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Chemical and Spectroscopic Characterization of Humic Acid Isolated from Ilgin Lignite, Turkey

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Abstract

To isolate humic acid (HA) from Ilgin Lignite (IL), alkali extraction method was used. Extracted samples were analyzed for elemental composition (C, H, N and O) by Elemental Analyzer. Spectroscopic techniques such as Fourier-transformed infrared (FTIR), ultraviolet–visible (UV), and fluorescence spectroscopy in emission and excitation modes were also used for characterization of IL-HA. Results obtained were compared with standard HA proposed from International Humic Substance Society (IHSS). Sulfur contents and C/N ratio of IL-HA (1.44) were found to be higher than that of standard IHSS-HA (0.76). The FTIR evidence suggested that IL-HA has an aromatic structure due to the presence of intense band at 1609.3 cm^{-1}. E_4/E_6 values of IL-HA and IHSS-HA were found to be comparable but, E_4/E_6 value of IL-HA (3.9) is slightly lower than that of the IHSS-HA (4.9). Results of present study might be useful for practical and commercial applications of IL-HA on basis of their humification parameters.

Keywords: humic acid, isolation, lignite, elemental composition, spectroscopic characterization

Nomenclature

A_{440}	Milori index, area under fluorescence emission spectra at the excitation wavelength of 440 nm
DI	Deionized water
E_4/E_6	Ratio of absorbances at 465 and 665 nm
FA	Fulvic acid
FTIR	Fourier transform infra red

HA	Humic acid
HAs	Humic acids
HSs	Humic substances
IHSS	International Humic Substance Society
IHSS-HA	Humic acid provided from International Humic Substance Society
IL	Ilgın Lignite
IL-HA	Humic acid obtained from Ilgın Lignite
NOM	Natural organic matter
UV	Ultra violet

1. Introduction

Humic substances (HSs) which are the most dominant fraction of natural organic matter (NOM) in soil are a series of different molecular weight, light-brown to black-colored, complex and heterogeneous organic polymers formed by secondary synthesis reactions (Stevenson 1994). They can be classified into three main fractions based on their solubilities in alkaline and acidic extraction solutions. These are HA, the fraction soluble in alkaline solutions but insoluble in acidic solutions; fulvic acid (FA), the fraction soluble in both alkaline and acidic solutions; and, the humin, insoluble in both solutions (Stevenson 1994).

Among these fractions, the predominant is HA which is very active in interacting with organic and inorganic chemicals when compared with others (Kishi 1988; Senesi 1993). This substance has effects in soil conservation, for water-holding capacity and for the complexation of metals in terrestrial and aquatic systems (Stevenson 1994; Tarhan 2011; Hayes & Graham 2000; Hayes & Malcolm 2001). Because the process of humification of organic matter has controlled structural characteristics and composition of HS in the environment, these effects are well known to base on the chemical properties of humic structure which are depended on soil type (Chiou *et al.* 2001; Saito *et al.* 2005; Piccolo 2000; Rosa *et al.* 2002; Baldock *et al.* 1992; Qideau *et al.* 2001; Martin-Neto *et al.* 1998; Dai *et al.* 2002).

Lignite is usually used for the production of HA which is in the form of alkali-soluble humate salts (Peuravuori 2006). The characteristics of lignite (size, chemical composition and functional groups) may differ considerably, depending on the origin and age of the material (Stevenson 1994; Fong & Mohamed 2007). Pertinent studies have been carried out to characterize HA from soils, waters and sediments (Fong & Mohamed 2007; Chen *et al.* 1978; He *et al.* 2008; Kim *et al.* 1990), however, HA extracted from IL has not been studied, especially with regard to degrees of humification. Because humification properties determine suitability of material for use as a HA source. In this study, humification properties of IL will be investigated for the first time.

The goal of this work is to characterize the HA fraction isolated from IL, Konya, Turkey in the frame of chemical and spectroscopic techniques and to compare with standard HA proposed from IHSS in terms of humification properties and suitability for use as a HA source.

2. Material and Methods

2.1 Chemical and reagents

All chemicals and solvents used were of analytical grade and were obtained from Merck® (Darmstand, Germany) and Sigma-Aldrich® (St. Lousis, USA) and were used without further purification. Deionized water (DI) was used to prepare all solutions. IL samples were collected from the Turkey Coal Enterprises, Ilgın, Konya. In order to compare, information of standard HA proposed from IHSS (cat. no. 1S104H) was used.

2.2 Extraction of IL-HA

Prior to laboratory analysis, samples were air-dried at room temperature and sieved with nylon sieve to an adequate size (< 80 µm). The HA fraction was isolated from IL using the alkaline extraction method and was purified according to the method schemed in Fig. 1 (Tarhan 2011). Air-dried and sieved lignite samples were extracted by solution 1 M KOH using a sample extractant ratio of 1:10. The mixtures were shaken mechanically under N_2 gas in capped plastic bottles for 24 h at 80 °C. The alkaline supernatant solution was then separated from the residues by centrifugation at 5000 rpm for 30 min. The extraction procedure was repeated two times on the residues, which were finally discarded. The combined alkaline supernatants (HA+FA) were acidified with 5 M HCl to pH 1, allowed to stand for 24 h in a refrigerator to permit coagulation of the HA fraction, and then centrifuged at 5000 rpm for 30 min. The HA precipitates were purified by dissolving them in a minimal volume of 0.4 M KCl and 0.1 M KOH under N_2 gas, centrifugation at 5000 rpm for 30 min to remove the residues, and acidification of the alkaline supernatants with 5 M HCl to pH 1. The suspensions were left for 24 h at room temperature, and then centrifuged at 5000 rpm for 30 min. The purification steps were repeated three times. The precipitated HA fraction was recovered with distilled water, dialyzed using a membrane made from natural cellulose having a molecular weight cutoff of 12,000–14,000 Da until free of Cl- ions, dried at 105 °C, and stored at RT in plastic vials placed in a desiccator containing P_2O_5.

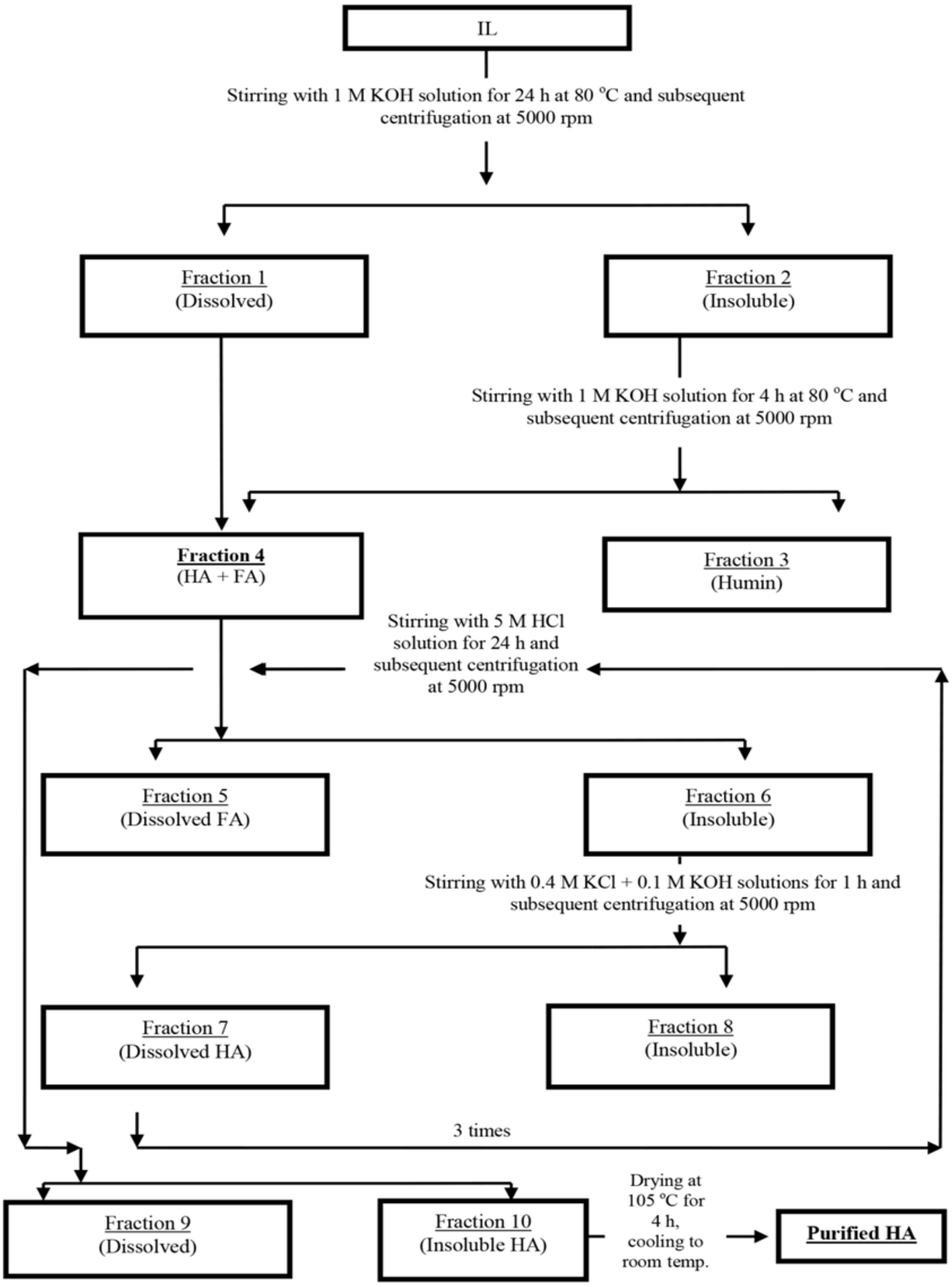

Figure 1. Extraction and purification procedure of IL-HA (Tarhan 2011)

2.3 Characterization of HA

2.3.1 Elemental analysis

Elemental analyses of IL-HA were performed using a Costech ECS 4010 elemental analyzer and the content of oxygen was taken as a difference from 100%.

2.3.2 FT-IR spectroscopy

The IR spectroscopic analysis was made with a Perkin-Elmer Spectrum 100 series FT-IR spectrophotometer on a spectral range of 650-4000 cm^{-1} with ATR probe.

2.3.3 Acidic functional groups

Total acidity and carboxylic groups were determined according to the Schnitzer & Gupta (1965); and Wright & Schnitzer (1959). In order to measurement of total acidity, the IL-HA was treated with a barium hydroxide solution under N_2 atmosphere for 24 h. The $Ba(OH)_2$ remaining in the solution after the reaction is then back titrated with a standard acid solution. For the titration of carboxylic groups, the IL-HA was handled for 24 h with calcium acetate solution in excess which causes the release of acetic acid. The CH_3COOH released is then titrated with a standard base solution. Phenolic OH group was calculated by difference between total acidity and the acidity of the carboxylic groups.

2.3.4 UV-Vis spectroscopy

Spectroscopic analyses were performed on a Lambda 25 model Perkin Elmer double beam spectrophotometer using 1 cm quartz cells. All measurements were achieved 24 hour after preparation of solutions. The following spectroscopic index was achieved:

- *E_4/E_6 ratio:* 200 mg of extracted and purified IL-HA was dissolved in 1000 mL 0.05 N $NaHCO_3$ and the absorbance at 465 and 665 nm were measured using the spectrophotometer (Tarhan 2011; Chen *et al.* 1977; Fuentes *et al.* 2006).

2.3.5 Fluorescence spectroscopy

Fluorescence spectra were recorded with a LS 25 model Perkin Elmer fluorescence using quartz cells. The emission and excitation slits were set to 10 nm, using scan speed of 300 nm min^{-1}. The following spectroscopic index was achieved:

- *Milori Index:* 200 mg of extracted and purified IL-HA was dissolved in 1000 mL 0.05 N $NaHCO_3$ and the emission spectra were collected within a range of 460-650 nm at the excitation wavelength of 440 nm, and the total areas under these spectra (A_{440}) were calculated (Fuentes *et al.* 2006; Milori *et al.* 2002).

3. Results and Discussion

3.1 Elemental analysis

Table 1. Elemental Characterization results of HA samples

	Elemental composition					**Atomic Ratios**			
Sample	C	H	O	N	S	H/C	C/N	O/C	Ref.
IL-HA	65.31	3.79	28.78	0.68	1.44	0.06	96.04	0.44	This work
IHSS-HA	63.81	3.7	30.5	1.23	0.76	0.06	51.88	0.48	(IHSS)

[a] Elemental composition is given moisture-free basis.

The elemental composition and atomic ratios of the HA samples are listed in Table 1. In order to comparative characterization, the H/C, the C/N and the O/C atomic ratios of HAs were calculated. The C, H contents and H/C, O/C ratios of HAs were found to be close to the each other. The S contents of IL-HA were higher than that of IHSS-HA. This may be due to high pyrite content of IL-HA. The contents of O and N of IHSS-HA were found little higher than that of IL-HA. The higher C/N ratio of IL-HA attributed that the nutrient profile of IL-HA may be lesser than that of IHSS-HA. The O/C ratio of IL-HA was little lower than that of IHSS-HA. This situation showed that IL-HA contained almost the same amount of O-containing groups and the degree of the oxygen functionality with IHSS-HA. Also same H/C ratio of HAs indicated that the content of aliphatic carbon and the aromatic structures were similar. According to these results, we can say that IL-HA contained almost similar elemental properties as IHSS-HA.

3.2 FT-IR spectroscopy

The FT-IR spectra of IL-HA studied was shown in Fig. 2. Main absorption bands are in the regions of 3400-3000 cm^{-1} (H-bonded OH groups), 2940-2900 cm^{-1} (aliphatic C-H stretching), 1750-1720 cm^{-1} (C:O stretching of COOH), 1620-1600 cm^{-1} (aromatic C:C, COO-, H-bonded C:O), 1420-1332 cm^{-1} (O-H of phenol and alcohol groups), 1280-1230 cm^{-1} (C-O stretching and OH deformation of COOH), 1220-1210 cm^{-1} (C-O and O-H of COOH, C-O of aryl ethers and phenols), 1040 cm^{-1} (C-O stretching of polysaccharide or Si-O of silicate impurities), and 794-770 cm^{-1} (benzene rings with 2 or 3 adjacent H). The spectrum evidently showed that IL-HA has predominance of OH, COOH and COO- groups which are the most characteristic features of soil humic materials.

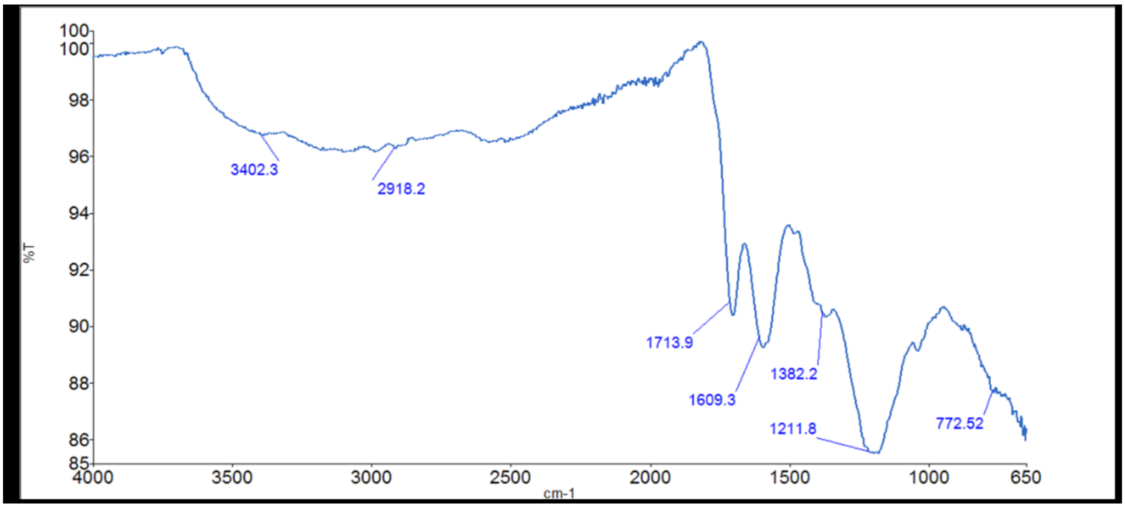

Figure 2. FT-IR spectra of the IL-HA

The peak at 3402.3 cm^{-1} may be assigned to O-H groups. The presence of aliphatic groups in IL-HA was confirmed by the peaks at 2918.2 cm^{-1}. IL-HA was characterized by stronger absorption at 1713.9 cm^{-1} which implies the high carboxylate group capacity. The aromatic character of IL-HA was indicated by the intense band at 1609.3 cm^{-1}. The spectrum of IL-HA was also characterized by the absorption at 1211.8 cm^{-1} (C-O and O-H of COOH, C-O of aryl ethers and phenols). The results of FT-IR spectra indicated that IL-HA has an aromatic structure expected.

3.3 Acidic functional groups

The values of total acidity, COOH and phenolic OH group contents of HAs are summarized in Table 2. The content of the total acidity and the COOH group is proposed to represent an important indicator of humification (Rosell *et al.* 1989). Compared with IHSS-HA, lower total acidity and lower content of COOH groups were found in IL-HA. It could be explained by that the low COOH content in IL-HA was caused from it has a higher degree of condensation. As shown in Table 2, IL-HA was found to contain much more phenolic OH compared with IHSS-HA. This result may due to lignite origin of IL-HA as indicated by Saito & Hayano (1980).

Table 2. Acidic functional groups of the HA samples

Sample	**Total acidity (meq/g)**	**COOH (meq/g)**	**Phenolic OH (meq/g)**	**Ref.**
IL-HA	6.94 ± 0.20	2.55 ± 0.33	4.39 ± 0.51	This work
IHSS-HA	9.77	7.46	2.31	(Ritchie & Perdue 2003)

3.4 UV-Vis spectroscopy

The spectroscopic characteristics determined for HA samples are given in Table 3. The spectroscopic ratio between absorbance at 465 and 665 nm (E_4/E_6) in aqueous solution were determined. E_4/E_6 ratio is inversely correlated with the degree of humification, structural condensation, molecular weight of HA and desirably from 3 to 5 of this ratio (Tarhan 2011; Chen *et al.* 1977; Fuentes *et al.* 2006). According to the data in Table 3, E_4/E_6 values of IL-HA and IHSS-HA were close to each other but, E_4/E_6 value of IL-HA (3.9) was slightly lower than that of the IHSS-HA (4.9), demonstrating a good humification degree. To the result, IL-HA was shown to be characterized by high aromatic condensation and to have high in molecular weight when compared with IHSS-HA.

Table 3. The ratios of specific spectral absorbance between spectral absorbances at particular wavelengths for IL-HA and IHSS-HA

Sample	**E_4/E_6^{a}**	**A_{440}^{b}**	**Ref.**
IL-HA	3.9	9796	This work
IHSS-HA	4.9	8540	(Fuentes *et al.* 2006)

[a] Ratio of absorbances at 465 and 665 nm.
[b] Milori index: area under fluorescence emission spectra at the excitation wavelength of 440 nm.

3.5 Fluorescence spectroscopy

Among the different fluorescence indexes that could be used to characterize of HAs, Milori index, which increases in line with the humification degree, has proven to be well correlated to the humification degree of humic features having different origin (Fuentes *et al.* 2006; Milori *et al.* 2002). As can be observed from Table 3, area values of IHSS-HA and IL-HA have close to each other, as expected, area values of IL-HA is slightly higher than that of the IHSS-HA since it has more polycondensed systems than that of the IHSS-HA. This result suggested that IL-HA is characterized by a higher content of polycondensed aromatic ring systems that are capable of a high degree of conjugation in unsaturated aliphatic structures, and the presence of electron-withdrawing substituents in aromatic rings such as carbonyl or carboxyl groups (Fuentes *et al.* 2006; Senesi *et al.* 1991).

4. Conclusions

HA fraction isolated from IL was investigated for elemental and spectroscopic analysis. From the present study we concluded as following:

i) In this study, humification properties of IL have been investigated for the first time and suitable of IL as a HA source have been exposed.

ii) The obtained findings reveal that IL-HA is characterized by a high degree of humification and a high content of O-containing functional groups.
iii) IL-HA showed almost similar characteristic properties with IHSS-HA according to the chemical and spectroscopic results obtained from this work.
iv) According to this study, we recommend that IL-HA is suitable to use as a HA source like IHSS-HA for commercial and agricultural applications.

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