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Spectroscopic studies of humic acids from subsurface sediment samples collected across the Aegean Sea

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Abstract

Natural humic acids are biogenic, structurally complex and heterogeneous, refractory, acidic, yellow-to black-coloured organic polyelectrolytes of relatively high molecular weight. They occur in all soils, sediments, fresh waters, and seawaters. Humic acids represent the largest portion of nonliving soil organic matter. In the present paper, humic substances were isolated from marine subsurface sediment samples collected across the Aegean sea (in Greece) and especially from a marine area extending northwards of the Samothraki plateau towards the north-eastern part of the island of Crete. In a following step, humic preparations were studied using infrared and fluorescence spectroscopy (emission, excitation and synchronous-scan excitation spectra were obtained). The infrared spectra suggested functional chemical groups such as OH^- , C-H aliphatic, C=C, C=O/COO⁻, salts of carboxylic acids, and also, in some cases, silicate anions or C-O from alcohols, esters and ethers. Fluorescence emission, excitation and synchronous scan excitation provided some valuable information concerning a probable origin (marine and/or terrestrial) for the isolated humics.

Keywords: Fluorescence spectroscopy; Functional group; Humic substances; Infrared spectroscopy; Marine sediments.

Introduction

Natural humic substances comprise a wide class of naturally occurring, biogenic, structurally complex and heterogeneous, refractory, acidic, yellow-to black-coloured organic polyelectrolytes of relatively high molecular weight that occur in all soils, sediments, fresh waters, and seawater (THURMAN, 1986; SCHNITZER, 1978; STEVENSON, 1982). Humic acids are polycarboxylic acids with phenolic, alcoholic and carbonyl groups, aromatic rings and free radicals (SCHNITZER, 1978; SCHNITZER

& KHAN, 1972). They are very important constituents of sediments which perform in many functions, such as: (a) metal scavenging, concentration and transport (MANSKAYA & DROZDOVA, 1968); (b) growth regulation; (c) toxic material concentration and transport; (d) sediment formation (MANSKAYA & DROZDOVA, 1968).

Marine humates are of a rather dubious origin (RICE & MACCARTHY, 1991). Marine origins (NISSEMBAUM & KAPLAN, 1972; NISSEMBAUM, 1974), continental origins (YEN & TANG, 1977), or both (ROGERS & KOONS, 1968) have been suggested.

Humic substances can be studied using spectroscopic methods such as infrared and fluorescence spectroscopy. The most interesting portion of the infrared spectrum for the structural and analytical study of molecules is the medium IR region, between 4000 and 400 cm^{-1} . Fluorescence spectroscopy includes the three conventional modes of emission, excitation and synchronous scan. The former two fluorescence methods show limited applicability in resolving the spectra of complex polymers satisfactorily while the latter is characterized as a non-separative, non-destructive, highly sensitive technique, with limited sample preparation and as offering the opportunity to select various $\Delta\lambda$ that allow the spectral separation of overlapping fluorescence signals into individual components (MIANO & SENESI, 1992).

Materials and Methods

Humic substances were isolated from relatively enriched in organic carbon marine subsurface sediment samples from the Aegean sea (Table 1, Fig. 1). Humic substances were isolated following the pre-treatment step (freeze-drying), extraction step (removal of the alkaline soluble fraction), acidification step (acidification of the alkali-soluble extract to $\text{pH} \leq 2$ with HCl and precipitation), then centrifugation and finally purification of humic acids (THURMAN, 1988).

Humic substances were studied using (a) infrared spectroscopy with an IR spectrophotometer Perkin Elmer model 399 B on KBr pellets (1mg sample and 400mg KBr, spectrometry grade), and (b) fluorescence spectroscopy, using a Perkin Elmer LS-5 luminescence spectrophotometer on aqueous solutions of 100mg/l sample adjusted to pH8 with 0.05N NaOH. Emission spectra were recorded over the range 300-550nm at a fixed excitation wavelength of 360nm at pH7. Excitation spectra were obtained over a scan range of 270-500nm, by measuring the emission radiation at a fixed wavelength

Table 1
Burial depth, and corresponding water depth of each sediment sample.

| Sample Number | Burial Depth (cm) | Water Depth (m) |
|---------------|-------------------|-----------------|
| 1 | 4-7.5 | 182 |
| 2 | 336-339 | 182 |
| 3 | 40-45.5 | 995 |
| 4 | 40.5-44 | 690 |
| 5 | 45-54 | 720 |
| 6 | 226-228.5 | 365 |
| 7 | 16.5-19.5 | 340 |
| 8 | 243.5-252 | 340 |
| 9 | 37-39.5 | 368 |
| 10 | 177.5-183 | 368 |
| 11 | 24-31 | 415 |
| 12 | 196-206 | 415 |
| 13 | 104-110 | 568 |

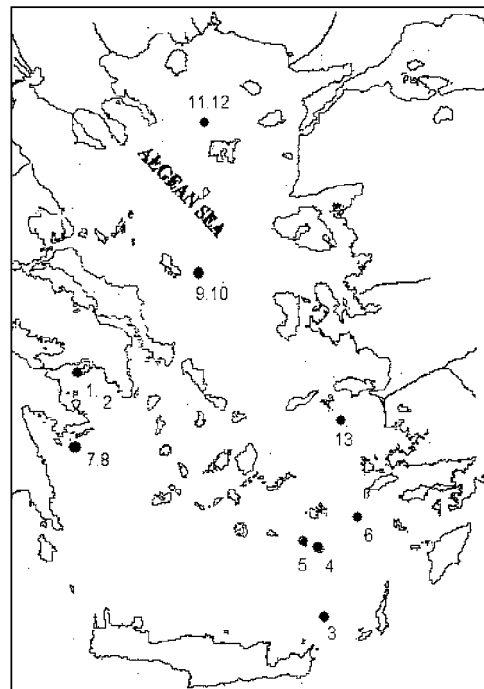


Fig. 1: Map showing the sampling sites.

of 520nm at pH 7. Synchronous-scan excitation spectra were obtained by measuring the fluorescence intensity while simultaneously

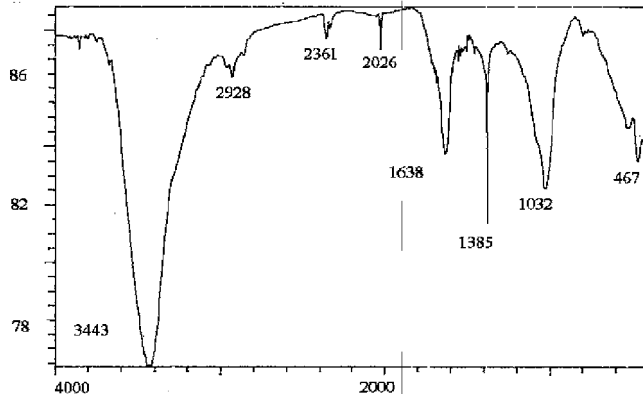
scanning over both the excitation and emission wavelengths, and keeping a constant, optimized wavelength difference, $\Delta\lambda = \lambda_{em} - \lambda_{exc}$ (18nm) between them (SENESI, 1990).

Results and Discussion

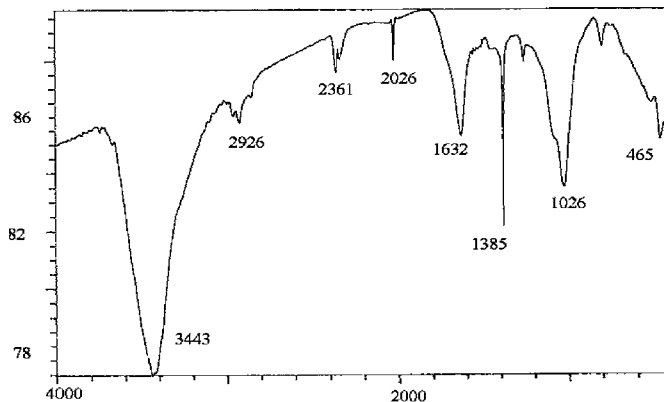
Infrared Spectroscopy

According to STEVENSON & GOH (1982), MACCARTHY & RICE (1985), VANDENBROUCKE *et al.* (1985), SENESI *et al.* (1989), and SENESI (1992), the absorption bands could be interpreted as the following: (a) the 3400 cm^{-1} band is the one where a strong absorption due to hydrogen

bonding hydroxyl groups occurs; (b) the 2920 and 2860 cm^{-1} absorption bands are attributed to the asymmetric and symmetric stretching vibrations, respectively, of aliphatic C-H bonds in methyl and/or methylene units; (c) the band at 1710 cm^{-1} , which in terrestrial humic acids appears as a deep shoulder, is assigned to C=O stretch of carboxyl and carbonyl groups; (d) the 1600-1650 cm^{-1} band is assigned to aromatic C=C double bonds conjugated with C=O and/or COO; (e) the absorption at 1540 cm^{-1} is the amide band due to peptidic linkages; (f) the band at 1385 cm^{-1} is assigned at O-H deformation and C-O- stretch of phenols, COO- group vibrations, C-H deformation of CH_3 ; (g) the



Sample 3



Sample 5

Fig.2.: Selected I.R. spectra.

absorption around 1220 cm^{-1} is assigned to the C-O stretching vibration and OH bending deformations, due mainly to carboxyl groups; (h) an intense band around 1035 cm^{-1} is assigned to alcoholic and polysaccharide CO stretch and OH deformation and, eventually, Si-OH bend in silicate impurities. In the present study, the IR spectra recorded show that significant bands occur. The IR absorption characteristics resemble each other significantly while the relative intensities of specific bands vary.

Thus, through a thorough study of the IR spectra, it appears that all samples show characteristic peaks for the following: hydroxyl, methyl, methylene, aromatic bond, carbonyl, carboxyl, phenol, alcohol, polysaccharide and silicate impurities. In addition, samples 4 and 10 show the characteristic peak for amide. Comparing the recorded spectra of the samples 7, 9 and 11 with those of 8, 10 and 12 respectively, it is found that hydroxyls, aromatic bonds, and phenols decrease with burial depth increase. Selected IR spectra are shown in Figure 2.

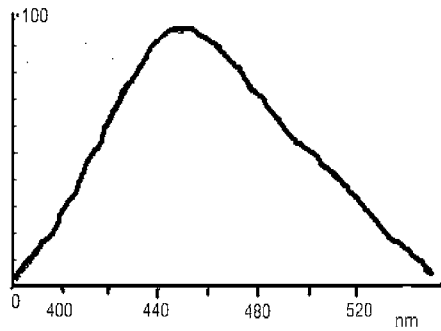
Fluorescence spectroscopy

Marine humates are of a rather dubious origin (RICE & MACCARTHY, 1991). Debris of vascular plants, commonly found in coastal sediments, can be a significant source of sedimentary humic substances (ERTEL & HEDGES, 1985). Marine humates could be of a marine origin, on the basis of their genetic relation with degraded planktonic material (NISSEMBAUM & KAPLAN, 1972; Nissebaum, 1974) or of a continental one via lignin formation on the continents (YEN & TANG, 1977). Both origins might also be valid (ROGERS & KOONS, 1968). Interpretation of fluorescence emission, excitation, and synchronous-scan excitation spectra suggests the probable origin of the isolated humic acids (SENESI, 1990; BOTO & ISDALE, 1985; SAAR & WEBER, 1980; RYAN & WEBER, 1982). In humic polymers, the most efficient fluorophores are var-

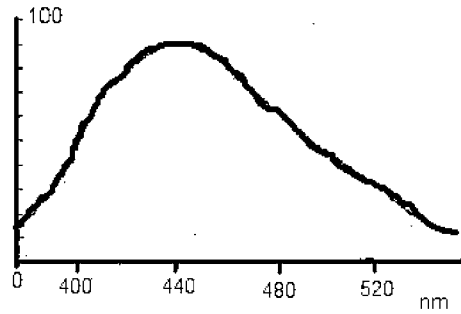
Table 2
Possible origin for humic acids isolated.

| Sample Number | Possible origin |
|---------------|-----------------|
| 1 | marine |
| 2 | marine |
| 3 | terrestrial |
| 4 | terrestrial |
| 5 | terrestrial |
| 6 | marine |
| 7 | marine |
| 8 | terrestrial |
| 9 | marine |
| 10 | terrestrial |
| 11 | marine |
| 12 | marine |
| 13 | marine |

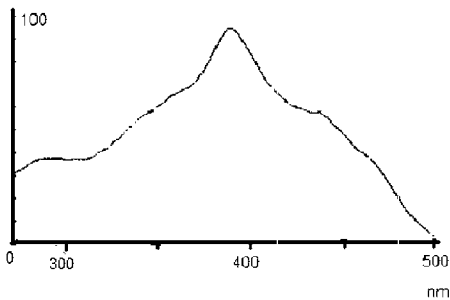
iously substituted, condensed aromatic rings, and/or highly unsaturated aliphatic chains (MIANO *et al.*, 1998; SENESI *et al.*, 1991a; SEITZ, 1981). Data from the literature (SENESI, 1990; VISSER, 1983; MIANO *et al.*, 1988, SAAR & WEBER, 1980; RYAN & WEBER, 1982) indicate that: (i) as far as emission spectra are concerned, humic acids isolated from various terrestrial sources typically show a maximum-intensity wavelength varying between 435 and 465nm, while humic acids originating from marine samples generally show a maximum emission intensity which varies within a lower wavelength range, 410-450nm; (ii) as far as excitation spectra are concerned, the major excitation peak for terrestrial humic acids commonly occurs at a wavelength of about 390nm, with a number of secondary peaks or shoulders occurring on either side of the major peak, although at lower (360-345nm) or higher wavelengths (455-470nm), while humic acids of marine origin are characterized by one main excitation peak at variable wavelength (from 385 to 320nm); (iii) as far as synchronous-scan excitation spectra are concerned, the spectra of soil humic acids exhibit two major peaks whereas the marine humic acids give the most structured spectra. Thus, a



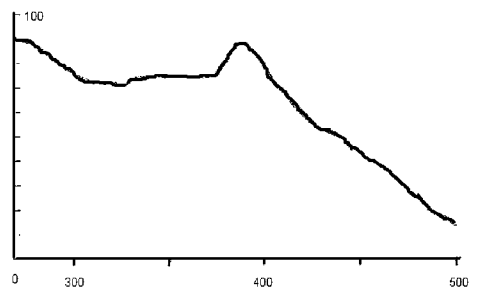
Sample 3(a)



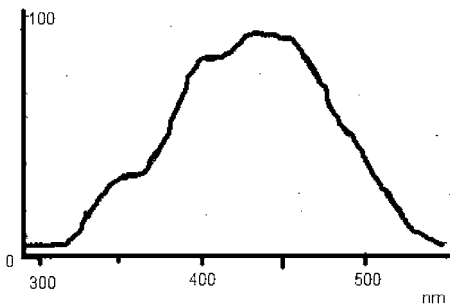
Sample 12(a)



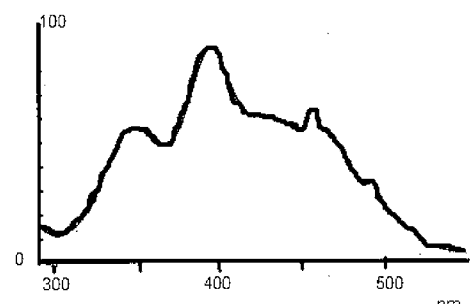
Sample 4(b)



Sample 1(b)



Sample 4(c)



Sample 1(c)

Fig. 3.: Selected fluorescence spectra (a: emission, b: excitation, c: synchronous scan).

thorough study of the recorded fluorescence spectra allows us to suggest that samples 1, 2, 6, 7, 9, 11, 12 and 13 are most probably of a marine origin while 3, 4, 5, 8 and 10 are of a terrestrial origin (Table 2). Selected fluorescence spectra are shown in Figure 3.

However, the study of any fluorescence spectrum does not allow the identification of individual structural components responsi-

ble for the macromolecule fluorescence of humic acids. Each spectrum is the sum of individual spectra of several fluorophores present in the complex and heterogeneous humic acid compound. The only way to be able to suggest structural components of a potential fluorescence contribution would be by applying various other techniques, such as degradation means, etc, to the humic acid

macromolecule methods, (SCHNITZER & KHAN, 1972; SENESI, 1990; SENESI *et al.*, 1991).

Conclusion

The spectroscopic methods that have been applied to the humic acids isolated from marine subsurface sediment samples from the Aegean Sea, allowed us to suggest the presence of various chemical units as well as the marine or terrestrial origin of each sample.

Thus, in all humic acid preparations there is a presence of hydroxyl, methyl, methylene, carbonyl, carboxyl, phenol, alcohol, polysaccharide groups, aromatic bond and silicate impurities. Occasionally, there is amide as well.

In two of the cores studied (samples 7, 8 and 9, 10) a different humic acid composition and origin was found between the upper and lower part. This composition differentiation could be attributed to the variation in oxygen content, particle size, sedimentation environment and relative productivity of terrestrial and marine environments (VANDENBROUCKE *et al.*, 1985). In a third core (samples 11 and 12), humic acids from the top and bottom part show variable composition, although they are of the same origin. This shows the impact on humic acid composition of the three first previously mentioned parameters.

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