

Estimation of Physiological Activity of Humic Substances of Oxidized Coal (Buryatia)

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Abstract

Structural parameters of humic acids of oxidized coal from the deposits of Buryatia were determined using quantitative ¹³C NMR spectroscopy. Estimation of physiological activity of humic acids of oxidized coal was carried out under the conditions of agrotechnical tests. An interconnection between the characteristics of physiological activity and structural parameter Φ of humic acids was discovered; it was assumed in this connection that ¹³C NMR spectroscopy would be a promising tool to predict biostimulant characteristics of humic acids.

INTRODUCTION

Humic substances (HS) of fossil coal of different type and degree of weathering possess different levels of physiological activity (PA), which is connected with the presence of active oxygen-containing groups in the organic structure of coal. A comparative estimation of the PA of HS of Buryatian coal deposits containing humic acids at a level up to 76 % was carried out using a rapid procedure to determine the structural parameters of humic acids with the help of quantitative ¹³C NMR spectroscopy [1]. Investigation of the structural features of HS of oxidized coal on the basis of their ¹³C NMR spectra, along with the results of their biological tests, is necessary for further prediction of their PA.

Using the indicated procedure, we obtained the characteristics of humic acids (HA) extracted from the oxidized coal of Buryatian deposits, estimated their PA, tested their biological action

under the conditions of laboratory and field experiments with crop plants.

EXPERIMENTAL

The HA preparations were obtained from coal sampled from the upper, most strongly oxidized beds and open pits of coal deposits in Buryatia. Technical and elemental composition of coal samples was determined using the standard procedures [2, 3]. Elemental analysis of the samples of HA was carried out with an automatic CNH analyzer of Karlo Erba company, model 1106 (Italy). The IR spectra of HA samples of oxidized coal were recorded with Specord-75 spectrometer within the wave-number range 400–4000 cm⁻¹. The ¹³C NMR spectra were recorded with DRX-500 spectrometer of Bruker Company. Elemental analysis and IR spectroscopy help obtaining the general information about the structure of HA

allowing one to determine the set of fragments of macromolecules; these examinations are necessary to confirm the results of NMR spectroscopy. ^{13}C NMR procedure allows one not only to determine the frequency and intensity of absorption lines of the fragments of macromolecules but also to establish quantitative parameters of their interactions with the surrounding atoms. The structural parameters for HA samples of oxidized coal treated in the solution of NaOD were established under the quantitative conditions of experiments.

Mean time necessary to obtain the parameters of fragment composition is 18 h. ^{13}C NMR spectra of HA of oxidized coal samples from the deposits under investigation were recorded with the frequency of 125.76 MHz.

RESULTS AND DISCUSSION

The elemental composition of HA extracted from the samples of oxidized coal from the upper layers of Buryatian deposits and the ash content are presented in Table 1. The HA of coal are similar to the HA of southern black soil in the concentrations of elements [3].

The following absorption bands were determined in the IR spectra of HA of oxidized coal, cm^{-1} : 1600–1625 (C=C conjugated groups in aromatic structures); 2960, 2870 (CH_3) and 2930, 2850 (CH_2); 1700–1725 (C=O of carboxyl groups), 1250 (OH of phenol groups), 1720 (C=O).

Comparative analysis of the IR spectra of HA of coal and black soil points to the similarity of the main absorption bands. At the same time, the IR spectra of oxidized coal contain the bands of stretching vibrations of aliphatic groups at 2960 and 2870 cm^{-1} (CH_3).

The IR spectra of oxidized coal clearly exhibit the absorption bands of aromatic structures. Absorption bands related to the vibrations of atoms in aliphatic chains in the IR spectra of oxidized coal from the Gusinoozerskoye and Tunkinskoye deposits are less intensive than those exhibited by the samples from the Zagustayskoye deposit. This is an indication that the HA of the former deposits are enriched with benzene-like components.

On the basis of the results of analysis of ^{13}C NMR spectra of HA in oxidized coal from the Gusinoozerskoye, Tunkinskoye and Zagustayskoye deposits and HA of black soil, intensive signals within the range 165–179 cm^{-1} were determined. The signals between 50 and 100 cm^{-1} correspond to polysaccharide groups. The presence of aromatic fragments in the HA of oxidized coal, which is also characteristic of the HA of black soil, is confirmed by intensive signals within the region 100–160 cm^{-1} [3, 4] (Fig. 1). The signals within the region 140–150 cm^{-1} correspond to quaternary aromatic carbon atoms in condensed systems.

For the HA of oxidized coal from the deposits of Buryatia, a complex structural parameter Φ was determined; it characterizes the ratio of the amount of active functional groups and $\text{C}_{\text{alk}}\text{O}$ fragments to the aromatic component in HA. In addition, this parameter also determines the ratio between hydrophilic and hydrophobic properties of HA macromolecules and depicts the reactivity of separate active centres of HA in the interactions with plant cells [1]. The content of carbon atoms in the structural fragments of oxidized coal sampled from the upper beds and from open pit regions of Buryatian deposits for comparative tests of PA of HA, along with

TABLE 1

Elemental composition and ash content of humic acids from oxidized coal of Buryatian deposits

| Deposit | Ash concentration*, % | Concentration, mass % daf | | | | |
|-----------------|--------------------------|---------------------------|-----|-----|------|-----|
| | | C | H | N | O | S |
| Gusinoozerskoye | 7.0 | 50.5 | 3.5 | 1.9 | 43.1 | 1.0 |
| Tunkinskoye | 6.4 | 53.4 | 3.8 | 2.0 | 40.0 | 0.8 |
| Zagustayskoye | 8.5 | 51.2 | 3.7 | 2.0 | 41.9 | 1.2 |

*Analysis was carried out by V. D. Tikhova (NIOCh, SB RAS, Novosibirsk).

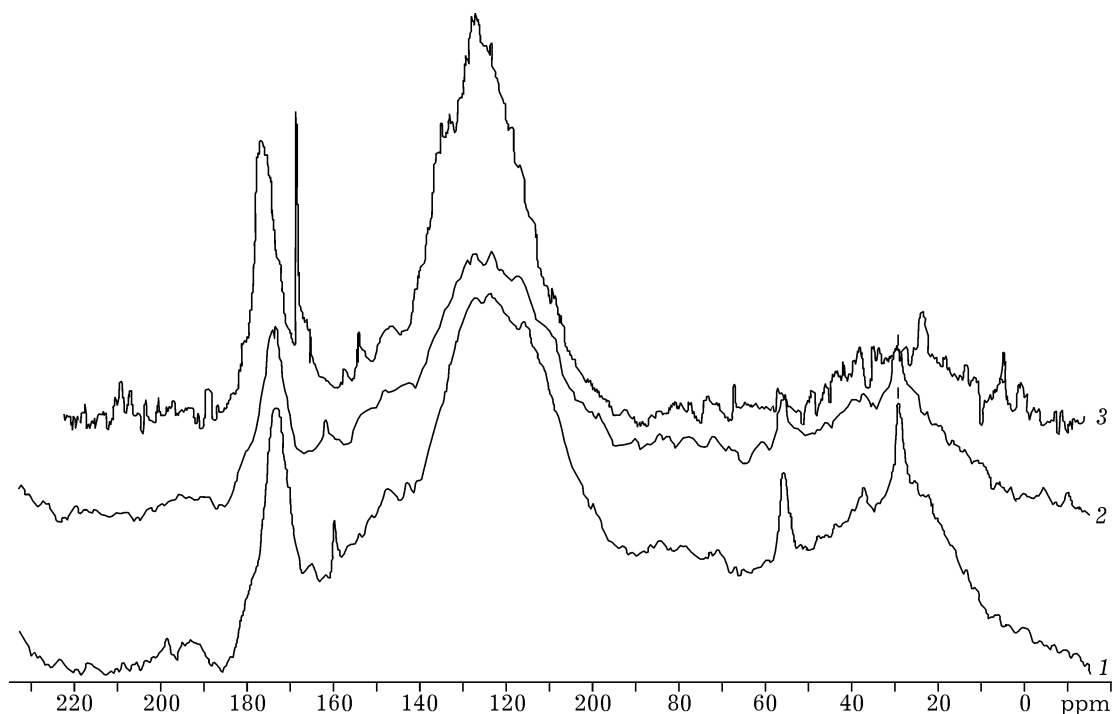


Fig. 1. ^{13}C NMR spectra of humic acids of oxidized coal from the Gusinozerskoye (1), Tunkinskoye (2), Zagustayskoye (3) deposits in Buryatia.

the data of laboratory and field tests of HA preparations, are listed in Table 2.

Aliphatic and aromatic carbon atoms bound with ester oxygen atoms and belonging to various functional groups are clearly exhibited and quantified in the ^{13}C NMR spectra. In

comparison with the HA of oxidized coal from the Zagustayskoye deposit, the HA of coal from the upper beds of the Gusinozerskoye and Tunkinskoye deposits exhibit prevalence of carbon atoms in $\text{C}_{\text{ar}}\text{O}$ fragments, unlike for $\text{C}_{\text{alk}}\text{O}$ fragments, while the ratio of aliphatic

TABLE 2

Carbon atom content of structural fragments of humic acids from oxidized coal and the indices of their physiological activity

| Coal sample | Sampling site | Carbon atom content, % | | | | | | | | | | Φ_1 | Δ_1^* , % | Δ_2^{**} , % |
|------------------|---------------|------------------------|--------------------------|------|---------------------------|--------------------------------|----------------------------------|---------------------------------|-----------------------|-------------------------|-------------|----------|------------------|---------------------|
| | | C=O | C_{quin} | COOH | C_{inorg} | $\text{C}_{\text{ar}}\text{O}$ | $\text{C}_{\text{ar}}\text{C,H}$ | $\text{C}_{\text{alk}}\text{O}$ | CH_3O | C_{alk} | f_a^{***} | | | |
| Gusino-ozerskoye | Upper bed | 4.7 | 3.3 | 9.6 | 20 | 15.0 | 46.1 | 5.4 | 0.8 | 13.1 | 61.1 | 0.70 | 28.0 | 49.5 |
| | Open pit | 1.2 | 1.6 | 12.8 | 3.3 | 11.6 | 51.0 | 2.1 | 1.0 | 15.4 | 62.6 | 0.52 | 26.9 | 42.0 |
| Tunkinskoye | Upper bed | 1.5 | 1.2 | 11.9 | 27 | 14.2 | 43.0 | 7.0 | 2.1 | 16.4 | 57.2 | 0.75 | 28.6 | 49.1 |
| | Open pit | 4.1 | 2.4 | 12.0 | 28 | 10.3 | 49.2 | 2.9 | 0.3 | 16.0 | 59.5 | 0.51 | 25.5 | 39.5 |
| Zagustay-skoye | Upper bed | 3.5 | 1.4 | 9.8 | 15.0 | 7.9 | 31.8 | 9.0 | 1.4 | 20.2 | 39.7 | 0.80 | 30.0 | 49.7 |
| | Open pit | 2.1 | 2.9 | 11.3 | 15.2 | 9.3 | 46.4 | 2.3 | 0.3 | 10.2 | 55.7 | 0.49 | 26.2 | 36.0 |

Note. Chemical shift, ppm: C=O 185–220, C_{quin} 186–180, COOH 165–179, C_{inorg} 162–168, $\text{C}_{\text{ar}}\text{O}$ 140–160, $\text{C}_{\text{ar}}\text{C,H}$ 106–140, $\text{C}_{\text{alk}}\text{O}$ 58–106, CH_3O 54–58, C_{alk} 0–54; $f_a = 106–160$.

*An increase in the green mass of garden radish under the action of the solutions of humic acids with a concentration of 0.0001 % (laboratory tests, repeated 6 times).

**An increase in the dry mass of corn; the same solutions (field tests, repeated 4 times).

***Aromaticity degree ($\text{C}_{\text{ar}}\text{O} + \text{C}_{\text{ar}}\text{C,H}$).

to aromatic component for the HA of oxidized coal from the upper beds of the Gusinozerskoye and Tunkinskoye deposits is 1.5–2 times less than that for the HA of oxidized coal from the Zagustayskoye deposit. This is an evidence of insufficient content of condensed aromatic fragments in the latter coal.

The value of structural parameter Φ_1 for HA extracted from the upper, most strongly oxidized beds of coal deposits of Buryatia is rather high (0.7–0.8) and close to the corresponding values for black soil (0.85–0.95) [5].

It was established in vegetation tests that the parameters of PA of HA of oxidized coal differ depending on sampling sites. For instance, PA of HA from oxidized coal of the upper beds is higher than that of open-pit oxidized coal (by 1.1–3.8 and 7.5–13.7 % in laboratory and field tests, respectively); however, substantial differences in growth and quality characteristics were detected. In addition, PA of HA from oxidized coal of upper beds is quite comparable with that of southern black soil (according to the data of some authors, PA of the latter is 50 to 62 % [6]). The reliability of experimental results increases due to the use of a large number of plants in each recurrence.

On the basis of statistical analysis of the results of investigation, a stable (over two statistical samples: laboratory and field tests) and significant interconnection was established between PA characteristics and structural parameter Φ_1 of HA. The statistical indices of this interconnection are characterized by equation $y = a + bx$ where y is the PA parameter of HA (Δ_1 , Δ_2 , see Table 2), x is the parameter of fragment composition characterizing the structure of HA (Φ_1).

The following values were obtained for statistical indices in the laboratory and field tests: $a = 20.33$, $b = 11.46$, $r = 0.95$, $s = 0.58$, $n = 6$ and $a = 18.65$, $b = 40.82$, $r = 0.94$, $s = 2.2$, $n = 6$, respectively, where r is the correlation coefficient, s is standard deviation, and n is the number of points (Fig. 2).

Physiological activity of HA extracted from the upper, most strongly oxidized coal beds of different deposits of Buryatia is approximately the same, which allows us to consider them as stimulants for plant growth and development.

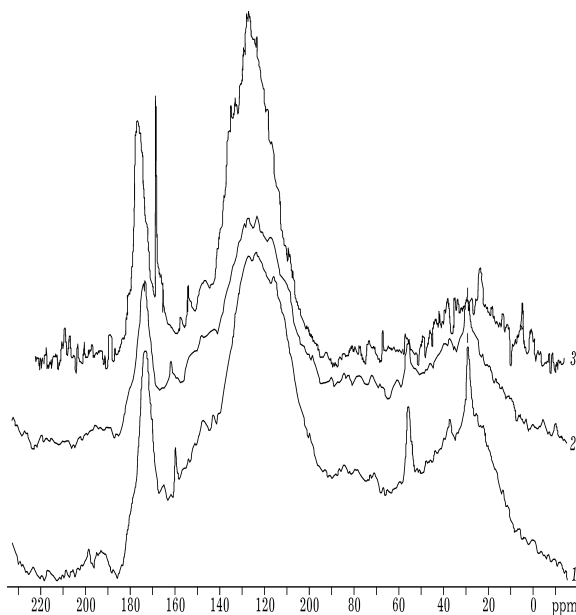


Fig. 2. Interconnection between the index of PA and the structural parameter Φ_1 : a – laboratory tests (Δ_1 – an increase in the dry mass of garden radish under the action of solutions of humic acids with a concentration of 0.0001 %), b – field tests (Δ_2 – an increase in corn crop; the same solutions).

CONCLUSIONS

It was established by means of elemental analysis, IR and NMR spectroscopy that the HA of oxidized coal of deposits in Buryatia are close in structure to the HA of black soil possessing high physiological activity.

Structural parameter Φ_1 was determined by means of quantitative ^{13}C NMR spectroscopy for HA of oxidized coal; this parameter is connected with PA and is characterized by rather high values (0.7–0.8), which are close to those for black soil (0.85–0.95).

It was established in agrotechnical tests that the HA extracted from coal of the upper, most strongly oxidized beds exhibit almost identical level of PA (an increase in corn crop is 49.1–49.7 %, depending on a deposit), which is close to the PA of southern black soil (an increase in corn crop is 50 to 62 %).

An interconnection between PA indices and structural parameter Φ_1 of HA was discovered, which is an evidence that the ratio of hydrophilic to hydrophobic fragments in the structure of HA is one of the decisive factors of their PA.

The results of ^{13}C NMR spectroscopy can be used to predict biostimulant properties of HA.

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