

Fluorescence and Electron Absorption Studies of the Structure of Humic Acids Extracted from the A Horizon of Soils

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Abstract

Fluorescence and electron absorption spectra have been recorded for humic acids (HA) extracted from different sampling depths R of the A horizon of black earth and saline land. It was established that the fluorescence bands red shifted and that the absorption intensity increased with R for both samples. It was concluded that the aromaticity of humic acid samples increases with R .

INTRODUCTION

Irrespective of their origin, humic acids (HA) are high-molecular nitrogen-containing organic acids containing aromatic groups in the molecule. The structure of the macromolecule was derived by analyzing the results of numerous studies by direct and indirect methods; it involves a nucleus (aromatic carbon skeleton) and peripheral polysaccharide and polypeptide chains [1, 2]. The specific features of humic acid macromolecules are their polydisperse character and variability of the elemental and molecular composition [1, 2]. Interest in the conformational properties of HA is dictated by the critical role these acids play in the formation of the agrochemically significant structure of soil, determining the physical and chemical properties of the latter. Moreover, the conformational properties of HA are of interest in view of the ability of HA to decrease the action of ecologically toxic agents by binding pollutants.

Electron spectroscopy has long been used to derive information on the conformational pro-

perties of HA. Absorption spectroscopy is the most popular method. For any HA, its electron absorption spectrum is nearly monotonous, with the absorption coefficient decreasing oppositely to the wavelength. Using the $E_4 : E_6$ parameter (chromaticity coefficient) for structural studies of HA is one of the greatest achievements of absorption spectroscopy; the parameter is defined as the ratio of optical densities at $\lambda = 460$ and 650 nm. It is commonly accepted that the $E_4 : E_6$ parameter is a quantitative measure of the relationship between the nucleus and the periphery, which depicts the aromaticity of the HA molecule [1, 2].

Recently, the fluorescence technique became widely used for investigating the properties of HA [3–6]. The simple procedure of sample preparation and express analysis are the advantages of this method. Moreover, this method is preferable to the absorption technique because the luminescence spectrum contains bands in a definite spectral range. The parameters of the fluorescence bands convey direct information about the degree of conjugation

in the HA macromolecule (this is indicated by changes in the position of the maximum or of the first moment of the fluorescence band) and about the degree of polydispersity (the dependence of the position of the fluorescence band maximum on the excitation wavelength) [4, 5]. Transformations of the fluorescence spectra of HA samples from the same kind of soil provide information about changes in the electronic structure of HA in different conditions of soil keeping and tillage [6].

Investigation of the electronic (structural) properties of HA extracted from different depths of soils has always been a challenge to both practice and theory of soil science [1–3, 7, 8]. As a rule, data on the electronic properties of HA molecules isolated from soil samples taken at different depths were obtained from electron absorption spectra [1, 2, 7]. The step δ used for sampling was at least 15 cm. However, using the fluorescence technique for investigating HA molecules extracted from different depths of the A horizon alone is not reported in the literature.

The goal of the present work was to investigate the electronic (structural) properties of HA molecules isolated from soils at different depths R with $\delta = 2\text{--}5$ cm within the A horizon ($R = 0\text{--}17$ cm) using the fluorescence technique and to compare the thus obtained information with the data obtained from the absorption spectra. For investigation we took the samples isolated from black earth and saline soils, which had been kept for 20 years under atypical natural conditions. Experiments revealed that for both samples from the A horizon, the fluorescence bands exhibited a monotonous bathochromic shift at increased values of R . It was concluded that the aromaticity of the HA samples increased with R .

EXPERIMENTAL

Humic acids were isolated from the weakly leached virgin black earth of the forest-steppe formed under the meadow steppe of the Altai region and from the medium-columnar meadow-steppe black earth formed under the solonchuk motley-grass-fescue-couch-grass steppe of Barabinsk lowlands. Before extraction, these

soils had been kept for 20 years as monoliths under a layer of sod, highly podzol soil with a second humus horizon; the layer formed under typical vegetation of the southern taiga (fir with admixtures of cedar and birch). The conditions of soil transplantation for the experiment and the main results were reported previously [8]. Humic acids were isolated by the standard methods [7] except that rigid treatment of HA was excluded at the stage of purification. The concentration of the aqueous solutions of HA was 100 mg/l. For better solubility, an alkaline medium (pH 12.3) was employed. The fluorescence spectra were obtained with an N_2 laser spectrometer ($\lambda_{\text{excit}} = 337$ nm) [9] in standard 4 cm³ quartz cells. The absorption spectra were recorded with a Bruker spectrophotometer. Because of noticeable absorption exhibited by all solutions [optical density (OD) ~ 0.5], the experimentally observed fluorescence spectra were corrected by including a factor $(K_{\text{OD}}^R)^{-1}$:

$$K_{\text{OD}}^R = 1 - 10^{-\text{OD}} \quad (1)$$

$$\text{OD}_R = (\Sigma \text{OD}_{\lambda_i})_R / (\Sigma \text{OD}_{\lambda_i})_0 \quad (2)$$

Here $(\Sigma \text{OD}_{\lambda_i})_R$ and $(\Sigma \text{OD}_{\lambda_i})_0$ are the sums of absorption coefficients within the spectral range 300–800 nm for HA samples taken from the depths $R = R_i$ and $R = 0\text{--}3$ cm, respectively.

The spectral data were processed with Origin 6 software. The temperature of experiments was (21 ± 1) °C.

The following parameters were used to observe the changes in the fluorescence spectra: 1) intensity ratio α of luminescence at $\lambda = 440$ and 460 nm (qualitative parameter, or the parameter of the contour shape of the fluorescence spectrum):

$$\alpha = I_{440}/I_{460} \quad (3)$$

2) first moment M_1 (conventional spectral parameter, centre of gravity of the spectrum):

$$M_1 = \Sigma I_i \lambda_i / \Sigma I_i \quad (4)$$

where I_i is the intensity at a wavelength λ_i in the contour of the fluorescence spectrum.

RESULTS AND DISCUSSION

Figure 1 shows the fluorescence spectra of the aqueous solutions of HA extracted from

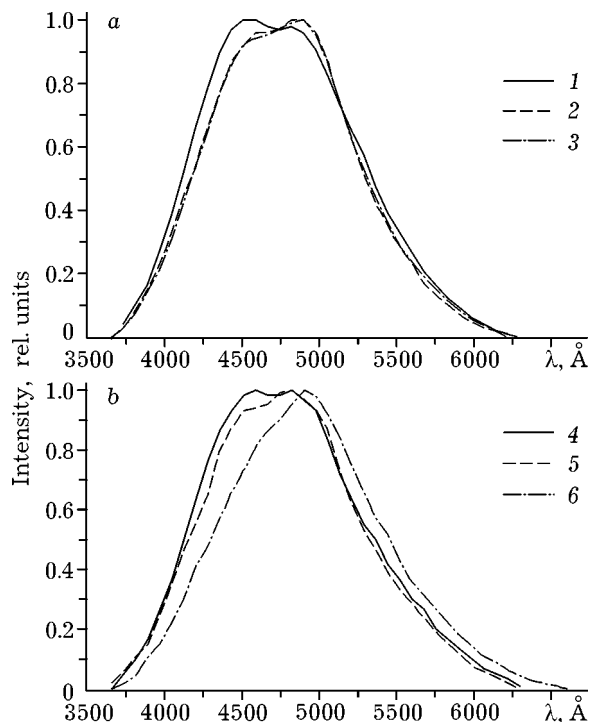


Fig. 1. Luminescence spectra of alkaline solutions of HA isolated from different depths of soils: *a* - black earth, *b* - saline land. *R*, cm: 1-3 (1), 3-6 (2), 14-17 (3), 0-3 (4), 6-9 (5), 14-16 (6).

different soil sampling depths *R* of black earth (see Fig. 1, *a*) and saline land (see Fig. 1, *b*). The spectra are normalized to the maximum. (In order to avoid congestion, the spectra are shown only for three values of *R*.) It follows that the shape of the spectral contours undergoes changes: the fraction of the red component of the spectrum increases with *R*. These changes are explicitly indicated by the dependences of the parameters α and M_1 on *R* (Figs. 2 and 3).

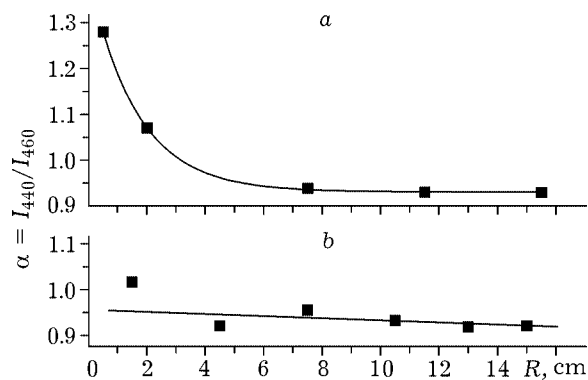


Fig. 2. *R* dependence of the parameter α of the luminescence spectra of alkaline solutions of HA: *a* - black earth, *b* - saline land.

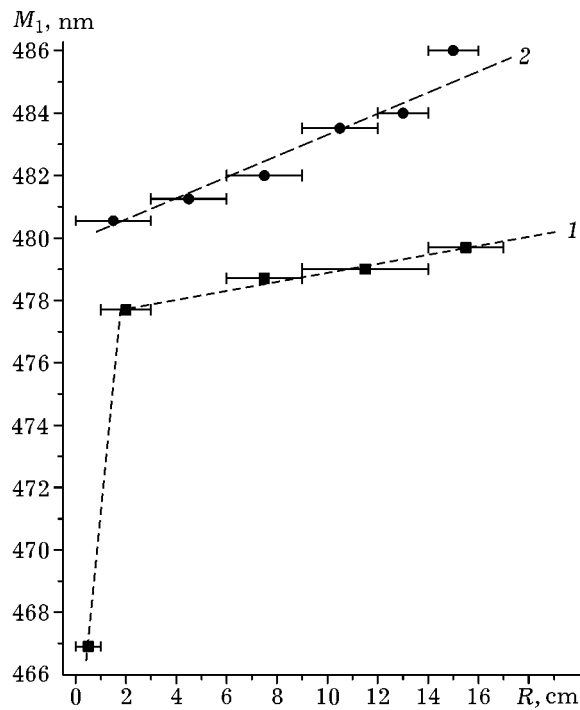


Fig. 3. *R* dependence of the first momentum of the luminescence spectra of alkaline solutions of HA: 1 - black earth; 2 - saline land. On the abscissa, the segments indicate the step of soil sampling for the given value of *R*.

The bathochromic shift of the fluorescence contour at increased values of *R* can be explained by higher conjugation in HA molecules extracted from larger depths or by the conformational changes of HA molecules due to changes in intermolecular interactions (H-bonding, hydrophobicity, etc.). Higher conjugation seems to be the most probable reason since it was shown [8] that the H/C parameter decreases at larger depths. The latter circumstance is usually considered to be evidence in favour of the increased aromaticity of HA molecules [2, 7]. It is noteworthy that the presence of a bathochromic shift in the fluorescence spectra of HA molecules of the same type is currently regarded as an indisputable argument in favour of higher conjugation in the molecule [3, 10].

It follows from Figs. 1 and 3 that the luminescence spectrum of HA extracted from saline land is red shifted relative to the spectrum of HA extracted from black earth. This means that the samples isolated from saline land contain more highly conjugated structural fragments than those extracted from black

earth. Because of this, for the HA molecule isolated from saline land, the first momentum is higher in magnitude.

Figure 3 reveals steeper descent of the dependence of M_1 at $R > 1$ cm for samples isolated from saline land. Since the bathochromic shift is due to higher conjugation in the HA molecule, this means that HA molecules become more differentiated according to the degree of conjugation in saline compared to black earth soil. This conclusion agrees with the known principle of soil science stating that black earth is less prone to changes [1, 2, 7]. An explanation lies in the fact that black earth possesses the largest number of stable aromatic structural units linked by aliphatic bridges than

any other type of soil does. Accordingly, the probability of changes (cleavage of chemical bonds in the stable units) under identical conditions is lower for HA molecules isolated from black soil than for HA molecules isolated from other kinds of soil.

The absorption spectra of HA samples isolated from different soils are shown in Fig. 4. Their appearance (contour) is much the same as for the spectra reported in [1, 2, 11]. Figure 5, *a*, *b* shows the R dependences of optical density OD at $\lambda = 337$ nm for aqueous solutions of HA extracted from black earth and saline land, respectively. One can see that the dependences of OD on R for HA samples extracted from black earth are monotonous, un-

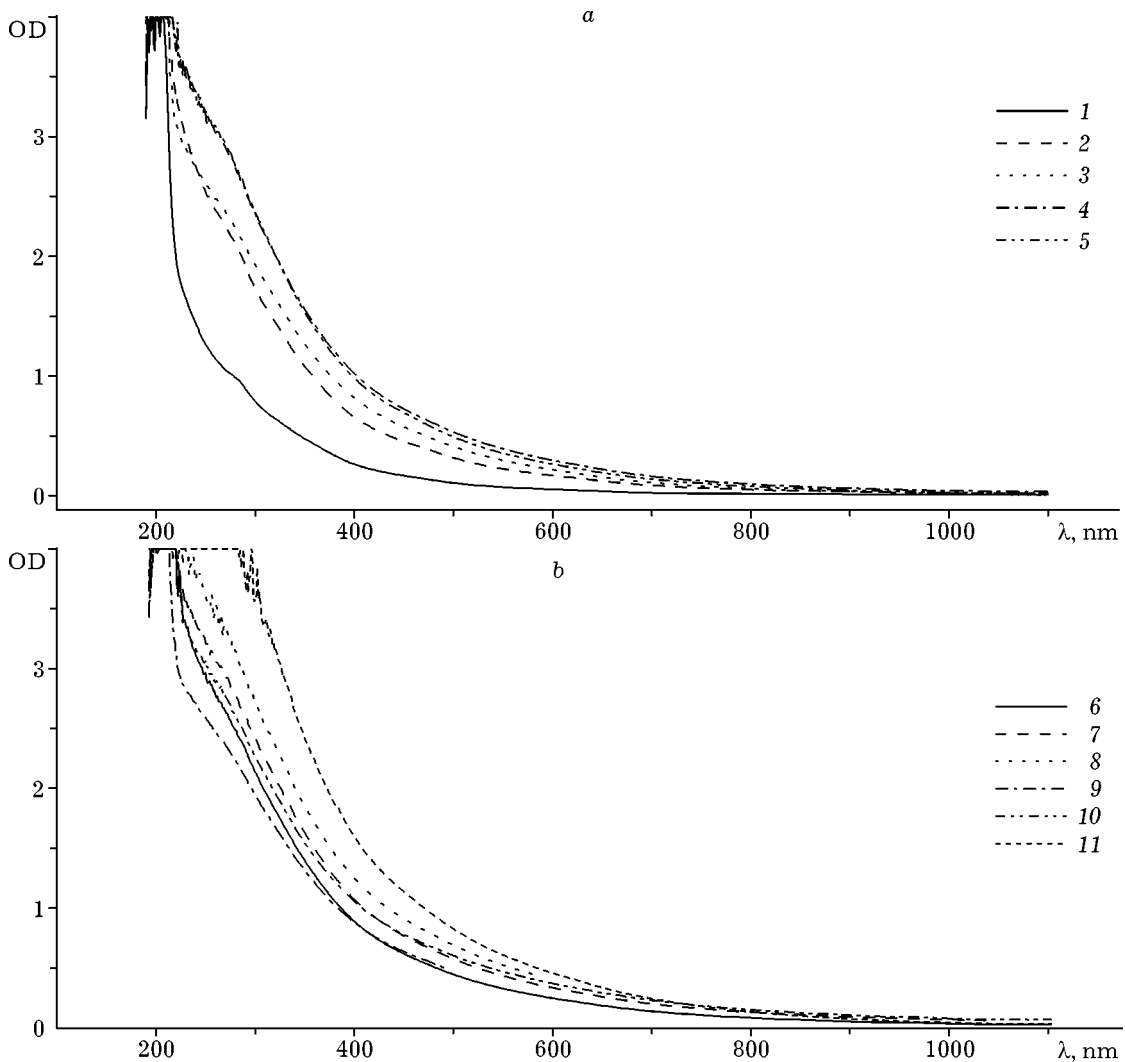


Fig. 4. Electron absorption spectra of alkaline solutions of HA isolated from different depths R of soils: *a* - black earth, *b* - saline land. R , cm: 0-1 (1), 1-3 (2), 3-6 (3), 9-14 (4), 14-17 (5), 0-3 (6), 306 (7), 6-9 (8), 9-12 (9), 12-14 (10), 14-16 (11).

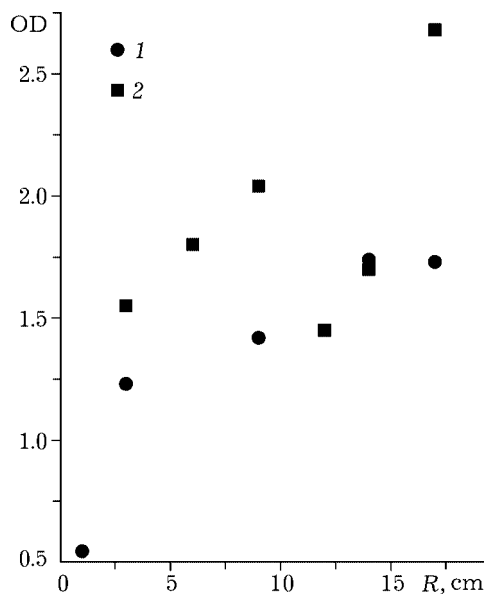


Fig. 5. R dependence of optical density of alkaline solutions of HA at $\lambda = 337$ nm: 1 - black earth, 2 - saline land.

like those for samples extracted from saline land. Similar results (monotonous and non-monotonous dependences) were obtained earlier, but experiments were performed with $\delta > 15$ cm [2, 7].

The non-monotonous $OD(R)$ dependences have never been interpreted, probably because the shape of the absorption spectra of HA has not been studied. Today we have no generally accepted interpretation of the shape of HA absorption spectra. Irrespective of the kind of soil from which HA molecules were extracted, the spectrum shape is always similar to the one shown in Fig. 4. The similarity of absorption spectra, independently of soil genesis, could be indicative of the fact that in reality we observe the scattering (but not absorption) spectra. Indeed, since HA molecules are very large ($M > 50\,000$ D), a real solution of HA can be a colloid system and therefore be light scattering (Rayleigh law). In this case, the scattering intensity decreases in proportion to the wavelength to the fourth power, which does not contradict the experimental data at a qualitative level. However, numerous experimental data (ours and those described in the literature) show that the spectrum shape does not change when the concentration decreases or when separate (smaller) fractions of HA are

registered (for example, see [11]) and is independent of the method of filtering the aqueous solutions of HA. Finally, for aqueous solutions of various biological molecules with molecular masses comparable to the molecular mass of HA, the absorption spectra have distinct maxima (for example, the absorption spectra of various dehydrogenases or cytochrome [12]). Thus it is maintained that the contribution of scattering to the observed absorption spectrum is small, and that the latter is due only to absorption.

The values of α and M_1 in the fluorescence spectra and optical density in the absorption spectra of a humic acid sample extracted almost from the surface ($R = 0-1$ cm) of black earth differ strongly from those for $R = 1-17$ cm. This means that the electron properties (in particular, the degree of conjugation) of the corresponding HA molecules are also quite different. At present, the lack of detailed information makes it difficult to ultimately state whether this is a unique phenomenon inherent in black earth only or a universal phenomenon characteristic of any kind of soil. Within the framework of our experiment, we assumed that this effect is characteristic of black earth only. Indeed, Figs. 2-5 show that the dependences of the spectral parameters of luminescence and absorption are linear for samples isolated from saline land ($R = 0-3$ cm) but non-linear for samples isolated from black earth.

Based on these results we can discuss whether a reliable and unambiguous interpretation can be achieved for data obtained by luminescence and absorption spectroscopy. For this, we re-examine the results associated with the observed difference between changes in the luminescence and absorption spectra. In soil science, the higher optical density in the absorption spectra of HA is usually explained by the higher degree of conjugation [2, 7]. If we accept this explanation, then for HA samples isolated from depths of 9-12 and 12-14 cm of saline soil, it follows from absorption spectral data that conjugation decreases in comparison with the sample taken from the depth of 6-9 cm. On the contrary, as can be seen from fluorescence data, conjugation increases. In fact, one should not expect any correlation between

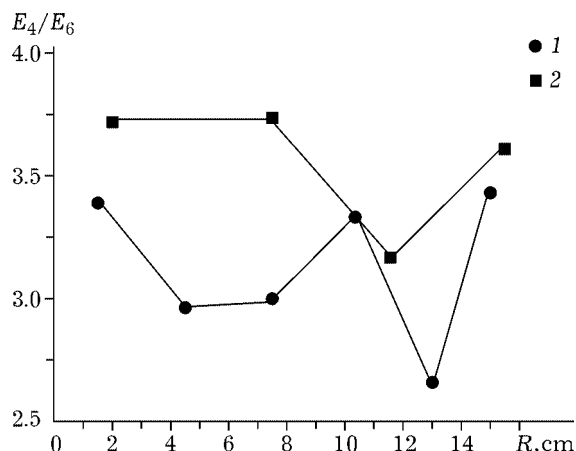


Fig. 6. Dependence of the chromaticity parameter ($E_4 : E_6$) of alkaline solutions of HA on R : 1 - black earth, 2 - saline land.

the data obtained by the fluorescence and absorption methods. This is explained by the following reasons.

As indicated above, characteristic bands are absent from the absorption spectra. This may be explained by the fact that the absorption spectrum has contributions from all molecular fragments of HA, while the fluorescence spectrum has contributions only from specific groups (fluorophores). It is likely that no *a priori* correlation can exist between changes in the whole set of absorbing fragments and separate specific fluorophores. Thus the information obtained by different methods can lack even qualitative correlation, because it refers to changes in the state of different molecular fragments. This accounts for the fact that the fluorescence spectral data reveal monotonous changes in the structure of HA at greater depths, while the absorption spectral data point to non-monotonous changes. Thus we arrive at the conclusion that more unambiguous information can be obtained from the fluorescence data and that this method is preferable to the absorption technique in studies of the properties of humic acids.

An additional argument in favour of the fluorescence method and against electron absorption spectroscopy is the absence of a clear-cut dependence of chromaticity on the depth for both samples (Fig. 6). There are no dependences at all, so it seems impossible to make any particular conclusions about changes in

chromaticity and accordingly about the degree of aromaticity. Similar examples of a large scatter of the chromaticity parameter for HA molecules were observed for one horizon over a uniform territory at distances of up to 20 m [2, 7]. This situation is rather typical for the absorption method, which confirms the above statement about the difficulty of its wide application for obtaining reliable and unambiguous data on the conformational properties of HA.

CONCLUSIONS

The fluorescence technique now seems to be preferable to the absorption method in studies of the depth-dependent properties of HA. This should be taken into account when choosing a suitable technique for investigating the structure of HA.

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