75	UDL
Total cobalt (Co) (mg L^{-1})	ICP-OES	UDL	UDL	UDL	UDL
Total nickel (Ni) (mg L^{-1})	ICP-OES	30,43	23,26	27,40	UDL
Total sulfur (S) (%)	Elemental analysis device	UDL	UDL	UDL	UDL
Total sodium (Na) (%)	Flame photometry	2,36	2,42	2,42	0,84

UDL: Under Detection Limit Mn<0,03 ppm, Pb <0,09 ppm, Cd<0,03 ppm, Co<0,01 ppm, Ni<0,14 ppm

FT-IR Analyzes

When the spectra of all samples are evaluated, it is seen that they give very close peaks at very close wavelengths. The peaks in the middle range between 3400-3200 cm^{-1} are O-H stress peaks. Bending peaks of C-H bonds are the most common bonds in the strong peak structure. Because of the overlap of O-C and C-N peaks, slightly shouldered peaks are observed between 1000-900 cm^{-1}. The weaker peaks below 800 cm^{-1} belong to N-H peaks.

Table 3. Observable ranges of some intramolecular vibration types in the IR region

Vibration type	Wave number range, cm^{-1}
C-H strain	2700-3300
C-H bending	1300-1500
O-H strain	3000-3700
O-H stretch (overtone)	6700-7100
N-H strain	3000-3700
N-H stretch (overtone)	6300-7100
N-H bending	700-900
C-O strain	900-1300
C-N strain	900-1300
C=C stress	1600-1700
C=O stress	1600-1900

The IR spectrum of the liquid potassium humate sample shown in Figure 1(d) only displays peaks related to O-H stretching and H-O-H shift, indicating the presence of water. After heating the liquid sample at 100°C for 10 hours, solid humic acid was obtained, and the IR spectrum was taken again. This time, IR peaks consistent with other solid samples were observed, as depicted in Figure 1(f).

Upon examining the overlaid graph in Figure 1(k), it is evident that all samples exhibit a consistent correlation, displaying peaks and shoulders at the same wavelength (Figure 1).
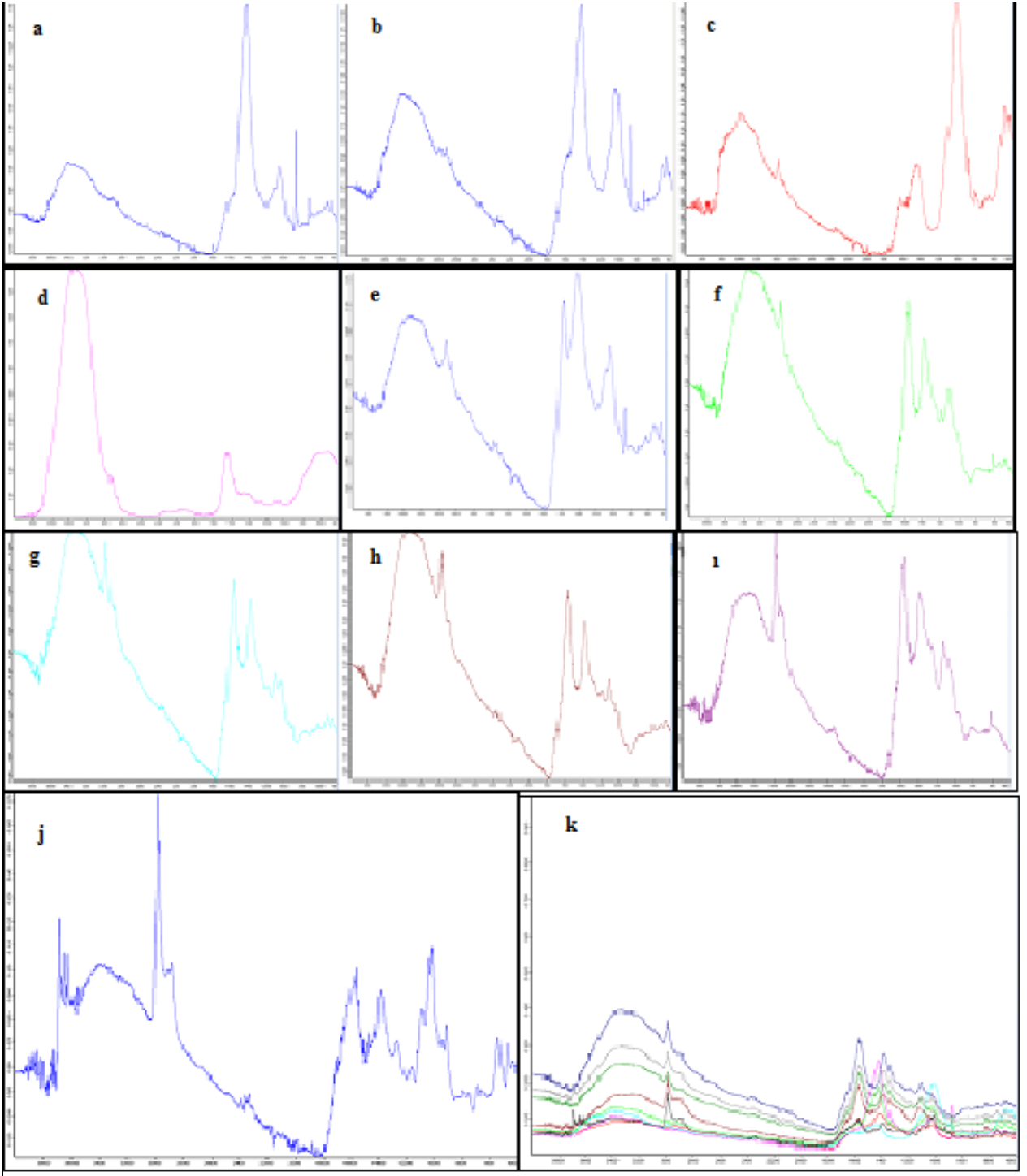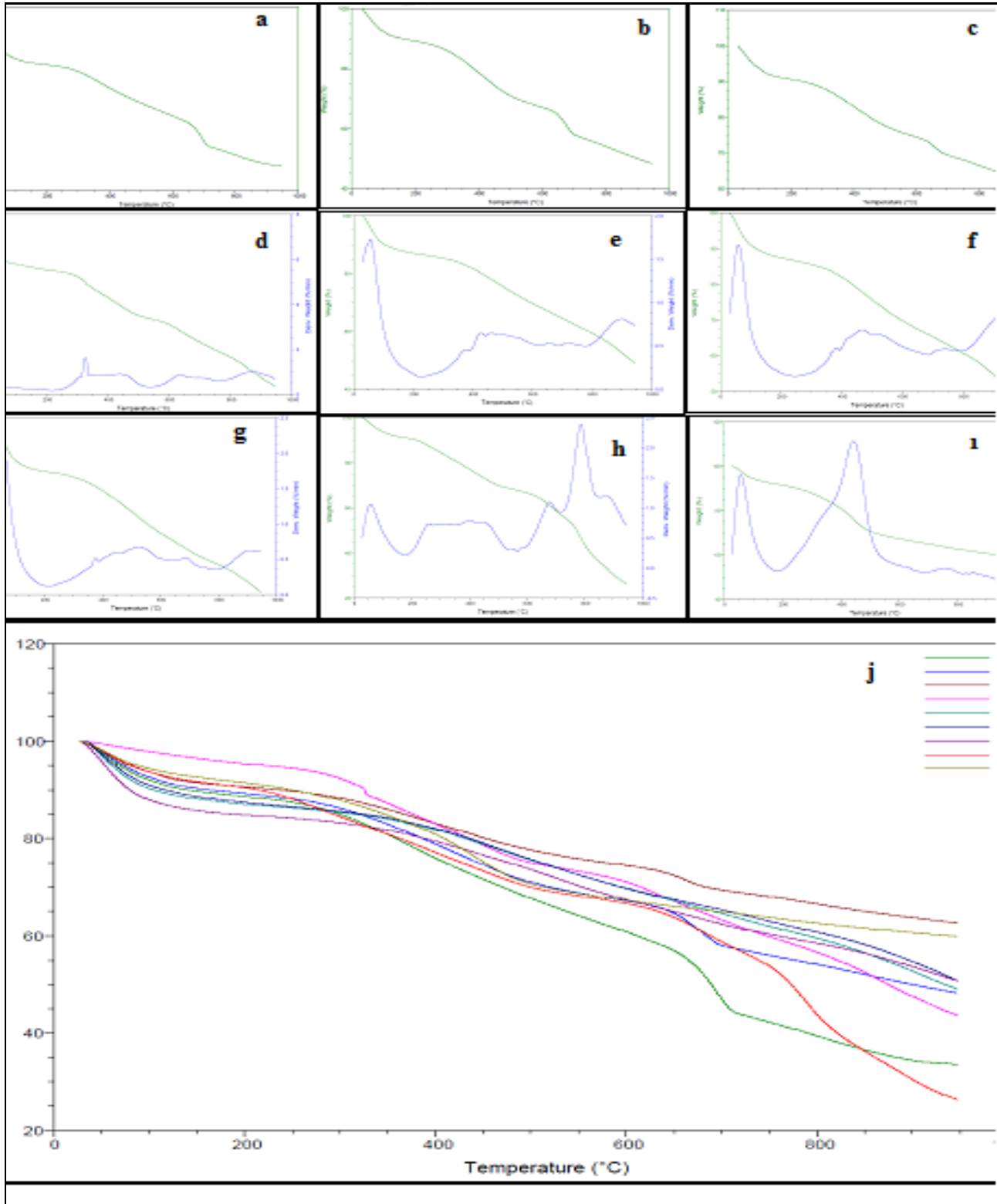

Figure 1. FT-IR spectrums of all samples a) Leonardite No. 1 b) Leonardite No. 2c) Leonardite No. 3 d) Liquid potassium humate No.4 (liquid phase IR) e) Liquid potassium humate no.4 (solid phase IR) f) Potassium humate No. 5 g) Potassium No. 6 humat h) potassium humate no. 7 i) potassium humate no. 8 j) humic acid standard k) Superimposed IR spectra of all samples. In all FTIR tables, the x-axis is given from left to right between 4000-4000 cm$^{-1,}$ and the y-axis is given between 0-0.900 A from bottom to top.

Thermal analysis curves

When the thermal analysis graphs are interpreted, it is clear that the 10-15% mass loss observed at 100°C is water. A mass loss of 7-10% is observed between 100-350°C and 35-40 % between 350-950°C. At 950°C, 35-50% of the mass of the structure is still solid.

Figure 2. TGA curves of all samples a) Leonardite No. 1 b) Leonardite No. 2 c) Leonardite No. 3 d) Liquid potassium humate No. 4 e) Potassium humate No. 5 f) Potassium No. 6 humat g) potassium humate no. 7 h) potassium humate no. 8 i) humic acid standard j) the same axis representation of all TGA curves

When the thermal analysis graphs are interpreted, it is clear that the 10-15% mass loss observed at 100°C is water. A mass loss of 7-10% is observed between 100-350°C and 35-40 % between 350-950°C, at 950°C, 35-50 % of the mass of the structure is still solid.

13C-NMR spectra

^{13}C-NMR analyses were carried out in the central laboratories of the Middle East Technical University with a 300 MHz Solid State High Power Bruker Avance device, using a 4 mm MAS probe and at least 500 mg of powder samples, with a rotation speed of 8500 Hz in approximately 8 hours.

As expected, mixed signals were observed in the ^{13}C-NMR spectra due to the high number of carbons in our samples. However, we observe that the same signals among these signals reinforce each other and form a peak. The divisions cannot be observed due to the large number of C peaks caused by the strong signals. Despite the confusion of the signals, it is seen that the signals become stronger at close points where the samples give very similar spectra in all spectrum shapes.
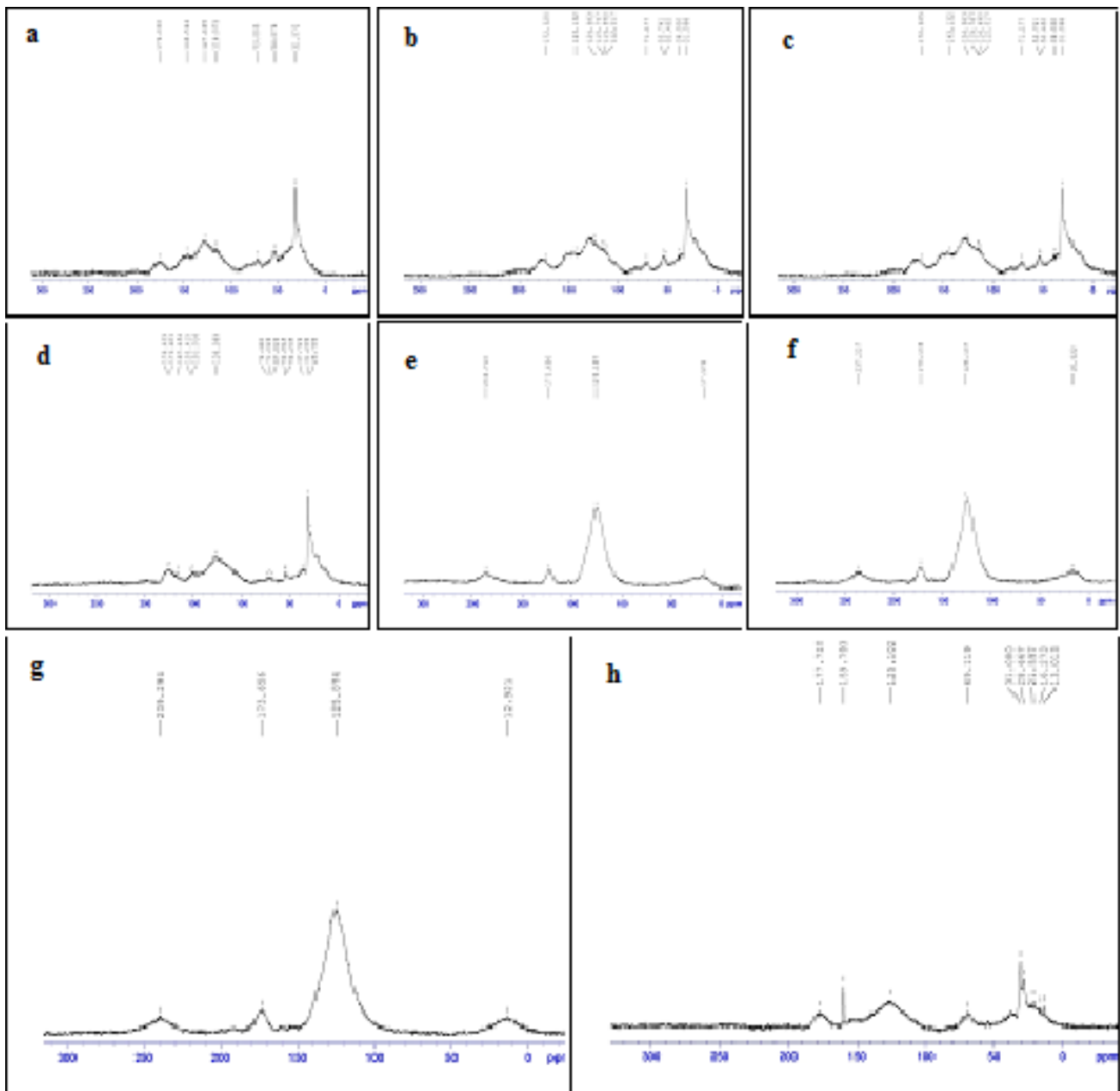
Figure 3. The carbon NMR spectra of the samples a) Leonardite No. 1 b) Leonardite No. 2 c) Leonardite No. 3 d) Liquid potassium humate No. 4 e) Potassium humate No. 5 f) Potassium No. 6 humat g) potassium humate no. 7 h) potassium humate no. 8. In all NMR tables, the x-axis is from left to right from 300 to 0 ppm.

Conclusions

Eight commercial products selected within the scope of the study were examined primarily with their chemical and physical properties. In the analysis of humate substances, it is common to look for a proportional relationship between the analysis of humic and fulvic acids and the analysis of organic matter. The humic acid + fulvic acid analysis result is the total percentage of both acids in the humate substance. Organic matter is an analysis that yields results based on the amount of the carbon element in the structure. In addition to carbon, humate substances also contain oxygen, hydrogen, nitrogen, and small amounts of metal elements. Humate substances are known to have different compositions depending on their source. Therefore, the amount of humic acid + fulvic acid is often greater than the amount of organic matter. However, a fixed correlation cannot be established between the two analysis results.

When the FT-IR spectra are evaluated, it is seen that they give very similar intensity peaks at very close wavelengths. The intermediate intensity peaks between 3200-3400 nm are O-H stress peaks. C-H stress peaks overlap with these peaks, creating flatter and more variable peaks. Because of the overlap of O-C and C-N peaks, peaks with slight shoulders are observed between 1000 and 900 nm. The weaker peaks below 800 nm belong to N-H peaks. When looking at the graph where the spectra are combined, it is seen that all samples show a suitable correlation with peaks and shoulders at the same wavelength. The IR spectrum of the liquid humic acid sample shows only O-H stretching and H-O-H shift peaks. Thus, only the spectrum of water was obtained. When the liquid sample was kept at 100°C for 10 hours, solid humic acid was obtained and the IR spectrum was taken again and IR peaks compatible with the other samples were observed. When FT-IR analysis is evaluated, it can be interpreted that the sample is not a homogeneous liquid humic acid solution, but a liquid suspension.

The general characteristics of the samples in the thermal analysis graphs can be summarized as follows; Samples contain an average of 10-15% moisture. 35-55% of the sample content became gas at 950 °C. The remaining 35-50% remains in solid form. The fact that most of it remained solid because of the analysis indicates a high carbon content and a multi-ring supramolecular structure.

It can be seen that mixed signals are collected in the ^{13}C-NMR spectrum due to the high number of carbons. However, we see that the same signals among these signals reinforce each other and form a peak. Due to the high number of C in the peaks, the breaks cannot be observed clearly due to strong signals. Despite the confusion of the signals, it is seen that the signals become stronger at points close to each other where the samples give very similar spectra. This situation shows that the number of C in the molecule is high.

All chemical and physical analyses carried out within the scope of the project show that humic acids are large macrocyclic supra molecules with high organic matter, and low solubility, but capable of interacting with elements with electropositive properties. They display these properties in different intensities depending on the humic acid content in natural humin sources.

In many studies conducted in our country and around the world, humic acid applications not only increase plant productivity but also transfer N, P, K, Ca, Mg, Fe, Cu, Mn, Zn, etc. from the soil to the plant. It provides the transfer of substances. It increases the transition speed of elements. Greater penetration of the elements affects the plant's nutrient uptake and thus its productivity. The findings we obtained within the scope of the project confirm that humic acid and humic substance applications will increase especially the yield and plant nutrient uptake. ^{13}C- NMR results show the abundance of the carbon element in the structure, i.e., high levels of organic matter.

Within the scope of these findings, it would be very beneficial to use natural humic sources, especially leonardite, or products prepared by adding humic substances by taking into account soil and plant analyses. The use of organic fertilizers containing less humic substances, such as animal manure and compost, by further enriching them is important for increasing organic matter, which is the most important effect of organic fertilizers.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

Funding Information

Acknowledgement

Author Contribution

AE: selection of samples, preparation of samples for analysis, interpretation of analyzes, writing of the final report EK: preparation of samples for analysis, interpretation of analyzes KA: selection of samples,

preparation of samples for analysis RMP: selection of samples preparation of samples for analysis.

References

Anonymous, (2018). Republic of Türkiye Ministry of Agriculture and Forestry. Regulation on Organic, Mineral and Microbial Fertilisers Used in Agriculture. Products of organic origin. Annex 3 (5-6-7).

Aşik, S. (2008). Some chemical properties of some liquid humic acid soil conditioners [master's thesis]. Ankara University Faculty of Agriculture.

Chen, Y., & Avnimelech, Y. (1986). *The role of organic matter in modern agriculture* (25). Springer Science & Business Media.

Enev, V., Pospíšilová, L., Klučáková, M., Lıptaj, T., & Doskočıl, L. (2014) Spectral Characterization of Selected Humic Substances Soil & Water Res., *9*(1), 9–17. https://doi.org/10.17221/39/2013-SWR

Erdik, E. (1998). Spectroscopic Methods in Organic Chemistry, Gazi Office Bookstore. Nicholas, R.E. (1968). Hermann, Paris. 147-153.
https://doi.org/10.1055/b-003-108602

Nieweś, D., Biegun, M., Huculak-Mączka, M., Marecka, K., Kaniewski, M., Zieliński, J., & Hoffmann, J. (2023). Extraction of humic acid from peat, lignite, and the thermal behavior of their mixtures with ammonium nitrate. *Journal of Thermal Analysis and Calorimetry*, *148*(23), 13175-13188. https://doi.org/10.1007/s10973-023-12326-7

Schnitzer, M., & Khan, S.U. (1978). Soil Organic Chemistry. Elsevier Book Series 8, 1-319.

Shuangdui Y, Naiyu Z, Juan Li, Yanan W, Yue L, Mengyao C, & Qiuyan Y., (2021) Characterization of humic acids from original coal and its oxidization production www.nature.com/scientificreports.https://doi.org/10.1038/s41598-021-94949-0

Ukalska-Jaruga, A., Bejger, R., Debaene, G., & Smreczak, B. (2021). Characterization of soil organic matter individual fractions (fulvic acids, humic acids, and humins) by spectroscopic and electrochemical techniques in agricultural soils. *Agronomy*, *11*(6), 1067. https://doi.org/10.3390/agronomy11061067

Yildiz, A., Genç, Ö., & Bektaş, S. (1997). Methods of Instrumental Analysis, Hacettepe University Press, a-64.

Yonebayashi, K., & Hattori, T. (1988). Chemical and biological studies on environmental humic acids: I. Composition of elemental and functional groups of humic acids. *Soil Science and Plant Nutrition*, *34*(4), 571-584. https://doi.org/10.1080/00380768.1988.10416472