

Spectroscopic Investigations of Soil Humic and Fulvic Acids from Okch'ön Basin

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Three humic acids (HA) and one fulvic acid (FA) are extracted from soils of the Okch'ön Basin (Koyesan, Yõngkwang and Taejõn), and are purified and characterized using ¹H, ¹³C NMR, and IR spectroscopic methods. The results are compared with one another and with commercial humic acid (Aldrich Co) and aquatic humic acid from Gorleben underground aquifer in Germany. The IR and ¹H and ¹³C NMR spectral features are found to be nearly identical, suggesting that humic substances formed in the Okch'ön Basin have quite similar chemical properties. These humic substances from Okch'ön Basin soils have undergone low degree of aromatic condensation and have high contents of aliphatic functionalities including carbohydrates.

Introduction

Humic substances are naturally occurring organic matter which play many important roles in terrestrial and aquatic environments¹. They can bind with organic pollutants, such as pesticides and herbicides, and catalyze in the degradation of such pollutants. They have a high affinity for metal cations ($Z \geq 2+$) and strongly influence the bioavailability of trace heavy metal ions in soils²⁻⁴. In underground aquifers, humic substances can either aid or retard in the migration of trace heavy metal cations, depending on the filtration and sorption properties of aquifer systems concerned^{5,6}.

Chemical equilibrium models are used to describe the chemical species formed between humic substances and trace metal ions in aquatic system⁷. However, the thermodynamic description of such reactions is made complicated by the complex nature of humic substances. A summary of the earlier studies indicated that humic substances are made up of a mixture of substances that have similar chemical properties². The bulk properties of humic substances are found to be relatively invariant, and attempts at fractionation to obtain simpler subfractions were met with little success⁸. Furthermore humic substances isolated from different regions are not found to be identical and are said to vary depending upon such factors such as the nature of soil or sediment, climatic and botanical environments, and age and depth of burial¹.

Earlier we reported the results of the chemical characterization study (elemental analysis, total acidity, molecular weight distribution) of the humic acids extracted from soil samples obtained from the outskirts of the town of Koyesan in the Ch'ungbuk province⁹. As an extension, additional samples of humic and fulvic acids from Koyesan and humic acids from two other regions (Taejõn and Yõngkwang) in the Okch'ön Basin were obtained, and they were investigated using both the chemical and spectroscopic methods. The chemical characteristics (elemental analysis, total acidity and molecular weight distribution) were found to be largely similar to one another, and herein report the spectroscopic data (IR, ¹H and ¹³C NMR) of these humic substances. The main objective of this investigation is compared the spectroscopic properties

of these three humic and one fulvic acids obtained from three areas and to identify those features that characteristic of the humic substances of the Okch'ön Basin covering a wide region of the Ch'ungch'õng and Chõlla provinces.

Materials and Methods

Materials and Reagents. Three humic acids (HA) and one fulvic acid (FA) were extracted from soil samples obtained from the following sites: (i) Tõkp'yõng-ri area on the outskirts of Koyesan, Ch'ungbuk Province; (ii) Taeduck Science Town area in the northern outskirts of Taejõn City; (iii) Yõngkwang-gun, on the western shores of Yellow Sea in Chõnnam Province. All three sites lie on the Okch'ön Basin, and have similar botanic and climatic environments. The site (i) is a part of the Okch'ön Metamorphic Belt and is covered with low uranium-bearing black shales¹⁰. The site (ii) and (iii) lie on the jurassic granite, and the soils are found to be rather acidic (pH 4.9-6.2) sandy clay loam. The detailed geology of these sites are given in the literature¹¹.

Soil samples were collected from the topsoils (0-5 cm) of approximately 0.01 square kilometer area, and made up of a composite of 25 subsamples taken from exposed surfaces. The humic substances were extracted and purified from air-dried soil by a procedure described below. Briefly, 1.5 kg of air-dried soil was shaken for 16h at room temperature in 15 L of 0.1 M HCl to decompose free carbonates and remove alkaline earth metals. The residue was separated from the supernatant by centrifugation (850×g, 1h), and was subsequently neutralized to pH 7 using 1 M NaOH. Subsequently, 15 L of 0.1 M NaOH was added to the residue and the resulting suspension stirred slowly under N₂ atmosphere for 24h. The residue was removed from the dark coloured supernatant by centrifugation. The HA fraction was separated by acid precipitation (pH 1) with 6 M HCl, leaving the FA fraction in the supernatant. The precipitate was redissolved in 0.1 M NaOH, and 0.2 g NaF/g HA was added and left overnight to remove silicate impurities. The solution was acidified to pH 1 to allow HA to coagulate. After centrifugation, the precipitate was washed. The protonated final product of HA was obtained after freeze-drying, and was

kept in a vacuum desiccator over P_2O_5 . The FA fraction was isolated from the supernatant by sorbing it on a column of previously purified XAD-8 resin. The FA was eluted from the column with 0.1 M NaOH, and NaF was added to the solution and left overnight to remove traces of silicate impurities. After adjusting pH of the solution to 1, the FA was adsorbed on XAD-8 resin once again. The resin was first washed with a small volume of 0.1 M HCl to remove the remaining trace impurities, eluted with 0.1 M NaOH, and then passed through a cation-exchange column of Dowex 50X8 (H^+) to remove Na^+ . The protonated final product of FA was freeze-dried¹².

Spectroscopic Studies. The infrared spectra were recorded with KBr pellets (1 mg of FA or HA per 100 mg of KBr) in a BOMEM mode MB 100 FT-IR spectrophotometry. KBr (FT-IR Grade, Aldrich Co.) was dried by heating and was kept under vacuum in a desiccator prior to use, to minimize the effect of water absorption. A linear baseline correction was applied to each spectrum using 3800 cm^{-1} , 2000 cm^{-1} , and 860 cm^{-1} as zero absorption points to facilitate in the comparison of the spectra.

The 1H NMR spectra of the humic and fulvic acid solutions in 5 mm sampled tubes were recorded on a Bruker AM 300 operating at 300 MHz. The sample solutions were prepared by dissolving approximately 100 mg of the acids in 0.5 M NaOD (50 mg/ml), gently shaken for several hours, centrifuged at 3800 rpm for 15 minutes using a benchtop centrifuge and filtered through glass wool into NMR tubes. Practically all of the material was soluble under these conditions. Spectra of sufficient signal to noise ratio required ~ 300 scans using a pulse width of 45° and recycle time of 1.7s. The chemical shifts were quoted relative to the internal water peak at 4.8 ppm and deuterium signal of the D_2O solvent was used as a lock. The ^{13}C NMR spectra were recorded using a Bruker AM 300 instrument operating at 75.47 MHz. More concentrated (HA's; 220 mg/ml sample, FA; 120 mg/ml) solutions in 1 M NaOD were prepared into 10 mm NMR tubes in a similar manner. The conditions were chosen for obtaining quantitative spectra: the spectra were obtained using inverse-gated decoupling¹³, with the decoupler off except during the acquisition time of 0.2s. A pulse width of 45° , and the total delay time of 2.0s were chosen to minimize distortions due to the nOe (nuclear Overhauser enhancement) effect on different carbon types and lack of complete relaxation of all carbons^{14,15}. Around 3×10^4 FID's (free induction decays) were signal averaged for each spectrum, and 50 Hz line broadening function was applied to enhance the signal to noise ratio. After Fourier transformation, the baseline of the spectrum was modified using the Bruker software to match and subtract parabolic functions. Uncertainty of the measurements in the peak area was approximately $\pm 8\%$, depending on the extent of spectral resolution and baseline distortion.

Results and Discussion

Humic substances from the Okch'on Basin. The humic and fulvic acids from their respective areas are named in the following manner: (a) Koyesan-HA and Koyesan-FA ($\sim 1.2\text{ g HA}$ and 300 mg FA per 1.5 kg dry soli), (b) Yöngkwang-HA ($\sim 850\text{ mg HA}$ per 1.5 kg dry soil), and (c) Taejön-

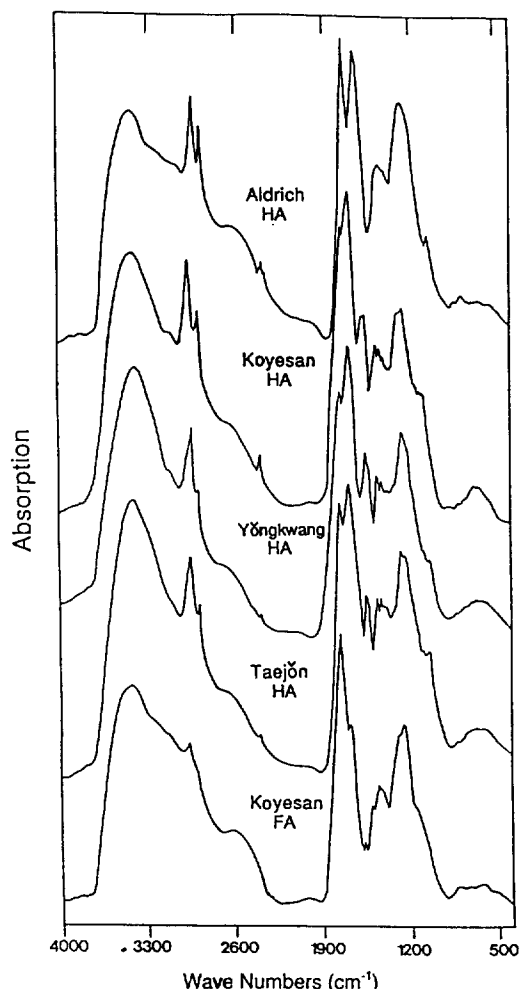


Figure 1. Infrared spectra of the Okch'on Basin soil humic and fulvic acids and Aldrich humic acid.

HA ($\sim 1.0\text{ g HA}/1.5\text{ kg dry soil}$). The Aldrich-HA is the commercially available humic acid from the Aldrich Co. The Gohy-573-HA, provided by G. Buckau of the Technical University of Munich, was isolated from the Gorleben underground aquifer in Germany¹⁶. The elemental analysis data gave the percentage carbon, hydrogen, oxygen, and nitrogen to be around 54-56% (C), 4-5% (H), 34-36% (O), 4-5% (N), and they are all within the range of values quoted in the literature¹⁷. The molecular weight distribution patterns were found to be similar to those given in the earlier study⁹. The total acidity was found to vary (range $2.7\text{--}5.1\text{ meq g}^{-1}\text{ HA}$; $7.1\text{--}7.4\text{ meq g}^{-1}\text{ FA}$) depending on the site and sampling period.

IR spectra. The IR spectra of Koyesan-HA and FA, Yöngkwang-HA, Taejön-HA and Aldrich-HA are shown in Figure 1. The characteristic absorption bands typical of humic substances are observed in all the IR spectra. The strong bands are evident in the regions of 3400 cm^{-1} (H-bonded OH), 2900 cm^{-1} (aliphatic C-H stretching), 1720 cm^{-1} (C=O stretching of COOH and ketonic C=O), $1600\text{--}1660\text{ cm}^{-1}$ (aromatic C=C and H bonded C=O), 1420 cm^{-1} (C-H bending of CH_2 or CH_3 groups), and 1240 cm^{-1} (C-O stretching and OH deformation of COOH) which are also observed in other data given in the literature¹⁸⁻²⁰. Humic substances have been classified into three general types according to Stevenson

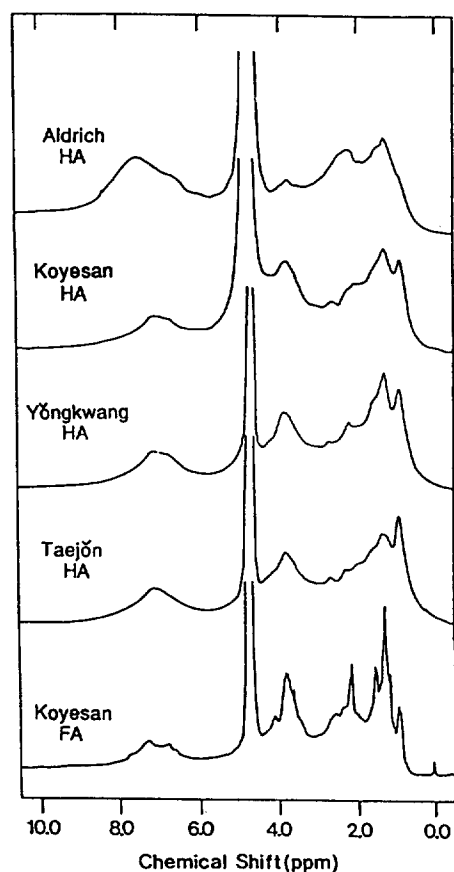


Figure 2. ^1H NMR spectra of the Okch'ön Basin soil humic and fulvic acids and Aldrich humic acid.

and Goh²⁰.

Accordingly both Aldrich-HA and Gohy-573-HA displayed similar spectra features that were typical of Type 1: In addition to the bands described above, the band at 1615 cm^{-1} is about equal in intensity with the band at 1720 cm^{-1} . By contrast, the three HA's from the Okch'ön Basin all showed features typical of the Type III: The absorption in the $1600\text{-}1660\text{ cm}^{-1}$ range is very strong and centered near 1650 cm^{-1} , while the band near 1720 cm^{-1} is relatively weaker. The bands are evident near 1540 cm^{-1} , which is known to attribute to the peptide linkage of proteins. The low-molecular-weight Koyesan-FA, like other FA²⁰, was of Type II: the band intensity near 1720 cm^{-1} is very strong, while the band intensity in the $1600\text{-}1660\text{ cm}^{-1}$ range is rather weak and is centered near 1640 cm^{-1} .

^1H and ^{13}C -NMR Spectra. The ^1H NMR spectra of the humic and fulvic acids are shown in Figure 2. All these spectra contain a broad peak at 6.0-9.5 ppm (aromatic), a strong peak near 3.8 ppm (carbohydrate), and intense peaks near 2.3, 1.2 and 0.8 ppm (aliphatic CH_2 , CH_3), similar those given in the literature^{21,22}. The strong peak at $\sim 4.8\text{ ppm}$ is due to HOD formed from the exchange of acidic proton of humic substances and D_2O solvent. The spectral data analyzed quantitatively according to the manner described in the literature²³. The humic and fulvic acids from the Okch'ön Basin seem to have high percentages of aliphatic protons (0.4-3.3 ppm; $\sim 54\%$). The percentages of aromatic proton (6.0-9.5 ppm) accounted for only 19% in Koyesan-HA compared to

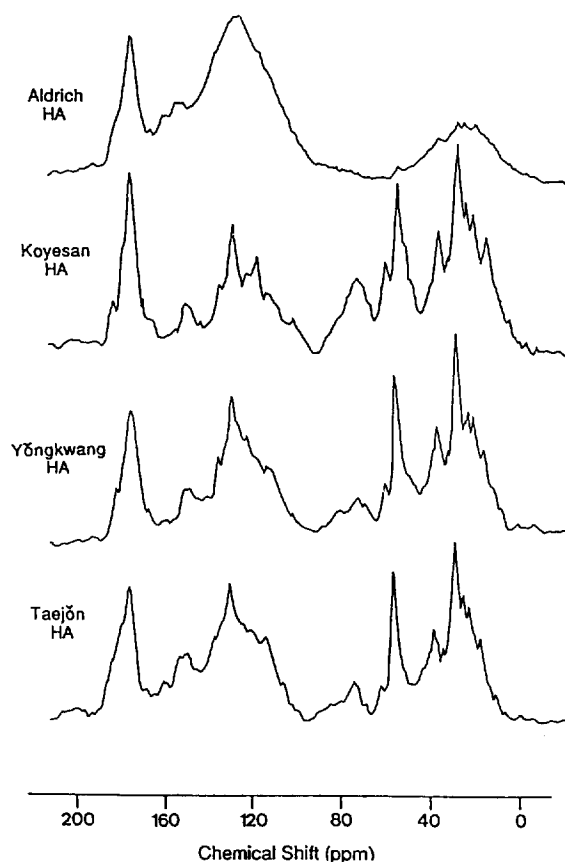


Figure 3. ^{13}C NMR spectra of the Okch'ön Basin soil humic acids and Aldrich humic acid.

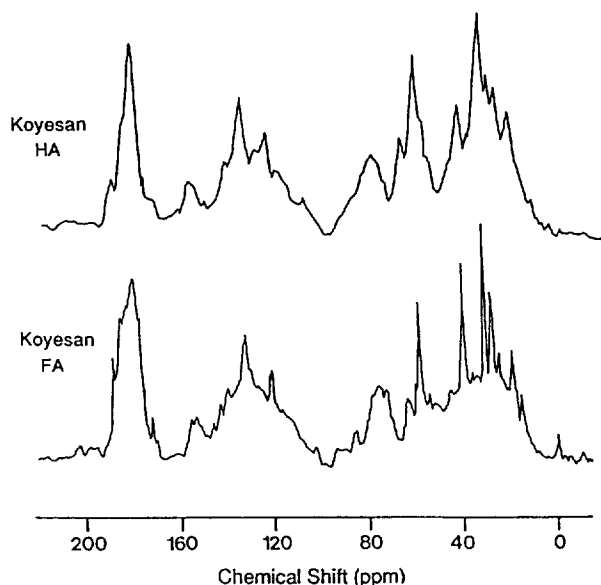
about 30-39% in Gohy-573-HA and Aldrich-HA, suggesting that the soil humic substances from the Okch'ön Basin have undergone a low degree of aromatic condensation. The relatively large intensity of the peak centered at 3.8 ppm suggests that polysaccharide components are present in these humic substances.

Further informations were obtained from the ^{13}C NMR spectral data of these humic substances. The ^{13}C NMR spectra of Koyesan-HA, Yöngkwang-HA, Taejön-HA, and Aldrich-HA are shown in Figure 3. In addition, the ^{13}C NMR spectra of Koyesan-HA and Koyesan-FA extracted from the same source material are shown in Figure 4 for comparisons. The ^{13}C -NMR spectra of the Okch'ön Basin humic substances showed a number of familiar and distinct peaks in both the aliphatic (0-105 ppm) and aromatic (105-165 ppm) regions. The peaks at 20, 25, 27 and 31 ppm are most likely aliphatic carbons of alkyl chains^{23,24}. The peaks at 20 ppm are characteristic of terminal methyl groups, while that at 31 ppm is the long $(\text{CH}_2)_n$ alkyl chains, even though other alkyl carbons may also occur at this region. The peak at 40 ppm may include contributions from alkyl carbons and amino acid carbons²⁵. The Okch'ön Basin humic acids have rather high N content (up to 5%), and they showed up as amino acid carbon in the 50-70 ppm region of the spectrum²⁴. The sharp peak at 58 ppm can be assigned as the methoxyl group (OCH_3). The sharp peak at 62 ppm and the broader resonance centered around 74 ppm are observed in virtually all of the spectra of Okch'ön Basin humic acids, and are attributed to the

Table 1. Relative intensities of various carbons in humic substances determined by solution ^{13}C NMR spectra

Chemical shift regions (δ) ppm	Assignment	Relative intensities (%)					
		Koyesan HA	Koyesan FA	Yongkwang HA	Taejŏn HA	Aldrich HA	Gohy-573-HA
I (0-47)	aliphatic carbons	32 \pm 2	26 \pm 2	26 \pm 2	31 \pm 2	17 \pm 2	25 \pm 2
II (47-105)	alcohols, amines, carbohydrates, acetals	27 \pm 2	24 \pm 2	20 \pm 2	20 \pm 2	9 \pm 1	9 \pm 1
III (105-145)	aromatic carbons	22 \pm 1	24 \pm 1	32 \pm 2	29 \pm 2	47 \pm 2	44 \pm 2
IV (145-165)	phenolic carbons	5 \pm 1	6 \pm 1	9 \pm 1	7 \pm 1	12 \pm 1	10 \pm 1
V (165-190)	carboxyl carbons	14 \pm 1	20 \pm 1	14 \pm 1	13 \pm 1	15 \pm 1	12 \pm 1

Solution-state with $D_1=1.90\text{s}$.

**Figure 4.** ^{13}C NMR spectra of Koyesan-HA and Koyesan-FA.

presence of polysaccharides in soil humic substances²³. The wide range of aromatic resonances (105-165 ppm) centered around 131 ppm are observed, which indicates a variety of substituents on the aromatic rings. The peak at 131 ppm is assigned as the alkyl-substituted aromatic carbons and aromatic $\text{CH}^{24,26}$, and 120 ppm as the aromatic CH that are ortho or para to O-substituted groups²⁶. The small peaks observed around 154 ppm are indicative of the presence of O- and N- substituted aromatic carbons of phenolic OH or aromatic NH_2 types²⁷. The carbons of carboxyl groups, including amides and esters all contributed towards a broad peak around the 176 ppm region. These peak assignments are in good agreement with the chemical shift data of substituted benzenes given in the literature²⁸. By contrast, the ^{13}C NMR spectra of the Aldrich-HA and Gohy-573-HA, displayed broad resonances in both the aliphatic and aromatic regions.

The ^{13}C NMR spectral data of the Koyesan-HA, Koyesan-FA, Yongkwang-HA, Taejŏn-HA, Aldrich-HA and Gohy-573-HA were further analyzed quantitatively in the manner described in the literature²⁹, namely divide these spectra into five regions as outlined in columns 1 and 2 of Table 1. The division of the spectra into five regions is somewhat arbitrary. The uncertainties were estimated by making three replicate measurements. The average results obtained for the

Okch'ŏn Basin humic substances, Aldrich-HA and Gohy-573-HA are shown in the columns 3-6 of Table 1. This results show that the humic substances from the Okch'ŏn Basin contain substances that rich in aliphatic functionalities (region I), including polysaccharides (region II), which is in good agreement with the ^1H NMR spectra data (see Figure 2). The relatively low intensity in the aromatic region (region III) suggests that Okch'ŏn Basin humic substances have undergone a low degree of aromatic condensation, compared to the Aldrich-HA and Gohy-573-HA, even more aged paleosol-HA from southern Italy (radiocarbon dating 6000-30,000 years)³⁰.

Conclusion

In this study, we have compared the spectral data of several soil humic and fulvic acids obtained from different regions in the Okch'ŏn Basin. The Okch'ŏn Basin is approximately 80 km wide and stretches from the southwest coast in the Chŏnnam Province to the east coast in the Kangwon Province, dissecting the Korean Peninsula in the northeastern direction¹¹. The humic substances obtained from different regions in the Okch'ŏn Basin displayed spectral and many of the chemical features similar to one another. Such similarities suggest that the factors which play major roles in the formation of humic substances such as nature of soil, climatic and botanic environments, and age are quite similar in much of the Okch'ŏn basin, and indeed in many parts of the peninsula. Therefore, similar humic substances may be obtained in other parts of the country. A careful analysis of the IR and NMR spectral data show that the humic substances from the Okch'ŏn Basin have low aromaticity and high carbohydrate content. Such humic substances are known to be formed in regions with low rates of microorganisms (wet conditions, cold weather)³¹. The two environmental factors which can cause the formation of humic substances having low aromaticity and high carbohydrate content are: i) The effects annually occurring torrential downpour of summer monsoon rain, which causes surface wash-off of much soil organic matters that are formed during the hot summer when the conditions are ideal for microbiological activities. During the subsequent dry autumn and very cold winter period, newly deposited organic matter is expected to undergo a much slower rate breakdown. ii) Most of the agricultural land is flooded to grow rice, and humic substances formed in such wetlands are expected to be similar.

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