

LASER DIAGNOSTICS OF HUMIC SUBSTANCES AS NATURAL DETOXICANTS AND FLUORESCENT INDICATORS OF POLLUTANTS IN WATER

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ABSTRACT

Chaining constantans of the pyrene and uranyl (which present two classes of ecotoxicants in the natural and effluent waters) by the humic substances were evaluated using the method of fluorimetry. The influence of these pollutants on the fluorescent characteristics and photophysical parameters of the humic substances acquired with the technique of laser fluorimetry was revealed and thus the perspectives of using humic substances as fluorescent indicators of the pollutants in the water were highlighted.

INTRODUCTION

In remote laser sensing of water mediums there are lots of problems concerning the investigation of the fluorescence of the humic substances (HS). HS are present in different concentrations in all natural water reservoirs and carry out many ecological functions – concentration of chemical elements and energy, heat conditions regulation, chemical solutions transfer [1]. One of the most important functions of HS is combining with solutions and elements harmful for the living organisms [2, 3].

This paper is aimed at defining the molecular photophysical parameters of the chosen toxicants and HS fluorophores using the nonlinear and kinetic laser fluorimetry methods. Our task was to find out the possibility of using HS as a fluorescent indicator of presence of toxicans in natural waters.

MATERIALS AND METHODS

As a model toxicants we've chosen the representative of polyaromatic hydrocarbons - pyrene, and the representative of the heavy metal salts – uranyl.

The photophysical parameters measurements of HS and toxicants were made on a laser spectrometer [4]. For excitation the 4-th harmonic (wavelength 266 nm) of YAG:Nd³⁺ laser radiation was used. Duration and frequency of the impulses reiteration are 10 ns and 10 Hz correspondingly. Impulse energy is 0.3 mJ, the photon flow density of the excitation radiation on 266 nm can be changed in $5 \times 10^{23} \dots 10^{26} \text{ cm}^{-2} \text{ c}^{-1}$ limits without changing the distribution of intensity in time and cross-section of the beam. For the corrected spectras measurements we used the lamp spectrofluorometer Fluoro-Max 4 (Jobin Yvon).

For the experiment we've chosen HS of the different genesis:

- CHP, CHPm, LHS – preparations isolated from the coil and the leonardite;
- RF – preparation isolated from the peat;
- SR – preparation isolated from the river water dissolved organic matter;
- POW – commercial preparation «Powhumus»;
- THS - commercial preparation «Tekhnoexport».

RESULTS

Fluorescence spectra and photophysical parameters of HS preparations, pyrene and uranyl

With the lamp spectrofluorimeter FluoroMax 4 we obtained fluorescent spectra of HS preparations (Fig. 1) and ecotoxicants pyrene and uranyl (Fig.s 2 and 3) separately. Also we measured photophysical parameters of HS preparations (Table 1) and toxicants (Tables 2) using nonlinear and kinetic fluorimetry methods [5 - 7].

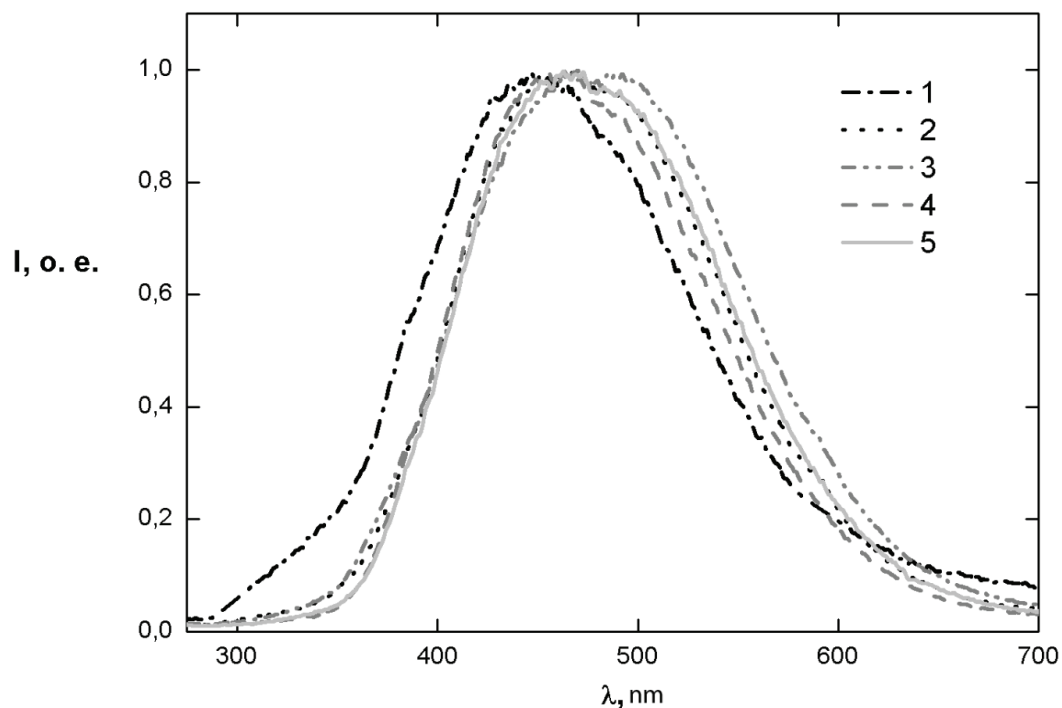


Fig.1 The corrected fluorescence spectra of HS preparations obtained with FluoroMax4 spectrofluorimeter (excitation wavelength 266 nm): 1-RF, 2-POW, 3-SR, 4-CHP, 5-CHPm

Table 1. Photophysical parameters of HS preparations measured by nonlinear and kinetic fluorimetry methods (excitation wavelength 266 nm).

Preparation	$\sigma \cdot 10^{16}, \text{cm}^2$	τ, ns	$\eta \cdot n/C, \text{mol/g}$
RF	0.65 ± 0.05	2.5 ± 0.5	8 ± 2
POW	2.7 ± 0.2	2.4 ± 0.5	6 ± 2
SR	3.5 ± 0.3	2.5 ± 0.5	4 ± 1
CHP	0.5 ± 0.04	3.0 ± 0.5	37 ± 5
CHPm	0.35 ± 0.03	2.7 ± 0.5	66 ± 8

σ , τ – absorption cross-section and the lifetime of the excited state of the HS fluorophore
 η , n – the fluorescence quantum yield and the fluorophores concentration.
 C – mass concentration of HS

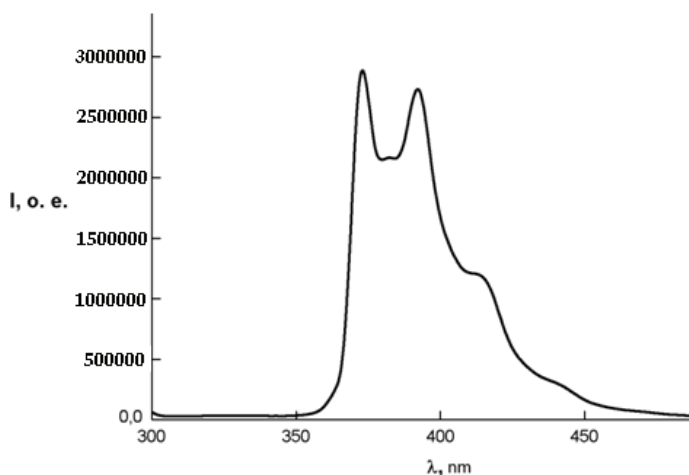


Fig. 2. Pyrene fluorescence spectra.

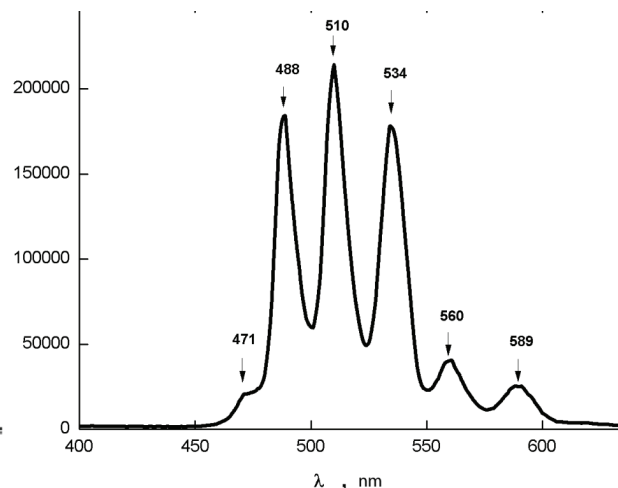


Fig.3. Uranyl fluorescence spectra.

Table 2. Photophysical parameters of pyrene and uranyl, obtained with the nonlinear and kinetic fluorimetry methods (excitation wavelength 266 nm).

Parameter	Pyrene	Uranyl
σ	$(0.7 \pm 0.1) \cdot 10^{-16} \text{ cm}^2$	$(3.0 \pm 0.3) \cdot 10^{-17} \text{ cm}^2$
τ	$85 \pm 3 \text{ ns}$	$1.8 \pm 0.1 \text{ } \mu\text{s}$

Photophysical parameters of pyrene and uranyl differ from HS: fluorophore excited state lifetime is much longer than the laser pulse: $\tau \gg \tau_p$

HS and pyrene interaction

Chaining constant

As one can see from Fig.4, the fluorescence intensity of pyrene decreases (pyrene concentration is 60 $\mu\text{g/l}$) while the HS concentration rises from 1 mg/l to 10 mg/l .

Using the Stern-Vollmer relationship [2]

$$F_0/F = 1 + K_{oc} \cdot C_{HA},$$

where F_0 и F – pyrene fluorescence intensity with and without HS, we can calculate the chaining constant K_{oc} :

$$K_{oc} = (3.2 \pm 0.3) \times 10^5 \text{ l/kg for LHS sample;}$$

$$K_{oc} = (3.6 \pm 0.4) \times 10^5 \text{ l/kg for THS sample.}$$

Pyrene's influence on the photophysical parameters of HS

Photophysical parameters of HS (3 mg/l), obtained by the nonlinear and kinetic fluorimetry methods:

$$\sigma = (3.5 \pm 0.2) \cdot 10^{-16} \text{ cm}^2 \text{ – without pyrene}$$

$$\sigma = (2.1 \pm 0.3) \cdot 10^{-16} \text{ cm}^2 \text{ – with pyrene (60 } \mu\text{g/l)}$$

$$\tau = 3 \pm 0.5 \text{ ns – without pyrene}$$

$$\tau = 5 \pm 0.5 \text{ ns – with pyrene (60 } \mu\text{g/l)}$$

The influence of the pyrene on the photophysical parameters of the HS shows that one can use HS as a natural fluorescent indicator of presence of ecotoxicants in the water medium.

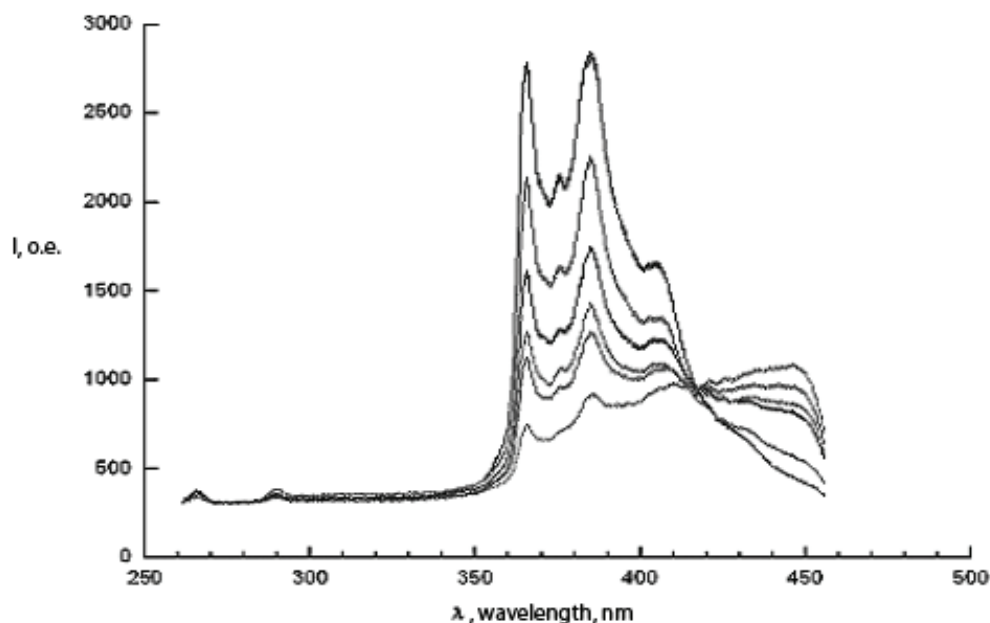


Fig. 4. The pyrene (concentration 60 mkg/l) fluorescence suppression by the THS preparation: the upper spectrum corresponds to the lowest THS concentration (1 mg/l), the lowest – to the highest (10 mg/l).

HS and uranyl interaction

Because of presence of functional (carboxyl in particular) groups in the HS macromolecule, it can bind with the uranyl molecule UO_2^{2+} . Using the data from table 3 [1 - 3] one can calculate the optimal HS concentration.

Table 3. Functional groups in HS macromolecules preparations

Preparation	-COOH, mmol/g	-ArOH, mmol/g
CHP	44.2±0.2	1.1±0.1
CHPm	3.6±0.1	4.4±0.2

Two $-COO^-$ groups can bind with one molecule of the uranyl UO_2^{2+} . There for can be calculated the concentrations of uranyl and humic substances at which the interaction between them take place. A good fluorescent signal from uranyl is noticeable at concentrations of about 10^{-5} mol/l. So the concentration of $-COOH-$ groups should be about $2 \cdot 10^{-5}$ mol/l, which means mass concentration of CHP - 5mg/l.

At Fig.5 one can see the influence of the uranyl on the fluorescence intensity of HS. Table 4 shows the influence of the uranyl on the photophysical parameters of the HS fluorophore. Those were obtained by nonlinear and kinetic laser fluorimetry methods (during measurements of the saturation curve the signal was registered in detector's strobe with 50ns width and zero delay from the laser pulse).

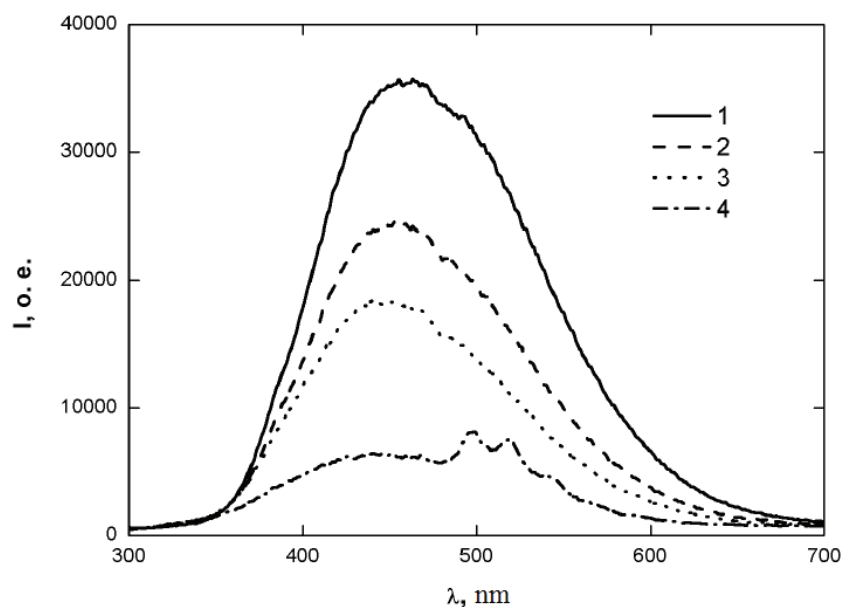


Fig. 5. The influence of the uranyl on the fluorescence intensity of HS. The samples with concentration of HS 50 mg/l (concentration –COOH- groups $2 \cdot 10^{-4}$ mol/l) and concentrations of uranyl $1 \cdot 10^{-5}$, $2 \cdot 10^{-5}$, $3 \cdot 10^{-4}$, $4 \cdot 5 \cdot 10^{-4}$ mol/l were used. The ratio of uranyl ions to concentration of –COOH- groups couples was there for 1:10, 1:2, 1:1, 5:1. Type of HS used: CHP.

Table 5. Influence of uranyl on photophysical parameters of HS (concentration of –COOH- groups $2 \cdot 10^{-4}$ mol/l)

UO_2 concentration	$1 \cdot 10^{-5}$ mol/l	$1 \cdot 10^{-4}$ mol/l	$5 \cdot 10^{-4}$ mol/l
σ_{CHP} , cm^2	$(0.50 \pm 0.03) \cdot 10^{-16}$	$(1.0 \pm 0.1) \cdot 10^{-16}$	$(1.5 \pm 0.2) \cdot 10^{-16}$
τ_{CHP} , ns	3.0 ± 0.5	3.7 ± 0.5	2.5 ± 0.5
σ_{CHP_m} , cm^2	$(0.70 \pm 0.04) \cdot 10^{-16}$	$(1.3 \pm 0.1) \cdot 10^{-16}$	$(1.4 \pm 0.2) \cdot 10^{-16}$
τ_{CHP_m} , ns	2.7 ± 0.5	3.7 ± 0.5	3.5 ± 0.5

With growth of uranyl concentration fluorophore intensity decreases and simultaneously fluorophore absorption cross section increases. Mechanisms are under investigation now.

CONCLUSION

In our experiments the influence of HS (preparations, extracted from leonardite, peat and from water dissolved organic matter were used) on the intensity of fluorescence bands of ecotoxicants' (represented by pyrene and uranyl) is shown.

Chaining constantans of the pyrene and uranyl with humic substances were evaluated using the method of fluorimetry.

The influence of pyrene and uranyl on the photophysical parameters of HS using laser fluorimetry technique is shown for the first time. This makes possible to use HS as a natural fluorescent indicator of an ecotoxicants' presence in the water medium.

Difference in behavior of HS fluorescence in cases of pyrene and uranyl (uranyl influences more than pyrene) is connected with the chemical properties: uranyl exists in solution as an ion, while pyrene

isn't charged (electro neutral). Interaction with uranyl leads to coagulation of HS; in case of pyrene coagulation doesn't take place.

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