Detoxifying Abilities of Humic Substances: The Influence of UV Pretreatment

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Humic substances

- **Humic substances (HS) are the products of extremely complex chemical and microbial processes of the decomposition and secondary synthesis of plant and animal residues in soil and water. HS are distributed throughout the environment. Many of their properties are known, but its exact structure and function are still in question.**

The functions of humic acids in the biosphere and geosphere

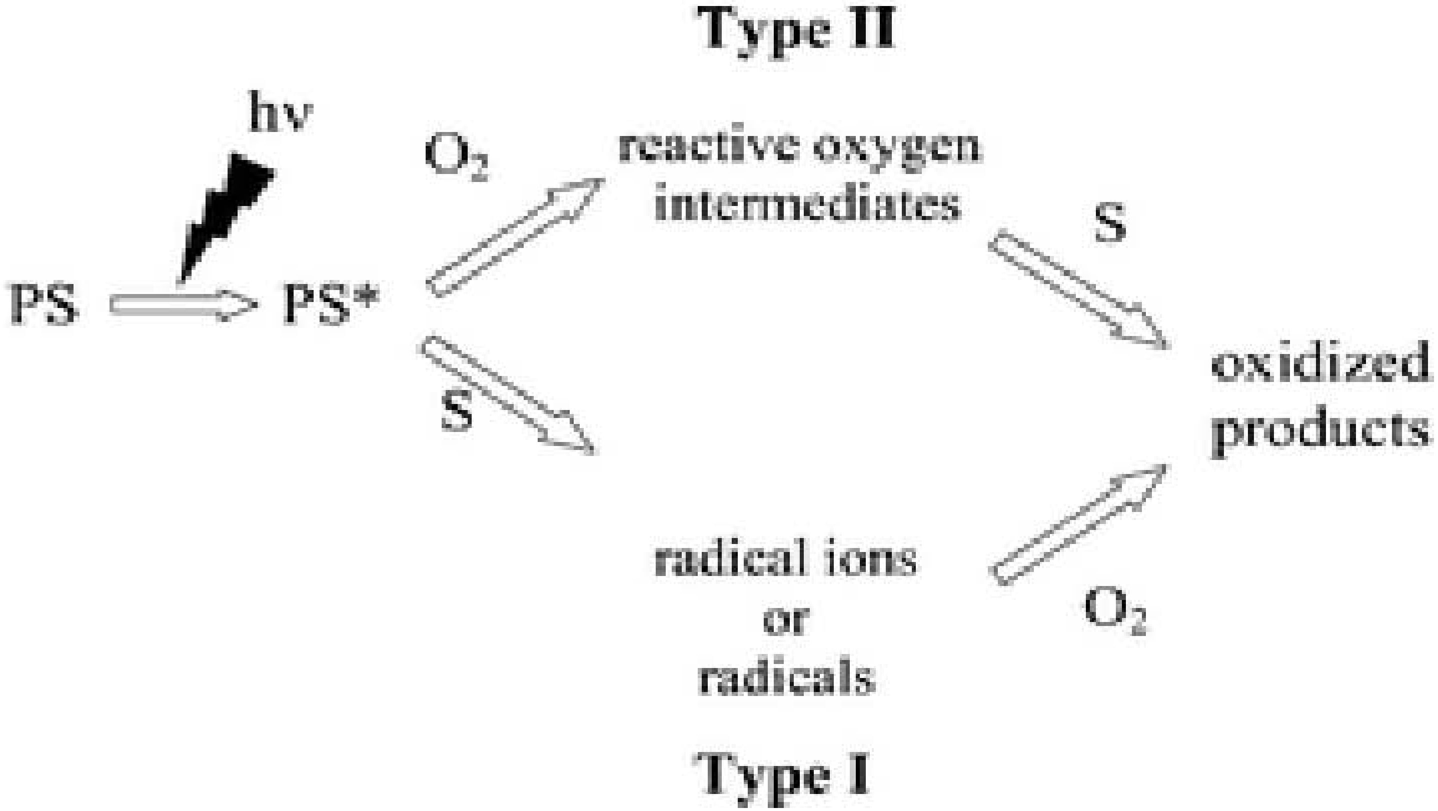
- accumulation
- transport
- regulation
- protection
- photostabilizing and photosensitizing
- producing active forms of oxygen after irradiation
- physiological

Humic substances can decrease or increase bioavailability and toxicity of compounds.

- Moreover, the photosensitizing and photoquenching effects of humic substances on some chemicals are also known.
- When HS absorbs UV or solar irradiation, reactive oxygen intermediates are formed which may attack chemicals in the environments and initiate their degradation. Also, the possibility of an UV screening by humic substances on chemicals cannot be excluded since the energy-transfer and charge-transfer between the chemical and humic substances can deactivate the excitated chemical molecules. Therefore, HS can either enhance or decrease the toxicity of compounds after irradiation. Our knowledge about these processes is still very limited.

Table 1. Overview of photosensitizers

Compound group	Name	Site of action by prokaryotic cells
Phenothiazines	Methylene blue	DNA interaction
	Toluidine blue	Membrane
Macrocyclic molecules	Acridine Phthalocyanine Porphyrin	Membrane /cytosolic
Natural products	Furanocoumarin	DNA intercalation
	Perylenequinonoid /hypericin	Inhibitor of protein kinase C
	Humic acids	


Type II
hν
O_2
reactive oxygen intermediates
S
PS
PS*
S
oxidized products
radical ions or radicals
O_2
Type I

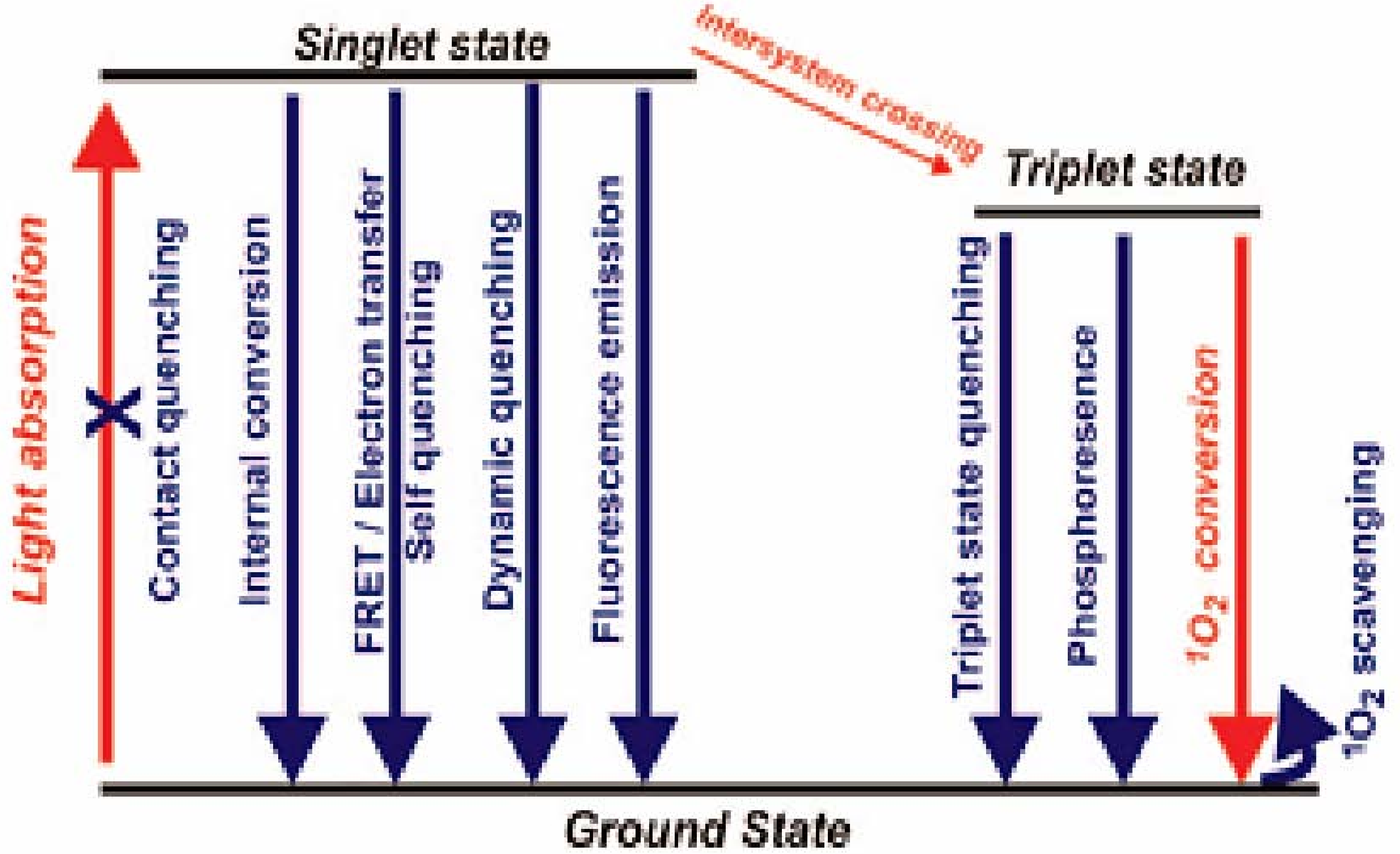
The photosensitization mechanisms

$$\mathrm{PS} \xrightarrow{h\nu} {}^{1}\mathrm{PS}^{*} \xrightarrow{\mathrm{ISC}} {}^{3}\mathrm{PS}^{*}$$

$${}^{3}\mathrm{PS}^{*} + {}^{3}\mathrm{O}_2 \rightarrow \mathrm{PS} + {}^{1}\mathrm{O}_2$$

$${}^{1}\mathrm{O}_2 + \mathrm{Sub} \rightarrow \text{oxidized Sub}$$

Simplified energy level diagram showing potential singlet oxygen deactivation pathways. Typical pathway to singlet oxygen generation is shown in red.



The aim of this work

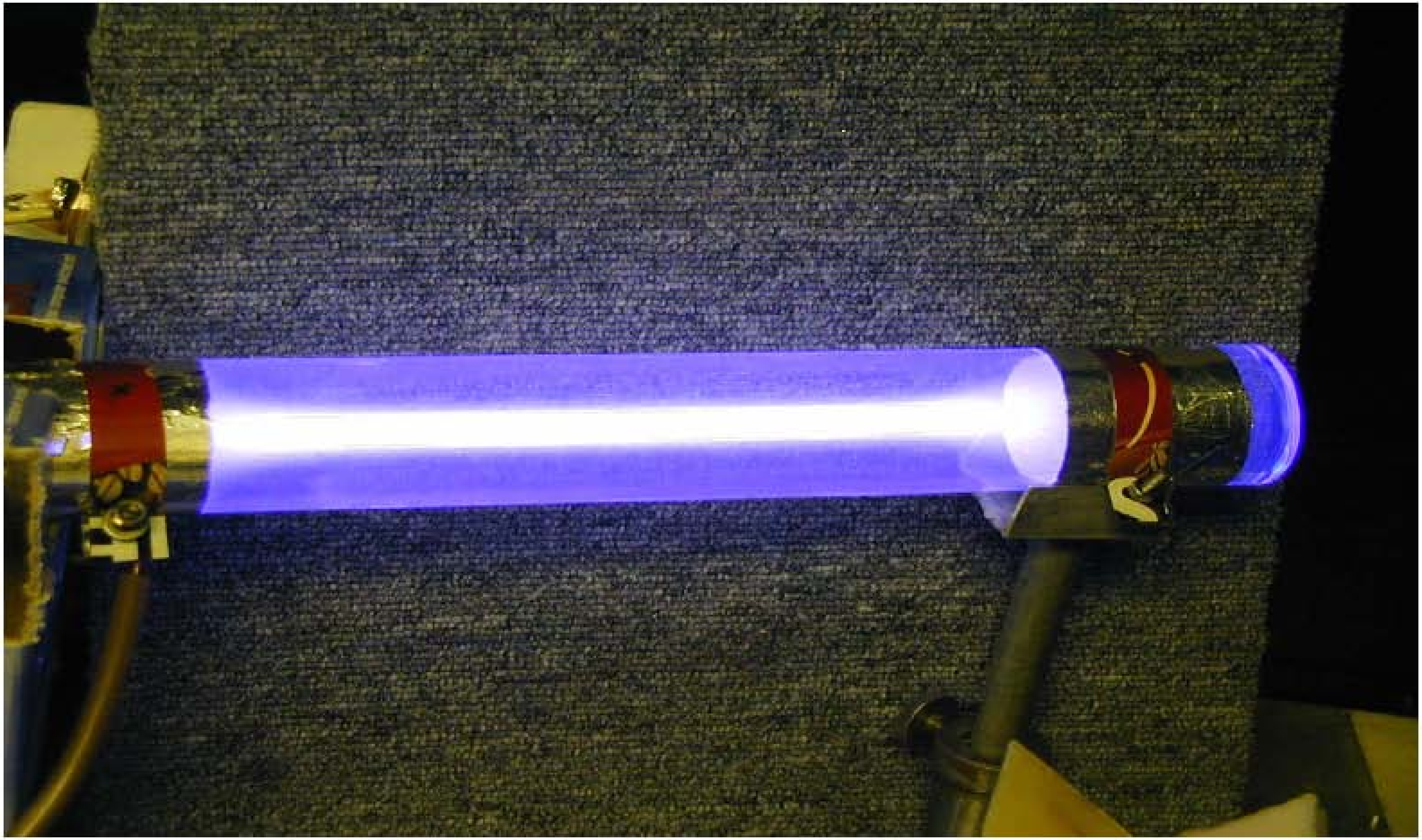
The aim of this work was to use a bioluminescence test system to study the effect of two factors on solutions of new substituted furocoumarins (sensitizers for phototherapy), phenols and common phenoxyl herbicides: UV radiation from different sources, and humic substances. The toxicity of solutions was assessed using the bioluminescence assay, which is based on lyophilized luminous bacteria *Photobacterium phosphoreum,* produced at the Institute of Biophysics Siberian Branch of Russian Academie of Science (Krasnoyarsk, Russia).

Methods and radiation sources for investigations

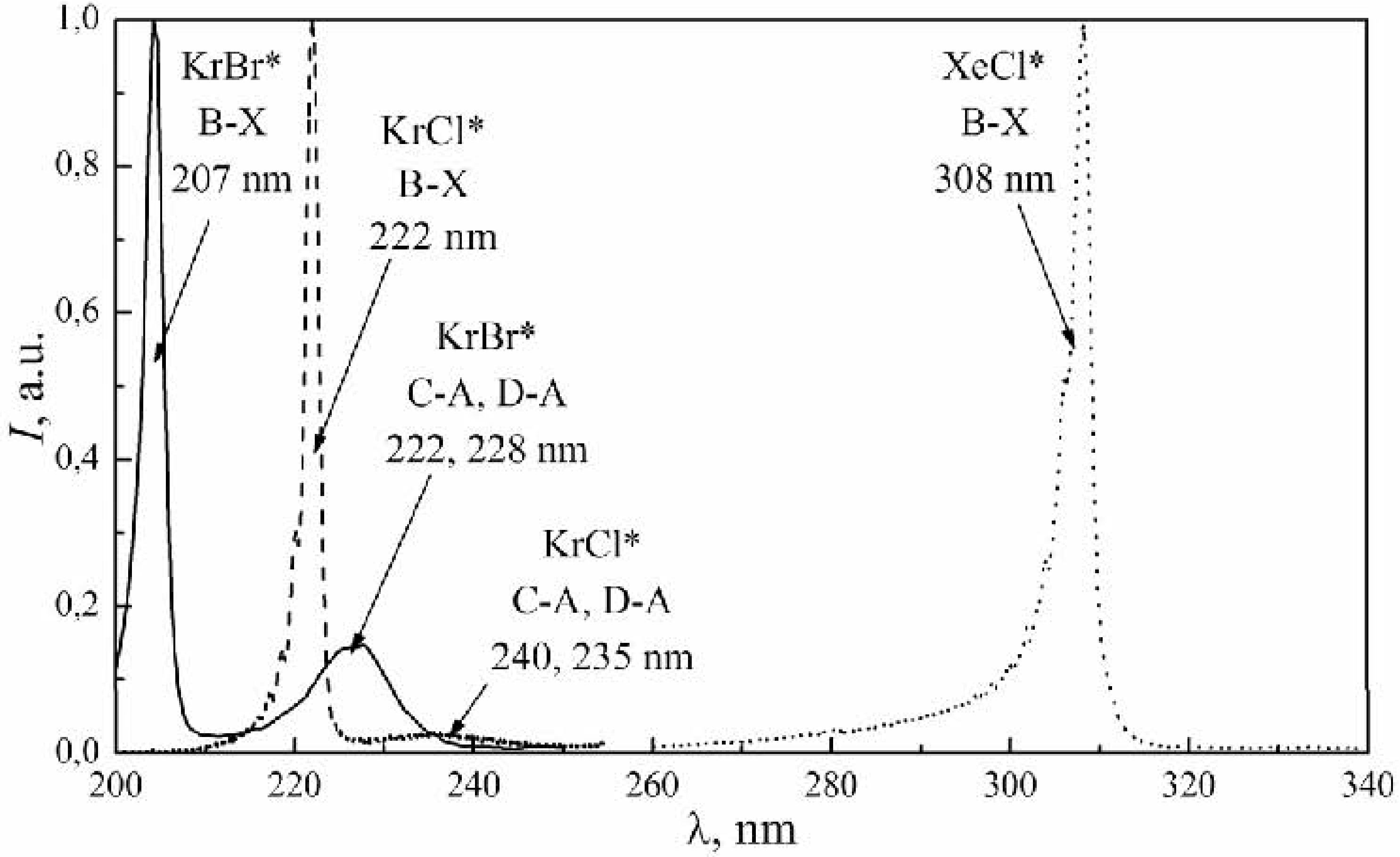
- absorption and fluorescent spectroscopy
- OKH-11M high-pressure mercury lamp
- exilamps on KrCl* (222 nm), XeBr* (283 nm), and XeCl* (308 nm) working molecules, ($\Delta\lambda$=5-10 nm, W=18 mW/cm^2, f=200 kHz, pulse duration 1 microsec) developed at the Institute of High-Current Electronics SB RAS, in the lab headed by Professor V.F. Tarasenko.

E.A. Sosnin, T. Oppenlander, V.F. Tarasenko, J. Photochem. Photobiol. C: Photochem. Rev., 7 (2006), 145-163.

A photograph of KrCl* excilamp



Typical emission spectra of rare gas halide exciplexes



Samples of humic substances

We used a Gumat-80 preparation (Gumat OOO, Irkutsk, Russia). The preparation was obtained by mechanochemical reaction of oxidized brown coal (Cheremkhovskoe field, Russia) with alkali (KOH, NaOH). The concentration of humic substances was selected so that the intensity of bioluminescence differed from the control by no more than 20%.

The other samples of humic acids (HA) fractions were obtained from Fluka Chemical Co and prepared from peat of Tomsk region.

The West Siberia totally and Tomsk Region especially are promising regions for development the peat industry.

- **Our Vasuygan Bog is the largest bog in the world.**
- **Peat and sapropel are especially valuable raw materials to produce humic acids.**

Results: furocoumarins

We have shown that efficient detoxification of an alcoholic solution of investigated furocoumarins (synthesized in Taras Shevchenko Kiev National University, Ukraine) occurs as a result of addition of humic substances. We have observed a dependence of the detoxification both on the structure of the studied compounds and on the characteristics of the source. After photoexcitation the bond lengths in the furocoumarins change and the humic substances detoxify the irradiated solution more efficiently.

Bioluminescence

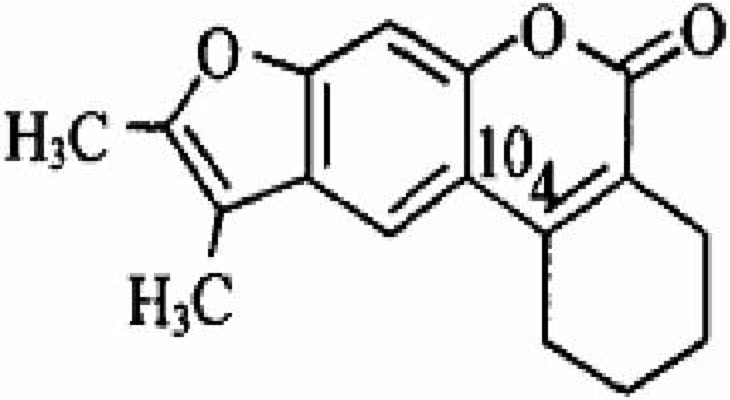
The intensity of bioluminescence in the control run I_0 was compared with the intensity of bioluminescence I in the presence of psoralene molecules at a concentration C_{ps}. The toxicity of the solutions was estimated by the value of the bioluminescent index $BI = I/I_0$.

The degree of detoxification of the psoralene solutions using humic substances and UV irradiation was characterized by the detoxification coefficient:

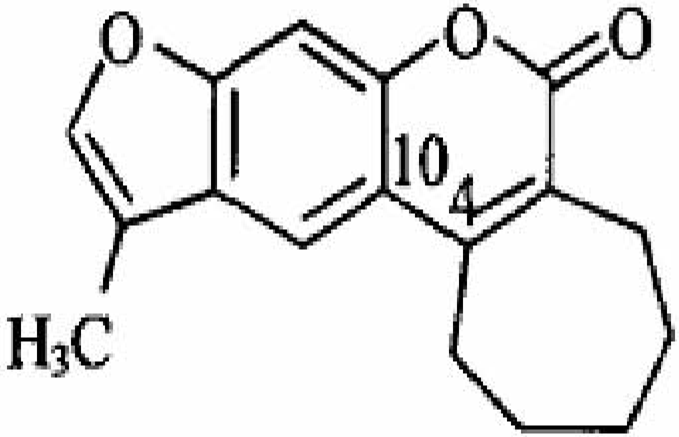
$K = BI_h/BI^0$, where BI_0 is the bioluminescent index of the psoralene solution; BI_h is the maximum bioluminescent index of the psoralene solution when humic substances are added and/or when exposed to UV radiation. Values of $K > 1$ are evidence for detoxification of the solutions.

Substituted furocoumarins: 4',5'-dimethyl-3,4-cyclohexyl psoralene (FC1) and 4'-methyl-3,4-cycloheptyl psoralene (FC2)

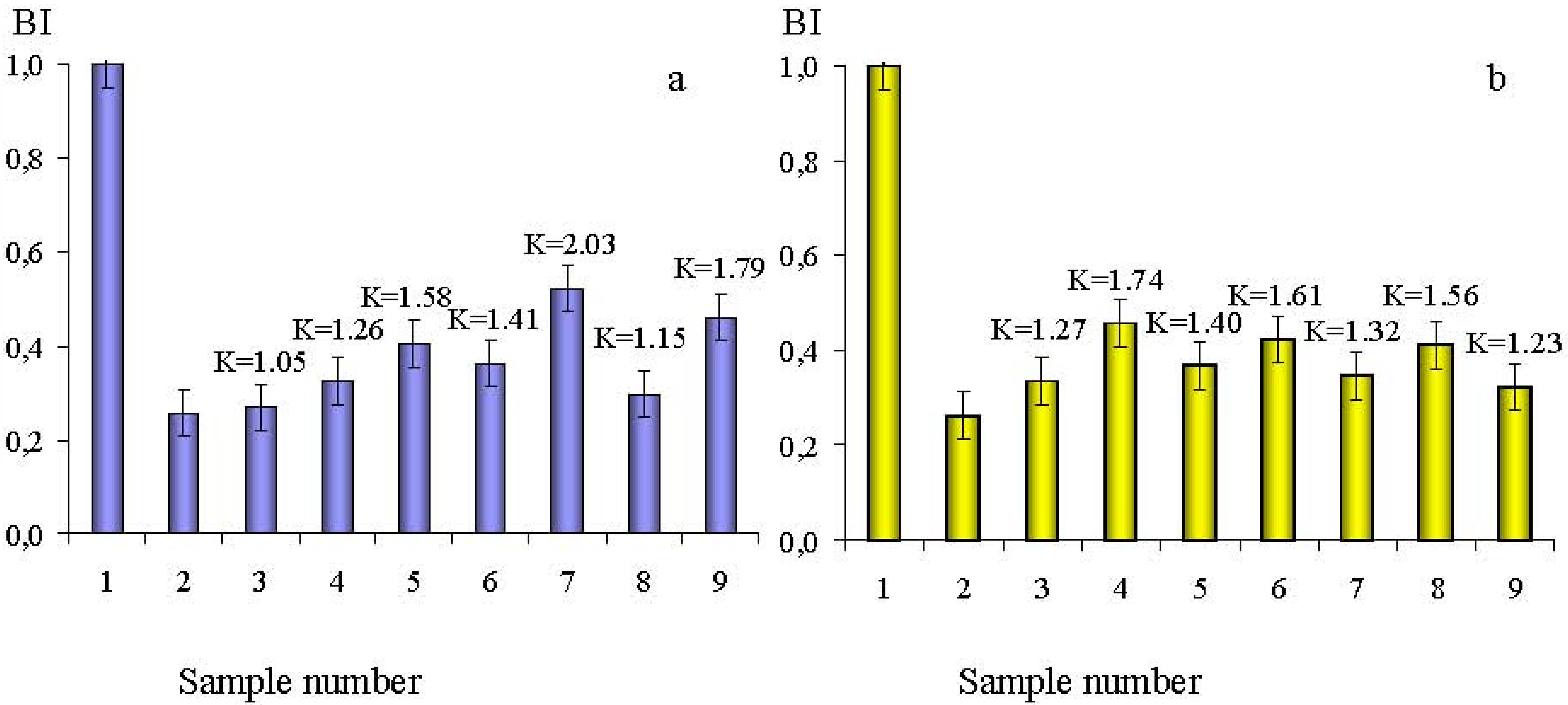
FC1



FC2



Bioluminescence index (BI) and detoxification coefficient (K) of the compounds FC1 (a) and FC2 (b): 1) control; 2) unirradiated; 3) unirradiated + humate; 4) irradiated by a mercury lamp (15 min); 5) irradiated by a mercury lamp (15 min) + humate; b) irradiated (308 nm, 15 min); 7) irradiated (308 nm, 15 min) + humate; 8) irradiated (222 nm, 15 min); 9) irradiated (222 nm, 15 min) + humate.



Organic ecotoxicants in water solutions

- **Cresols: 4-methylphenol, 2- methylphenol, c=10^{-4} mol/L, «*Aldrich*»**

  Phenol and some of its derivatives are widespread organic pollutants in the environment:

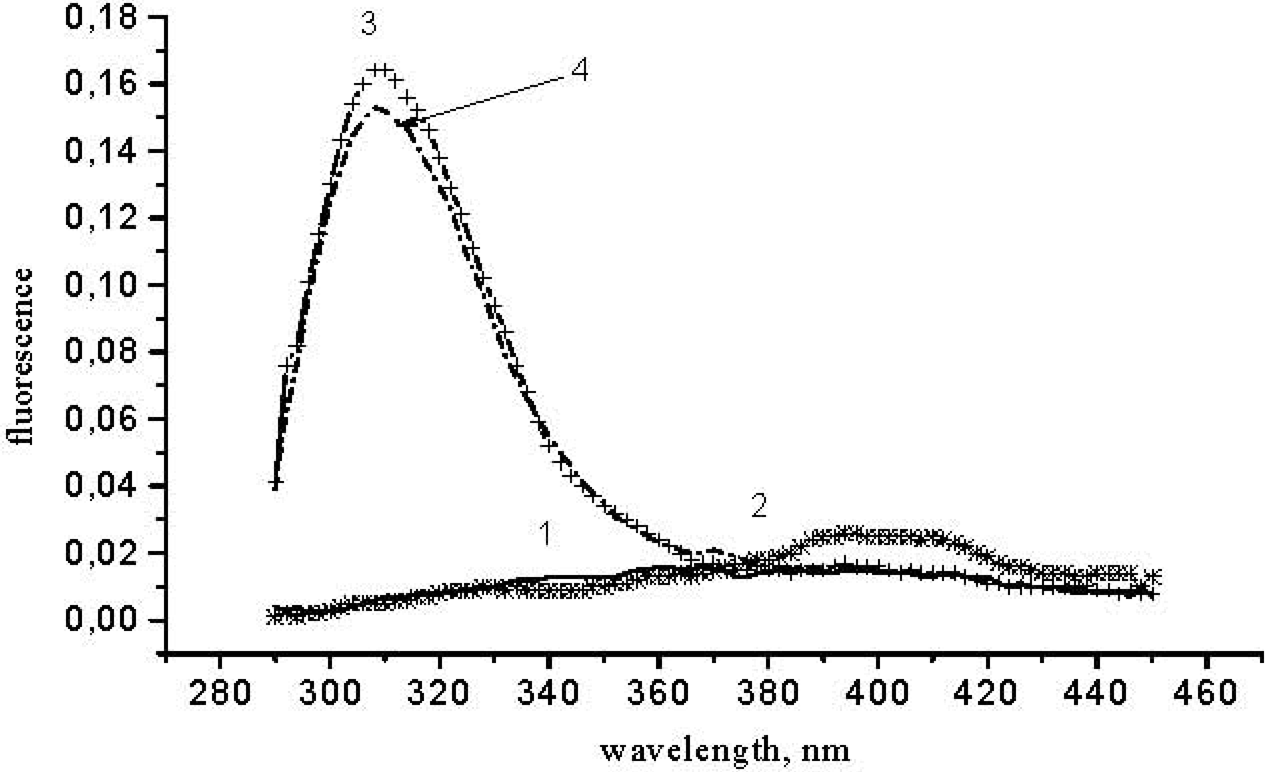
  Verhaar H., Solbe J., Speksnijder J., van Leeuwen C., Hermens J. Classifying environmental pollutant , *Chemosphere* 2000, *40*, 875.

  Elin E. S. Phenolic compounds in the biosphere. Novosibirsk. Ed. SB RAS, 2001, 392 p., (in russian).

- **After UV treatment of 222 nm excilamp irradiation the *p*-cresol was completely photodegraded.**
- **The transformation from the highest electronic excited states is the most probable route of *p*-cresol decomposition by irradiation by 222 nm. With the increase in the energy of the photoexciting quantum, the probability of the population of the photodissociative triplet state increases and OH bond rupture in the *p*-cresol molecule may occur.**

- **The UV pre-treatment of *p*-cresol solution (30 min) decreased the speed of biodegradation, especially by using XeCl* excilamp and mercury lamp.**
- **The *o*-cresol, contrariwize, is practically not biodegradable (Fig. 4) under current experimental condition and only after the UV pre-irradiation was obtained the degradation (the best result by KrCl* lamp and 20h biotreatment).**

Fluorescence spectra ($\lambda_{excitation}$=280 nm) of *p*-cresol (10^{-3} mol l^{-1}) after 7 calendar days biodegradation: without irradiation (1), pre-irradiation by KrCl* (2), XeCl* (3) excilamps and mercury lamp (4)



- **Based on the results of the present photochemical-biological studies of cresols, the influence of the photochemical pretreatment on the biodegradation is highly dependent on the structure of the compounds being treated and other process parameters.**

- **Humic acid did not increase the efficiency of cresols photolysis in contrary to earlier date for phenol at 222 nm irradiation. The results of our work have shown that humic acids have photostabilizing properties with respect to cresols.**

Conclusion

- This study illustrates the potential of UV as pre-treatment for biological treatment in order to enhance the biodegradability of reluctant xenobiotics.
- We believe that the increased efficiency of phenols photolysis in the presence of HA is explained by a higher reactivity of irradiated HA (caused by the presence of radical particles, broken aliphatic chains).

- **The results of our work have shown that depending on the excitation wavelength and type of pollutant compounds HA in the presence of phenols can manifest both photosensitizing and photostabilizing properties with respect to ecotoxicant.**

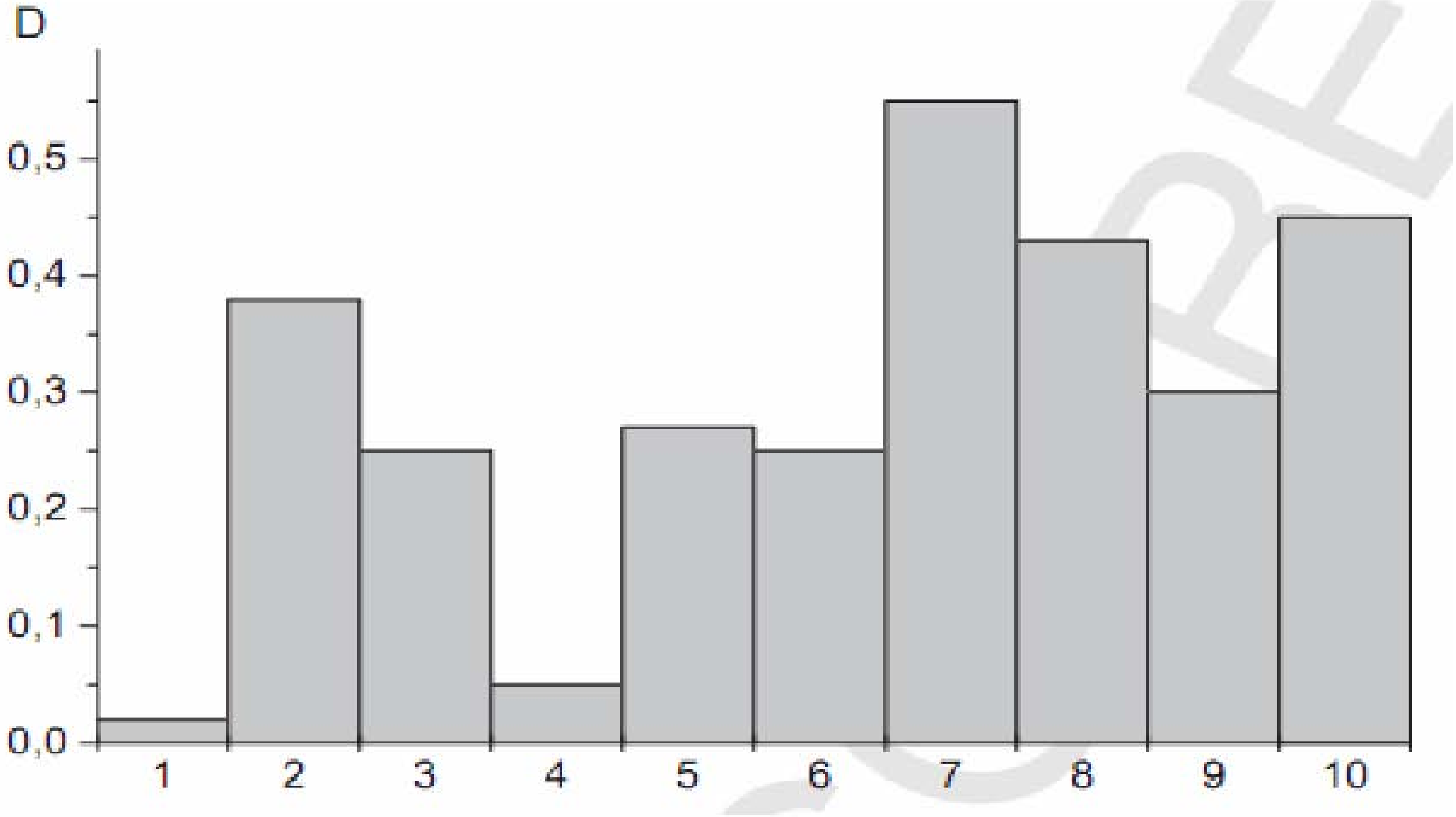
Bioluminescence assay

It was shown that the most effective photoinduced detoxification of cresols and their mixture was observed using XeCl excilamp and mercury lamp. The KrCl excilamp irradiation and the combination of UV-radiation and humic acids are charakterised by the lesser detoxification.

Herbicide 2,4-D

The degradation of herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in water by the combination process of UV-irradiation, humic acids and activated sludge treatment has been studied. The photoreaction rate of all irradiated samples was lowest for the sample irradiated at 308 nm (the XeCl excilamp) in the absence and in the presence of humic acids, and highest for the sample irradiated at 222 nm (the KrCl excilamp). HAs apparently catalyzed the formation some different not toxic photoproducts of 2,4-D after UV-irradiation treatment by excilamps.

The comparison of the absorbance intensity at 256 nm of 2,4-D in water (1) without (1–5) and in the presence (6–10) of HAs with no preliminary UV-irradiation (1, 6) and pre-irradiation for 30 min: 2, 7 – by the KrCl lamp; 3, 8 – by the XeBr lamp; 4, 9 – by the XeCl lamp; 5, 10 – by the Hg lamp.



Conclusion

One possible method for detoxification is use of humic substances: the products of natural transformation of various organic matter in soil. Under natural conditions, detoxification by humic substances can occur in the presence of optical radiation in the visible and UV ranges, so study of the mechanisms of photoinduced detoxification of pollutants in the presence of humic substances is of particular importance.

It seems expedient to use a bioluminescent test for monitoring the toxicity of solutions exposed to UV radiation and treated with humic substances, along with luminescent monitoring of the photodecomposition of the pollutants and other biological important compounds.

Acknowledgements

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