

ACIDITY OF HUMIC ACID RELATED TO ITS OXYGEN-CONTAINING FUNCTIONAL GROUPS

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Acidic properties of polyprotic acids, such as humic acid, can be described quantitatively with potentiometric titration interpreted with the modified Henderson-Hasselbalch equation. Oxygen-containing functional groups such as carboxylic, phenolic, and alcoholic groups are the most important groups that contribute to acidity of humic acid. Those groups in isolated soil humic acid are selectively blocked by methylation with $\text{CH}_3\text{I}/\text{Ag}_2\text{O}$ in dimethylformamide. The obtained values for acidic group contents, the apparent and intrinsic dissociation constants for underivatized and derivatized HAs, provide direct evidence on the oxygen-containing functional groups contribution to HA acidity, especially the contribution of alcoholic hydroxyls, which is usually neglected and ascribed to phenolic groups content.

Key words: humic acid; acid-base titration; Henderson-Hasselbalch equation; oxygen-containing functional group content

КИСЕЛОСТА НА ХУМИНСКАТА КИСЕЛИНА СПОРЕД СОДРЖИНАТА НА КИСЛОРОДНИТЕ ФУНКЦИОНАЛНИ ГРУПИ

Киселинските својства на полипротичните киселини како што е и хуминската киселина, можат квантитативно да се опишат со потенциометриска титрација интерпретирана со помош на модифицираната Хендерсон-Хаселбахова равенка. Кислородните функционални групи, како што се карбоксилните, фенолните и алкохолните групи, се најважни групи кои придонесуваат за вкупната киселост на хуминската киселина. Овие групи во изолирана почвена хуминска киселина се селективно блокирани со метилација со $\text{CH}_3\text{I}/\text{Ag}_2\text{O}$ во диметилформамид. Добиените вредности за содржината на киселите групи, привидните и карактеристичните константи на дисоцијација за недеривитизираните и деривитизираните хумински киселини, овозможуваат директен увид во придонесот на кислородните функционални групи за киселоста на хуминските киселини, а посебно во придонесот на алкохолните групи, кој обично е запоставен и се припишува на содржина на фенолните групи.

Клучни зборови: хуминска киселина; ацидобазна титрација; Хендерсон-Хаселбахова равенка; содржина на киселите групи

INTRODUCTION

Humic acids (HA) are heterogeneous macromolecules derived from chemical, physical and biochemical degradation of organic matter [1]. Their significance comes from the ability to bind and mobilise metals and anthropogenic organic contaminants [2, 3]. The presence of HA in terrestrial and aquatic ecosystems have been shown to

increase photo-degradation of contaminants, decrease biological uptake and toxicity of heavy metals, and increase water solubility of highly insoluble organic compounds. However, the understanding of the mechanisms of their environmental behavior has been hindered by the fact that they are assemblage of large polyfunctional molecules such as lipids, carbohydrates, aromatics, etc. Thus, the interaction of HA with different contaminants de-

depends on the types and amounts of its functional groups [1].

Due to the huge environmental importance of those functional groups, efforts have been made to determine their content in HA, as well as their contribution to the overall humic proton/metal interaction. These ionizable sites are oxygen, nitrogen or sulfur atoms. Thus, the commonly encountered ionizable functional groups include carboxylic acids, phenols, alcohols, ammonium ions, and thiols. To a lesser extent, sulfonic acids and "active methylene" compounds (with $-\text{CO}-\text{CH}_2-\text{CO}-$ structural moiety) are also encountered [4].

The major binding sites are attributed to the oxygen-containing functional groups – carboxylic and phenolic groups, which contribute to the total acidity (TA) of humic substances. Phenolic groups, determined as a difference between TA and carboxyl acidity (CA), are usually assumed to be the only weak acidic groups, while the contribution of alcoholic groups in carbohydrate entities and enols is usually neglected [4]. Before all the weakly acidic groups are assumed to be phenols, the structural implications of such an assumption should be examined. There is no direct evidence for the contribution of alcoholic hydroxyls to the total humic acidity, and the assumption that due to low content of those acidic groups they are relatively unimportant in comparison with phenol groups has never been systematically investigated. Numerous methods of analysis for determination of HA oxygen-containing functional groups have been published [4]. Those methods include direct and indirect potentiometric titrations [5], thermometric titrations, nonaqueous titrations, infrared spectroscopy [6], and ^{29}Si -nuclear magnetic resonance (NMR) [7]. Beside those methods, some authors studied the differentiation between various hydroxyl groups of HA by derivatization [8, 9] or by applying ^{13}C -NMR spectroscopy after using ^{13}C -enriched derivatization agents [10, 11]. These modification procedures, resulting in HA with selectively blocked functional groups, could be used for the estimation of the effect of each blocked functional group on the total humic acidity. The modified HA derivatives, when compared to unmodified HA, could be used for investigation of the influence of the blocked functional groups on the acidic properties of HA.

In the present work, we estimated the contribution of carboxylic, phenolic, and alcoholic hydroxyl functional groups on the total humic acidity by potentiometric titration of humic acid with selectively blocked carboxyl and phenol groups.

EXPERIMENTAL

Extraction, purification and characterization of humic acid

HA was obtained following the procedure suggested by IHSS [12] from a well-humified organic horizon of old beech-forest soil (10 cm depth), in the autumn of 2003, using standard grinding equipment. The soil was air-dried and sieved to pass a 2.0 mm sieve.

Briefly, HA was extracted from the soil with 0.1 mol dm^{-3} NaOH at 1:10 solid to solution (mass/volume) ratio under N_2 for 4 hours. The suspension was then centrifuged at 4500 rpm for 30 min, the supernatant was acidified with 6 mol dm^{-3} HCl to pH 1.0, and the suspension was allowed to stand overnight. Precipitated HA was separated from fulvic acid by centrifugation at 4500 rpm for 30 min. The HA precipitate was redissolved in 0.1 mol dm^{-3} KOH, under N_2 . Solid KCl was added to attain 0.3 mol dm^{-3} (K^+), and the suspension was centrifuged at high speed to remove suspended solids. The HA was reprecipitated with 6 mol dm^{-3} HCl to pH 1.0. The HA precipitate was suspended in a solution of 0.1 mol dm^{-3} HCl/ 0.3 mol dm^{-3} HF. In order to minimize the ash content, this procedure was repeated three times. The acid was then dialyzed against distilled water until the Cl^- ions were eliminated. Bound metal ions were removed by putting the HA solution (at pH 8 adjusted with 0.1 mol dm^{-3} NaOH) into a $300 \times 10 \text{ mm}$ column with ion-exchange resin Dowex 50W-X8 (H^+ -form) at a flow rate of 0.5 ml min^{-1} . The protonated HA was diluted in a volumetric flask and stored at 4°C . The mass concentration of the stock was determined at 1 g l^{-1} , by dry weight of measured volume of well homogenized HA suspension, and it was stored in the dark, at 4°C .

The elemental composition (C, H and N) of isolated HA was determined directly with Vario El 3, while oxygen was obtained by the difference. UV/VIS spectra of underivatized HA (4 mg) dissolved in 100 ml 0.05 mol dm^{-3} NaHCO_3 solution was recorded on a Secomam Anthelie 306 spectrophotometer, using wavelengths ranging from 200 to 1000 nm. FT-IR spectra of HA and methylated HA were recorded on KBr pellets [HAs (1.0 mg) and KBr (150.0 mg)] using a Bomem Hartman & Braun MB-Series FT-IR spectrophotometer. TA (the Baryta method) and CA (the Ca-acetate method) were determined according to Schnitzer and Gupta [13]. Phenolic acidity was determined by the difference between TA and CA.

Methylation of humic acid

Chemical modification specific to carboxyl and phenol groups was achieved by $\text{CH}_3\text{I}/\text{Ag}_2\text{O}$ methylation in dimethylformamide (DMF) solution [9, 14]. Very strong methylating agents, such as, sodium hydride-methyl iodide, are applied for complete derivatization of all hydroxyl groups in HS, and are capable of derivatizing even hindered and tertiary hydroxy groups [8]. However, methyl iodide with silver oxide derivatize only carboxyl and phenol groups and thus, can be used to differentiate between those groups on one hand, and aliphatic hydroxyl groups, on the other hand [14]. Due to its selectivity and efficiency, the method was suitable for functionally assessment of very heterogeneous humic macromolecule.

We synthesized methylated HA starting from the isolated soil HA. The procedure was based on that of Schnitzer and Skinner [14] but was modified and is thus described in details. HA (300 mg) and DMF (40 ml) were put in a 250-ml flask. Ag_2O (900 mg) and CH_3I (8 ml) were added to the resulting suspension. The flask was stopped and shaken for 48 h at room temperature. The suspension was centrifuged for 15 min at 3000 rpm. After separating the supernatant, the centrifuged solid was washed with methylene chloride and this solution was mixed with the supernatant liquid. After solvent evaporation and drying of the solid, the methylation was repeated. Twice methylated derivative (MHA) with methyl esters of carboxyl groups and methyl ethers of phenolic groups was obtained (Fig. 1).

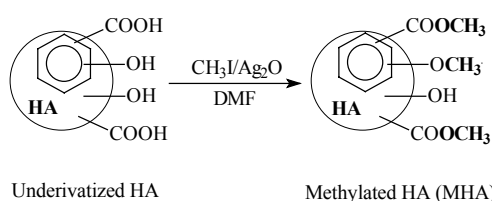


Fig. 1. Reaction scheme for the synthesis of methylated HA

Potentiometric titrations and data treatment

A suspension of HA was prepared in 0.1 mol dm^{-3} NaCl by diluting an appropriate volume of the stock suspensions with $25.00 \text{ ml } 0.2 \text{ mol dm}^{-3}$ NaCl into a 50 ml volumetric flask and bringing the volume up to 50 ml with decarbonated water. The whole volume was transferred into a titration vessel. The mass concentration of the resulting suspension was 496.8 mg dm^{-3} . A suspension of MHA was prepared in the same manner as the HA

suspension. The suspension (40.0 ml) was transferred into a titration vessel. The mass concentration of the resulting suspension was 900.0 mg dm^{-3} . Potentiometric measurements were made with Hach sension 3 pH-meter (precision of 0.1 mV or 0.001 units of pH) using Hach gel-filled combination glass electrode (51935-00). Throughout the titrations an N_2 atmosphere was maintained over the solution, which was thermostated in a water bath at $25.0 \pm 0.1^\circ\text{C}$. After introduction of the electrodes, burette tip and the N_2 conducting tube, the titration vessel was closed and maintained at a slight over pressure of nitrogen in order to exclude atmospheric CO_2 . Titrations were performed from pH 3 to 11 with standardized, CO_2 -free solution of NaOH ($0.0970 \text{ mol dm}^{-3}$ for the HA suspension titration and $0.0074 \text{ mol dm}^{-3}$ for the MHA suspension titration, with the same NaCl concentration as the titrated humic acids suspensions). The potential was read only when its variation was smaller than $0.555 \text{ mV min}^{-1}$, which was monitored by the drift control of the pH meter.

The acid-base properties of HA, the acidic group content (C_{AG}), and the average values of apparent pK (pK_{app}), were evaluated by potentiometric titration with the modified Henderson-Hasselbalch interpretation. The modified Henderson-Hasselbalch equation is usually used for analyzing potentiometric titration curves for weak polyacids:

$$\text{pK}_{\text{app}} = \text{pH} + \log((1 - \alpha)/\alpha),$$

where pK_{app} and α are the negative logarithm of the apparent acid dissociation constant and the degree of neutralization, respectively. The degree of neutralization, α , of the HA, at each point of alkali addition, is defined by the equation:

$$\alpha = ([\text{base}] + [\text{H}^+] - [\text{OH}^-])/C_{\text{AG}},$$

where $[\text{base}]$, $[\text{H}^+]$, and $[\text{OH}^-]$ are the molarities of the added base, free hydrogen ion, and hydroxide ion, respectively, and C_{AG} is the acidic group content (the total concentration of titrable acid groups), in mmol g^{-1} , determined by a modified Gran relationship [15]. The $[\text{H}^+]$ and $[\text{OH}^-]$ ions were obtained from the pH values, assuming that the activity coefficient is unity. Therefore, $\alpha = 1$ at complete neutralization. Plots of pK_{app} versus α for a monomeric acid yield a unique line with a slope of zero. However, if the molecule exists as a polymeric assembly, such as humic macromolecule, a plot of pK_{app} versus α can give a quantitative measure of the nonideal behavior by measuring any deviation from a straight line of the zero slope

[16]. The intrinsic dissociation constant (pK_{int}) is obtained by extrapolating the pK_{app} versus α curve to $\alpha = 0$ and reading off the pK_{app} value as the negative logarithm of the intrinsic dissociation constant, while the average pK_{app} value corresponds to the pK_{app} value at $\alpha = 0.5$.

RESULTS AND DISCUSSION

The elemental composition of isolated soil HA is: C (%) 55.74, O (%) 36.55, H (%) 5.45, N (%) 0.86 and S (%) 1.40. All the obtained data were consistent with typical values for soil HA [1].

Since phenolic substances, aniline derivatives, benzoic acids, polyenes, and polycyclic aromatic hydrocarbons, which are main precursors or components of terrestrial HS, show absorption in the region of 270 – 280 nm, molar absorptivity at this wavelength yields estimation of the degree of aromaticity ($Ar.$), extent of humification, and molecular weight (Mw) [17]. According to Chin et al. [17], the following equations provide quick estimation of HA size and their aromatic contents:

$$Ar. (\%) = 0.05 \cdot \epsilon_{280} + 6.74$$

$$Mw = 3.99 \cdot \epsilon_{280} + 490$$

The data derived from the UV/VIS spectrophotometric measurements for isolated HA were consistent with the typical values for humic substances [17], and are shown in Table 1.

Table 1

Parameters derived from UV/VIS spectrophotometric measurements of HA sample.

	ϵ_{280} ($\text{l mol}^{-1} \text{cm}^{-1}$)	$Ar.$ (%)	Mw
Humic acid	263	20	1540
Literature values range [17]	122 – 919	12.6 – 27.4	–

The FTIR spectrum of unmodified HA sample (Fig. 2) exhibits the typical major peaks for humic acid: OH stretching vibration and possibly NH stretching vibration ($3300 - 3500 \text{ cm}^{-1}$), CH stretching vibration from $-\text{CH}$, $-\text{CH}_2$ and $-\text{CH}_3$ ($2800 - 3100 \text{ cm}^{-1}$), $-\text{C}=\text{O}$ stretching from $-\text{COOH}$ (1700 cm^{-1}), aromatic $\text{C}=\text{C}$ stretching vibration and/or asymmetric $-\text{COO}^-$ stretching vibration ($1600 - 1650 \text{ cm}^{-1}$), $-\text{OH}$ and $-\text{CO}$ deformation vibration from alcoholic and phenolic $-\text{OH}$ and/or $-\text{COO}^-$ symmetric stretching (1400 cm^{-1}), and $-\text{CO}$ stretching vibration and OH bending vibration of $-\text{COOH}$ ($1200 - 1220 \text{ cm}^{-1}$).

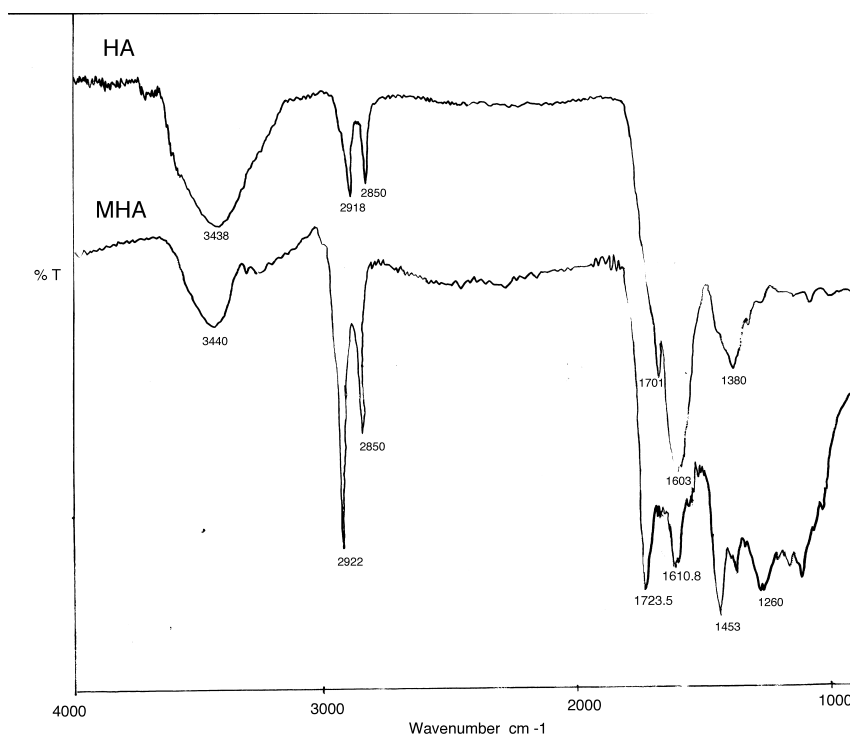


Fig. 2. FT-IR spectra of the unmodified humic acid (HA) and methylated humic acid (MHA)

The FTIR spectrum of MHA (Fig. 2) indicates that methylation of the carboxyl and phenol groups occurred, as seen from the differences in the IR absorption before and after the derivatization. There are three important IR regions related to this derivatization study: (1) 3400 cm^{-1} absorption due to OH stretching vibrations in carboxyl, phenol, and hydroxyl groups; (2) 2920, 2850 and 1450 cm^{-1} absorption due to aliphatic C—H vibrations, and (3) 1720 cm^{-1} absorption due to stretching vibration of $\text{C}=\text{O}$ ester groups. Absorption in the 3400 cm^{-1} region decreased in the spectra of MHA (compared to a broad and strong peak of unmodified HA) as less hydroxyl groups are present, indicating effective derivatization. However, the hydroxyl OH stretching region is not completely eliminated by the methylation, indicating that hydroxyl groups have been methylated only partially. This peak may be assigned to the alcoholic hydroxyls which are not methylated under these conditions. Higher OCH_3 content of the MHA derivative is also indicated by the stronger bands for C—H stretching at 2920 and 2850 cm^{-1} , and C—H bending at 1450 cm^{-1} , than in HA. The intensity of the band at 1724 cm^{-1} , attributable to stretching vibration of the $\text{C}=\text{O}$ ester groups, was increased by methylation, as could be seen in Fig. 2, which confirms the carboxyl groups esterification. The methylation also changed the shape of the 1100 – 1450 cm^{-1} region of the MHA derivative, indicating that esters and ethers formations occurred.

The results of the potentiometric titration of original and methylated HAs show that C_{AG} was decreased after derivatization due to formation of methyl esters of carboxyl groups and methyl ethers of phenolic groups.

Table 2

The acidic group contents, phenol group contents, total (TA) and carboxyl acidity (CA), average pK_{app} values, and intrinsic dissociation constants (pK_{int}) of unmodified HA and methylated humic acids (MHA)

	HA	MHA
$C_{\text{AG}} / \text{mmol g}^{-1}$	4.63 ± 0.25	0.2 ± 0.04
TA / mmol g^{-1}	4.68	0.20
Phenolic OH / mmol g^{-1}	1.88	—
CA / mmol g^{-1}	2.80	—
Average pK_{app}	4.08	5.46
Intrinsic pK_{int}	3.8	5.5

The difference between C_{AG} values for HA and MHA could be assigned to the content of both carboxyl and phenolic groups, while the obtained C_{AG} value for MHA provide estimation of the remaining weakly acidic group content. Thus, the value of $0.2 \pm 0.04 \text{ mmol g}^{-1}$ could be assigned to alcoholic groups in carbohydrate entities and enols. The contribution of those weakly acidic groups to TA is only 4.3 % (0.20 mmol of weakly acidic groups compared to 4.68 mmol of total acidity groups), whereas those same groups contribute even 10.6 % to the phenolic groups in HA (0.20 mmol of weakly acidic groups to 1.88 mmol of phenolic groups). This confirms that phenolic content is usually overestimated when it is determined by the difference between TA and CA.

The Henderson-Hasselbalch plot (pH vs. $\log((1-\alpha)/\alpha)$) is shown in Fig. 3 for underivatized and derivatized HAs. For HA, the decrease in pH is not linear, confirming that HA possesses more than one type of functional ionizable group [18]. However, a linear decrease in pH for MHA is observed, which corresponds with polymeric compounds bearing acidic groups that are chemically identical, as polyacrylic acid [3, 18]. This confirms the significant reduction in the number of different ionic group types in humic structure after applied derivatization. The pH at $\log((1-\alpha)/\alpha) = 0$, corresponds to the average pK_{app} values of HA and MHA, which are summarized in Table 2.

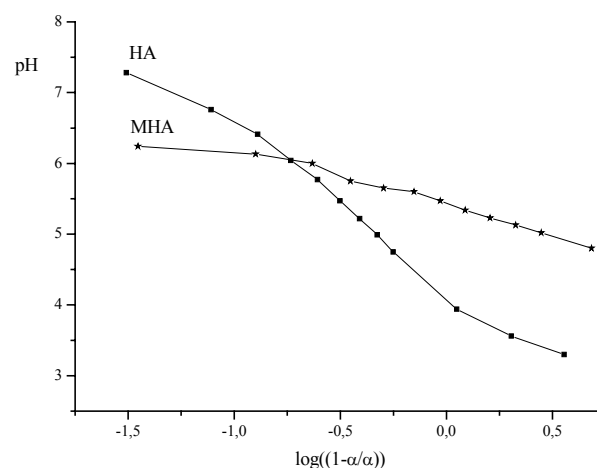


Fig. 3. Henderson-Hasselbalch plot of HA and MHA

The average value of pK_{app} for HA is 4.08, which is in agreement with other reports [18]. The underivatized HA is stronger acid than MHA, as it possesses free carboxylic, phenolic and alcoholic groups. The higher pK_{app} value for MHA ($pK_{\text{app}} =$

5.46) compared to HA, confirms the blocking of carboxyl and phenolic groups.

Fig. 4. shows the comparison of α vs. pK_{app} curves of HA and MHA. The curve for MHA is almost constant and independent of α , suggesting that deprotonation occurs in one step, while the curve for HA is typical for polyacids as it depends on α . The change in pK_{app} over the range of α values is larger for HA ($\Delta pK_{app} = 2.0$ pK units) compared to MHA ($\Delta pK_{app} = 0.2$ pK units). This parameter can give a quantitative measure of the deviation from a straight line of a zero slope which is characteristic for monomeric acid [16]. Thus, higher pK_{app} distribution was found in unmodified HA, polyprotic acid with the greater number of different ionizable groups, while MHA is comparable to monomeric acid.

The intrinsic dissociation constant, pK_{int} is increased after derivatization (Table 2). Its value is also related to the different chemical structure of the acid. It is known that pK_{int} is related to the dissociation constant when there is no electrostatic interaction from any other ionized group on a particle, whereas the pK_{app} contains an additional contribution related to the extra work when transferring the proton from the polyanion to the bulk. Thus, pK_{int} is related only to the chemical nature of the ionizable groups. The pK_{int} value that was determined implies that MHA possesses weaker acidic groups in a free form than unmodified HA.

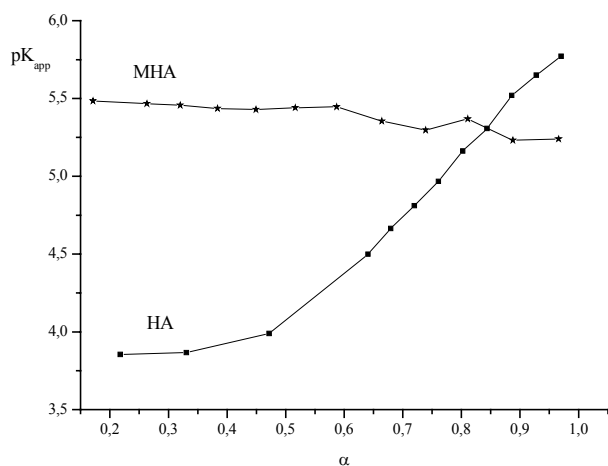


Fig. 4. pK_{app} vs. α plot for HA and MHA

CONCLUSION

Selective blocking of carboxyl and phenol groups combined with potentiometry can provide direct estimate of the effect of blocked and unblocked functional groups on total humic acidity.

FTIR spectra indicates that the applied methylation with CH_3I/Ag_2O in DMF causes the intended structural changes. The values of pK_{int} , pK_{app} and pK_{app} distribution increase by derivatization, confirming the reduction in the number of free ionizable groups. Their values are related to the different chemical structure of the acids: lower values for the underivatized HA bearing free carboxylic, phenolic, and alcoholic groups, and higher values for MHA bearing free alcoholic groups. The C_{AG} of MHA, 0.2 ± 0.04 mmol g^{-1} , is assigned to alcoholic and enolic groups. The values obtained show that the phenolic groups content of isolated soil HA, determined by the difference between TA and CA, is overestimated for even 10.6 %.

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