

FLUORESCENCE OF AQUEOUS SOLUTIONS OF COMMERCIAL HUMIC PRODUCTS

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We have studied the spectral luminescence characteristics of aqueous solutions of humic products obtained from different raw material sources, and their behavior as the excitation wavelength increases from 270 nm to 355 nm. We have identified differences in the spectral properties of industrial humic products from coalified materials, lignin-containing organic waste, and humic products from plant raw material (peat, sapropel, vermicompost). We have shown that humic products from plant raw material have spectral properties closer to those for humic substances in natural water or soil than humic products from coalified materials.

Keywords: *humic products, high molecular weight polymers, absorption, fluorescence, fluorescence quantum yield.*

Introduction. Today use of environmentally friendly and safe natural substances is becoming increasingly needed in various sectors of industry and agriculture. The use of humic substances is promising: natural high-molecular weight polymers of irregular structure that have been formed in the biosphere (soils, peats, coals, natural waters) as a result of the decomposition of dead biomass. Since the biosphere functions of natural humic substances are common knowledge [1–5], many commercial companies have begun to produce a variety of commercial humic products as organic fertilizers, plant growth stimulators, reducing agents for disturbed soils, and sorbents for toxic pollutants. The main raw material for production of humic products is peat and some kinds of brown coals (mainly leonardite). An advantage of this raw material is its accessibility in various regions of the world. Humic acids in such raw material are represented in the form of high-molecular weight, water-insoluble fractions. Extraction of humic acids occurs by monovalent sodium, potassium, or ammonium cations in alkaline medium. In this case, dilute solutions of humic acid salts (humates) are obtained, which then are concentrated by evaporation. Such a technology results in good processing characteristics of the product but at the same time also results in a high cost for the material. "Dry processing of brown coals" is economically more promising for obtaining dry humic products. Most leading producers around the world use this technology because it is cheap. At the same time, the products obtained have a number of disadvantages such as variable product composition, incomplete solubility in water, the content of inactive insoluble components, and an insignificant amount or absence of fulvic and low-molecular weight acids. In a number of humic products, lignohumates are isolated: humic products obtained by oxidative/hydrolytic degradation of lignin-containing raw material, simulating the natural process of humification of plant wastes of various origins.

Despite accumulated positive experience using commercial humic products [6–10], some questions remain unanswered. The use of different raw material sources and different processing methods for obtaining the humic products results in considerable variety in their properties. So it has become important to develop analytical methods for classification of humic products. Spectral methods, having such advantages as high sensitivity, speed, and the option for contactless implementation, have been used for a long time in environmental studies, including for diagnostics of humic substances in natural water [11–14]. Natural humic substances fluoresce when excited by UV or visible radiation. The luminescence maximum is in the blue region of the visible spectrum, and depends on the excitation wavelength. Humic substances in natural waters or originating from soil have a fluorescence maximum at about 450–460 nm when excited

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TABLE 1. Description and Chemical Composition of Studied Humic Products

Raw material source, country of manufacture	Product designation	C_{WS} , % dry weight of product	C_{HA}/C_{WS} , %	C_{ASF}/C_{WS} , %
Coal, Russian Federation	BC-EnNa	18.6	13.3	4.9
Coal, Russian Federation	BC-EnK	52.2	25.3	26.7
Coal, Russian Federation	Bc-HumNa	21.1	19.6	1.5
Humalite, USA	Hu-UsaNa	16.5	13.8	2.0
Humalite, USA	Hu-BsolK	11.8	6.4	5.2
Lignite, USA	Li-BorgK	19.5	1.3	17.9
Leonardite, Germany	Le-PhK	24.1	18.9	5.0
Peat, Russian Federation	Pe-FlexK	27.7	22.8	4.7
Peat, Russian Federation	Pe-IXPNa	7.4	2.8	4.3
Peat, Russian Federation	Pe-EdaNa	23.7	19.7	3.4
Sapropel, Russian Federation	Sa-BigK	13.4	8.8	4.3
Lignosulfonate, Russian Federation	OW-LhNa	25.8	2.6	22.5
Lignosulfonate, Russian Federation	OW-LhK	26.4	4.8	21.3
Vermicompost, Russian Federation	OW-GulK	41.2	26.5	14.7
Lignite, USA	Li-FA	23.7	9.1	14.6

at $\lambda = 270$ nm [13]. If the excitation wavelength is changed from 270 nm to 310 nm, the maximum of the fluorescence band in natural humic substances is shifted to shorter wavelengths (a "blue shift") [15, 16].

Commercial humic products in aqueous solution also can fluoresce, but their spectral luminescence properties have been poorly studied to date. Studies of commercial humates have shown that analysis of the fluorescence spectra can be the first step in determining their structure, since the features and characteristics of the fluorescence bands carry information about the composition, structural details, and genesis [17]. For example, the fluorescence spectra of commercial humates from coal, when excited by laser radiation at $\lambda = 355$ nm, have a characteristic shape matching the visible spectrum of humic acids from coal, as well as a fluorescence emission maximum at 470–480 nm. In humates obtained from lignin and peat, the maxima in the emission spectrum are blue-shifted by 20–30 nm relative to the maximum of the band for humic acids in coal.

Practically all the spectral measurements described in the literature have been done for commercial products: humic substance standards from the International Humic Substances Society (IHSS). The fluorescence spectra of IHSS humic acids have a luminescence maximum in the 500–520 nm region, while the fluorescence spectra of IHSS fulvic acids have a luminescence maximum in the 445–465 nm region [18]. It has been shown that IHSS humic substances isolated from water or from soil can be distinguished from the fluorescence spectra measured at different pH values [19]. The typical fluorescence quantum yield for IHSS humic acids is a few percent; for fulvic acids, these values are even lower [20]. For humic acids from Fluka, the position of the maximum in the fluorescence spectrum varies within the range 450–540 nm, depending on the pH and concentration of the solution [21]. Study of the luminescence kinetics in different ranges of the fluorescence spectrum for Fluka humic acids and Aldrich humic acid sodium salt [22] made it possible to identify two types of fluorophores with luminescence maxima at 460 nm and 520 nm, the fluorescence intensity ratio of which depends on the concentration and pH of the solution. In contrast to humic substances in natural water, solutions of Aldrich humic acid sodium salt did not show any appreciable hypsochromic shift of the fluorescence spectrum: the position of the maximum in the fluorescence spectrum remained constant as the excitation wavelength varied from 270 nm to 355 nm [14]. Furthermore, the fluorescence quantum yield of the Aldrich humic acid salt is lower than the typical values for natural humic substances, and in contrast to the quantum yield for natural humic substances, does not depend on the excitation wavelength for the spectra in the range 270–355 nm [13, 14].

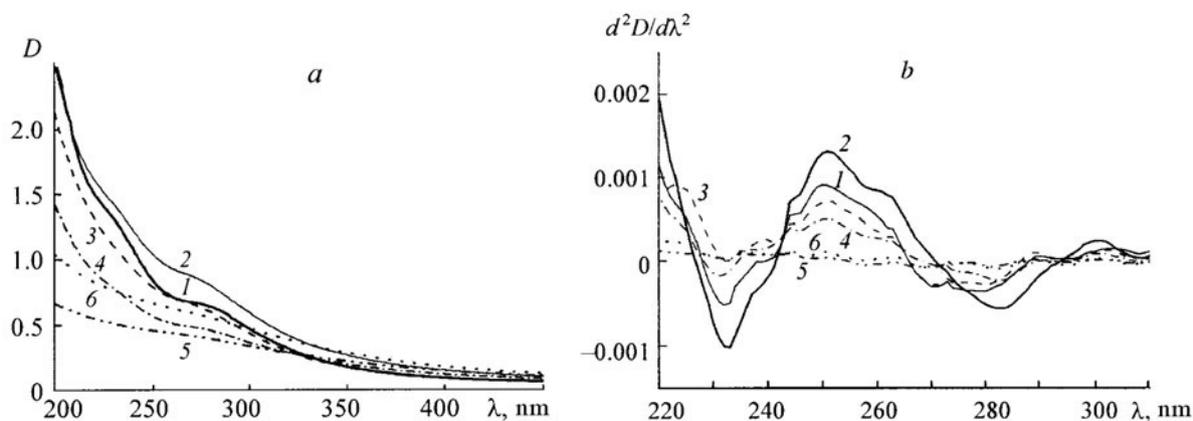


Fig. 1. Optical density spectra (a) and spectral dependence of the second derivative of the optical density (b) for aqueous solutions of humic products: lignohumate OW-LhNa (1), sapropel Sa-BigK (2), vermicompost OW-GulK (3), peat Pe-EdaNa (4), humalite Hu-BsolK (5), leonardite Le-PhK (6).

Humic products obtained by industrial technologies differ with respect to many properties from commercial humic substance products isolated by laboratory methods [23]. This is reflected in their spectral luminescence properties. Systematic measurements of the spectral luminescence properties of a number of commercial humic products were made for the first time in [24, 25]. Differences from analogous characteristics of natural humic substances have been identified with respect to their fluorescence quantum yield and the position of the wavelength of the emission maximum. This work continues the investigation of commercial humic products begun in [24, 25]. We have measured and analyzed the absorption spectra and their second derivatives, fluorescence excitation and emission spectra for a large series of products obtained by different manufacturers from different raw material sources. In this case, we have identified features of the spectral characteristics as a function of the genesis of the organic matter and the composition of the product, and we have also evaluated the feasibility of using spectral methods to qualitatively characterize commercial humic products.

The Experiment. *Commercial humic substance products.* The objects of investigation were humic products in the form of humic acid salts: sodium and potassium humates, obtained by domestic and foreign manufacturers from seven different groups of raw material sources: coalified materials (brown coal, lignite, leonardite and humalite), peats, sapropel and commercial organic waste (Table 1). Brown coal and lignite are among the youngest low-carbon low-grade fossil coals; leonardite and humalite are the oxidized forms of such coals. The humic product from commercial waste is synthesized from lignosulfonate (waste from paper and pulp plants).

In aqueous solutions of the humic products we determined the composition of the organic matter: total content of water-soluble carbon (C_{WS}) and mobile humic substances: humic acid carbon (C_{HA}) and carbon in substances of the acid-soluble fraction (ASF) (C_{ASF}). ASF substances in aqueous solutions of the humic products are basically represented by fulvic acids and nonspecific organic compounds (low molecular weight organic acids, phenol compounds, amino acids, carbohydrates). The total water-soluble carbon content was measured by the Tyurin bichromate method [26] after evaporation of a known volume of an aqueous extract of the product. The humic acid carbon content was measured by the same method, after they were precipitated by sulfuric acid at pH 2, centrifuged, and then dissolved in base. C_{ASF} was measured in the acid filtrate after precipitation of the humic acids [26]. Table 1 gives the chemical composition of the studied humic products.

Spectral measurements and calculation of the fluorescence quantum yield. We used solutions of the humic products of concentration 0.02 g/L in distilled water, pH 5.9–6.6. The absorption spectra in the 200–700 nm range were measured on a Unico double-beam spectrophotometer in quartz cuvetts with 1 cm optical path length. For making the fluorescence measurements, the solutions were additionally diluted five-fold with distilled water, the pH values of the dilute solutions were measured in the range 6.0–6.2. The fluorescence emission spectra were recorded with step 1 nm on a Solar CM2203 fluorimeter in standard quartz cuvetts for fluorimetry with $\lambda_{ex} = 270, 310, \text{ and } 355$ nm. The

fluorescence excitation spectra were recorded at $\lambda_{\text{det}} = 450$ nm. The fluorescence quantum yield Φ_{FL} was calculated by the method used previously for samples of natural water in [14]. As the reference compound, we used an aqueous solution of quinine sulfate since the shape of its spectral line and the position of the fluorescence maximum are close to those of the humic substances. The fluorescence quantum yield of quinine sulfate in a 0.05 M H_2SO_4 solution is $\Phi_{\text{FL}}^0 = 0.546$ [27].

Experimental Results and Discussion. *Absorption spectra of aqueous solutions of humic products.* Humic substances absorb UV and visible light; their optical density decreases monotonically with an increase in wavelength. In the visible region of the spectrum, the absorption of the humic products used is low, so in Fig. 1a we give the absorption spectra in the range 200–450 nm. The spectra of some products have features including the appearance of weak maxima in the interval 230–280 nm. These features can be seen much more clearly in the spectra of the second derivative of the optical density (Fig. 1b), as extrema in the negative region. Lignohumates have two maxima in the absorption spectra, at 230 nm and 285 nm, while products of plant origin from sapropel Sa-BigK, vermicompost OW-GulK, three products from peat, and a sample from lignite Li-BorgK have maxima at 230 nm and in the range 270–280 nm. Many low molecular weight phenol compounds, products of the decomposition of lignin from plant tissues, absorb in this region. The features of the absorption spectra in this region are observed only for humic products from the youngest organic materials with high content of ASF substances, which have not gone through the coalification stage (Table 1). This suggests that in the technological process for obtaining the humic product from raw material that has retained fragments of the lignin structure, low molecular weight phenol substances go into the product. In raw material that has passed through the deeper coalification stages, these fragments are not retained and the absorption spectra of the humic products obtained from it do not have pronounced maxima over the entire wavelength range. Therefore the absorption spectra of aqueous solutions of the samples in the UV region can be a characteristic indicator for identification of the raw material source of the humic product. Furthermore, since many phenol carboxylic acids have high physiological activity, the presence of absorption peaks in the UV region can also indirectly characterize the potential effectiveness of the humic product as a plant growth stimulator.

Fluorescence spectra of aqueous solutions of the humic products. Humic substances can fluoresce when exposed to UV and visible radiation. The fluorescence excitation spectra for aqueous solutions of some of the studied humic products are shown in Fig. 2. We see that the fluorescence excitation spectra of the products from plant raw material are different from the spectra of humic products originating from coal.

The fluorescence spectrum of the humic products is a broad band at 350–600 nm with maximum depending on the origin of the humic substances (Fig. 3). The position of the fluorescence maximum can also depend on λ_{ex} , which suggests the presence of several fluorophore groups in the composition of the substance. As we see from Fig. 3 and Table 2, for most samples of humic products, the fluorescence maximum (λ_{max}) is located in the 480–500 nm region, except for those which contain a high fraction of ASF substances (BC-EnK, Li-BorgK, and FA).

For solutions of humic products from sapropel, peat, vermicompost, and the indicated products from coals, the fluorescence maximum occurs in the shorter wavelength region, 430–450 nm, while the fluorescence maximum for humic products from lignosulfonate is located in the UV range (360 nm). For these humic products, and also for humic products from lignosulfonate, predominance of ASF substances is typical (see Table 1): fulvic acids, and also low molecular weight carboxylic and phenol carboxylic acids, which make a substantial contribution to the emission spectra. Therefore for such humic products, the fluorescence maximum is shifted from the long-wavelength values typical for aromatic fluorophores of humic acids toward the shorter-wavelength values typical for fulvic acids.

Humic substances from natural water or soil have a fluorescence maximum at about 450–460 nm for $\lambda_{\text{ex}} = 270$ nm [13]. Based on the nature of the fluorescence spectra of humic products of plant origin that are the most similar to them, their fluorescence maxima approximately coincide. In contrast, for humates from coalified materials (brown coal, lignite, leonardite, and some products from humalite), the fluorescence maximum is shifted 40–50 nm toward longer wavelengths relative to the fluorescence maximum of dissolved organic matter of marine or soil origin, and is located at about 500 nm.

Fluorescence quantum yield. The fluorescence quantum yield Φ_{FL} of commercial humic products for $\lambda_{\text{ex}} = 270, 310, \text{ or } 355$ nm is 0.3–2.2% (Table 2). For comparison: for humic substances from marine and river natural water, $\Phi_{\text{FL}} = 2\text{--}5\%$; for humic substances of soil origin, 0.1–0.3% [13, 14] when excited at the same wavelengths in the UV range. For most humic products, the fluorescence quantum yield decreases as λ_{ex} increases in the UV range.

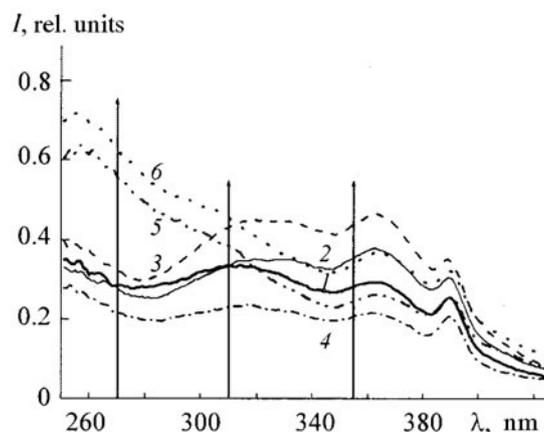


Fig. 2. Fluorescence excitation spectra for solutions of humic products, $\lambda_{\text{det}} = 450$ nm. The numbers have the same meaning as in Fig. 1. The arrows indicate the position of the excitation wavelengths for detection of the emission spectra.

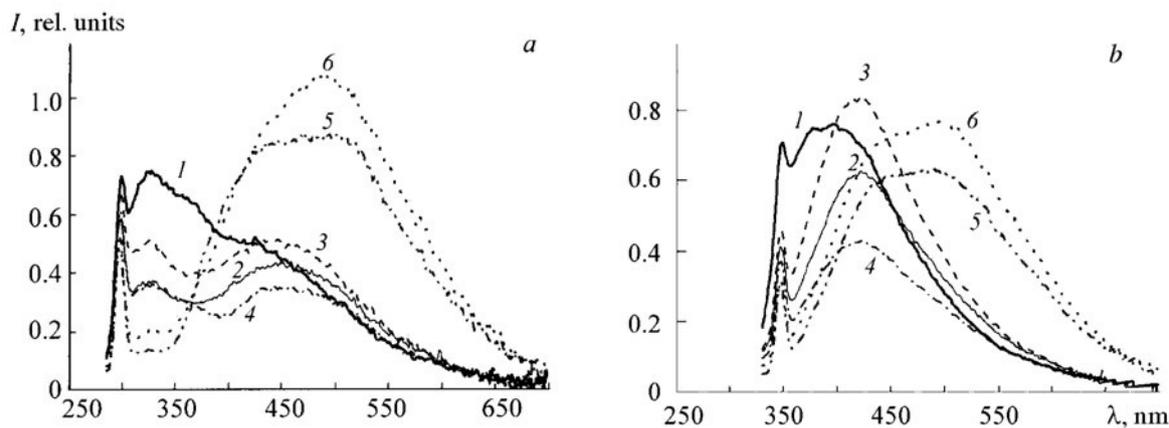


Fig. 3. Fluorescence spectra for solutions of humic products, $\lambda_{\text{ex}} = 270$ nm (a) and 310 nm (b). The numbers have the same meaning as in Fig. 1.

An exception is humic products for which the source was peat and sapropel: the quantum yield for these humic products practically does not change (the product from sapropel Sa-Plod and from peat Pe-Eda) or monotonically increases with an increase in λ_{ex} from 270 nm to 355 nm (the product from peat Pe-IXP).

For humic substances from natural waters or soils, we observe a dependence of Φ_{FL} on excitation wavelength opposite to that observed for commercial humic products of coal origin, and specifically: the fluorescence quantum yield increases with an increase in λ_{ex} , and sometimes quite significantly [16]. For example, for humic substances originating from natural water from a lake in the Belomorsk region, the quantum yield increases two-fold when λ_{ex} changes from 270 nm to 355 nm. Humic products from raw material of plant origin demonstrate fluorescent properties close to the properties of natural humic substances: a dependence of the maximum in the emission spectrum and the fluorescence quantum yield on the excitation wavelength.

Hypsochromic shift of the emission spectra as the excitation wavelength increases. For natural humic substances, we observe a short-wavelength (blue) shift [13–16] of the maximum in the fluorescence spectrum with an increase in the excitation wavelength within a certain interval. The "blue shift" for natural humic substances can be up to 40 nm for a change in λ_{ex} from 270 nm to 310 nm. With a further increase in λ_{ex} up to 355 nm, the wavelength

TABLE 2. Position of the Fluorescence Maximum and the Fluorescence Quantum Yield of Solutions of Humic Products in Water for Excitation at Three Wavelengths

Raw material source	Product designation	λ_{ex} , nm			λ_{ex} , nm		
		270	310	355	270	310	355
		λ_{max} , nm			Φ_{FL} , %		
Coal	BC-EnNa	475 (326)	433	456	0.60	0.66	0.77
Coal	BC-EnK	464	437	455	0.27	0.35	0.46
Coal	BC-HumNa	495	478 (440)	486	0.15	0.17	0.18
Humalite	Hu-UsaNa	485 (300)	485 (448)	491	1.05	0.99	0.90
Humalite	Hu-BsolK	509 (434)	485 (445)	485	1.10	1.02	0.94
Lignite	Li-BorgK	465 (328)	427	456	0.66	1.15	2.15
Leonardite	Le-PhK	495	483	480	0.91	0.91	1.00
Peat	Pe-FlexK	508	488	498	0.29	0.30	0.36
Peat	Pe-IXPNa	453 (328)	431	451	0.98	0.94	0.97
Peat	Pe-EdaNa	435 (328)	422	452	0.40	0.51	0.65
Sapropel	Sa-BigK	450 (330)	422	451	0.26	0.46	0.81
Lignosulfonate	OW-LhNa	329	379 (399)	446	0.46	0.72	0.98
Lignosulfonate	OW-LhK	365	369	440	0.47	0.66	0.47
Vermicompost	OW-GulK	438 (328)	424	445	0.43	0.81	1.45
Fulvic acid	FA	460	440	468	1.89	2.00	1.69

Note. In parentheses: position of the second short-wavelength maximum.

of the maximum in the emission spectrum increases and its value can exceed the wavelength of the maximum in the fluorescence spectrum for $\lambda_{\text{ex}} = 270$ nm. A "blue shift" was observed in the fluorescence spectrum for samples of natural humic substances of marine, river, and soil origin [13, 16], but was observed for most commercial humic products.

From Table 2 we see that for most of the products from coalified materials (coal, lignite, leonardite), no shift in the emission maximum toward shorter wavelengths is observed, while this effect is seen for products from peat and sapropel. Thus the "blue shift" in the emission spectra is 21–39 nm for products from peat and 12–20 nm for products from sapropel, with a typical value of 20–40 nm for humic substances from natural water. Note that a shift in the fluorescence maximum toward shorter wavelengths as the excitation wavelength increases from 270 nm to 310 nm is observed for the same products (humic products from peat and sapropel), for which λ_{max} is located in the 430–450 nm range typical for the luminescence maximum of natural humic substances. In contrast to samples from plant raw ma-

terial (peat and sapropel), for humic products from coals the fluorescence maximum is found in the longer wavelength region (480–515 nm) and is not shifted as the excitation wavelength changes. This experimental observation again demonstrates the similarity between humic products from plant raw material and natural humic substances, and is evidence for greater heterogeneity of the composition for natural humic substances and humates from plant raw material (peat and sapropel) compared with products from coal.

Conclusions. The absorption spectra of aqueous solutions of commercial humic products and natural humic substances are similar: the optical density monotonically decreases in the range 200–700 nm. For some products from plant raw material, having retained fragments of the lignin structure, we observe features including the appearance of weak maxima in the range 230–280 nm. The absorption spectra of aqueous solutions of humic products in the UV region (both the optical density spectrum and its second derivative) may be a characteristic indicator for identification of the raw material source of humic products, and since many phenol carboxylic acids have high physiological activity, the presence of features in the absorption in the UV region can also indirectly characterize the potential effectiveness of humic products as plant growth stimulators.

The shape of the fluorescence spectrum for humic products from peat, vermicompost, and sapropel is similar to that of natural humic substances (fluorescence maximum at 430–440 nm for excitation at 310 nm), while the maximum for humic products from coalified materials is shifted by 40–50 nm toward longer wavelengths relative to the maximum for natural humic substances. Lignohumate synthesized from organic waste during "artificial humification" is characterized by a fluorescence spectrum with maximum at 370 nm which has a different shape than other humic products.

The fluorescence quantum yields for humic products and natural humic substances are similar and equal to a few percent, but with a change in the excitation wavelength in the UV range, the values of Φ_{FL} change differently for natural and commercial humic substances. For products from peat or sapropel, Φ_{FL} increases with an increase in excitation wavelength, which is also observed for natural humic substances, or it remains constant. For all the rest of the commercial humic products, Φ_{FL} decreases as the excitation wavelength increases.

For commercial humic products from peat, vermicompost, and sapropel, we observe a "blue shift" of the emission spectra as the excitation wavelength increases from 270 nm to 310 nm, which was detected earlier for humic substances of natural origin. For all the humic products from coalified materials, except for one product from leonardite, the position of the maximum in the spectrum either does not change or increases as the excitation wavelength changes within the indicated range.

Thus commercial humic products from peats, vermicompost, and sapropel have spectral properties similar to those of humic substances from natural water or soil. On the other hand, commercial humic products from coalified materials and also organic waste have spectral characteristics distinguishing them from humic products from plant raw material and from natural humic substances. Consequently, spectral methods can be used for rapid classification of humic products without preliminary sample preparation.

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