Fluorescence of Humic Acids Formed in the Early Stages of Forest Litter Decomposition

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Fluorescent spectroscopy is a non-destructive analytical method which supplies information about the structure of a system.

Our aim was to study, using the fluorescence technique, unmaturated humic acids derived from the decomposition of forest litter. The authors think that the results obtained will bring new data on the early stages of plant material humification.

The composition of fluorophores and the changes in their proximity are reflected in the spectrum of macromolecule fluorescence. The changes may be brought about by conformed changes of the macromolecules caused by different environmental factors, which in effect block or create conditions convenient for the energy transfer inside the macromolecules /LAKOWICZ, 1986/.

Materials

We studied humic acids extracted from various forest litters decomposing under natural conditions /Table 1/.

The materials, after exhausting extraction in alcohol-benzene, were treated with 0.1 M NaOH in nitrogen. The alkalic extract obtained was acidified to pH 1 in 0.5 M H_2SO_4; the humic acid gel was centrifuged and dialysed until the sulphuric ion reaction stopped.

Humic acid fluorescence assays were made in a Hitachi MPF-4 spectrofluorometer.

Corrected fluorescence excitation and emission spectra were recorded. Frontal observations /cell surface at 45^{o} angle to the exciting band/ were made. In spite of certain drawbacks, this type of cell positioning has the advantage of rendering fluorescence of high optic density solutions concentration-independent. The entire excitation band is absorbed near the cell surface, thus minimizing the inner filter effect. This procedure is particularly recommended for testing suspensions and macromolecular solutions /LAKOWICZ, 1986; EISIGER and FLORES, 1979; PUZYNA, 1984/.

Table 1
Forest litter - source of investigated humic acids

Type of forest and geographic coordinates	Sample number	Sort of litter	Decomposition time - years	Humification coefficient /after Springer/
Tilio-Carpinetum /sanctuary/	1	hornbeam leaves	0.5	23.2
	2	hornbeam leaves	3.0	29.0
	3	oak leaves	0.5	16.8
$53^{o}05'N$; $18^{o}34'E$	4	oak leaves	3.0	23.1
	5	pine needles	1.0	15.0
	6	pine needles	6.0	23.1
Cladonio-Pinetum	7	pine needles	1.0	16.2
$53^{o}31'N$; $18^{o}00'E$	8	pine needles	5.0	24.0
Leucobryo-Pinetum	9	A_oL podzol sample		22.4
$54^{o}14'N$; $16^{o}18'E$	10	A_oH podzol sample		81.2

Parameters used to describe fluorescence excitation and emission spectra

Excitation spectra

1. Band location
2. i_{315}/i_{370} maximum excitation band intensity ratio observed at wavelengths of λ = 400 nm and λ = 520 nm, respectively.
3. i_{315}/l, maximum band intensity /i_{315}/ to its half-width /l/ ratio observed at λ = 400 nm.
4. "Slope" of curves, expressed as: i_1/i_2; i_3/i_4; i_5/i_6.

Emission spectra

1. Fluorescence intensity /I/ at selected wavelengths.
2. Values of intensity ratios I_2/I_1; I_1/I_4; I_3/I_5; I_3/I_6.
3. Total light emission /Σ I/ related to the area beneath the spectrum curve and brought about by excitation with a given wavelength.
4. Differential spectrum - a difference between fluorescence spectra, resulting from excitation of fluorescence caused by λ_{ex1} followed by λ_{ex2} /$\lambda_1 < \lambda_2$/.

Conclusions

HAs derived from forest litter after various times of decomposition are similar in their fluorescence properties.

All the HAs show three excitation bands: 260 nm, 315 nm and 370 nm.

The time of forest litter decomposition is reflected in the shape of the first two excitation bands, particularly the λ = 315 nm one.

The excitation within this range is related to both the short wave /350-400 nm/ and long wave /480-600 nm/ emissions.

The principal emission band of Fl has its maximum at 500 nm and results mainly from short wave excitation /- 260 nm/ and to a lower extent, from excitation at 315 nm.

"Young" HAs show a larger proportion of short wave emission in Fl.

The studies show neither distinct transformations to take place in the main HA skeleton structures during humification, nor any transformations occurring within a peribd shorter than 6 months.

References

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