Comparative study on humic substances isolated in thermal groundwaters from deep aquifers below 700 m

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Main objectives of this study were to investigate whether humic substances (HS) exist in hot groundwater from deep basinal aquifers waters and to provide basic knowledge on humic (HA) and fulvic acids (FA) isolated from these fluids. Water sampling and HS isolation was carried out in three consecutive years from wells tapping the aquifers of the Pannonian Basin (Southeast Hungary). The isolated humic acid and fulvic acid samples were studied by elemental analysis, Fourier transform infrared (FTIR) spectroscopy, and Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI-FT-ICR/MS). We found that modified sampling (acidification of water samples either immediately or after cooling down) had no traceable effect on the properties of isolated HAs and FAs. The quantity and quality of humic materials did not change in three years. The analytical results clarified distinctive chemical characters of HS in the deep aquifer groundwater compared to the Suwannee river fulvic acid (SRFA) as a reference. In addition, we stated that aliphatic content of isolated HAs and FAs increased with increasing aquifer depth and temperature, but the aromaticity and heteroatom content decreased in both fractions.

INTRODUCTION

Major components of natural organic matter in natural waters are humic substances (HS) as the refractory decomposition products of biological material (Thurman, 1985; Perdue and Ritchie, 2003). The interest in studying HS from shallow to deep aquifers has increased recently (Artinger *et al*., 2000; Buckau *et al*., 2000; Hertkorn *et al*., 2002; McIntyre *et al*., 2005; Einsiedl *et al*., 2008). Most geochemical studies of thermal waters are focused on isotope composition of the water as well as major and minor dissolved ions or trace elements (Varsányi *et al*., 1997; Kralj, 2004; Yalcin, 2007; Parello *et al*., 2008; Fekete *et al*., 2009). The investigation of organic compounds in thermal waters has attracted much less attention apart from some exceptions (Kárpáti *et al*., 1996, 1999; Varsányi *et al*., 2002; Di Gioia *et al*., 2006; González-Barreiro *et al*., 2009; Fekete *et al*., 2009). The origin of HS isolated from groundwater of different depths on the basis of elemental and isotopic composition as well

as spectroscopic characteristics was discussed in some studies (Wassenaar *et al*., 1990; Grøn *et al*., 1996; Artinger *et al*., 2000). Thurman (1985) studied several properties of HS isolated from $Ca(HCO_3)_2$ and $CaSO_4$-rich groundwater originating from different depths in sandy, dolomitic or limy aquifers. He found that groundwater containing organic carbon concentration greater than 1 mg/L either may originate from aquifers receiving recharge from organic-rich waters or may be in contact with sediments rich in kerogen. However, the investigation of HS from deep groundwater or geothermal water has received relatively little attention compared with other environments (Thurman, 1985). The isolation of humic acid (HA) and fulvic acid (FA) fractions from thermal waters circulating in deep aquifers of the Pannonian Basin has been carried out recently (Kovács *et al*., 2010). Based on our knowledge, our group was the first who isolated HS from deep thermal waters. However, thermal waters from Pannonian Basin were studied earlier by Sajgó *et al*. (1998), Kárpáti *et al*. (1999) and Fekete *et al*. (2009) that found several aromatic families in hot waters with outflow temperature over 70–80°C. They suggested that the appearance and abundance of aromatic hydrocarbons, alkyphenols and fatty acids in thermal waters is control-

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led by water temperature. Observed compounds were generated from organic materials available in aquifers.

One objective of this study is to investigate whether HS really exist in thermal waters or not. Catalytic synthesis of HS by natural clays or oxides is well known (Shindo and Huang, 1982, 1985; Wang and Huang, 1989, 2000). On the other hand, Giannakopoulos *et al*. (2009) recently demonstrated that a humic acid-like polymer (polycondensate) can be synthesized with high yield via oxidative polymerization of simple organic acids at pH > 9 under ambient O_2, with no need for a catalyst. In our concept, the acidification of water samples can inhibit polymerization/polycondensation reactions of organic precursors to produce humic-like polymers. A modified sampling methodology based on acidification at different times after recovery of thermal water samples can reveal if HS was originally present in thermal water. Furthermore, the effect of acidification at different times on the properties of isolated HS is important to be studied. Consequently the changes in conditions such as temperature and presence of oxygen do or do not cause any observable alteration of the properties of humic substances after the groundwater is pumped out. Additional aim is to provide basic data and knowledge on humic and fulvic acids isolated from deep thermal waters. In this study we investigated the chemical similarities and/or differences of isolated HAs and FAs as a function of the aquifer's depth and temperature.

Fig. 1. Location of the wells indicated by the borehole depths.

MATERIALS AND METHODS

Sampling area

The sampling area is located near the town of Makó in the southeastern part of Hungary, within the Pannonian Basin (Fig. 1). The water samples were from Makó Through. The western margin of this area is well known for its non-conventional gas accumulations in tight sandstone. Pannonian Basin is a sedimentary basin characterized by a thinned crust and filled with sediments of low heat conductivity which favorize high geothermal gradients (approximately 50°C/km) with a heat flow of 90–100 mW/m^2 (Dövényi *et al*., 2002). The bulk of the basin consists of Late Miocene, Pliocene and Quaternary clastic sediments deposited under brackish to freshwater conditions and containing type III kerogen in the dispersed form of lignite (Varsányi *et al*., 1997; Hetényi, 1992). Two main hydrological systems are associated with geothermal fluids. One of them is in the Upper-Pannonian (Late Miocene and Pliocene) units consisting of clastic porous sandstones between depths of 500 and 2500 m (Erdélyi, 1979). Varsányi *et al*. (1997, 1999) described in detail the geochemistry and hydrogeology of geothermal waters of the Pannonian Basin. They found that the formation waters located in the center of the Pannonian Basin
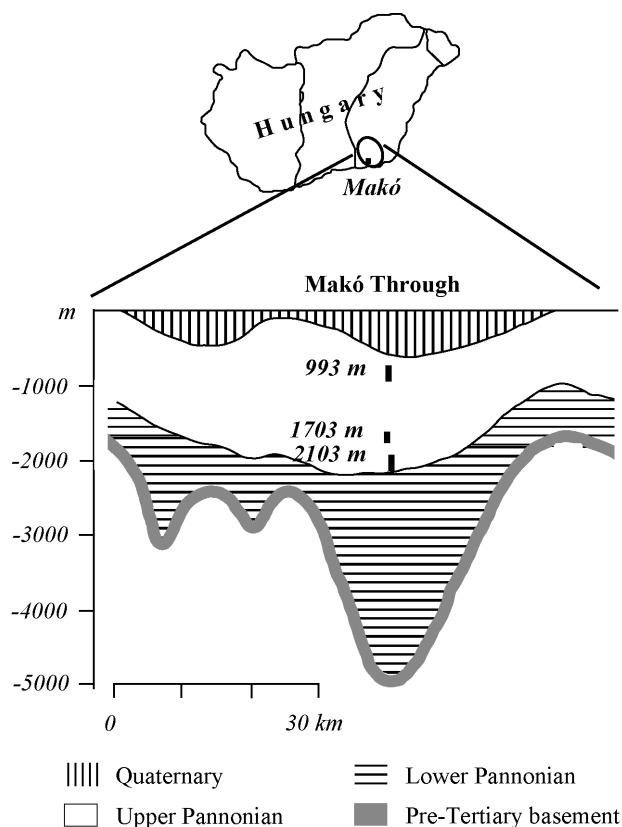
at depths between 75 and 2500 m are meteoric in origin, infiltrated during the last glacial period (Würm glacial, 70,000 to 10,000 years before present).

Samples of thermal water

Samples of thermal water from depths of 993, 1703 and 2103 m were taken in February 2006, January 2007 and February 2008. Some information on the wells and water samples are reported in Table 1. Thermal waters in this area were studied earlier by Sajgó *et al*. (1998) and Kárpáti *et al*. (1999) (samples M-a, M-b and M-c). Two of them are also studied in this paper (M-a, M-b samples), but M-c well (1948–2268 m) was not accessible, so a near well (1968–2099 m) was selected for this study. The top sample (M-a; 752–884 m) was devoid of aromatic hydrocarbons and dissolved C_{2+}gas; the middle one (M-b; 1622–1684 m) contained many aromatic homologues and dissolved C_{2+}gas but free of fatty acids and alkylphenols; and the deepest and hottest sample (M-c) contained aromatic homologues and enriched alkylphenols and fatty acids (details in table II of Sajgó *et al*., 1998; and tables 2–4, figures 2, 5b, 6a, 7 in Kárpáti *et al*., 1999).

Water temperatures were measured at the well exit and

Table 1. Physical and chemical data of wells and thermal waters sampled in this study

No.	Borehole depth	Perforated depth	Water temperature[a]	pH	Na^{+}	HCO$_3^-$	TDS[b]	TOC[c]
	(m)		(°C)			(mg/L)		
1	993	752–884	41.4 (1.5)	8.01 (0.15)	334 (20)	936 (269)	1343 (40)	8.5
2	1703	1622–1684	75.5 (0.9)	7.72 (0.06)	600 (20)	1630 (69)	2438 (47)	17.6
3	2103	1968–2099	83.2 (2.6)	7.48 (0.06)	1027 (31)	2684 (52)	3878 (80)	138.8

[a]*Measured in situ at the surface;* [b]*Total dissolved solid;* [c]*Total organic carbon.*
The mean and standard deviation in parenthesis were calculated from results of chemical analyses done on samples collected in 2006 and 2007 and from additional databases of previous water samplings.

presumably bottomhole temperatures are 15–35°C higher than the temperature values measured at the surface (Fekete *et al.*, 2009). These waters are NaHCO$_3$-type with pH values higher than 7 and more than 1000 mg/L of total dissolved solid (TDS) content. The water samples (30–100 L) were collected in containers initially washed with the sample. Continuous water production of wells ensured that non-stagnant groundwater was taken. In order to investigate the effect of time elapsed after groundwater was pumped out on the properties of water samples as well as isolated HA and FA fractions, the start of isolation procedure was changed. In the case of 2006 and 2007 sampling, the water samples were preserved by adjusting the pH during their cooling (1-day standing). In 2008 sampling, the acidification was performed immediately after the sampling, which means that potential reactions were completely excluded. The acidification at different moments of the isolation process can reveal the presence of HS in thermal waters. In addition, water samples (0.5 L) were also taken without acidification in 2008 in order to carry out measurement without any chemical modification of the water. The acidified samples were stored below 5°C for a few days until processing.

Isolation of humic and fulvic acids

The HA and FA were isolated according to the procedure recommended by International Humic Substances Society (IHSS) (http://www.humicsubstances.org/aquatichafa.html). This protocol has a long tradition within the scientific community and reference materials are available. This allows a direct comparison to existing data on HS from various environments. Based on this method, quantities of low-ash (<1.0 weight %) aquatic HS can be obtained by adsorption chromatography on Amberlite XAD-8 resin, ion exchange and freeze-drying (Thurman and Malcolm, 1981). Isolation procedure started with the acidification of the water sample (pH = 2.0) which made dissolved HA and FA enough hydrophobic to be adsorbed on the XAD-8 resin. After alkaline elution, the eluate was acidified to separate HA from FA since HA precipitates at pH ~ 1 and FA remains in solution. After cation-exchange using Varion KSM resin, H^{+}-saturated HA and FA were obtained. The Suwannee River fulvic acid (SRFA, 1R101F) was obtained from IHSS as a reference material.

Analytical methods

The UV-vis spectra of the original water samples were recorded using a double ray spectrophotometer (Uvikon 930) just after receiving samples at the laboratory. The Na^{+} concentration as well as total organic carbon (TOC) content of water samples were determined within a few weeks after the sample collection using Horiba Jobin Yvon ULTIMA 2 C ICP-OES spectrometer and a Beckman 915B instrument, respectively. The alkalinity, i.e., HCO$_3^-$ concentration was determined on the basis of the values of the *p*- and *t*-alkalinity from the titration of the original water samples to fixed pH end points, i.e., pH 8.3 and 4.5. The $p < 1/2t$ alkalinity relationship was found to be valid to determine HCO$_3^-$ concentration. The mean and standard deviation of temperature, pH, Na^{+}, HCO$_3^-$ and TDS were calculated from results on 2006 and 2007 sampled water as well as additional databases of previous water samplings (personal communication with Dr. Z. Kárpáti) (Table 1). TOC determination of water samples was carried out during the 2008 sampling; these results can be found in Table 1.

The isolated HA and FA were investigated by elemental analysis, FTIR spectroscopy, and ESI-FT-ICR/MS. Elemental analysis (N, C, H, S) was performed on a NA 1500 NCS analyser (Fisons Instruments) at 1010°C and O content was calculated as difference. FTIR spectra were recorded with a Perkin-Elmer 1600 Series spectrophotometer using the KBr pellet technique. Ultra-high resolu-

Table 2. The quantification of humic substances isolated from deep aquifers

No.	Borehole depth	HA	FA	Total humic content	HA:FA	HS:TOC	Absorbance at 420 nm
	(m)			(mg/L)			
1	993	1.9	1.0	2.9	2:1	1:3	0.025
2	1703	2.1	0.9	3.0	2:1	1:6	0.020
3	2103	6.9	2.6	9.5	3:1	1:15	0.056

Humic and fulvic acid concentrations in the waters (HA and FA, respectively), their sums (total humic content) and their ratios (HA:FA) determined by gravimetry after isolation, as well as the ratios of total humic content to total organic content (HS:TOC), and the absorbance values measured at wavelength 420 nm in thermal waters. The standard deviation of the quantification of the humic contents is ±0.2 based on the water sampling from 2006–2008 (3 year).
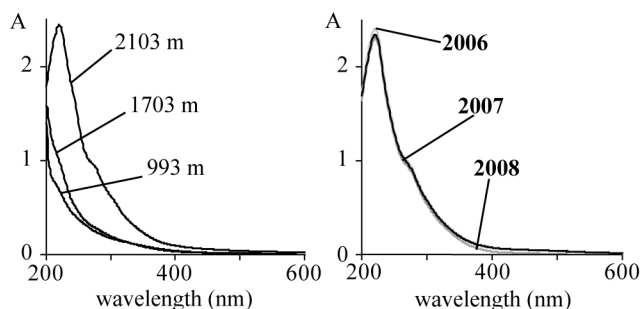

Fig. 2. UV-vis absorption spectra of water samples taken from different depths during the 2006 sampling (left), and taken from depth of 2103 m in 2006, 2007 and 2008 samplings (right).

tion mass spectra were acquired at the Helmholtz Zentrum München with a Bruker APEX Qe Fourier transform mass spectrometer equipped with a 12 Tesla superconducting magnet and an Apollo II electrospray source. Negative ion ESI coupled with FT-ICR/MS allows for the direct detection of acidic compounds without pre-chromatographic isolation. Ultra-high resolution and mass accuracy ensure detailed identification of compounds of extremely complex mixtures such as humic substances (Koch and Dittmar, 2006; Sleighter and Hatcher, 2007; Hertkorn *et al*., 2008). The samples were dissolved in a mixture of methanol and water directly before the analysis and measured at 10 mg/L. The HA solutions were prepared by using small amount of concentrated NH_4OH to complete the dissolution. The spectra were externally calibrated on arginine clusters and then internally recalibrated with fatty acids. The molecular formulae were batch-calculated by a software tool, written in-house (Gáspár *et al*., 2009). Chemical constraints were applied: the maximum number of elements C(100) H(unlimited) O(80) N(5) S(2), tolerance of mass error by ^{13}C isotope search and formulae assignment was ≤1 ppm, N-rule and thresholds for molecular element ratios (0 < H/C < 2.5, 0 < O/C < 1, etc.). Additional details can be found in Kovács *et al*. (2010).

Analysis of water samples

The chemical analyses results show relatively small deviations suggesting the consistency of inorganic composition through time at least in a short period (Table 1). However, distinctive differences can be identified with the depth of wells, related to diagenetic processes. The TDS as well as Na^+ and HCO_3^- contents increase with depth. CO_2 produced by diagenesis of organic matter increases the dissolution of carbonate present in the sediments as well as enhances the hydrolysis of feldspars, which is one the most important Na^+ source (Fekete *et al*., 2009). The water samples show significant UV absorption (Fig. 2), indicating high content of dissolved organic matter as expected from the greater TOC values (Table 1). To illustrate the uniformity of organic matter content in three consecutive years, UV-vis spectra of water samples taken from the same well (of 2103 m depth) are shown in Fig. 2. These spectra do not indicate any mentionable changes in quantity of UV-absorbing organic matter.

HA and FA content of water samples

The IHSS method was adopted successfully for all water samples. However, in the case of thermal water from 2103 m depth, the procedure had to be carried out carefully. After alkaline elution, difference in appearance of the HA precipitates in FA solutions was experienced when pH was adjusted to 1.0 (not shown). Fine flocculated precipitate formed in the samples from both 993 and 1703 m depth. Contrary to them, a sticky HA precipitate was generated in the thermal water from 2103 m depth. The adhesive character of hydrophobic HA precipitate made the separation and further purification steps difficult because of adhering HA particles to the glass- and plastic wares.

The isolation method allowed the gravimetric determination of HA and FA concentration in water yielding a good reproducibility (the standard deviation of the humic contents is ±0.2) even in hot waters (Table 2). The total humic content of all thermal water samples was

Table 3. Elemental composition of HAs and FAs isolated from thermal waters in deep aquifers and SRFA

Humic acids	Borehole depth	C	H	N	S	O	O/C	H/C
	(m)			(wt%)				
1	993	58.78	4.28	2.22	1.70	33.02	0.42	0.87
2	1703	63.35	4.95	1.97	1.20	28.53	0.34	0.94
3	2103	64.40	5.04	1.84	1.32	27.40	0.32	0.94
Fulvic acids	Borehole depth	C	H	N	S	O	O/C	H/C
	(m)			(wt%)				
1	993	53.19	4.13	1.28	1.70	39.70	0.56	0.93
2	1703	56.03	4.85	1.01	1.31	36.80	0.49	1.04
3	2103	58.01	5.48	0.74	0.90	34.78	0.45	1.13
	SRFA	53.04	4.36	0.75	0.46	43.91	0.62	0.99

C, H, N, S and O amounts expressed as weight percentages and their H/C and O/C atomic ratios are given. The standard deviation of elemental analysis for C is ± 1.03, H is ± 0.26, N is ± 0.23, S is ± 0.42 and O is ± 1.41, respectively. The data for the elemental composition of SRFA are obtained from IHSS.

higher than 1 mg/L. Based on this observation it can be supposed that these waters either are associated with organic-rich sediments or receive organic-rich recharge waters (Thurman, 1985). In all cases, the HA content was higher than the FA content in thermal waters, their ratios (HA:FA) range from 2:1 to 3:1. This finding is contrary to that usually observed in surface waters, where FA content is generally higher than HA with the ratio of 1:3 (Perdue and Ritchie, 2003). In addition, the ratio of total HS content to TOC (HS:TOC) decreased with increasing temperature (Table 2). TOC values from Table 1 were used for the calculation. On the basis of the changes of these ratios it can be concluded that HS constitute the decreasing proportion of organic carbon content of thermal waters with increasing aquifer depth, which may suggest the HS decomposition in the deeper aquifers. On the other hand, it can be hypothesized that FA fraction is more sensitive to decomposition than the HA fraction as the relative amount of FA decreased with increasing aquifer depth. The absorbance at 420 nm of our water samples increased as total humic content (which may represent the colored substance) increased (Table 2). Linearity can be seen between the total humic content and the UV-vis absorption. Here we note that in previous studies the HA contents of thermal waters were measured spectrophotometrically at 420 nm using a Fluka humic acid as standard (Varsányi *et al.*, 1997, Kárpáti *et al.*, 1999).

Elemental analysis

Table 3 shows the elemental composition of HS isolated from thermal waters and SRFA. In all samples HAs have higher C and N, but lower O abundances than in
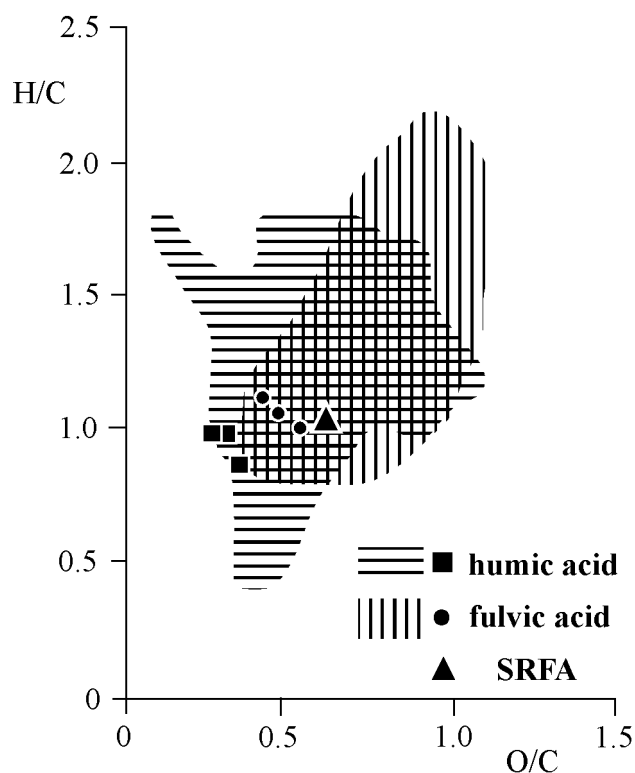

Fig. 3. The characteristic patterns of humic (410 samples; horizontal stripes) and fulvic acid (214 samples; vertical stripes) from different sources in the van Krevelen diagram (adapted from Rice and MacCarthy, 1991). The positions of humic (black squares) and fulvic acids (black circles) isolated from thermal waters in deep aquifers of different depths, as well as that of SRFA (black triangle).

Table 4. Infrared absorbance values belonging to wavenumbers of 2925, 1710 and 1620 cm⁻¹ to represent the aliphatic, carboxyl and aromatic content of the HAs and FAs isolated from thermal waters of different depths

Humic acids	Borehole depth (m)	$A_{2925\ cm-1}$ (aliphatic)	$A_{1710\ cm-1}$ (carboxyl)	$A_{1620\ cm-1}$ (aromatic)	aliphatic/carboxyl	aliphatic/aromatic	carboxyl/aromatic
1	993	0.11	0.52	0.34	0.21	0.32	1.53
2	1703	0.18	0.79	0.42	0.23	0.43	1.88
3	2103	0.17	0.87	0.36	0.20	0.47	2.42

Fulvic acids	Borehole depth (m)	$A_{2925\ cm-1}$ (aliphatic)	$A_{1710\ cm-1}$ (carboxyl)	$A_{1620\ cm-1}$ (aromatic)	aliphatic/carboxyl	aliphatic/aromatic	carboxyl/aromatic
1	993	0.15	1.41	0.53	0.11	0.28	2.66
2	1703	0.19	1.41	0.42	0.13	0.45	3.36
3	2103	0.17	1.34	0.28	0.13	0.61	4.79
	SRFA	—	—	—	0.25	0.56	2.22

The standard deviation values of absorbance values at 2925, 1710 and 1620 cm⁻¹ are ±0.03, ±0.16, ±0.10 in case of HAs, and ±0.03, ±0.12, ±0.05 in case of FAs, respectively. The ratio of aliphatic to carboxyl ($A_{2925cm-1}/A_{1710cm-1}$), aliphatic to aromatic ($A_{2925cm-1}/A_{1620cm-1}$), and carboxyl to aromatic ($A_{1710cm-1}/A_{1620cm-1}$) were reported to compare the IR characteristics of the HS samples. The ratios of SRFA were determined on the basis of IR spectrum published on IHSS website.
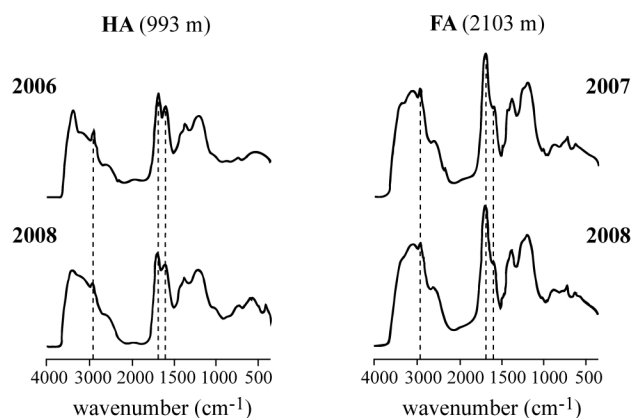
Fig. 4. FTIR spectra of humic (left) and fulvic acids (right) isolated from thermal waters at 993 m and 2103 m depths in different samplings (the years of the samplings are in parenthesis); dashed lines assign the wavenumbers of 2925, 1710, and 1620 cm⁻¹.

polycondensation reactions of organic precursors did not cause any alteration on elemental composition of isolated humic materials. Based on the constant elementary composition of HS we can assume that HS are originally present in thermal waters as refractory compounds.

The van Krevelen diagram is suited to differentiate HAs and FAs of aquatic and terrestrial origins and investigate the changes occurring in the transformation processes of organic matter (van Krevelen, 1950; Visser, 1983). Extensive and statistically analyzed study for several hundreds of HAs and FAs can be found in Rice and MacCarthy (1991). A diagram adapted from this paper can represent the typical pattern of humic substances in the light of our HS samples (Fig. 3). In Fig. 3 the calculated ratios of over 400 samples of HAs and over 200 samples of FAs from different sources cover a significant area of the diagram. Besides their overlapping (0.7 < H/C < 1.8 and 0.4 < O/C < 1.0), FA samples tend to occupy the region with higher O/C and H/C values, while HA samples are characterized by lower atomic ratios. The H/C ratio generally appears to be a qualitatively useful parameter for comparing the aromatic/aliphatic character of humic materials (Rice and MacCarthy, 1991). The O content and O/C ratio in turn indicate mainly the enrichment/depletion of oxygen containing functional groups such as carboxyl and phenolic groups. The positions of HAs and FAs investigated in this study in the van Krevelen diagram are within the range characteristic of HS. This observation confirms the previous finding that some preferred composition, or a relatively narrow range of com-

corresponding FAs. This fact is in good agreement with previous studies of HAs and FAs in various sources, such as soils, peat and freshwater (Rice and MacCarthy, 1991). The data for the elemental composition of SRFA are obtained from IHSS (http://www.ihss.gatech.edu/elements.html). The elemental composition of HS did not change significantly during the repeated samplings (2006–2008). In addition, the modified sampling method tested in 2008 for preventing the potential polymerization/

positions, exists for HS in nature (Rice and MacCarthy, 1991). The lower ratios of HS in thermal waters indicate higher aromaticity and depletion in oxygen than usually experienced for humic materials in soil and freshwater (for soil HA O/C: 0.50 ± 0.09, H/C: 1.04 ± 0.25; for soil FA O/C: 0.78 ± 0.16, H/C: 1.35 ± 0.34; for freshwater HA O/C: 0.60 ± 0.08, H/C: 1.12 ± 0.17; for freshwater FA O/C: 0.75 ± 0.14, H/C: 1.10 ± 0.13) (tables 3 and 5 in Rice and MacCarthy, 1991).

The H/C ratios of HS isolated from our thermal water samples increased and O/C decreased as function of the water temperature (Table 3). It can be supposed that the aliphatic content of HS increased and/or their aromatic content decreased with increasing temperature. On the other hand the amount of the oxygen-containing functional groups decreased in both HAs and FAs with increasing thermal water temperature. In addition, the amount of other heteroatoms, especially N content, decreased in both humic fractions with increasing temperature. The differences of atomic ratios are derived from distinct molecular characteristics which can be further detailed by ESI-FT-ICR/MS.

FTIR spectroscopy

The FTIR spectra of the isolated organic materials are essentially similar to each other; the main difference is that the relative intensity of some bands differs slightly (Fig. 4). The absorption bands were identified based on published data (Stevenson, 1994; Barančíková *et al.*, 1997). The absorbance values belonging to different wavenumbers, i.e., 2925, 1710 and 1620 cm^{-1} (marked with dashed lines in Fig. 4) can be used for representing the aliphatic, carboxyl and aromatic content of the HSs (Lis *et al.*, 2005). The shape of the HAs and FAs spectra isolated from the same well during different samplings are very similar suggesting that the functional group composition of humic substances did not change significantly over time. In addition, the modified water sampling in 2008 had no effect on the spectra. On the basis of this observation we assume that also the functional group composition was unchanged in HAs and FAs.

The average of FTIR absorbance values at the selected wavenumbers and their standard deviations from different samplings are listed in Table 4. One of the most conspicuous features is that absorbance at wavenumber of 1710 cm^{-1} is the highest in all cases, especially for FAs. It seems that most of the oxygen-containing functional groups are the carboxyl group and that FAs are reasonably enriched in carboxyl groups than HAs. This finding is confirmed by the fact that elemental analysis of FAs shows higher oxygen content. The differences in aliphatic and aromatic contents of HAs and FAs are not representative based on FTIR absorbance values and their standard deviation (Table 4). The aliphatic content is relatively

unchanged in FAs but their aromatic content show variation based on the absorbance values at 2925 and 1620 cm^{-1}, respectively. In case of HAs the aromatic content does not change virtually, but their aliphatic content slightly differs.

To compare the IR characteristics of the HS samples from thermal waters and SRFA the ratios of absorbance values were investigated as Lis *et al.* (2005) suggested. The ratio of aliphatic to carboxyl ($A_{2925cm^{-1}}/A_{1710cm^{-1}}$), of aliphatic to aromatic ($A_{2925cm^{-1}}/A_{1620cm^{-1}}$), and of carboxyl to aromatic ($A_{1710cm^{-1}}/A_{1620cm^{-1}}$) can be found in Table 4. Comparing with the FTIR spectrum of SRFA (http://www.humicsubstances.org/spectra.html), the ratios of absorbance values were determined. The ratio of aliphatic to carboxyl and the ratio of carboxyl to aromatic of HAs and FAs from the same thermal water show differences which agree with the higher carboxyl content and more aliphatic character of FAs compared to HAs. But the ratios of aliphatic to aromatic of the corresponding HAs and FAs are quite similar except those from the deepest well. This finding is consistent with the H/C ratios obtained by elemental analysis. The highest difference in H/C ratios can be found in HA and FA isolated from the deepest thermal water (Table 3). In other two samples (993 m and 1703 m), the differences in H/C ratios of HAs and FAs are not too significant being the ratios of aliphatic to aromatic quite similar. However, it is worth to note that the H/C ratio perfectly reflects the aromatic/aliphatic character of a molecule only in the case of hydrocarbons. Furthermore, the absorbance ratios of HAs and FAs changed as a function of the aquifer's depth and temperature (Table 4): the ratios of aliphatic to aromatic and that of carboxyl to aromatic increased, but the ratio of aliphatic to carboxyl was unchanged. On the basis of these findings it can be concluded that the HAs and FAs gradually become more aliphatic and/or have less aromatic character with increasing depth, while their carboxyl contents increase or remained unchanged. In this case, the decrease of oxygen content can be explained by decrease of other oxygen-containing functional groups than carboxyl groups, e.g., the phenolic ones.

In SRFA, the aliphatic to carboxyl ratio is higher while the carboxyl to aromatic ratio is lower compared to those measured in FAs from thermal waters. This may confirm higher carboxyl content and more aliphatic character of SRFA than of FAs in thermal water. The ratio of aliphatic to aromatic of SRFA is in the range of FAs isolated from thermal waters which corresponds to the H/C ratios.

ESI-FT-ICR/MS

Due to the complexity of HS and the ultra-high resolution capability of the FT-ICR/MS, the measurement of HAs and FAs typically produce very complex spectra (Kovács *et al.*, 2010). In our case the spectra denoted more
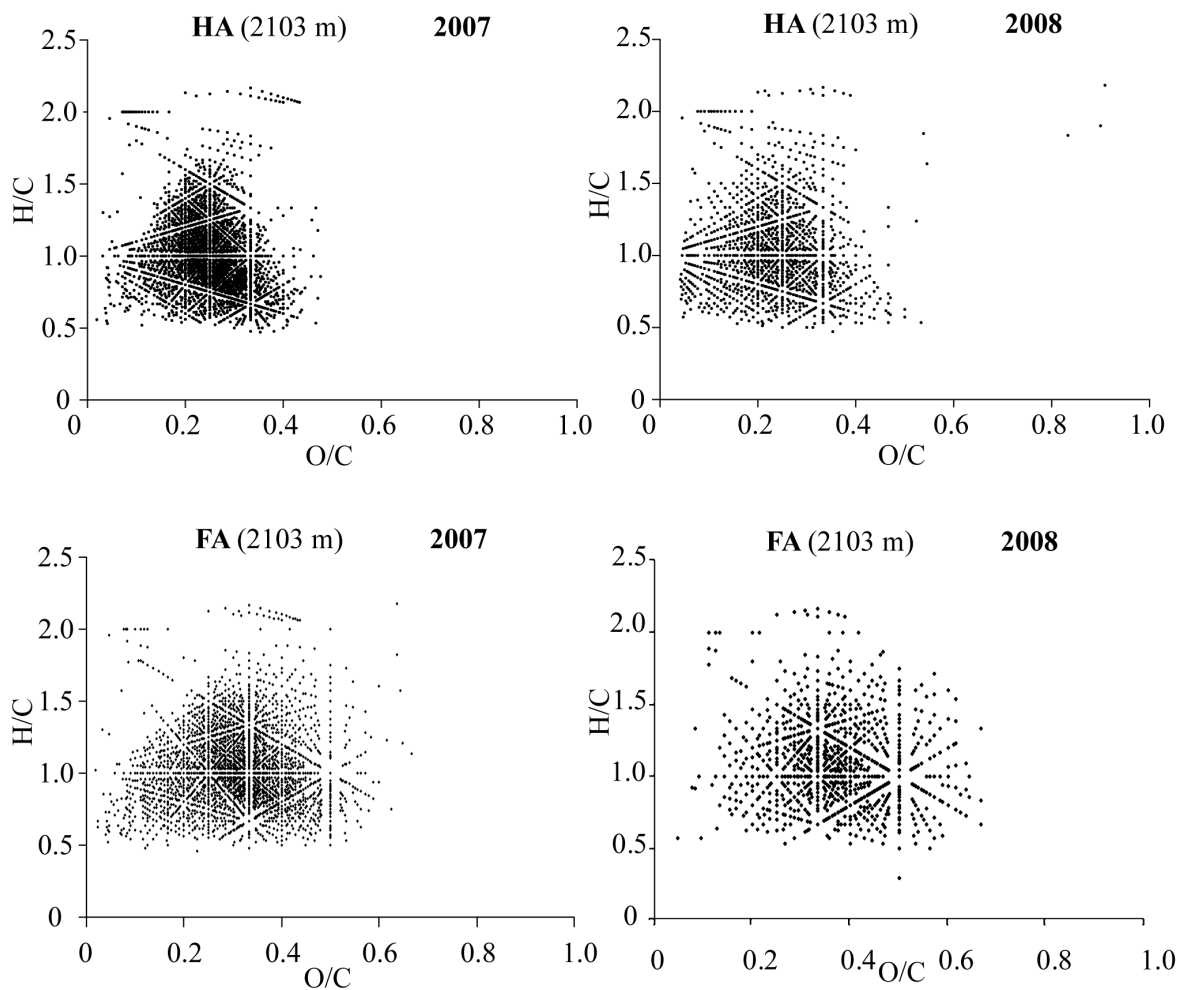
Fig. 5. The van Krevelen diagrams of humic acids isolated from thermal waters at depths of 993 and 2103 m in different samplings (the sampling years are in parenthesis).

than 20,000 base-line resolved signals. The ultrahigh-resolution mass spectrometric data was converted to molecular formulae. Based on the formula calculation and validation used in this study, 2700 peaks in average could be assigned to a unique elemental composition (Kovács *et al.*, 2010). The elemental compositions allow their H/C and O/C atomic ratios to be calculated and plotted on the van Krevelen diagram (Kim *et al.*, 2003). However, it has to always keep in mind, that assigned formulae and their positions in the van Krevelen diagram do not necessary denote one unique chemical structure but possible superposition of isomers (Hertkorn *et al.*, 2008). The H/C vs. O/C ratios of HAs and FAs isolated from thermal waters at 2103 m depth from different samplings are illustrated in the diagrams of Fig. 5. The covered areas by HA and FA samples are almost identical in the van Krevelen diagram. Based on the positions of the patterns of atomic ratios, compositional differences of HAs and

FAs from different samplings cannot be seen. The FT-ICR/MS measurements confirmed that the quality of HS did not alter considerably in a three-year period. Furthermore the change in the sample processing had negligible effect as it can be seen in case of HS isolated in 2007 and 2008, respectively (Fig. 5).

In Fig. 6 results derived from FT-ICR/MS measurements of HA and FA isolated from thermal water (from depth of 2103 m) as well as SRFA were reported to represent their differences and similarities, at molecular level. To date no data is available on high-resolution mass spectra of SRFA as reference measurement. 3D presentation of mass data on van Krevelen diagram (Fig. 6A) with *m/z* distribution (Fig. 6B) as well as the distribution of oxygen containing compound classes (CHO$_x$) (Fig. 6C) and aromaticity index (AI) distribution (Fig. 6D) are used to characterize the humic samples. In Fig. 6A it can be seen that the ratios of HA and FA at depth of 2103 m as
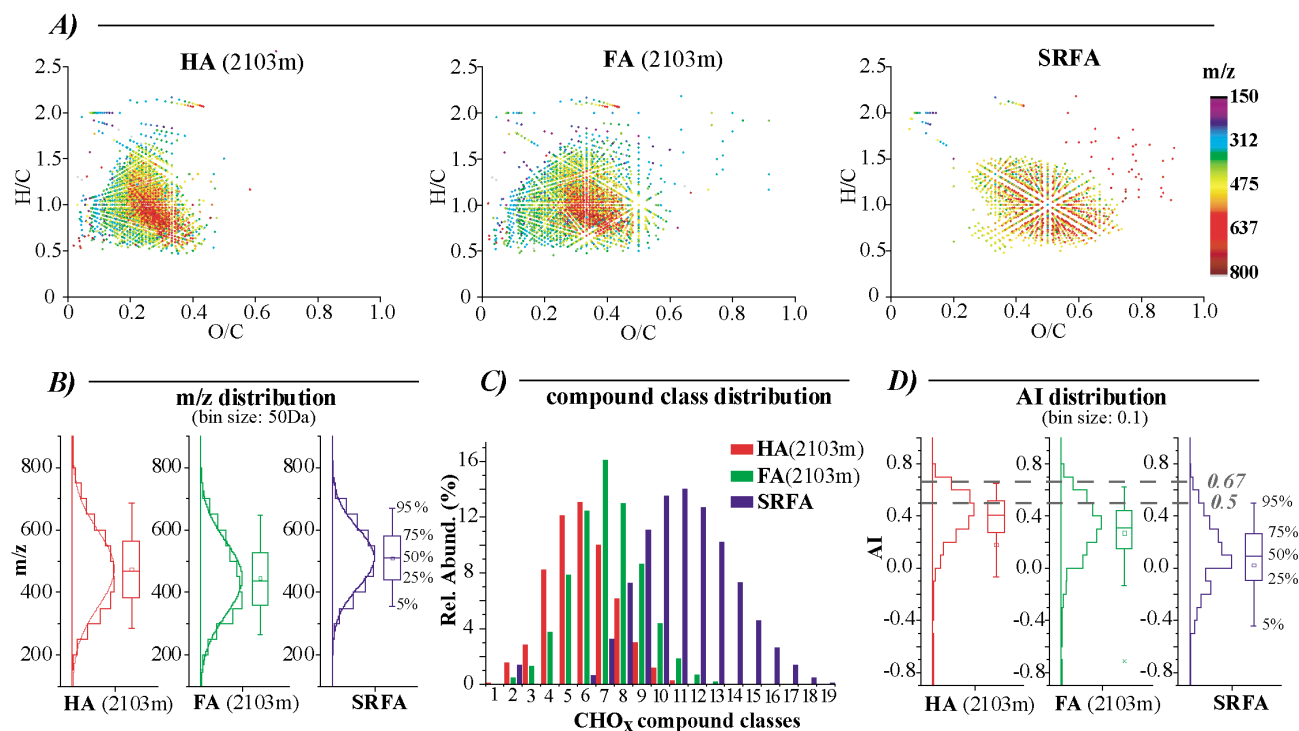
Fig. 6. 3D presentation of mass data on van Krevelen diagram (A) with m/z distribution (B) as well as the distribution of oxygen containing compound classes (CHO_x) (C) and aromaticity index (AI) distribution with two characteristics threshold values (AI > 0.5 and AI ≥ 0.67) (D) of HA and FA isolated from thermal water (from depth of 2103 m) as well as SRFA.

well as SRFA isolated from surface water differ each other. While the vast majority of the SRFA constituents, independently of the denoted masses, are concentrated around the O/C 0.3–0.7 and H/C 0.6–1.5 values, HA and FA from the deep aquifer denote a significant shift of their loci towards the oxygen depleted region of the van Krevelen diagram. In addition, the O/C ratios of FA tend to have higher values than HA in thermal water. However, the H/C ratios, indicating the variation in aromaticity/aliphaticity, differ less in these samples. These results are further detailed in Fig. 6C, where the assigned molecular formulae are separated into compound classes based on the number of O atom incorporated in the individual species. During data processing the relative abundance for each CHO_x compound class is summed over all nominal masses and then divided by the total relative abundance for all identified peaks. The results of the compound class analysis show Gaussian-like distributions where the most abundant CHO_x classes in SRFA and FA isolated from thermal water are O_{11} and O_7, respectively. The most abundant is the CHO_6 class for HA. These results agree with the results of elemental analysis because the oxygen contents and O/C ratios of SRFA, FA and HA in Table 3 show the same trend. Similar trends were observed in the cases of HAs and FAs at depths of 993 and 1703 m (Fig.

7). In order to demonstrate the presence of aromatic and condensed aromatic structures as well as to compare the aromaticity of the samples, the aromaticity index (AI) proposed by Koch and Dittmar (2006) was applied (Fig. 6D). A threshold value of AI > 0.5 is the most conservative calculation for the existence of aromatic structures. At the same time a threshold value of AI ≥ 0.67 provides an unambiguous minimum criterion for the presence of condensed aromatic structures in a molecule. These threshold values are indicated in the AI distribution diagram. AI is a measure for C–C double-bond density and considers the contribution of π-bonds by heteroatoms, since all heteroatoms are taken into account as potential contributors, the actual aromaticity in a molecule can be higher and AI must be regarded as the most conservative approach (Koch and Dittmar, 2006).

Based on the FT-ICR/MS measurements, the presence of condensed aromatic structures is negligible in all humic samples. Aromatic structures can be identified in all samples, but their presence is more significant in HAs, FAs isolated from thermal waters than in SRFA. Slight shift in the AI values can be seen between HA and FA isolated from thermal water in favors of HA which indicates more aromatic character of HA. This result is in accordance with the difference in H/C ratios by elemen-
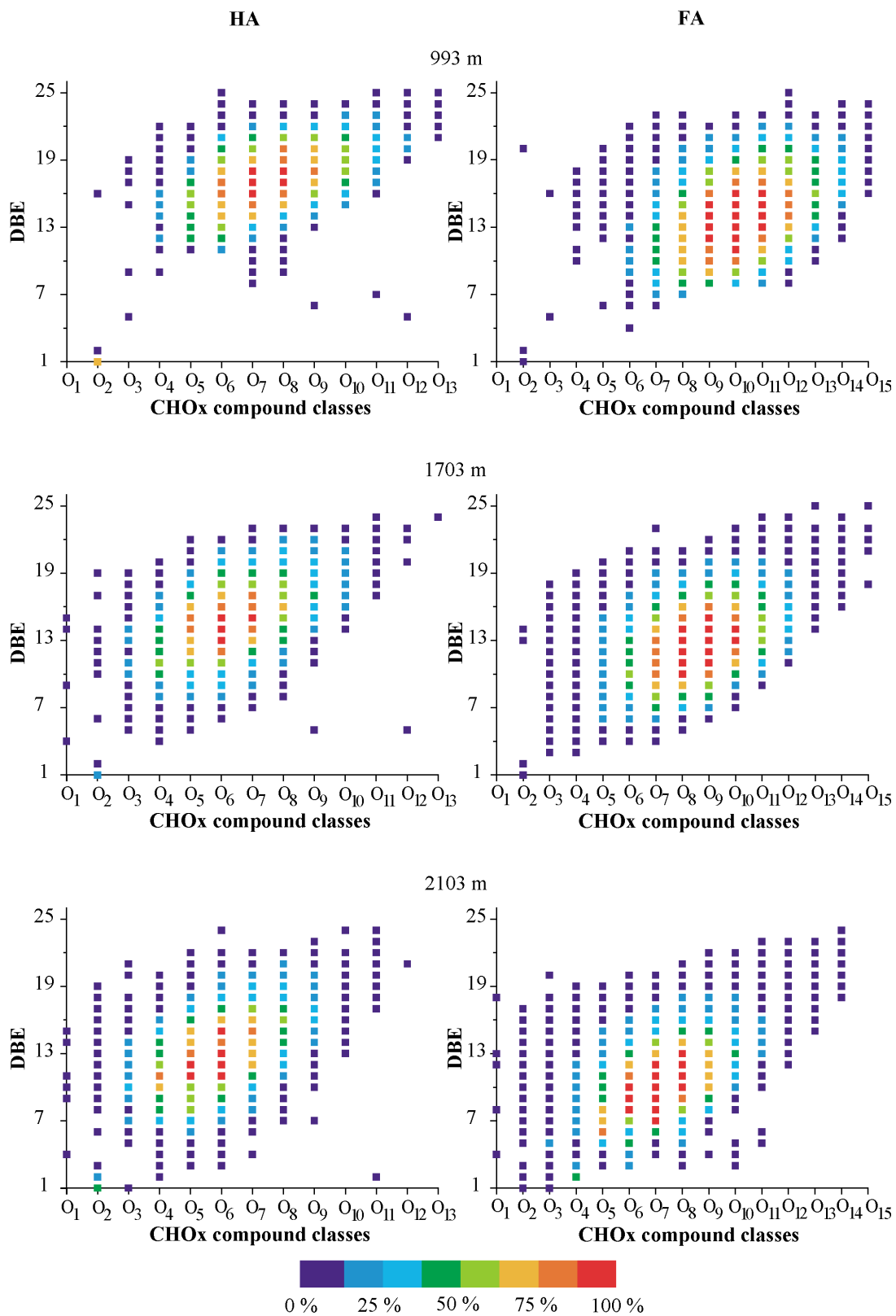
Fig. 7. Distribution of the assigned molecules with different DBE values within CHO$_x$ compound classes of humic and fulvic acids isolated from thermal waters at depths of 993, 1703, and 2103 m.

tal analysis (Table 3). At the same time, molecules with low AI values can be found in SRFA in a highest proportion suggesting the least aromatic character. However, this finding is not supported by H/C ratios from elemental analysis and the ratio of aliphatic to aromatic derived from FTIR spectrum (Table 3, Table 4). Here we note again that these threshold values indicate the most conservative approach. Overall, HS isolated from thermal waters show more aromatic character than SRFA on the basis of AI index calculated from assigned molecular formulae determined by ESI-FT-ICR/MS.

In addition, the distribution of m/z value of assigned molecular formulae was also investigated (Fig. 6B). Since only singly charged species were observed, the detected m/z values represent the actual molecular weight. The HS samples show almost identical mass distribution, however, HA and FA of thermal water origin have slightly lower mean values (475 and 445 Dalton, respectively) than SRFA with the mean of 520 Da. Similar trends were found for HS from depth of 993 and 1703 m. Here we have to note that these observed values obtained by ESI-FT-ICR/MS are generally lower than the usually found ones in literature where other methods were used. For example, Aiken *et al*. (1989) determined using various methods the average molecular weight of SRFA as 800 Da. This discrepancy between m/z values was justified by the existing charge suppression and competition that undergoes under the applied electrospray ionization conditions (Stenson *et al*., 2002). The lower m/z values for HS may suggest decomposition. Overall, the HS isolated from thermal waters can be characterized by lower heteroatom content and m/z values as well as more aromatic character than SRFA from surface water on the basis of ESI-FT-ICR/MS measurements. These results can support the presence of decomposition processes in deep aquifers suggested by the change of relative amount of HS.

In order to investigate the chemical and structural differences in HAs and FAs, the DBE (double bond equivalent) was investigated within the CHO_x compound classes. DBE is the sum of the number of rings and double bonds (Pellegrin, 1983). A decreasing number of H atoms (e.g., by addition of a ring or double bond) in a molecule increases unsaturation and hence leads to higher DBE values. However, DBE is independent of the number of O and S atoms. The distribution of the assigned molecules with different DBE values within the CHO_x compound classes of HAs and FAs from thermal waters is shown in Fig. 7. The DBE value ranges from 25 to 1 in these samples. It is worthy to note the presence of compounds with DBE of 1 within the O_2 compound class of HAs. These compounds are much less notable in FAs. Based on their compositional features it is possible that these are saturated fatty acids (Koprivnjak *et al*., 2009). The most abun-

dant compound classes are CHO_7 and CHO_6 for the HAs and CHO_{10}, CHO_8 and CHO_7 for the FAs as a function of the aquifer's depth (Fig. 7). This observation is also in good agreement with the change of bulk elemental composition (Table 3), since the compounds of less oxygen content with increasing abundance reflect the decrease in total O content with increasing water temperature. In addition, the maximum of the DBE values are 17, 15, and 12 in the most abundant compound classes of HAs as well as 14, 12, and 9 in case of FAs as temperature increased, respectively (Fig. 7). These results show descending presence of unsaturations and rings of the assigned molecules in HAs and FAs which can support the above-mentioned finding on the increasing aliphatic character as a function of the aquifer's depth and temperature. Additionally, compound classes of HAs and FAs with the same oxygen content can be characterized by different DBE values, which confirm significant qualitative differences between humic fractions. For example, the CHO_7 compound class with maximum DBE value of 17 and 9 is for HA from 993 m and for FA from 2103 m depth, respectively. This difference in DBE value with the same oxygen content assigns more aromatic character to HAs and more aliphatic character to FAs. Finally, we note that the increasing aliphatic character of HA and FA fractions as a function of the aquifer's depth can be attributed to the appearance of aromatic homologues and alkylphenols as described by Sajgó *et al*. (1998) and Kárpáti *et al*. (1999). Based on these results, we hypothesize that the decomposition processes with the increasing temperature can involve the breaking of aromatic units from the humic molecules resulting in the more aliphatic character of the residual part of the humic molecules with lower DBE values.

CONCLUSIONS

Humic materials isolated from thermal waters circulating in deep aquifers of the Pannonian Basin were studied during three consecutive years. The main dissolved ions (Na^+, HCO_3^- and TDS) of this $NaHCO_3$-type waters as well as the amount of their UV-absorbing organic matter did not change significantly over the sampling periods.

The water samples were acidified at different times in order to determine the presence of HS in deep thermal waters. We found that HS originally exist in thermal waters on the basis of the HA and FA isolation, which was carried out from water samples acidified immediately after thermal water was pumped out. Presumably the original pH of the thermal waters (pH 7.4–8.1) is too low to allow polymerization/polycondensation reactions of organic precursors to produce humic-like polymers without any catalyst. The changes in temperature and the presence of

oxygen did not cause any alteration in the properties of HS after the groundwater was recovered. Also, the characteristics of HS did not change during the sampling period.

The HAs and FAs of deep thermal waters are different from those of humic materials in aquatic and terrestrial surface environments. The elemental ratios of HAs and FAs are within the range characteristic of HS supporting the fact that some HS preferred composition range exists in nature. In addition, the humic materials can be characterized by lower H/C and O/C ratios, indicating higher aromaticity and depletion in oxygen than usually experienced for HS in soil and freshwater environments. Based on the IR absorbance values at 1710 cm^{-1} it can be suggested that most of the oxygen-containing functional groups are carboxyl groups and that FAs are reasonably enriched in carboxyl groups than HAs as observed in surface environments. The ratio of aliphatic to carboxyl of SRFA is higher while its ratio of carboxyl to aromatic is lower compared to those of FAs from thermal waters. This may confirm the presence of higher carboxyl content and the more aliphatic character of SRFA than those found in FAs in thermal water. However, the aliphatic to aromatic ratio of SRFA is in the range of those of the FAs which corresponds to the H/C ratios. The results derived from ESI-FT-ICR/MS measurements indicate lower heteroatom content and m/z values as well as more aromatic character of HS isolated from thermal waters than SRFA.

In addition, the aliphatic/aromatic character and heteroatom content of HA and FA changed as a function of the aquifer's depth. The decrease in the oxygen content of humic samples can be interpreted by the decrease of their oxygen-containing functional groups including phenolic ones on the basis of FTIR measurement. On the other hand, the increasing aliphatic character of HA and FA fractions can be attributed to the appearance of aromatic homologues and alkylphenols in thermal waters as described by Sajgó *et al.* (1998) and Kárpáti *et al.* (1999). These changes may reflect decomposition of HS, which can be supported by that fact that the relative amount of isolated humic fractions to TOC content of thermal waters decreased with increasing aquifer's depth. The HA and FA content has been found to be sensitive to increasing temperature and it could be a possible source for aromatic homologues and alkylphenols appeared only in hot waters. The HS of thermal waters is partly inherited and partly is generated from organic materials available in aquifers. HS is also transforming like kerogen during diagenesis and catagenesis and this process might be governed by temperature. The temperature effect on HS in thermal waters has been recently corroborated in simulations (Fekete *et al.*, 2012).

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