

FİTOPLANKTONLARIN HUMİK ASİT FLORESANSININ YAYINIMI

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ÖZET: Bu çalışmada Kuzeydoğu Akdeniz kıyılarından toplanan deniz suyu örneklerinde ve laboratuvarında üretilen fitoplankton kültürlerinde klorofil- α uyarma dalga boyu: 425 nm; yayınım dalga boyu: 660 nm) ve hümkik asitten uyarma dalga boyu: 360 nm; yayınım dalga boyu: 455 nm) kaynaklanan floresans ölçülmüştür. Fitoplankton kültüründe hücreler ölmeye başlar başlamaz hümkik asit floresansının oluştuğu gözlenmiştir. Diğer yandan Kuzeydoğu Akdeniz yüzey suları florometrik ve istatistiksel olarak analiz edildiğinde hümkik asitin yamalar halinde dağılım gösterdiği saptanmıştır. Genellikle hümkik asit floresansı su kolonunda klorofil- α konsantrasyonu ile ya aynı fazda ya da zıt fazda değişim göstermektedir. Klorofil-a ile hümkik asit derinlikte aynı fazda değişim gösterdiğinde bu ortamda sağlıklı fitoplankton kolonilerinden söz edilmelidir. Ölen fitoplanktonlardan hızla hümkik yapısında maddelerin oluşması Morris ve arkadaşlarının gözlemleriyle uyum sağlamaktadır (1-3).

THE EMISSION OF HUMIC ACID FLUORESCENCE BY PHYTOPLANKTON

ABSTRACT: The chlorophyll- α (excitation, 425 nm; emission, 660 nm)

and humic acid (excitation, 360 nm; emission, 455 nm) fluorescence of phytoplankton cultures and of samples of coastal sea water from the Northeastern Mediterranean have been measured. The phytoplankton culture generated humic acid fluorescence as soon as cells started to die. The observations of surface, Northeastern Mediterranean waters were analysed statistically and it has been found that humic acid was distributed nonrandomly in clumps. Generally the intensity of the humic acid fluorescence was observed to vary with depth either in phase or out of phase with the chlorophyll- α concentrations. It is suggested that when the intensity of humic acid and chlorophyll- α emission varied together in phase, healthy phytoplankton colonies were being observed. The rapid production of humic structures by dying phytoplankton is in agreement with the observations from Morris and his colleagues (1-3).

INTRODUCTION:

Humic acids are formed by the biochemical diagenesis of vegetation and traditionally this has been regarded as a slow process. Recently, however, Morris and his colleagues (1-3) demonstrated that the formation of fulvic and humic acids occurred in stages the first of which was complete in days or weeks. Morris extracted humic and fulvic acids from sediments and diatom debris, separated the acids according to their molecular weight and characterised them by their ultra violet absorption spectra. Such spectra contain no peaks and are consequently somewhat nebulous. It is of interest to strengthen Morris' contentions using a spectroscopic technique which characterises humic acids more precisely. Fluorescence spectrometry, in which humic acids give both excitation and emission maxima, enables one to characterise very dilute solutions of the acids quite accurately. Ghassemi and Christman (4) established that humic acids in coastal waters fluoresced with an excitation maximum at ~ 365 nm and an emission maximum at 450 - 460 nm. Balkaş et al., (5) showed that humic acids from lignites and soils gave the same fluorescence spectra as those from coastal waters and sediments and that the emission maxima shifted to longer wavelengths with increase in concentration. They ascribed the fluorescence to polynuclear aromatic structures. Hayase and Tsubota (6) reviewed the literature carefully and reported experiments which suggest that sedimentary humic and fulvic acids exhibit differing fluorescence spectra which also show systematic variations with molecular weight although their results gave no indication of the effects of concentration. Here we show that the characteristic fluorescence of humic acids is generated by phytoplankton colonies within a few days of culturing the colony. This result is used to interpret fluorescence measurements of humic acids in the relatively unproductive coastal waters of the Eastern Mediterranean.

EXPERIMENTAL:

Cultures of phytoplankton were maintained under sterile conditions at 25°C. The number of cells present was determined periodically by optical microscopy. Fluorescence spectra were

measured by a Turner Model 430 spectrofluorometer using a Xenon high pressure lamp and a 10 nm band pass. Humic acid concentrations were determined spectrofluorometrically using 360 nm excitation and monitoring 455 nm emission according to the procedure given by Balkas et al. (5). Commercial humic acid available from Fluka was used as a standard and care was taken to ensure that all intensity measurements were made in the region where concentration was directly proportional to intensity. Chlorophyll- α concentrations were also determined spectrofluorometrically using excitation at 425 nm and monitoring emission at 660 nm according to the procedure of Strickland and Parsons (7) and using coproporphyrin as a standard.

Samples of sea water were obtained from measured depths and locations in clean Nansen bottles and stored in sterile, plastic, sealed containers till fluorescence measurements could be made about twenty four hours later.

In statistical analysis of sea water analyses, the distributions of observed (from spectrofluorometry) humic acid concentrations were compared with those expected from a Poisson distribution viz, the distribution which would have been obtained had the samples been taken from an infinite population whose concentrations were scattered randomly about a mean value (8).

RESULTS AND DISCUSSION:

Figure 1 shows typical generation of humic acid fluorescence by *Nitzia Longissima* diatoms. The fluorescence was excited at 360 nm and emission was monitored at 455 nm, wavelengths characteristic of humic acid fluorescence (5). Obviously diatoms and phytoplankton in general also exhibit chlorophyll fluorescence but emission by chlorophyll does not contribute to the 'humic acid' fluorescence at the wavelengths we have used. Figure 1 shows that a solution of standard (Fluka) humic acid and nutrient in sterile sea water emitted 'humic acid fluorescence' which remained of constant intensity for at least 16 days. However, 4 days after 110 cells per ml of *N. Longissima* were added to this solution is 'humic acid' fluorescence began to increase markedly and continued to increase for at least a further twelve days.

Figure 1 also shows the number of living intact cells in the culture. It will be seen that the culture grew for some six days, after which there was a dramatic increase in mortality and after ten days only cell debris and no intact living cells could be observed. Thus Figure 1, which is typical of many such results, indicates that *N. Longissima* generated 'humic acid' fluorescence as soon as cells started to die. Probably all species of phytoplankton generate 'humic acid' fluorescence as is suggested in Figure 1 by the rise in emission when a culture of mixed phytoplankton was added to the sterile nutrient-humic acid solution. Figure 1 shows also that a similar population of mixed phytoplankton in sterile sea water with neither nutrient or humic acid generated 'humic acid' fluorescence from the start of culturing. That is, in the absence of nutrient some cells died during the first day of culturing. In a healthy colony of phytoplankton birth and death will be occurring simultaneously, growth being the excess of births over deaths. Our results suggest that the intensity of 'humic acid' fluorescence may be usable as a measure of death rate.

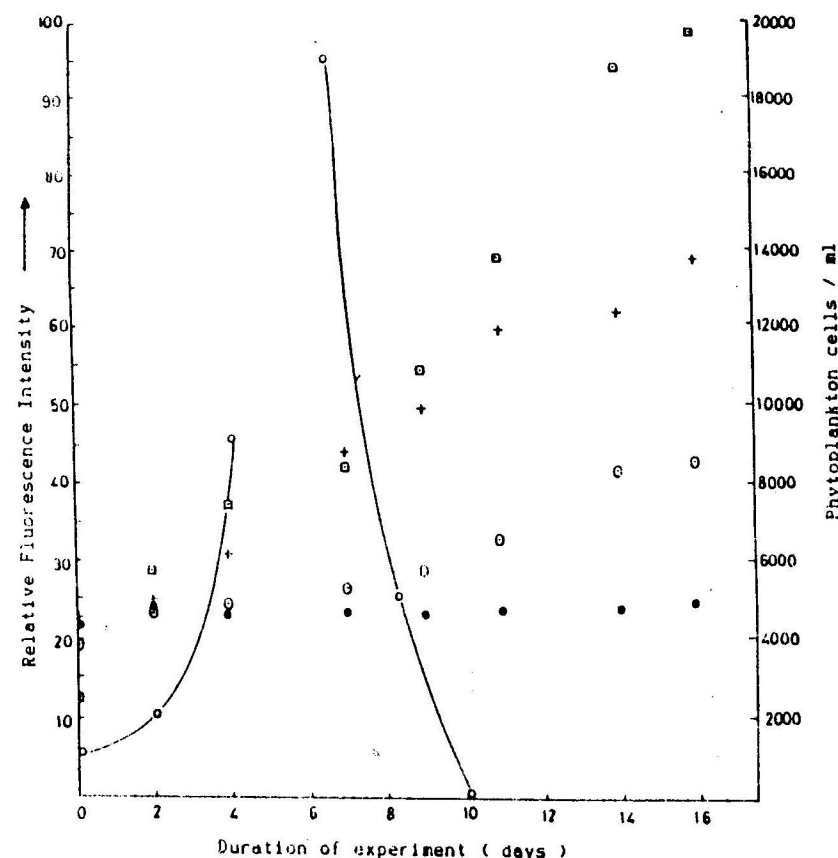


Figure 1. Development of fluorescence from phytoplankton culture

- = Mixed phytoplankton (Fluorescence)
- = A = Sterile sea water + 5 µg/ml Humic acid + 2% Nutrient (Fluorescence)
- = A + N. Longissima (Fluorescence)
- ⊕ = A + Mixed phytoplankton (Fluorescence)
- = A + N. Longissima (Counts)

Morris and his colleagues (2,3) showed that "highly unstable, water soluble compounds with a visible absorbance spectrum similar to that of typical humic substances are rapidly produced within days of the start of anoxic degradation of diatom debris". This was regarded as the first stage in the formation of humic material. It is probable that this is the process observed by our fluorescence measurements and that the water soluble compounds are therefore generated as soon as cells started to die, even more rapidly than Morris supposed.*

After Kalle's (9) observation of the fluorescence of sea water it became established (4) that the fluorescence spectrum was the same as that of humic acid. Figure 2 illustrates this. It shows

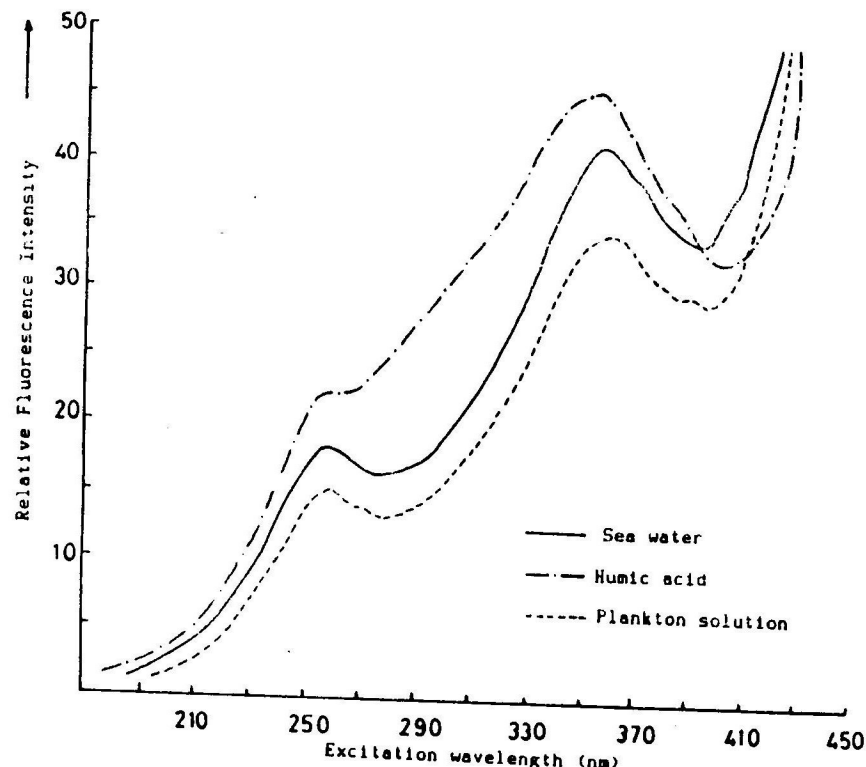


Figure 2. Fluorescence excitation spectra of sea water, humic acid and phytoplankton solution for 455 nm emission.

the excitation spectra - corresponding to 455 emission - of sea water from the Northeastern Mediterranean coast of humic acid and of a phytoplankton culture. The near identity of these spectra demonstrates that in each system the fluorescence is due to similar chemical structures. One repeats that these excitation spectra correspond to emission at 455 nm, at longer emission wavelengths both the phytoplankton and the sea water would give the distinctive fluorescence of chlorophyll.

These observations have led us to investigate the humic acid fluorescence of the coastal waters of the Eastern Mediterranean in the Antalya and Cilician basins of the southern coast of Turkey. It should be stressed that, despite the presence of three moderate sized rivers, these waters are relatively unproductive. The comparative absence of strong currents and tides and the large Secchi disc measurements which are often obtained suggested the region might be suited to a study of humic acid-phytoplankton relationships in coastal waters. Measurements of the intensity of humic acid fluorescence from samples of surface water taken from known locations were made over a period of one to two years. Had approximately constant amounts of fluorescing material been scattered randomly throughout the surface waters of the Eastern Mediterranean then plots of the intensity of humic acid fluorescence against the number of

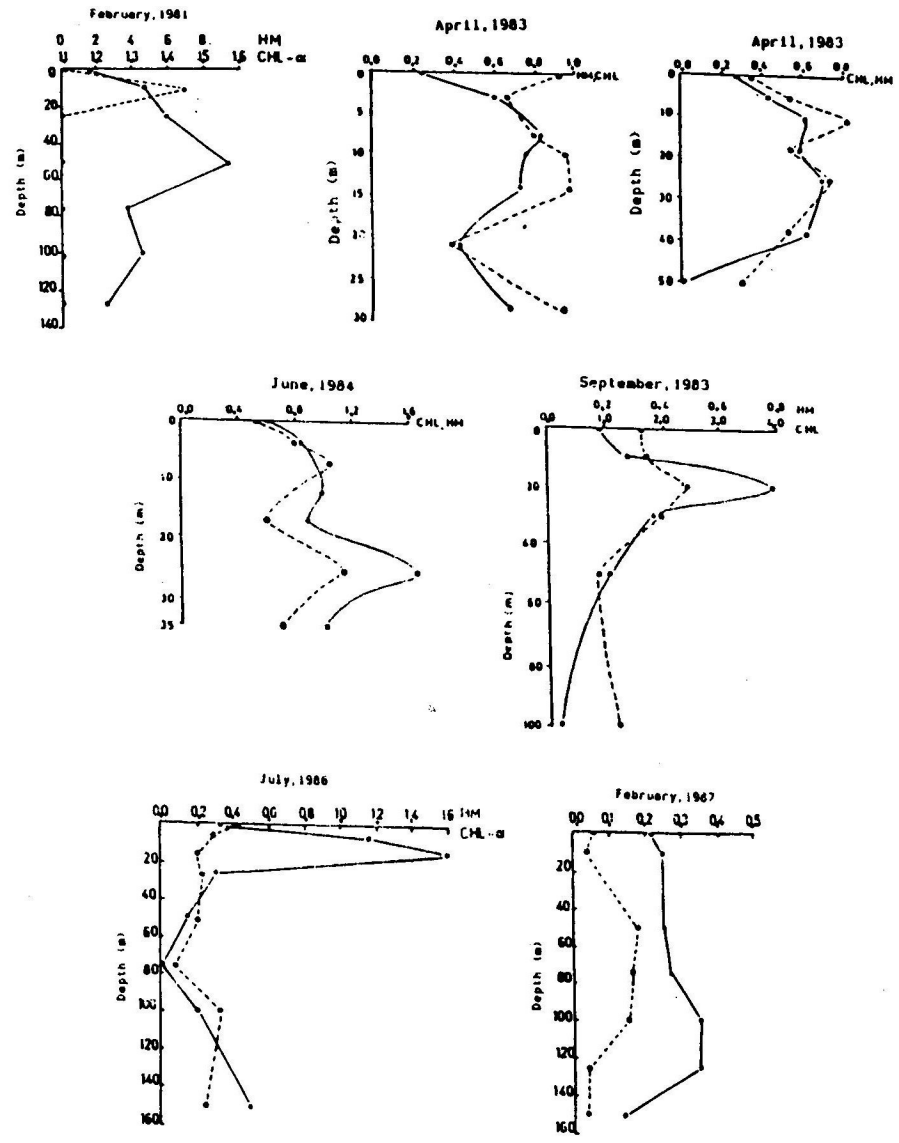


Figure 3a. Humic material and chlorophyll-α profiles in the Northeastern Mediterranean (In phase)
o - - - o Humic Material, mg/1 (HM)
● - - - ● Chlorophyll-α, μg/1 (CHL)

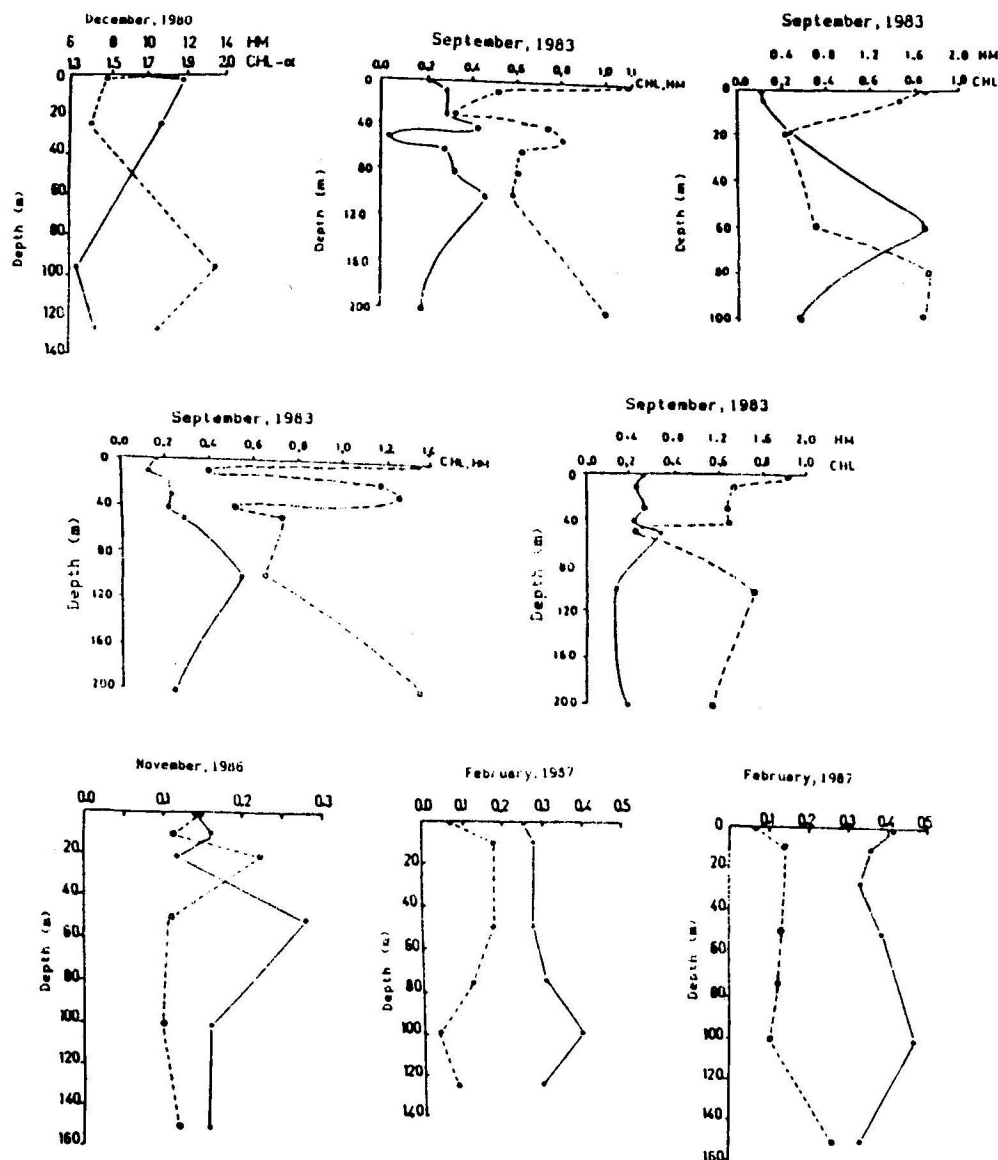


Figure 3b. Humic material and chlorophyll- α profiles in the Northeastern Mediterranean (Out of phase)

○ - - - ○ Humic Material, mg/l (HM)
● - - - ● Chlorophyll- α , μ g/l (CHL)

times the intensity was observed within a set of measurements would have given Poisson distributions. Generally more low results were observed than would have been expected for a Poisson distribution and skewed distributions indicative of clustering of the humic acid concentrations were obtained. Illustrative results are given in Table 1. The observations of surface waters suggest that in the Eastern Mediterranean coastal region humic acids occur nonrandomly in clumps.

*It is reasonable to enquire whether the generation of material similar in fluorescence to humic acid - and therefore possessing at least part of its characteristic structure - serves any natural purpose. Phytoplankton begin the lowest member of the food web, one would suspect that some of the higher members would have evolved to respond to the formation and polymerisation of humic material. It is established (10) that small concentrations of humic acid stimulate the growth of phytoplankton colonies and one may therefore speculate that in addition the presence of such material signals to the colony that cells are dying and vital members of the colony respond by giving birth.

Further insight into the distribution of humic coastal waters of the North eastern Mediterranean was obtained from depth profiles. Figure 3 shows how concentrations of humic acid and of chlorophyll- α varied with depth at selected locations in these waters. It will be seen that frequently (Figure 3a for example) the humic acid and chlorophyll- α concentrations were in phase, maximum and minimum concentrations of both compounds occurred at the same depths and the concentration of humic acid was approximately proportional to the concentration of chlorophyll- α . The laboratory studies of phytoplankton suggest that such results should be interpreted as observations of healthy colonies of phytoplankton, the concentrations of humic acid being a consequence of the more or less stable rate of mortality within the colony. Sometimes (Figure 3b for example) humic acid and chlorophyll- α concentrations were out of phase, humic acid maxima occurring at the same depths as minima in the chlorophyll- α concentrations. In these instances it is natural to regard the humic acid as arising from decayed or decaying vegetation.

The in phase and out of phase variations of humic acid and chlorophyll- α concentrations account for most of the depth profiles that have been observed in the Northeastern Mediterranean and it would seem that in these unproductive coastal waters much of the humic acid fluorescence of the sea generated from colonies of living phytoplankton and from decaying vegetation and that in consequence it is distributed nonrandomly in clumps. In more productive waters or where stronger currents and tides abound other descriptions of the coastal humic distribution are likely.

CONCLUSION:

Phytoplankton cultures emitted fluorescence identical to that generated by humic acids. This has been interpreted as meaning that chemical structures identical to some of those found in humic acids are formed as soon as phytoplankton cells start to die. The same phenomenon has been observed in phytoplankton colonies in coastal waters of the Northeastern Mediterranean. These waters are relatively unproductive and relatively untroubled by currents and tides and phytoplankton exists largely in isolated colonies.

Table 1. Typical distribution of humic acid concentrations from seventy six locations in the North Eastern Mediterranean

Concentrations of humic acid (mg/l)	0	1	2	3	4	5	6	7	8	9	10	12	13
Number of times observed	8	21	11	9	6	5	3	3	2	5	2	0	1
Number expected for a* Poisson distribution	3	9	15	17	14	9	5	2	1	0	0	0	0

*The Poisson distribution has the same mean, 3.3, as the observed values but expected numbers are given to the nearest unit

These colonies provide a major source of the humic acid fluorescence of the sea water, the more populous the colony, the greater the humic acid fluorescence. Thus both in the laboratory and in sea water, birth and death of cells may be observed by measurements of chlorophyll- α and humic acid fluorescence of healthy phytoplankton colonies. The rapid generation of humic acid structures from dying cells agrees with the results of studies of organic rich anoxic marine sediments made by Morris et al., (1,2,3) which indicated the formation of water soluble, unstable humic complexes within weeks of the deposition of planktonic debris.

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