LUMINESCENCE PROPERTIES OF HUMIC SUBSTANCES

V. M. Mazhul', L. S. Ivashkevich, D. G. Shcherbin, N. A. Pavlovskaya, G. V. Naumova, and T. F. Ovchinnikova

A detailed analysis of luminescence properties of humic substances and fulvic acids extracted from peat soil is carried out for the first time. Their fluorescence excitation and emission spectra, fluorescence lifetimes, phosphorescence excitation and emission spectra, and phosphorescence lifetimes at room temperature are investigated. The nature of chromophores of humic substances is discussed.

Key words: humic acids, fulvic acids, absorption spectrum, fluorescence, room-temperature phosphorescence, chromophore.

Presently, the scale of applications of humic preparations in agriculture, animal husbandry, veterinary science, and medicine is dramatically increasing. They make it possible to improve agricultural production, decrease the morbidity of farm animals, and improve their productivity and resistance to adverse environmental factors. Wide prospects of applications of humic preparations are opening up in medicine. Important advantages of humic preparations are their natural origin, the absence of toxic action on organisms and the environment, large stocks of raw materials, and a low cost of production.

Wide applications of humic preparations invite thorough investigations of the physico-chemical properties of their main components — humic acids (HA) and fulvic acids (FA) — using the most modern methods. Luminescence analysis with measurements of the fluorescence and phosphorescence characteristics of objects under investigation is a promising approach to investigation of the molecular properties of humic preparations. In the present work, a detailed luminescence analysis of HA and FA extracted from peat and soil is carried out for the first time.

Experimental. Extraction and purification of HA and FA were carried out according to [1]. Soils from various regions of Belarus were chosen as sources of HA and FA. Peat HA and FA were extracted and purified by the method of [2]. For this, we used lowland meadow peat with a decomposition degree of 50%. The ash content in the HA and FA samples was 3-5%.

Absorption spectra of HA and FA were recorded on a Uvicon 931 spectrophotometer (Germany). In fluorescence measurements, aqueous solutions of HA and FA (pH 8.0) with a substance concentration of 1 mg/mliter were used. Parameters of the room-temperature phosphorescence (RTP) of HA and FA were measured in a thin polyvinyl alcohol (PVA) film. To produce the film, a 4% aqueous solution of PVA containing HA or FA was spread on the horizontal surface of a glass plate and was dried slowly at room temperature. Chromophore concentration in the film was 1 mg per 1 g PVA. In measurements, a 10 × 10 mm square cut from the central portion of the film was used.


---

Institute of Photobiology, Academy of Sciences of Belarus, 27, F. Skorina Ave., Minsk, 220072, Belarus;
We studied the following luminescence parameters of the objects under investigation: fluorescence spectra, fluorescence excitation spectra, fluorescence lifetimes, RPT spectra, RTP excitation spectra, and RTP lifetimes.

Fluorescence lifetimes of HA and FA were measured on a pulsed spectrofluorimeter [3] designed at the A. N. Sevchenko Scientific Research Institute for Applied Physical Problems, Minsk. Fluorescence lifetimes were determined from an analysis of fluorescence decay curves obtained by the single photon-counting method. Other luminescence measurements were carried out using automated devices built at the Institute of Photobiology of the Academy of Sciences of Belarus (Fig. 1).

A spectrophosphorimeter, whose block diagram is presented in Fig. 1a, makes it possible to record phosphorescence spectra of objects under investigation with a quantum yield down to $10^{-6}$ within the spectral range 250-650 nm. The resolution time of the phosphoroscope is $10^{-3}$ sec.

An automated system for luminescence measurements (Fig. 1b) makes it possible to investigate the following characteristics within the range 250-700 nm: fluorescence spectra, fluorescence excitation spectra, degree of fluorescence polarization, phosphorescence decay kinetics within the range $10^{-3}$-20 sec, phosphorescence spectra, and phosphorescence anisotropy within a wide temperature range (77-373 K). The setup has a modular construction, which makes it possible to change the measurement mode (fluorescence or phosphorescence) in a short time. In the polarization measurements mode, polaroids of Iceland spar—polarizer and analyzer—are placed in front of optical windows of the cell compartment. A personal computer carries out analysis of spectral, kinetics, and polarization parameters of luminescence, and transformations of axes (normalization, scaling, and taking the logarithm), data accumulation, numerical smoothing of recorded signals, correction of spectra for the spectral sensitivity of the setup, evaluation of kinetic phosphorescence parameters based on single-, double-, or three-exponential decay character. The analysis of the accuracy of the recovery of parameters of the phosphorescence