Biogeocycle of the Pollutants in the Mediterranean Sea : Environmental Importance of Humic Acids*

by

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Abstract

Humic acids are pervasive organic compounds derived from the biochemical degradation of terrestrial and marine vegetation and perhaps of zooplankton under aerobic (oxic) conditions. Their presence has traditionally been used as an indicator of the likelihood of a body of water to foster productivity. They sequester metals, often forming insoluble complexes and their presence may well determine the concentration of trace metals present in a sea. Their chemical structures are poorly understood. There is evidence that humic acids from terrestrial and marine vegetation are different although both sets react with metals.

This paper presents the first attempts to determine the distribution of fluorescent humic material in coastal regions of the north eastern Mediterranean. The IR spectra of humic acids obtained from lignites, soils, rivers and the sea are compared.

The fluorescence of humic acids is quenched alkaline hypochlorite, implying that the fluorescence is due to polynuclear aromatic structures.

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Introduction

The results of the first stage of a study of the role of humic acids in the eastern Mediterranean are reported. Eventually, the aims are to determine the chemical structure of humic acids, their distribution throughout the region, and their function. The present investigation emphasizes IR spectra of humic acids and the measurement of their fluorescence in regions of the eastern Mediterranean (shown on Map 1).

Materials and methods

IR spectra were measured on a Beckmann Acculab 2 by the KBr disc technique. Fluorescence was measured using a Turner Model 430 instrument, generally employing 360 nm light for excitation and observing emission at 455 nm. (Wavelengths at which neither fluorescence from chlorophyll nor from hydrocarbon pollutants were seen in our samples). Fluorescence was measured on 5 ml samples of sea water, using appropriate solvents as controls. Samples either with high humic acid concentrations or taken from phytoplankton cultures were first passed through millipore filters to check whether filtering affected fluorescence. Reactions with alkaline hypochlorite were performed with excess reagent in test tubes at 60° C. Surface tension was measured by the rise of liquid in a glass capillary.

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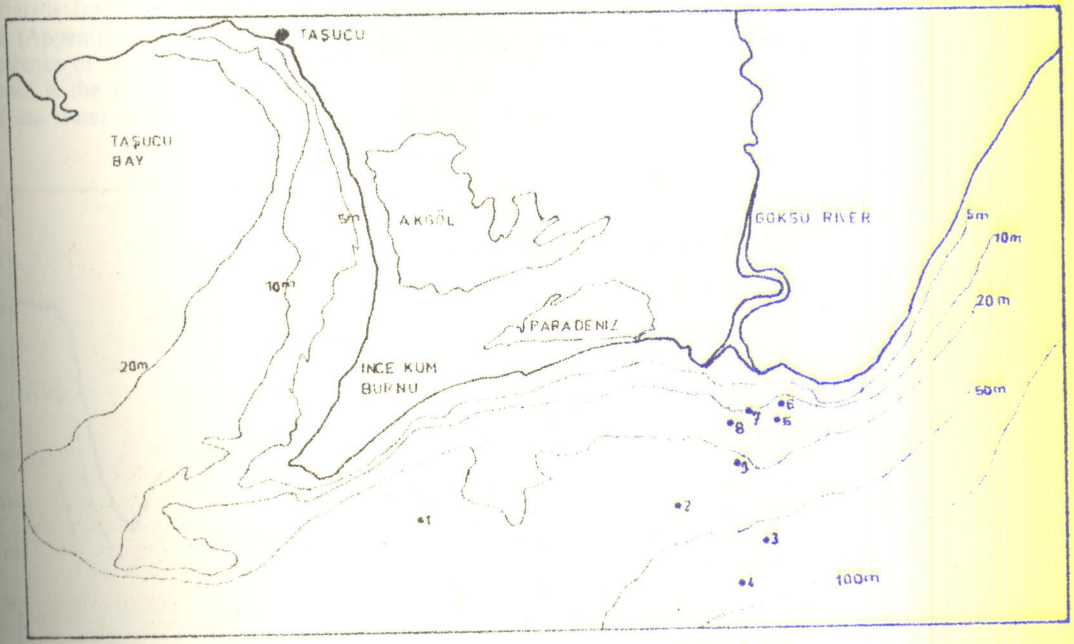
A preliminary Investigation of the Distribution of Humic Acids in the North Eastern Mediterranean

a. *Structure and Distribution of Terrestrial Humic Acids*



MAP 1: SAMPLING STATIONS IN GÖKSU RIVER DELTA (36°15'N, 34°00'E)

Figure 1 shows IR spectra of humic acids obtained from lignite, marine sediments and coastal waters, which were similar to those obtained by previous workers (SHAPIRO, 1957; GOH, 1969; RASHID, 1971; SCHNITZER, 1976; STUERMER & PAYNE, 1976). The spectra of humic acids are all similar to each other and also similar to spectra of tertiary lignites (VAN VUCHT *et. al.*, 1955; ELOFSON, 1957; BURAVAS *et. al.* 1970), except for enhanced absorption by carboxyl groups at 1700 cm^{-1}. These spectra are interpreted as representing aromatic material substituted by aliphatic groups, phenolic OH groups and carboxyl groups. One notes that absorption near 1400 cm^{-1} was generally higher than absorption near 1450 cm^{-1}; the former was ascribed to methylene groups adjacent to a carboxyl group, therefore some of the carboxyl groups may occur in the aliphatic part of the molecules, an interpretation consistent with the ^{1}Hnmr spectrum of a humic acid by STUERMER & PAYNE (1976). That the latter failed to obtain resolved C^{13} nmr spectra suggests that the molecules of humic acid are rather rigid.

Much simpler than the spectra of humic acids are the spectra of fulvic acids, which showed prominent absorption by OH groups and carboxylate anions, which SCHNITZER (1976) has interpreted as being due to the carboxylic acids of phenols.

The largest localized concentrations of humic acid found in the north eastern Mediterranean, large enough to be determined gravimetrically, are the lignite seams, which sometimes occur in the Toros mountains. Peaty soil in the Çukurova plain at Tarsus and af Kirikhan for example contains 10 % humic acids. Red Mediterranean soil found in the plain contains 1 % humic acids, while soils associated with limestones appear to contain much less. Fulvic acids generally occur in smaller quantities than humic acids.

EVANS (1976), in a survey of the beach geology of Cilicia, rarely noted any organic material occurring in beach sediment. About half of this sediment is calcium carbonate. Given our previous remarks, this beach is an unlikely site for humic material. We have found that as little organic matter as ~ 0.5 % may sometimes be recovered from these sediments; the material is acid soluble alkalii insoluble and gives IR spectra unlike those given of humic acids. Sediments from the delta of the Göksu River contained between 0.1 and 1 % humic acid. (Sediments from the mouths of the Seyhan and Ceyhan rivers have yet to be examined).

b. *Structure of Marine Humic Acids*

Phyto and zooplankton, unlike terrestrial vegetation, contain no lignin and it is likely that the marine humic acids, to which they give rise upon aerobic (oxic) biochemical degradation, have structures which differ from those of terrestrial humic acids. HATCHER *et. al.* (1980) have interpreted nmr spectra of fulvic acids from marine sediments in terms of modified carbohydrates: nevertheless, UNSAL & YILMAZ (Appendix Fig. 2) have shown that degradation of a phytoplankton culture is accompanied by the appearence of fluorescence very similar to that of terrestrial humic acid. We have assumed that this fluorescence is the consequence of the formation of marine humic acid and that fluorescence measurements of sea water show humic acid concentrations, either from a terrestrial source or from plankton.

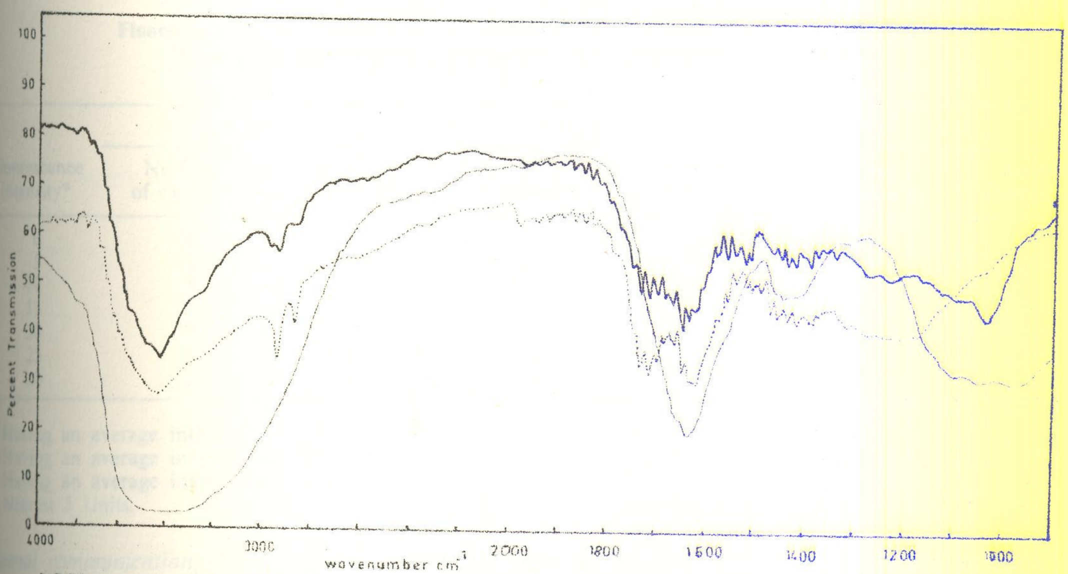


FIGURE 1 INFRA RED SPECTRA OF —— MARINE SEDIMENT HUMIC ACIDS
········ LIGNITE HUMIC ACIDS
- - - - COASTAL WATERS

c. *Distribution of Humic Acids in Waters of the North Eastern Mediterranean*

Humic acids occurring in coastal waters are generally of terrestrial origin and are carried to the sea by rivers. The proportion of humic acid derived from plankton increases with the depth of the water column. The north eastern Mediterranean is traversed by three rivers, the Göksu, the Ceyhan and the Seyhan and by various streams, all of which transfer humic acids from the land to the coastal region. Fluorescence measurements of water in a lagoon, fed continuously by the Ceyhan river, indicated summer concentrations of humic acid of the order of ppm. which is high for river water in the region. Table 1 A shows fluorescence measurements obtained from sea water in the vicinity of the Göksu River during a flood. Although the fluorescence of other river waters has yet to be measured, since flood water continues for several weeks each year, it is clear that throughout this time the sea is being supplied with humic acid. Table 1 B gives measurements of humic acid fluorescence in sea water from the vicinity of the Göksu river throughout the spring of 1980. The results are insufficient, but possibly future work will provide correlations between fluorescence and oceanographic parameters not yet apparent. The fluorescence intensities of the samples fit a modified Poisson distribution. That is, patches of humic acid appear to be distributed more or less randomly throughout the sea, the concentration of each patch varying about an average value which might be expected to vary seasonally.

Humic acid and the environment. Humic acid concentrations are a measure of the "health" of a river or sea. Large concentrations of humic acid indicate (1) the presence of decaying vegetation which diminishes oxygen available for respiration and (2) an increased rate of assimilation of nutrients by plankton (HAIR & BASSETT, 1973; PRAKASH & RASHID, 1968; UNSAL & YILMAZ, 1980:

TABLE 1 A

Fluorescence measurements of filtered surface waters from plume of Göksu river 14-16.4.1980

Fluorescence intensity	3	3	6	2	12	22	30	29	27	36	55
Salinity ‰	37.94	36.28	35.94	35.55	34.63	30.28	23.28	18.10	12.71	5.58	0.60

TABLE 1 B

Fluorescence of sea water near the Göksu River in March, April and May 1980
Results showed no simple correlation with oceanographic parameters

	MARCH		APRIL		MAY	
Fluorescence Intensity*	Number of samples	Poisson[a] Distribution	Number of samples	Poisson[b] Distribution	Number of samples	Poisson[c] Distribution
0	11	11	7	4.5	16	3.5
5	8	8	10	10	6	8.75
10	4	3	13	11	11	11
15	1	0.7	5	8	8	9.3
20	0	0.1	5	4.4	6	5.6
25	1	0	2	1.9	3	2.8
30	0	0	2	0.7	2	1.1

a) Having an average intensity of 3.5.
b) Having an average intensity of 11.
c) Having an average intensity of 12.5.
* Nearest 5 Units.

personal communication). In the north eastern Mediterranean, the growth of plankton appears to be controlled not by light, which is always reasonably intense, but by the availability of nutrients. Thus, productivity develops in late winter and early spring following the increase in nutrient concentrations induced by winter weather, whereas in boreal waters the spring bloom develops somewhat later when the light intensity increases. The rapidity of the development of productivity in the north eastern Mediterranean may well depend on the concentration of humic acid which is present when the supply of nutrients increases. Humic acids figure prominently in environmental studies by virtue of their interaction with metal cations. That they bind metals both strongly and weakly and that their concentration in the north eastern Mediterranean exceeds that of most trace metals, suggest that humic acids may often be regulating trace metal concentrations and possibly even the transfer of essential metals to plankton. The complexing of humic and fulvic acids has been studied extensively (SCHNITZER, 1978 ; O'SHEA & MANCY, 1978 ; RASHID, 1971, 1974 ; MANTOURA *et. al.* 1978). The subject is complicated and confused, although somewhat clarified by noting that humic acids in solution are colloidal electrolytes. The formation of excimers, indicated by fluorescence studies (Appendix), suggests that humic acids form micelles. This is supported by our measurements showing that 0.5-1 % of humic acid reduces the surface tension of water or of 0.1 M NaOH by ~ 25 % at room temperature, resembling soap.

It is well known that trivalent cations precipitate humic acids more readily than divalent or monovalent cations. This has been interpreted in terms of the number of available complexing sites in a humic acid molecule, which may be valid. But it seems better to regard the phenomenon as an example of the Schulze-Hardy rule governing the coagulation of lyophobic colloids. Table 2 shows some of our own determinations of the amounts of cation needed to cause coagulation of a humic acid. Differences in the coagulation concentrations of cations possessing the same ionic charge presumably can be explained in terms of changes in the fixed layer of charge on the colloid. Complex formation may well be important here. Complex formation and colloid coagulation effects might be distinguished by investigating the effect of varying the ionic strength of the electrolyte. The Verwey-Overbeek-Derjaguin description of a lyophobic colloid solution suggests that increase in ionic strength of the solution will usually decrease the amount of a coagulating cation needed to cause precipitation, while the Debye-Hückel theory of dilute

TABLE 2 A

Amount of metals needed for coagulation of 1 g of humic acid

Metal	Amount needed (g)
Fe^{+3}	0.23
Al^{+3}	0.51
Mg^{+2}	1.39
Ca^{+2}	1.23

TABLE 2 B

Dependence of coagulation of metal-humic acid complexes on the ionic strength of solution

	Fluorescence Intensity[b)]			
Ionic[a)] Strength	Fe^{+3}	Al^{+3}	Mg^{+2}	Fe^{+2}
0.00	2.5	2.5	46.0	10.5
0.09	11.7	6.5	46.5	6.5
0.20	14.5	11.0	48.0	7.0
0.30	17.5	23.5	56.5	—
0.40	26.5	—	—	5.5
0.51	39.2	27.5	—	—
0.72	43.5	49.0	61.0	—

a) $I = \dfrac{19.9273 \text{ S ‰}}{1000 - 1.00509 \text{ S ‰}}$

b) A known amount of Humic acid was titrated with metal ions. The precipitate was filtered through a millipore membrane filter. The precipitate was equilibrated with the solutions of various ionic strengths. The fluorescence intensity of each solution was measured after centrifugation.

TABLE 3

Variation of humic acid fluorescence with concentration : Typical results
Excitation at 360 nm. Emission maximum 520 nm. (High concentrations), 455 nm. (Low concentrations)

Concentration	A/2	A/4	A/8	A/16	A/32	A/64	A/128	A/256	A/512	A/1024	A/2048
Fluorescence Intensity 455 nm	1.8	31	76	104	95	67.5	43.9	24.9	13.5	9	6.9
520 nm	8.4	38	75	85.5	75	51	30	15.9	9	5	3.5
f	1	2.55	2.48	2.65	2.67	2.63	2.62	2.41	3.04	*	*
Concentration	B/2	B/4	B/8	B/16	B/32	B/64	B/128	B/256			
Fluorescence Intensity 455nm	9	48.5	85.5	93	71.5	43.5	20.0	10.5			
520 nm	19	53	78	75	52.5	29.9	13	6.5			
f	1.55	2.65	2.43	2.47	2.80	*	*	*			

A = 0.4 mg Humic acid per ml of solution buffered at Ph 9.2.

B = 0.45 mg Humic acid per ml of solution buffered at Ph 7.0.

f = (Fluorescence intensity at 455 m) $(T_{455})^{-1}$ $(1\text{-}T_{360})^{-1}$ where T_x = optical transmittance at x nm.

* = Optical transmittance inaccurate.

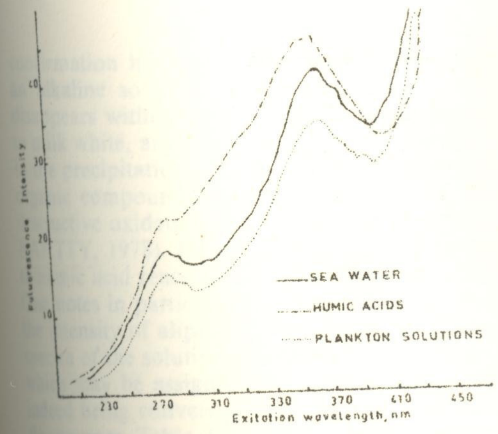


FIGURE 2: FULUORESCENCE EXCITATION SPECTRA OF SEA WATER, HUMIC ACIDS AND PLANKTON SOLUTIONS

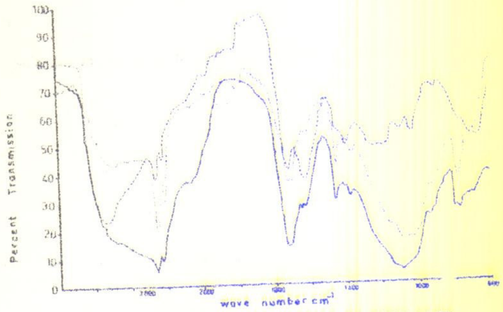


FIGURE 3: EFFECT OF HYPOCHLORITE ON HUMIC ACIDS

INFRA RED SPECTRUM _ _ _ OF UNTREATED HUMIC ACID

____ OF BROWN SOLID AFTER WEAK TREATMENT

.... OF YELLOW SOLID AFTER STRONG TREATMENT

electrolytes shows that a complex increases in solubility with the square root of the ionic strength. Table 2 B shows preliminary results which suggest that the coagulation of humic acids depends in a complicated way on the ionic strength and that both coagulation of a colloid and metal-humate complex formation are involved.

Conclusions

Humic acids occur in certain north eastern Mediterranean soils in concentrations of the order of 1 ppb. They are transported by rivers to coastal waters where concentrations are ~ 1 ppm. Such humic acids have chemical structures similar to tertiary lignites. Their fluorescence, which appears to be due to polynuclear aromatic structures, may be used to monitor their concentration in the sea where they behave as colloidal electrolytes. Humic acid concentration in north eastern Mediterranean waters is sufficiently high that it very likely regulates the concentration of trace metals as well as determines the rate of assimilation of nutrinents by plankton.

Acknowledgment

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Appendix : Fluorescence of Humic Acids

GHASSEMI & CHRISTMAN (1968) observed that humic acids give broad fluorescence with a maximum corresponding to excitation at 350 nm and emission centered at 450-460 nm. Our own results for very dilute solutions of humic acid agree with this. We have generally used an excitation wave length of 360 nm and observed emission at 455 nm. These maxima however do vary depending on the source of the material and the material, namely a fulvic or a humic acid. Figure 2 summarises our observations. It will be seen from Figure 2 that both sea water and soluble organic matter produced by plankton show similar humic acid fluorescence.

There is no direct evidence for the existence of polynuclear aromatics in humic acids but the similarity between UV and IR spectra of humic acids and solid fuels (which contain polynuclear aromatic structures), with which they may be associated, suggests that such structures may indeed be present. Some confirmation has been obtained by studying the loss of fluorescence with hypochlorite treatment. When an alkaline solution of a humic acid is treated with hypochlorite anions, the colour of the solution disappears within twenty minutes to an hour, passing from coffee brown, to cocoa, to orange, to yellow, to milk white, and decreasing in fluorescence. The reaction can be stopped at any stage with acid resulting in the precipitation of a solid possessing the same colour as the solution. Alkaline hypochlorite reacts with organic compounds in many ways but if the pH is high enough, the major reaction appears to be the destructive oxidation of polynuclear aromatic structures to give benzene polycarboxylic acids (CHAKRABARTTY, 1978). Figure 3 shows the IR spectra of solids precipitated by acidification of the hypochlorite-humic acid reaction mixture. These spectra are consistent with the course of reactions postulated above. One notes in particular the diminution of absorptions by aromatic structures at 1600 cm^{-1} compared with the intensity of aliphatic absorptions in the regions 3000-2900, 1450 and 1360 cm^{-1}; while UV absorption spectra of the solutions from which the solids were obtained showed increased absorption around 250 nm, which may be assigned to benzene rings, hence our inference that polynuclear aromatic structures were indeed being converted to benzenoid structures and that this was accompanied by the destruction of the fluorescence. Table 1 shows the dependence of fluorescence on the concentration of humic acid. It will be seen that this dependence was similar to that shown by many other systems. Fluorescence increased linearly with concentration, then non-linearly, maximized and fell to zero (OLSEN, 1975). The non-linear dependence on concentration is mainly due to the self-absorption of the fluorescent light by the humic acid solutions and this is not surprising since humic acid solutions absorb throughout the visible range. Table 3 shows that this explanation covers most but not all of the deviation from linearity. At highest concentrations, collisions must be increasingly competing with the production of fluorescence by returning excited molecules to their ground state. One might consider that collisions between humic acid molecules, excited by the absorption of light, with normal humic acid molecules form excimers. Table 3 shows that, at highest concentrations, the maximum intensity of fluorescence occurs at 520 nm rather than 455 nm, which indicates that excimer formation does occur. The highest concentrations are still very dilute, thus there must be regions of the solution where the local concentration of humic acid molecules was unusually high. This suggests that in those solutions the humic acid molecules were associating, for example, as dimers, or, recalling the colloidal properties of humic acids, perhaps in micelles.

Fluorescence is used to distinguish the liptinite-exinite group of macerals in solid fuels from the non-fluorescing huminite-vitrinite group. This is surprising since knowledge of the chemical structures suggests that the huminite-vitrinite group should contain more polynuclear aromatic material. Analogy with the present work suggests that the liptinite-exinite group fluoresces because it represents a very dilute solution of polynuclear aromatic material whereas, in the huminite-vitrinite group, the polynuclear structures are more concentrated and are packed in small stacks so that fluorescence is inhibited. Etching the surface of the liptinite-exinite group with alkaline hypochlorite reduces fluorescence; conversely it is possible that restricted etching of the huminite-vitrinite group might enhance fluorescence.

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