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Structural investigations of aquatic humic substances from different watersheds

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Abstract

Aquatic humic substances are amorphous organic macromolecules and precursors of mutagens formed on chlorination of raw water. Their isolation from River Mornos and Lake Yliki (primary reservoirs of Athens Water Supply and Sewerage Company) using columns of XAD-8 and IR-120 resins is performed in order to study their structural differences among reservoirs. Fractionation into humic and fulvic acids (HA and FA respectively) and spectroscopic examinations with FTIR technique is applied. In addition, THMFP is determined in every fraction of the reservoirs mentioned above. The presence of carboxylic acids and other functional groups such as alcohols, aromatic rings and aliphatic chains is indicated. Chloroform and chloropropanones detected can be considered to be products of humic acid, as they can be formed during chlorination of hydroxy-benzoic acid and β-keto-carboxylic acid. The chlorosubstituted acetonitriles detected are attributed to the reaction between chlorine and aminoacid.

Keywords: Aquatic humic substances (AHS); Humic acid (HA); Fulvic acid (FA); THMFP; FTIR spectroscopy; FT-IR spectrum ; Chlorination by-products (CBPs)

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1. Introduction

Humic and fulvic substances are a group of organic compounds with a very complex structure. They are formed in soil, water and sediment by the decay of dead organisms [1]. Because of the large number of different organic compounds composing the living organisms, the variety of these substances is large. So, the whole group of compounds is operationally divided into the so-called fulvic and humic fractions, for the sake of simplicity [2].

Fulvic compounds represent the low-molecular-weight fraction of the whole group of substances that are water soluble in all PHs. The range of their molecular weights varies from a few hundred to a few thousand [3]. On the contrary, humic acids are insoluble in strong acid (pH = 1) with fewer acidic functional groups than fulvic acids but a more significant degree of aromatic character (i.e. the presence of benzene rings in the structure) [4]. Their environmental importance is due to multiplicity of their properties.

- They can mobilize heavy metals by complexation and insecticides by adsorption [5].
- They may be adsorbed on surfaces of many particles, thus, influencing processes such as dissolution, coagulation, and crystal growth [6].
- They have acid-base properties (Figs. 1,2) with a wide range of pK values [7].

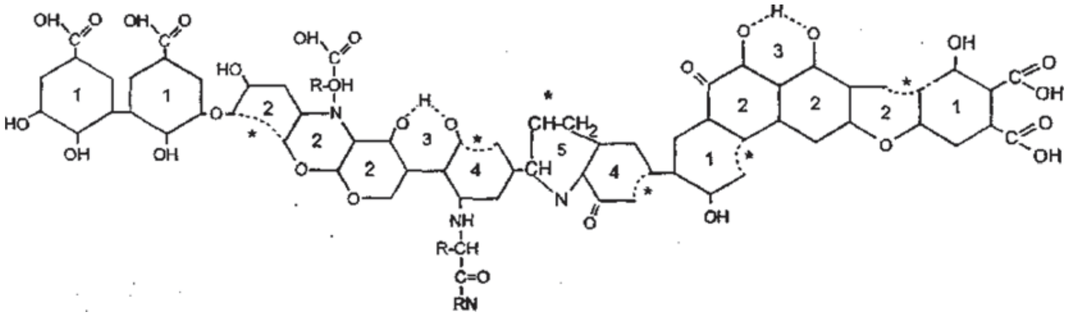

Fig. 1. Oxidized humic acid molecule. The organic structure of humic acid is naturally oxidized as shown by the asterisks, giving it a negative charge. Positive ions attracted to broken bonds at the site of the oxidation create sites for micronutrients and microflora to attach.

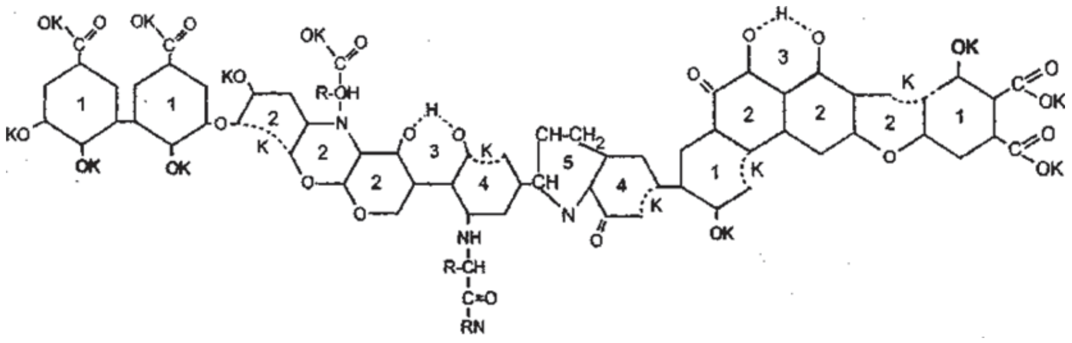

Fig. 2. Humic acid saturated with potassium. Treating leonardite ore with potassium hydroxide saturates the oxidized sited with potassium.

- Their degradation, during water chlorination process, leads to a number of different organic compounds (CBPs, chlorination by-products) which can cause serious health problems [5].

In the past, researchers made attempts to determine FA and HA molecular structures and interrelate with their chlorination by-products. THM formation potential (THMFP) is a test employed to measure the potential of THM precursors to produce THMs under specific conditions chosen to simulate waterworks processes. These conditions might be chlorine dosage, incubation time (usually one week), temperature, pH (usually 7) and bromide ion concentration [8].

EYDAP is the water supplier of Athens, the capital with 3.5 million people inhabitants. It uses two source waters with different qualities: the lakes Yliki and Paralimni and the river Mornos. During the present study, Yliki lake and Mornos river FA and HA molecular structures are studied and their potential to produce CBPs is examined. In order to evaluate precursors potential, THMFP processes are used (FA and HA are incubated under selected conditions and HClO is added, sufficient to oxidize them), but instead of THMs sum, the actual levels of THMs, haloacetonitriles and halopropanones are exhibited because the parameter bromide ion that determines the four THMs ratio is not applied during incubation process.

2. Materials and methods

2.1. Reagents and standards

- Acetone (MERCK HPLC purity)
- Methanol (Riedel de Haen, analytical grade 99%).
- MTBE (PESTANAL, Riedel de Haen)
- Chlorine (commercial product, NaOCl 4%)
- Sodium hydroxide (solid pellets, ACS, ISO, Riedel de Haen)
- Sodium sulfate Na_2SO_4 (ACS, ISO, Riedel de Haen)
- Potassium bromide KBr (ACS, ISO, Riedel de Haen)
- Ammonium chloride NH_4Cl (ACS, ISO, Riedel de Haen)
- Stock standard solutions (1 mg/ml) of analytes in methanol (THMs), in acetone (HANs, HKs, CH, CP)

2.2. Sample pretreatment

The resin DAX-8 was firstly wetted and stirred with methanol, left for 15 min in methanol, cleaned with distilled water, and then was used for filling the column [9]. Humic samples for FTIR analysis were isolated from water of the lake Yliki, and the River Mornos (raw water from Aspropyrgos plant). The preconcentration of the samples was made on resins DAX-8. 40 L of the samples passed through the resin and then eluted with NaOH 0.1 N (about 400 ml). To separate humics in fractions, the water sample was passed through column of XAD-8 resin to retain both humic (HA) and fulvic acids (FA)[10]. All humics were then eluted with 0.1 N NaOH. HCl 2M was added to acidify the eluate till pH = 1 immediately in order to avoid oxidation of humic substances HA precipitated overnight when the eluate was stored at 4°C. FA fraction was adjusted to pH 13 with NaOH and readsorbed on a column filled with resin IR-120 already protonated with HCl 2M and was pumped through resin IR-120. Distilled water was pumped through resin IR-120 till the effluent indicated conductivity was less than 250 mS/cm. FA was eluted with NaOH. FA was isolated after lyophilisation [11]. After elution of humics, the resin was regenerated with methanol, NaOH 0.1 N, HCl 0.1 N just before pumping sample into column. The quantitative elution as well as the regeneration were checked with absorbance measurement of the effluent at 254 nm. The flow rate for loading step was 3 bed volumes (BV)/h and flow rate for elution step 1.5 BV/h.

2.3. FT-IR

Infrared spectroscopy is a very powerful tool for determination of molecular structure because direct information about the presence of functional groups is easily provided. An infrared spectrum is obtained when a sample absorbs radiation in the region of electromagnetic spectrum known as the infrared. Energy is transferred from the incident radiation to the molecule, and a quantum mechanical transition occurs between two vibrational energy levels E_1 and E_2. The difference in energy $E_2 - E_1$ is related to the frequency ν (s^{-1}) of the electromagnetic radiation. Although this radiation is weak, it does supply sufficient energy for bonds in the molecule to vibrate by stretching or bending. The atoms of a molecule can be considered as linked by springs that are set in motion by the application of energy. The frequency of vibration ν (s^{-1}) of two atoms of reduced mass $\mu = m_1m_2/(m_1+m_2)$ connected by a spring with force constant k (Nm^{-1}) can be calculated from Eq. (1). The force constant is a measure of the resistance to stretching of the spring. For example, the reduced mass of the diatomic group with the double bond C=O is 6.86 amu, the force constant is 1200 nm^{-1} and the calculated frequency is 1725 cm^{-1} [12].

$$\nu\ (\mathrm{cm}^{-1}) = 130.3\sqrt{k/\mu} \qquad (1)$$

Energy absorptions are recorded as bands (peaks) on chart paper. Vibrations of certain functional groups such as OH, NH_2, CH_3, etc., always give rise to bands in the infrared spectra within well-defined frequency ranges. An infrared (IR) spectrometer subjects a compound to infrared radiation in the 5000–667 cm^{-1} (2 μm) range. As the molecule is subjected to the individual wavelengths in the 5000–667 cm^{-1} range, it absorbs only those possessing exactly the energy required to cause a particular vibration. Since different bonds and functional groups absorb at different wavelengths, an infrared spectrum is used to determine the structure of organic molecules.

Infrared spectra can be obtained by either dispersive or interferometric methods. Dispersive infrared spectrometers record the spectrum in the frequency domain whereas Fourrier Transform Infrared Spectrometers record the spectrum in the time domain. The latter result is an interferogram, which must be transformed to the frequency domain by means of a Fourier transformation to obtain the infrared spectrum [13].

2.4. Sampling

The solid sample was handled in the form of pressed discs (pressed-pellet technique). In an agate, or mullite mortar, few milligrams (20 mg) of the sample were ground together with about 100 times the quantity of a matrix material, the infrared transmitting material that was KBr (transparent to 400 cm^{-1}). The finely ground powder was introduced into a stainless steel die, usually 13 mm in diameter, which was evacuated for a few minutes with a vacuum pump to remove air from between the particles. The powder was then pressed into a disc between polished stainless steel anvils at a pressure of about 30 t/in^2.

2.5. Aquatic humic substances (AHS) chlorination

The spectra exhibited on the following pages indicate the functional groups of aquatic humic substances of Mornos River and Yliki Lake. Aromatic rings, aliphatic chains, carboxylic as well as carbonyl groups are the main parts of AHS. Their reaction with chlorine produces CBPs, the majority of them can be measured quantitatively using a gas chromatograph with Electron Capture Detector. After fulvic and humic acids isolation, 0.025 g/l fulvic or humic acids water solutions were adjusted to pH 7 with drops of 68.1 g/l KH_2PO_4 and 11.7 g/l KOH, then NaClO solution was added till 10 ppm chlorine concentration and stayed incubated for two days. NH_4Cl was added for chlorine oxidation before CBPs quantitative analysis in a gas chromatograph. The method employed for the determination of THMs, HANs, HKs, Chloral Hydrate and Chloropicrin was a modification of 551.1 EPA method developed by Nikolaou et al. [14].

2.6. Determination of CBPs

2.6.1. Glassware

All glassware was washed with tap water and a detergent, rinsed with tap water, ultrapure water (produced in an apparatus “ELGA PURELAB option”), acetone of HPLC grade, and baked at 200°C for 2 h.

2.6.2. Sample preparation

Methyl-t-butyl ether (MTBE) is the primary extraction solvent. A 35 ml sample aliquot is extracted with 2 ml of MTBE after addition of 2.5 g Na_2SO_4. One μl of the extract is then injected into a GC equipped with a fused silica capillary column and electron capture detector for separation and analysis. Procedural standard calibration is used to quantitate method analytes.

2.6.3. GC analysis

The determination of CBPs involves the use of a Trace 2000 Thermofinnigan gas chromatograph with a ^{63}Ni Electron Capture Detector (ECD), employing a capillary column AT-1 60 m × 0.25 mm i.d. × 0.25 μm. The procedure for the analysis of THMs is: splitless injection at temperature 175°C; carrier gas, helium; flow rate 2.0 ml/min. The oven temperature program includes initial temperature 37°C for 12 min, ramp to 52°C at 8.0°C/min and second ramp to 100°C at 10.0°C/min.

3. Results and discussion

3.1. FT-IR results

Fulvic and humic acids’ spectra indicated bands at about 2950, 1720, 1650, 1500, 1210, 1160 cm^{-1}.

A very strong and fairly broad band at 1720–1700 cm^{-1} was due to a carbonyl stretch in carboxylic acids. The C=O stretch normally appears to all carboxylic acids in solid and liquid states together with a O–C=O bending at 650 cm^{-1} as well as a C–OH deformation at 930 cm^{-1}.

A small band at 1650 cm^{-1} implies the presence of benzophenones (a stretch between carbon and oxygen in carbonyl). Benzophenones are present in all humic acids (Yliki and Mornos as well as Sigma-Aldrich standard, Figs. 9, 6 and 5, respectively) and yet, in Sigma-Aldrich solid sodium salt (Fig. 8 tilted humic acid standard after drying). As can be seen from Fig. 2 (humic acid saturated with potassium) benzophenones remain stable after neutralization reaction of humic acid with potassium hydroxide (or sodium hydroxide). At 1700–1650 cm^{-1} a band indicates the presence of aromatic ketones stretch (Yliki, Mornos as well as Sigma-Aldrich standard humic acids). On the other hand, fulvic acids respective bands (Figs. 4, 7 and 10) might be covered by the stronger band of carboxylic acids.

The benzene ring in aromatic compounds, normally, absorbs (stretching) at 1630–1430 cm^{-1} (medium, sharp band). Although spectra are not used for quantitative analyses, higher intensity related to this band indicates a higher proportion of aromatic rings to all humic acids.

At 1465–1440 cm^{-1} the band indicates the presence of CH_3 and CH_2 in aliphatic compounds (antisymmetric deformation). CH_3 symmetric deformation in aliphatic compounds appears at 1380–1370 cm^{-1}. Symmetric and antisymmetric stretching in aliphatic compounds appears at 2950–2850 cm^{-1}. The same bands appear smaller in Sigma-Aldrich solid sodium salt.

Esters and lactones are also present in Fig. 3 (possible structure of Suwannee River fulvic acid). Fulvic as well as humic acids’ spectra in this article indicate a possible presence of esters and lactones respectively (1280–1150 cm^{-1} antisymmetric stretch).

The bands at 1120–1080 cm^{-1} indicated C–O

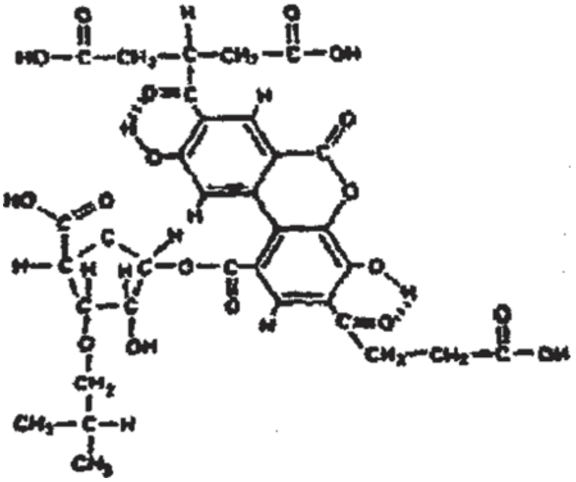

Fig. 3. Proposed average structural model of Suwannee River fulvic acid [19].

stretching and the presence of secondary alcohols.

On the other hand, spectrum of the humic acid (Sigma-Aldrich, sodium salt) indicated a broad band at 1390–1320 cm^{-1} because of the symmetric stretch between both oxygens and carbon of the carboxylic acid sodium salt (Table 1).

3.2. Aquatic humic substances (AHS) chlorination

Humic acid as a precursor of THMs might produce greater amounts of chloroform as well as other chloroacetonitriles and chloropropanones than fulvic acid when chlorine oxidizes fulvic and humic acids from Yliki Lake and Mornos River. Chlorination of 0.025 g/l fulvic or humic acids produced chloroform, chloroacetonitrile, dichloroacetonitrile, chloral hydrate, 1,1,1, trichloropropanone and 1,1, dichloropropanone (Table 2).

Table 1
FTIR bands of Sigma-Aldrich fulvic acid (FA) standard, Sigma-Aldrich humic acid (HA) standard, and Sigma-Aldrich humics standard sodium salt

	Sigma-Aldrich fulvic acid (FA) standard (cm^{-1}) Fig. 1	Sigma-Aldrich humic acid (HA) standard (cm^{-1}) Fig. 2	Sigma-Aldrich humics standard sodium salt (cm^{-1}) Fig. 5 (titled: humic acid standard after drying)
OH stretching	3420	3410	
H-bonded OH stretching	2685 broad	2680 broad	
Aliphatic CH stretching (symmetric and asymmetric stretching of CH_3 and CH_2)	2940 sharp	2920 sharp	
Aliphatic CH_2 and CCH_3 bending	1435 weak	1435 weak	
C=O stretching of COOH and ketones	1720 (very strong)	1718 (very strong)	
CO stretching and OH deformation of COOH and phenolic groups	1205 (very strong)	1205 (very strong)	
COO ~ asymmetric stretching (and stretching of aromatic C=C)	1630 (strong)	1630 (strong)	1600 (very strong)
COO~ symmetric stretching (and CH deformation. CO stretching of phenolic OH)	1388	1377	1380 (very strong)
Aromatic ring stretching	1560 weak	1560 weak	
COH bending, CO stretching of alcohols and ethers	1100	1095	

Table 2
Fulvic and humic acids chlorination by-products (μg/l)

	Mornos FA	Mornos HA	Yliki FA	Yliki HA
$CHCl_3$	13.0	66.3	20.0	68.0
CH_2ClCN	2.4	18.1	2.5	20.0
$CHCl_2CN$	n.d.	13.5	n.d.	0.15
CCl_3CN	n.d.	n.d.	n.d.	n.d.
$C_2H_3ClO_2$	n.d.	12.4	n.d.	0.3
$CHCl_2COCH_3$	n.d.	2.5	n.d.	n.d.
CCl_3NO_2 (chloropicrin)	n.d.	0.1	n.d.	n.d.
CCl_3COCH_3	n.d.	15.1	n.d.	0.2
$CHBr_3$	0.1	2.1	n.d.	5.0
$CHClBr_2$	1.5	6.0	n.d.	0.2
$CHCl_2Br$	1.4	6.5	n.d.	7.0
CH_2BrCN	n.d.	3.3	n.d.	5.0
$CHClBrCN$	n.d.	4.7	n.d.	0.1
$CHBr_2CN$	n.d.	0.2	n.d.	0.4

Bromoform, dibromoacetonitrile, bromoacetonitrile and bromochloroacetonitrile were also detected because traces of bromides may be found in NaClO solution. All CBPs mentioned above are small molecules with 1-3 carbon aliphatic saturated chains. The origin of these chains is, mainly, from aromatic carbons of AHS molecules because past bibliography shows a good correlation (coefficient of determination $R^2 = 0.7$) between TTHMFP and absorption at 254 nm (wavelength where benzene rings absorb) [15,16].

Research of the past indicated that humic substances chlorination form products with benzene rings that, gradually, lead to already known CBPs of 1-2 carbons in their molecule. Benzene rings of humic substances are bonded with functional groups such as carboxylic acid, hydroxide, and carbonyl group that make benzene rings more chemically active than benzene itself [12]. Reactions of dihydroxybenzoic acids with aqueous hypochlorite yield chloroform in reaction time less than an hour. The conversion of 2,4-2,6- and 3,5-dihydroxybenzoic acids to chloroform involves decarboxylation. 3-ketoglutaric acid (acetone-dicarboxylic acid $COOHCH_2CH_2(CO)CH_2COOH$) reacts rapidly with hypochlorite at near neutral PH giving quantitative yields of chloroform within a few minutes due to two carboxylate groups close to one carbonyl group. Acids with similar structures(acids with β-keto groups) can yield also dichloro- and trichloroacetones which, after enolization, hydrolize to corresponding halomethanes and acetic acid ion $CH_3COCCl_3 + OH^- \rightarrow CH_3COO^- + CHCl_3$ [17].

Citric acid $(COOH)(OH)C(CH_2COOH)_2$ yields chloroform too, because of its easily enolized structure: three carboxylate groups available close to one hydroxide on appropriate PH conditions. Chloroform, chloro-substituted acetone and chloro-substituted acetic acid by-production from humic substances chlorination is because of their similar moieties such as carbonyl or hydroxide close to carboxylates bonded to benzene rings (Fig. 3). On the other hand, chlorination of benzene derivatives such as anisole ($Ph-OCH_3$), or o-methoxybenzoic acid might lead to chlorophenol, chloroanisole and monochloroderivatives of o-methoxybenzoic acid respectively. Also, reactions of substituted Benzoic acids with aqueous hypochlorite did not yield chloroform or other usual CBPs such as HAAs but chlorophenols.

Products of humic substances degradation (after chlorine addition) might include benzene-mono- di- or tricarboxylic acids (e.g. benzoic acid), succinic acid, butanoic acid, propanoic acid, other aliphatic acids, acetic acid and their chloro-derivatives [18,19]. Other products of humic acid solutions chlorinated might be hydroxy-methyl-benzoic acids, 1,2,4-triazolidine-1-carboxylic acid, butyl-1,2-benzenedicarboxylic acid, 3-nitro-1,2-benzenedicarboxylic acid [20]. The vast majority of the products mentioned above seem not to be stable in a chlorine solution, and after 2 days of reaction the final products could be chloroform, chloral hydrate, chloropicrin, chloro-, and dichloropropanones and mono-, di- and trichloroacetic acids [21].

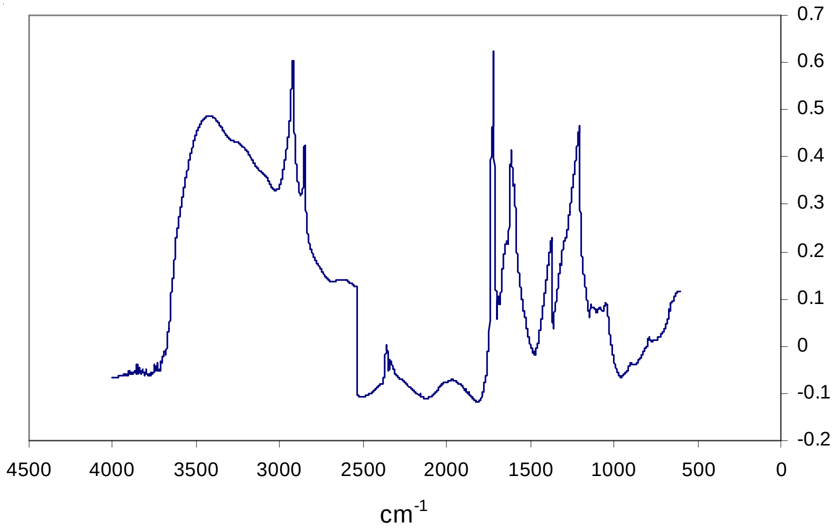

Fig. 4. FT-IR spectrum of SA (Sigma-Aldrich) standard fulvic acid.

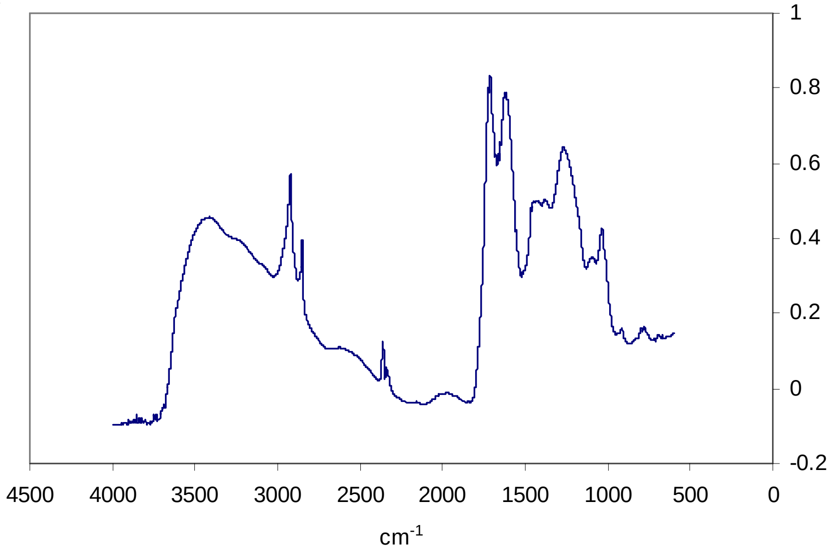

Fig. 5. FT-IR spectrum of SA (Sigma-Aldrich) standard humic acid.

Degradation of humic or fulvic acids by irradiation formed products such as benzene-, -di-, -tri-, -tetra-, -penta-, and -hexa- carboxylic acids, traces of n-C16 and n-C-18 fatty acids [22]. Pyrolysis GC/MS products of aquatic humic substances included pyridine, toluene, phenol and ethyl, methoxy as well as methyl derivatives, 2- and 3-furaldeydes, furanones, benzacetaldeyde, benzoic acid and others [23].

The presence of haloacetonitriles indicates ei-

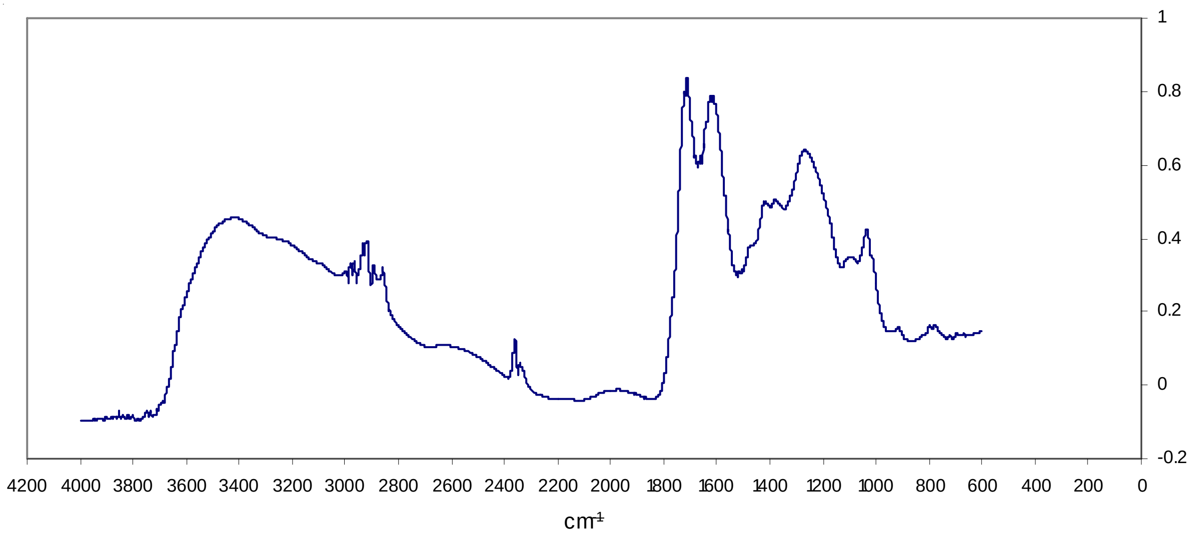

Fig. 6. FT-IR spectrum of Mornos humic acid.

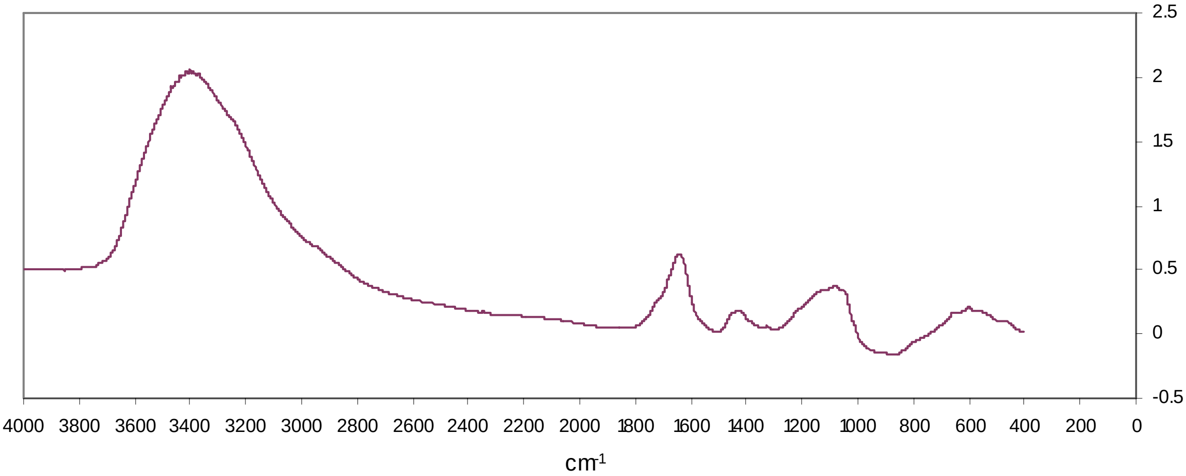

Fig. 7. FT-IR spectrum of Mornos fulvic acid.

ther aminoacids adsorbed in humic substances or a possibility of presence of nitrogen containing functional groups in the humic or fulvic acid molecules. Past research indicated that peptide materials associated with HA or FA are retained by XAD-8 resin [24]. The most possible degradation path of aminoacids (in the presence of chlorine) is to chloroform through dichloroacetonitrile $CHCl_2CN$ and dichloroacetaldehyde $CHCl_2CHO$–chloral CCl_3CHO [25] :

$$\text{R-}CH_2CH(NH_2)COOH \rightarrow CHCl_2CHO \rightarrow CCl_3CHO + CHCl_3$$

$$\text{R-}CH_2CH(NH_2)COOH \rightarrow CHCl_2CN \rightarrow CCl_3CN \rightarrow CHCl_3$$

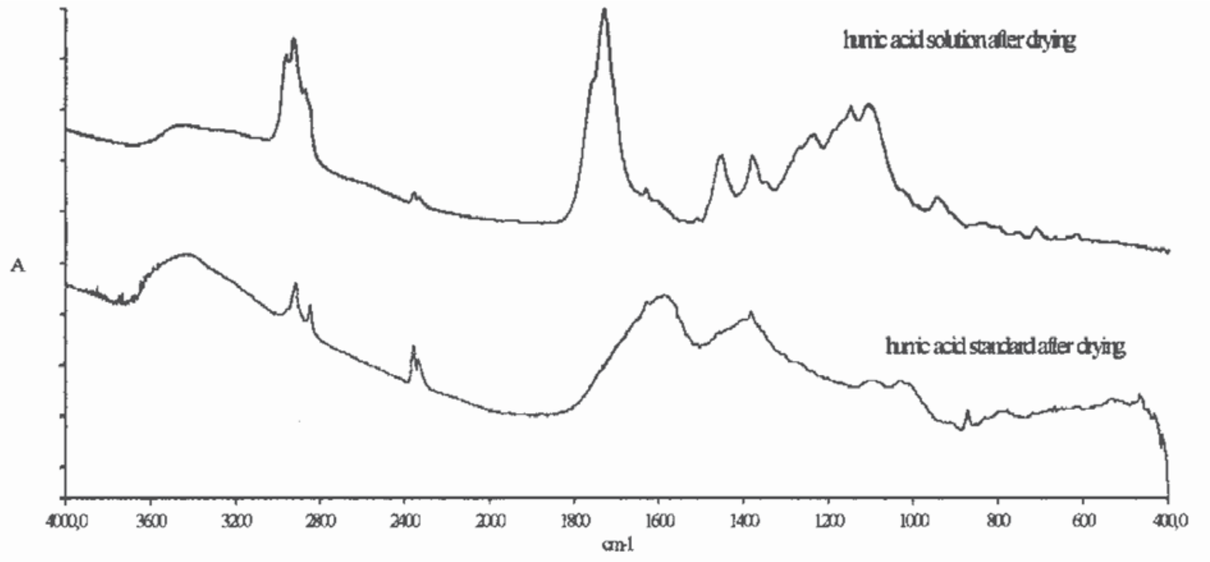

Fig. 8. FT-IR spectra of standard humics (humic acid solution in pH = 1 after drying) and standard humics sodium salt (humic acid standard after drying).

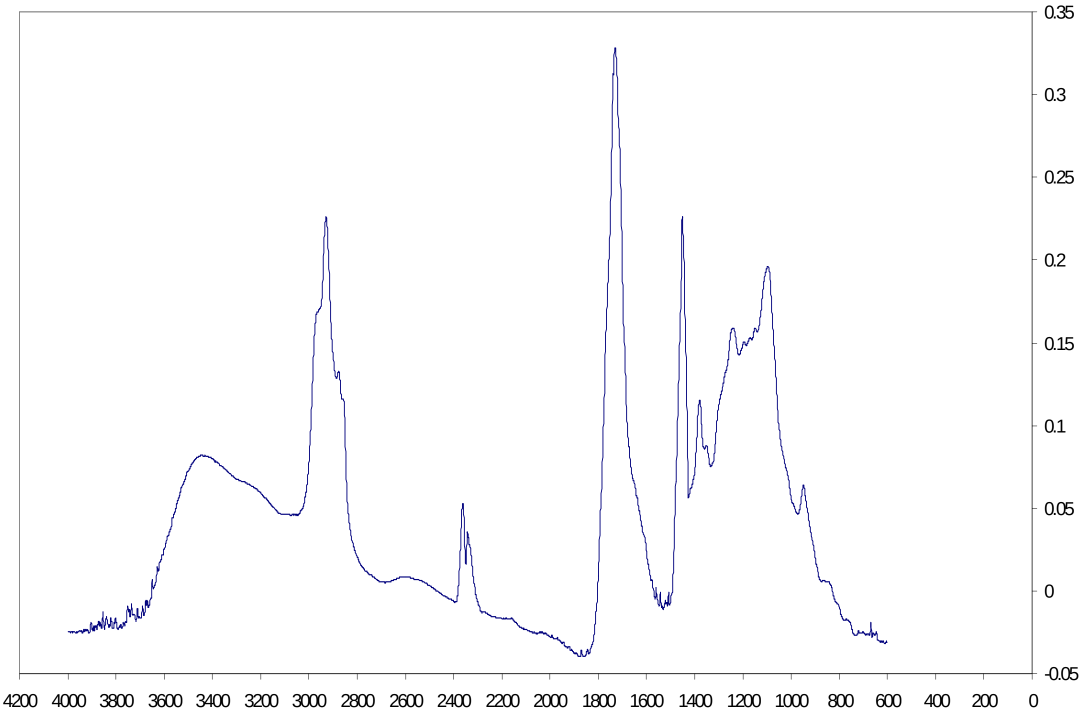

Fig. 9. FT-IR spectrum of Yliki humic acid.

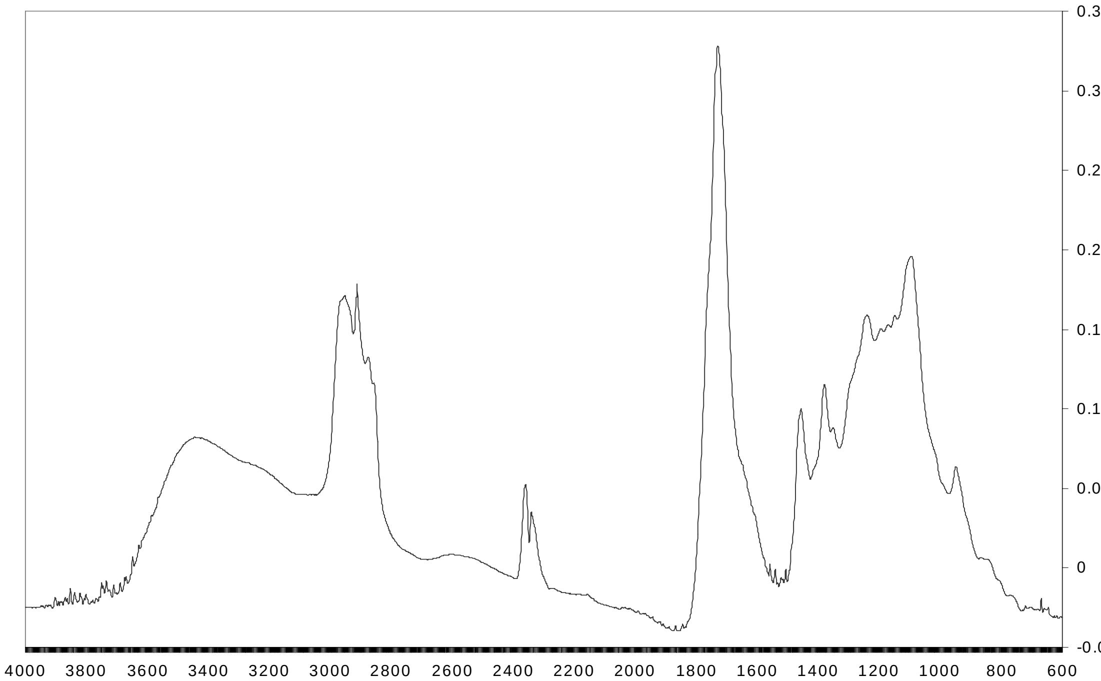

Fig. 10. FT-IR spectrum of Yliki fulvic acid.

Past research of the corresponding author indicated that chlorination of Mornos River raw water (sample volume 20 l) formed mainly 1 or 2 carbon haloderivatives (bromides concentration is 8–12 ppb) and ethyl acetate did not elute traces of by-products with molecules larger than those of 20 carbons from XAD-8 resin, such as methyl-substituted benzenes: 2-chloro-1,3,-bis (1-methylethyl) benzene, 1,2 dimethyl- 4-chloromethylbenzene, 1-phenyl, 3-chloro 2-propanone and other aromatic and aliphatic degradation products.

4. Conclusions

As can be seen from the above, fulvic and humic acids appear with the same wavelength bands at similar frequencies. The band with the highest intensity is that of the carbonyl stretch in carboxylic acids. Except for carboxylic acids, other functional groups may be alcohols, aromatic rings and aliphatic chains.

The major precursor to CBPs formation seems to be humic acid of Yliki Lake as well as Mornos River. Chloroform as well as chloropropanones seem to be a products of humic acid as they are formed during the chlorination of hydroxy-benzoic acid and β-keto-carboxylic acid. The first step of humic substances degradation during chlorine reaction is a break of their molecules to aliphatic chains as well as aromatic rings (these are called CBPs precursors). Aromatic carboxylic acids with hydroxy-substitutes are good chloroform and haloacetic acids precursors, and β-keto-carboxylic acids yield chloropropanones (during the second step of chlorine reaction that begins to yield CBPs after minutes of reaction). Chlorosubstituted acetonitriles might be chlorination products of aminoacids. These aminoacids may be adsorbed to humic substances.

Chloropicrin, chloral, trichloroacetonitrile, trichloroacetic acid yield, finally, chloroform. There are also traces of a great variety of other chlorosubstituted chemical compounds, aliphatic and aromatic ones, hardly recognizable by a mass spectrometer. Fatty acids, aromatic ketones, furaldeydes and furanones appear in traces.

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