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Molecular compositions of humic acids extracted from leonardite and lignite as determined by Fourier transform ion cyclotron resonance mass spectrometry

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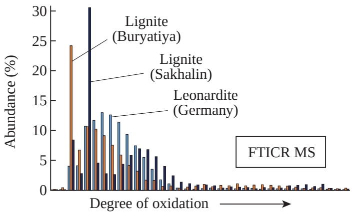
Fourier transform ion cyclotron resonance mass spectrometry reveals substantial differences in the structural motifs of lignite and leonardite humates, which can be used for the standardization and molecular systematics of humic substances and for the directed design of humate-based products.



Humates are widely used in agriculture as growth promoters and organic fertilizers. The major raw material for their production is lignite and, in particular, its most oxidized variety, leonardite, which is the richest source of humic substances (HS).[1–3] Recently, detailed information on the molecular compositions of HS from peat, soil and water reservoirs was obtained by Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS).[4,5] This ultrahigh resolution technique affords mass resolving power ($m/\Delta m_{50\%} > 350\,000$, where $\Delta m_{50\%}$ is the mass spectral peak full width at half-maximum peak height) and mass accuracy (<1 ppm) allowing for molecular formula assignment to the constituents of complex mixtures.[6–8] Electrospray ionization (ESI) FTICR MS is most frequently used for the compositional analysis of HS, which possess immense structural heterogeneity.[9] Despite active studies in this field, data on the molecular composition of HS from different coal deposits are still missing. They could be very useful for the comparative analysis of humates extracted from different types of coal and for inventorying the molecular components of coal HS in view of improving humate quality and developing new nonfuel applications of coal. Here, we report the results of the FTICR MS analysis of coal HS isolated from three types of lignite and discuss the common and unique features of their molecular composition.

Coal humic acids (CHA) were isolated from potassium humate produced from leonardite (Powhumus, Germany), lignite (Sakhalin Humate, Russia) and a sample of lignite from the deposit in Buryatia (Russia). The samples were designated as CHA-Pow, CHA-SH and CHA-GL, respectively. The CHA samples were isolated according to a standard IHSS procedure,[10] redissolved in 10% ammonia and diluted by HPLC grade methanol to a final concentration of 1 g dm^{-3} prior to FTICR MS analysis. The full-scan negative ion ESI FTICR mass spectra were acquired using a commercial 7 Tesla LTQ FT ultra mass spectrometer equipped with an Ion Max electrospray ion source (Thermo Electron Corp., Germany) located at the facilities of the Institute of Biochemical Physics of the Russian Academy of Sciences. Formula assignment was performed using the lab-made Transhumus software based on a total mass difference statistics algorithm.[11]

ESI FT-ICR MS resolved more than 4000 compositionally distinct $C_cH_hN_nO_oS_s$ compounds in CHA-Pow and CHA-SH and about 3000 in CHA-GL (Figure 1).

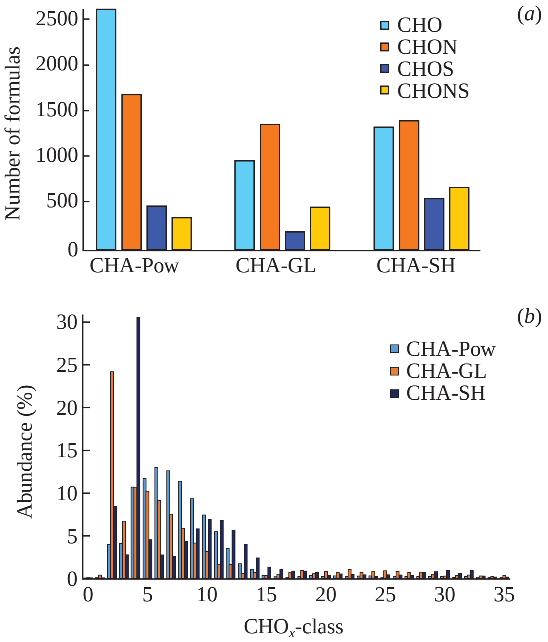


Figure 1 (*a*) The amount of different elemental composition assignments (CHO, CHON, CHOS and CHONS) made from the ESI FTICR MS data for the three CHA test samples and (*b*) oxygen distribution among different CHO$_x$ classes in these samples.

Figure 1(*a*) shows that the highest CHO content of compositions was characteristic of CHA-Pow extracted from highly oxidized lignite (leonardite), whereas the two samples extracted from lignite (CHA-SH and CHA-GL) were characterized by much lower CHO contents and higher contributions of CHON and CHNOS. The largest amount of CHON formulas in the CHA-GL sample corroborates well the elemental analysis data, which showed the highest nitrogen content of CHA-GL (2.9%), as compared to 1.3 and 1.7% in CHA-Pow and CHA-SH, respectively. Figure 1(*b*) shows the segregation of the assigned molecular formulas into compound classes with respect to the number of constituting O atoms (CHO_x classes). Again, substantial differences can be seen in O distribution among leonardite and lignite HA: while the CHA-SH and CHA-GL samples are dominated by CHO_4 and CHO_2 classes, respectively, the CHA-Pow is characterized by a maximum of oxygen distribution in the higher molecular weight species, which is consistent with the highest oxidation degree of this coal. A bimodal distribution of CHA-SH oxygen classes probably indicates the presence of two different groups of molecules: saturated (lipids, fatty acids) and aromatic (condensed tannins) compounds.

We have plotted all assigned formulas into Van Krevelen diagrams, which represent the plots of H/C *vs.* O/C ratios (Figure 2). These diagrams were introduced for a graphical-statistical analysis of coal genesis,[12] and they were extended later for the interpretation of FTICR MS data of HS.[13]

A comparison of the VK diagrams in Figure 2 shows that CHA-Pow had the most unique molecular composition due to the presence of densely populated regions with $1.0 < H/C < 1.5$ and $0 < O/C < 0.5$, which were scarcely populated in case of CHA-SH and almost void in case of CHA-GL. This is indicative of the presence of numerous lignitic and aliphatic carboxyl-containing compartments, which is in line with the observed oxygen distribution among molecular components of the three test samples [Figure 1(*b*)]. The presence of abundant aromatic compounds (including CHON, CHOS, and CHONS molecules) with $0.3 < H/C < 0.8$ and $O/C < 0.4$ can be seen for all of the three samples. The high abundance of heteroatoms in these compartments is indicative of the presence of heterocyclic compounds (*e.g.*, pyridine, pyrrole, and thiophene).[14] In opposite to CHA-Pow, lignite HA – CHA-SH and CHA-GL also possess highly oxidized sulfur-containing molecules [Figure 2(*b*),(*c*)] such as sulfonates. The highlighted regions in Figure 2(*d*) indicate unique features in the molecular spaces of all samples. As mentioned above, CHA-Pow possesses high density of lignitic and carboxyl-containing aliphatic molecules, and CHA-GL contains a black carbon region, which relates to condensed aromatic molecules in coal,[15] whereas CHA-SH is characterized by the presence of the most oxidized sulfonic compounds. All of the samples possessed about 400 common identifications [Figure 2(*d*)] related to tannin-like compounds constituting the most conservative humic region.

Table 1 Fractions of molecules with AI related to non-conjugated and conjugated aromatics in the test coal humic acids.

Sample of coal HA	Fraction with AI ⩾ 0.67 (%)	Fraction with AI ⩾ 0.5 (%)
CHA-Pow	10	33
CHA-GL	24	58
CHA-SH	16	41

For revealing the specific features of aromatic compartments present in each of the sample, the aromaticity index (AI) was calculated according to Koch and Dittmar.[16] A threshold value of $AI \geqslant 0.5$ indicates the presence of nonconjugated aromatic structures, whereas $AI \geqslant 0.67$ is an unambiguous criterion for the presence of condensed aromatic structures in a molecule. The corresponding values were calculated and presented in Table 1 as the percentage of compounds with $AI \geqslant 0.5$ and $AI \geqslant 0.67$.

The calculated aromaticity indices indicate that CHA-GL possesses the highest portion of molecules with condensed aromatic rings (24%), whereas CHA-Pow has the least contribution of these compartments (10%), and CHA-SH takes an intermediary position. At the same time, it can be clearly seen that aromatic compounds represent a very significant part of coal HA, which is in accordance with the chemical nature of coal.[15] It is of importance that the sample extracted from leonardite (CHA-Pow) is characterized by the lowest amount of aromatic structures.

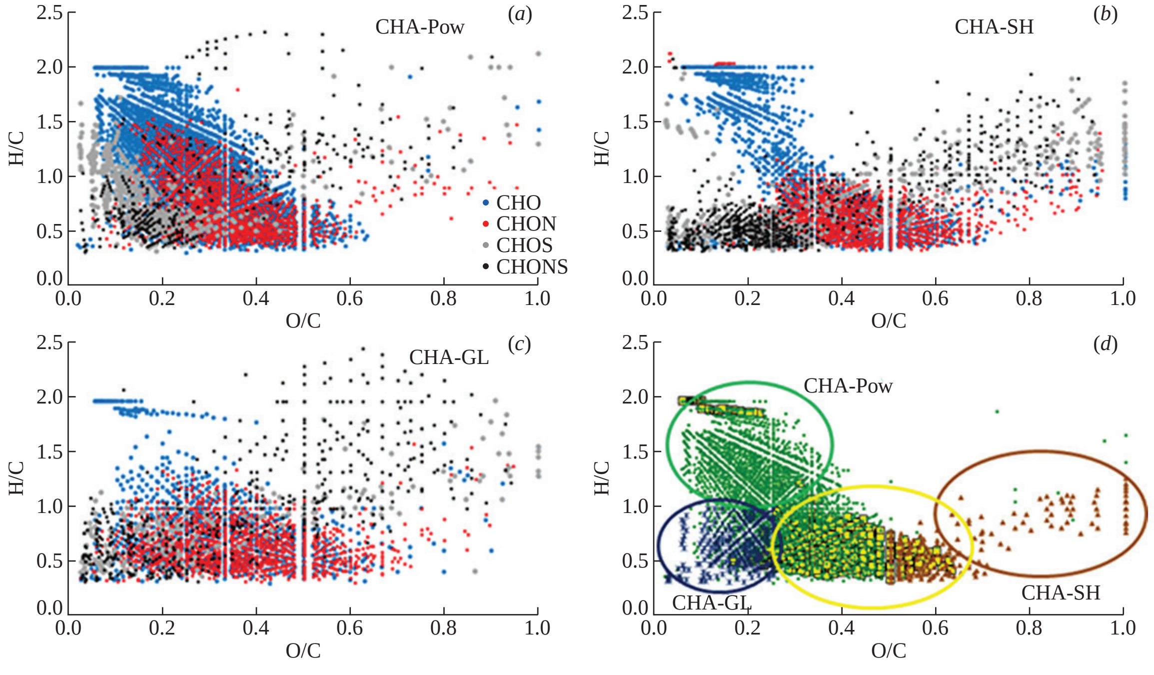


Figure 2 Van Krevelen diagrams for coal humic acids from three sources: (*a*) CHA-Pow, (*b*) CHA-SH, (*c*) CHA-GL; (*d*) common and unique molecular compartments in three test CHA: dark blue dots designate unique compositional region of CHA-GL, green – in CHA-Pow, brown – in CHA-SH, and yellow dots represent common compositions for all samples.

This observation along with its enrichment with oxygen containing and CHO-only compounds is consistent with the specific features of leonardite materials.

Thus, FTICR MS is a powerful method of structural analysis capable of distinguishing specific molecular motifs within complex organic matrices even of similar origins. This was demonstrated on the example of humic acids extracted from two lignite samples and the oxidized variety of lignite, leonardite. The identification of molecular constituents extracted by the same traditional alkaline technique showed very distinct molecular features of leonardite humates as compared to those of lignite. These structural differences can give peculiar chemical properties to HA isolated from different coals. We anticipate that the presented results might be used for both the standardization of coal humates and the directed design of new humate-based products.

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