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## Article

# Compositional features of Japanese Humic Substances Society standard soil humic and fulvic acids by Fourier transform ion cyclotron resonance mass spectrometry and X-ray diffraction profile analysis

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## Abstract

The composition of humic acids (HAs) and fulvic acids (FAs) from Inogashira (Umbric Andosol) and Dando (Dystric Cambisol) soils authorized as standard samples by the Japanese Humic Substances Society was characterized using Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) and X-ray diffraction (XRD) 11 band profile analysis. In FTICR-MS, the number of peaks that molecular formulas were assigned to was 2549–2913 for the FAs and 1943–2457 for the HAs. Molecular formulas with H/C and O/C ratios similar to condensed hydrocarbons were dominant in both the HAs, while those with H/C and O/C ratios similar to lignin were more abundant in the Dando HAs than in the Inogashira HAs. In both the FAs, molecular formulas having H/C and O/C ratios similar to lignin, condensed hydrocarbons, and tannins were dominant. The double bond equivalent (DBE), an index of the degree of unsaturation, was in the range of 0–30. However, larger DBE values of 26–30 were observed only in the Inogashira HAs with a larger degree of humification. The XRD results also showed that the Inogashira HAs contain larger amounts of condensed aromatic structures with a higher condensation degree. Relative content of carbon layer planes estimated from the peak area of the 11 band in the XRD profile was 1.00, 0.58, 0.15, and 0.17 for the Inogashira HAs, Dando HAs, Dando FAs, and Inogashira FAs, respectively. Size distribution of carbon layer planes based on the 11 band profile analysis, which could be estimated only for HAs, was 0.48–1.68 nm (4–37 rings) in the Inogashira HAs and 0.48–1.20 nm (4–19 rings) in the Dando HAs.

**Keywords:** Humic acid, Fulvic acid, Fourier transform ion cyclotron mass spectrometry, Condensed aromatic structure, X-ray diffraction profile

## Introduction

The Japanese Humic Substances Society (JHSS) authorizes two sets of humic acids (HAs) and fulvic acids (FAs) from representative types of Japanese soils, an Umbric Andosol (Inogashira) and a Dystric Cambisol (Dando), as standard soil humic substance samples. To characterize these samples, various types of physicochemical and chemical analyses, including ultraviolet-visible absorbance (Kuwatsuka et al., 1992), liquid- and solid-state nuclear magnetic resonance (NMR), electron spin resonance, and

X-ray photoelectron N1s spectroscopies (Watanabe et al., 1994, 2004), gas chromatography or gas chromatography/mass spectrometry (GC/MS) analyses of degradation products released upon acid hydrolysis, thermochemolysis (Watanabe et al., 2004), and ruthenium tetroxide (RuO<sub>4</sub>) oxidation (Ikeya et al., 2007a), etc., have been applied. According to those data published, the proportion of carbon (C) as aromatic C estimated from <sup>13</sup>C cross polarization/magic angle spinning NMR spectra was higher in the Inogashira HAs (35%) than in the Dando HAs (27%), which is in agreement with the

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higher degree of dark color, the larger proportion of nitrogen (N) as aromatic N estimated by XPS, and the larger yield of RuO<sub>4</sub> degradation products derived from polynuclear aromatic components in the Inogashira HAs. The proportions of C as carbohydrate C or alkyl C substituted with O or N, as well as the yield of neutral monosaccharides in acid hydrolysis, in the two HAs were similar to each other and larger than those in the two FAs. Differences in the C composition between the two FAs were generally small, e.g., the proportion of C as aromatic C was 33–34%. Evident differences were observed in the yields of products upon chemical and thermochemolytic degradations, including monosaccharides, benzenepolycarboxylic acids (BPCAs), and polynuclear aromatic component-derived BPCAs, all of which were larger in the Inogashira FAs than in the Dando FAs.

Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) can measure accurate mass to charge ( $m/z$ ) values of components in a complex mixture of high molecular weight compounds, owing to the ultrahigh resolving powers (>400,000 on average; Kramer et al., 2004, Sleighter and Hatcher, 2011). In general, using this technique, organic samples dissolved in an aqueous solution are ionized (either negatively or positively) by an electrospray ionization (ESI) source (or with an atmospheric pressure photoionization or chemical ionization source). The ions are transferred to an ion cyclotron resonance cell, where they resonate in the cell under a constant magnetic field and the cyclotron frequency is detected and measured. The obtained free induction decay signal is Fourier transformed, and then each individual frequency (which is inversely proportional to  $m/z$ ) is converted to its corresponding  $m/z$  according to the following equation:

$$f = B/(m/z) = zB/m \quad (1),$$

where  $f$  and  $B$  are the cyclotron frequency and the strength of the magnetic field, respectively, and  $m$  is the mass of the ion while  $z$  is the charge of the ion (Sleighter and Hatcher, 2011).

This technique has been utilized for characterizing the composition of aquatic and soil humic substances (Stenson et al., 2003; Kim et al., 2003; Sleighter and Hatcher, 2007; Dittmar and Paeng, 2009). Ohno et al. (2010) applied FTICR-MS for both the mobile and calcium HA fractions from 10 soils, assigned molecular formulas, and analyzed the composition of

the formulas by comparing them with biomolecules on a van Krevelen diagram. In the study, generally, the components assigned in the moderately refractory mobile HA fraction were plotted on the region of lipid and the boundary region of lignin and condensed aromatic structure and those in the refractory calcium HA fraction were plotted on the region of lipid and condensed aromatic structure.

Another technique having been applied to soil and artificial HAs recently includes the 11 band profile analysis in the X-ray diffraction (XRD) profile that can estimate the composition of condensed aromatic rings with regard to carbon layer plane size (Ikeya et al., 2011; Sultana et al. 2011). Ikeya et al. (2011) reported the presence of condensed aromatic structures including that consisting of 30 or 37 rings as the largest ones in various soil HAs using this XRD technique, which had not been found previously.

The information about JHSS standard samples should be accumulated in order to utilize them as reference samples effectively. In the present study, FTICR-MS and XRD profile analyses were applied for the samples. Molecular formulas assigned in FTICR-MS were characterized using the van Krevelen diagram and double bond equivalent (DBE) analysis, and relative content and/or size distribution of condensed aromatic rings were estimated by the XRD 11 band profile analysis.

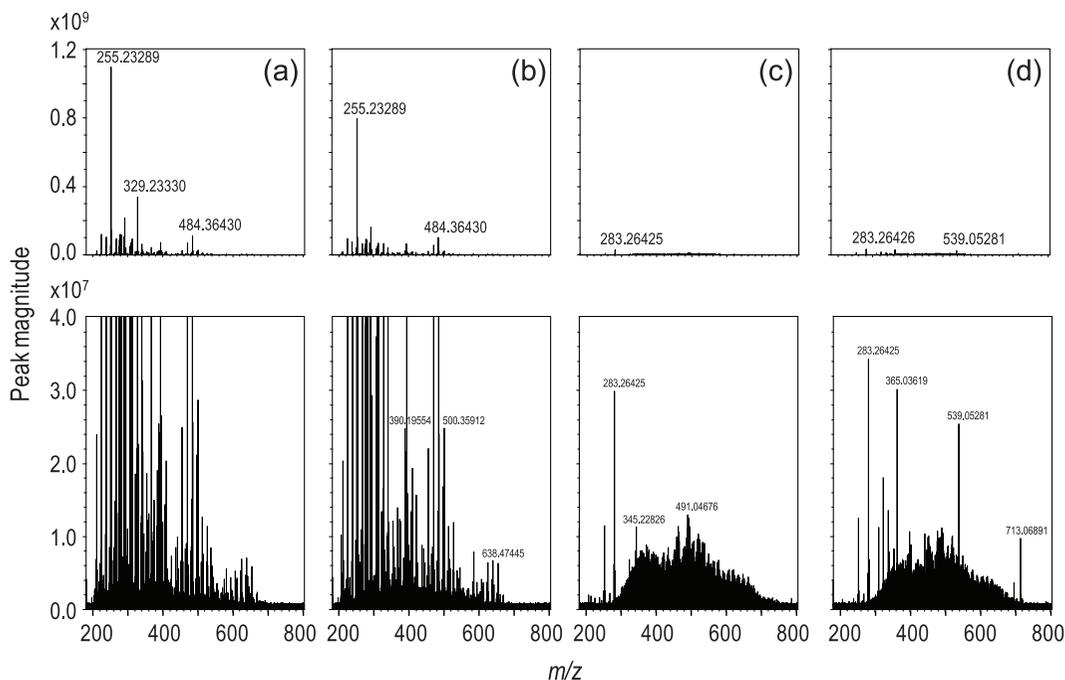
## Materials and Methods

### Samples

The HA and FA samples used were prepared from the A-horizon soil of an Inogashira Umbric Andosol profile (Fujinomiya, Shizuoka Prefecture, Japan) and a Dando Dystric Cambisol profile (Shitara, Aichi Prefecture, Japan) according to the International Humic Substances Society method (Kuwatsuka et al., 1992).

### Electrospray ionization (ESI) FTICR-MS

In preparation, the HA samples dissolved in 0.1 M NaOH were washed with chloroform/methanol (1:1) to remove lipids and then transformed into the protonated form by passing through a column packed with a protonated cation exchange resin (Dowex monosphere 650C, Dow Chemical Company, Midland, U.S.A.). The pretreated HA and non-pretreated FA samples were dissolved in



**Fig 1.** Entire (upper) and magnified (lower) negative ion mode ESI-FTICR mass spectra of Dando HAs (a), Inogashira HAs (b), Dando FAs (c), and Inogashira FAs (d).

water/methanol (1:1) containing 0.1% ammonium hydroxide to regulate pH at 8 and injected into an Apollo II ESI ion source of a 12 Tesla Apex Qe FTICR-MS (Bruker Daltonics, Bremen, Germany). Samples were introduced by a syringe pump at an infusion rate of 120  $\mu\text{L h}^{-1}$  and then analyzed in negative ion mode with  $m/z$  range of 200–2,000. The peaks were identified at an  $S/N \geq 4$ . The mass spectrum obtained was internally calibrated using aliphatic mono- and di- carboxylic acids in the samples, according to Sleighter et al. (2008).

Molecular formulas were assigned to the peaks using the Molecular Formula Calculator v.1.0 (©National High Magnetic Field Laboratory, Tallahassee, Florida) under the conditions of  $C_{0-100}$ ,  $H_{0-200}$ ,  $N_{0-7}$ ,  $O_{0-50}$ ,  $S_{0-3}$ , and  $P_{0-3}$  atoms, according to Stubbins et al. (2010). Permeable error between the actual and theoretically calculated  $m/z$  was  $<1.0$  ppm. The DBE (i.e., the number of rings plus double bonds in the structure) for the corresponding neutral molecular formula described as  $C_cH_hN_nO_oS_sP_p$ , was calculated using the following equation (Bae et al., 2011):

$$\text{DBE} = c - h/2 + n/2 + p/2 + 1 \quad (2),$$

where the lowest valence state for all atoms is assumed.

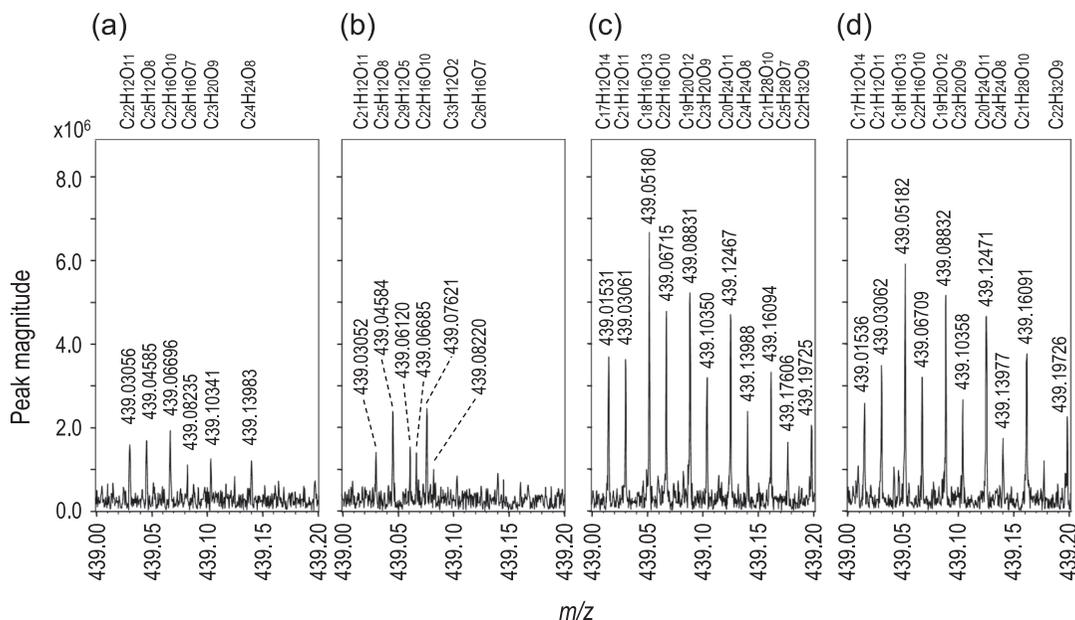
### X-ray diffraction profile analysis

X-ray diffraction profile analysis was conducted according to Ikeya et al. (2011). A 24.2-mg sample was set in a silicon holder and the XRD profile was measured using an X-ray diffractometer, XRD6100 (Shimadzu, Kyoto, Japan), under the following conditions: target, copper  $K\alpha$ ; wavelength, 0.154 nm; tube voltage/current, 40 kV/30 mA; scan mode, step scanning at  $0.1^\circ$ ; scan range,  $2\theta = 5\text{--}100^\circ$  (counting time, 6 s) and  $60\text{--}100^\circ$  (12 s). The 11 band, that is derived from the various sizes of carbon layer planes consisting of six-membered ring in an amorphous organic matter, in the obtained XRD profiles was analyzed using Carbon Analyzer DiHiGa Series 2007 software (Ryoka Systems Inc., Tokyo, Japan), which is based on the least-square fitting of the observed 11 band profile to a theoretically calculated 11 band profile (Ikeya et al., 2011). The total amount of carbon layer planes in arbitrary units (A.U.) per mg of sample was estimated from the area of the 11 band by the base line method.

## Results and Discussion

### ESI FTICR-MS analysis

The FTICR mass spectra of the HAs and FAs are



**Fig 2.** ESI-FTICR mass spectra expanded at  $m/z$  439.0–439.2 of Dando HAs (a), Inogashira HAs (b), Dando FAs (c), and Inogashira FAs (d). The numbers on each of the detected peaks are the observed  $m/z$  values, with their assigned molecular formulas given above.

shown in Fig. 1. The spectral pattern was similar between the two HAs and between the two FAs, respectively. In the spectra of the two FAs, most of the peaks were detected in the  $m/z$  range of 280–750 with similar magnitudes ( $1 \times 10^6$ – $1 \times 10^7$ ). In the spectra of the two HAs, on the other hand, some signals with high magnitudes ( $> 2 \times 10^7$ ) were observed in the  $m/z$  range of 227–394 and 456–500, with the majority of smaller peaks throughout the  $m/z$  range of 200–700. The peaks with high magnitude in the HAs are species that are more efficiently ionized by ESI and many of them were assigned to aliphatic mono- and di- carboxylic acids (discussed later) that are well known to have high ionization efficiencies (Sleighter et al., 2008). In contrast, most of the peaks in the  $m/z$  range of 200–280 detected in the HAs were not detected in the two FAs. Those compounds are the aliphatic compounds having H/C 1.6–1.9 and O/C 0.1–0.4 that were detected more in the HAs than in the FAs (discussed later with Fig. 3). Few peaks were detected with  $m/z$  higher than 700–750, likely because they could not be ionized by ESI amongst the complex mixture of other species having higher ionization efficiencies. Figure 2 shows the mass spectra expanded in the range of  $m/z$  439.0–439.2 of the HAs and FAs, which highlights that the spectra were successfully obtained with ultrahigh resolution

and each of the peaks were assigned to molecular formulas. In this region, 10 or 11 peaks were detected in the narrow space of 0.20 Da for the FAs.

The total number of peaks that could be assigned to molecular formulas was 2457 in the Dando HAs and 1943 in the Inogashira HAs. Those values for the Dando and Inogashira FAs were 2549 and 2913, respectively (Table 1). The peaks assigned accounted for 83–91% of all peaks detected, excluding those from <sup>13</sup>C isotopes and those of inorganic origin, which give higher mass defects (0.44–0.99 for  $m/z$  <400 and 0.6–0.99 for  $400 < m/z < 800$ ; Sleighter and Hatcher, 2011). One of the possible reasons for the higher number of peaks detected in the FAs than in the HAs might be that the components detected in the FAs have relatively similar ionization efficiencies, compared with those in the HAs. The components detected in the HAs seem to have more variable ionization efficiencies, as the aliphatic acids had much higher peak magnitudes than the other organic matter compounds (lower figures in Fig. 1). Another potential cause for more peaks to be detected in the FA samples is that the FAs have more functionalized components that can be ionized by ESI. It is likely that the HAs have more pure hydrocarbon components (without any oxygen functionalities), which will not be ionized by ESI.

**Table 1** Number of peaks in the FTICR mass spectra and elemental composition of bulk samples.

Sample name	----- Humic acids -----		----- Fulvic acids -----	
	Dando	Inogashira	Dando	Inogashira
Total number of peaks detected <sup>a</sup>	3087	2521	3358	3476
Number of peaks without <sup>13</sup> C and salt peaks ( <i>w</i> ) <sup>b</sup>	2724	2234	3054	3195
Number of formulas assigned ( <i>x</i> )	2457 (90) <sup>c</sup>	1943 (87)	2549 (83)	2913 (91)
Number of formulas consisting of C, H, O ( <i>y</i> )	1691 (62) <sup>d</sup>	1193 (53)	2120 (69)	2120 (66)
with N ( <i>z</i> )	722 (27) <sup>e</sup>	715 (32)	419 (14)	770(24)
with S and/or P	44	35	10	23
N (weight%) <sup>f</sup>	4.49	4.01	0.77	1.73
H/C (atomic ratio) <sup>f</sup>	1.18	0.93	0.88	0.95
O/C (atomic ratio) <sup>f</sup>	0.52	0.50	0.76	0.89

<sup>a</sup> *m/z* range of 200–750. Peaks that were outside the *m/z* range of the assigned formulas were excluded.

<sup>b</sup> <sup>13</sup>C isotopes and inorganic peaks unassigned due to high mass defects (0.44–0.99 for *m/z* <400 and 0.6–0.99 for 400 < *m/z* <800) were excluded (Sleighter and Hatcher, 2011).

<sup>c</sup> *x/w* × 100 (%).

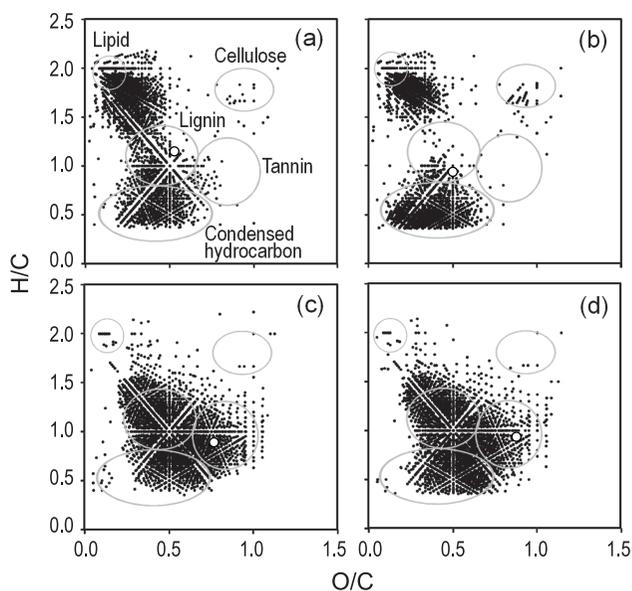
<sup>d</sup> *y/w* × 100 (%).

<sup>e</sup> *z/w* × 100 (%).

<sup>f</sup> Based on elemental analysis. Data were cited from Watanabe et al. (1994).

The number of the peaks consisting of C, H, O, and N was 715–722 in the two HAs and 770 and 419 in the Inogashira and Dando FAs, respectively. These values accounted for 27–32%, 24%, and 14%, respectively, of the peaks assigned (excluding those from <sup>13</sup>C and inorganics, Table 1). Similarity in these values between the two HAs corresponded to that in total N content, 4.0–4.5% (Table 1). The larger N content in the Inogashira FAs (1.7%) than in the Dando FAs (0.8%) also correlates to the difference in the proportion of the peaks assigned to molecular formulas including N between the two FAs.

Figure 3 shows the van Krevelen diagrams of the molecular formulas assigned in the HAs and FAs. Regions where the plots of lipids, cellulose, lignin, tannins, and condensed hydrocarbons appear are indicated in the figure. The peaks assigned to molecular formulas that are similar to condensed hydrocarbon regions as well as those having H/C 1.6–1.9 and O/C 0.1–0.4 were dominant in the Inogashira HAs. In addition to these two groups, many peaks having H/C 1.3–1.6 and O/C 0.1–0.4 and those having molecular formulas similar to lignin were observed in the Dando HAs. This difference in the region of H/C 1.3–1.6 and O/C 0.1–0.4 corresponds to the previous result of <sup>13</sup>C NMR where the aliphatic C (= alkyl C



**Fig 3.** van Krevelen diagrams of molecular formulas assigned to the FTICR mass spectra of Dando HAs (a), Inogashira HAs (b), Dando FAs (c), and Inogashira FAs (d). Open circles indicate the H/C and O/C of bulk samples measured by elemental analysis (Table 1). Circles indicate the lipid, cellulose, lignin, tannin, and condensed hydrocarbon regions as shown in Sleighter and Hatcher (2007).

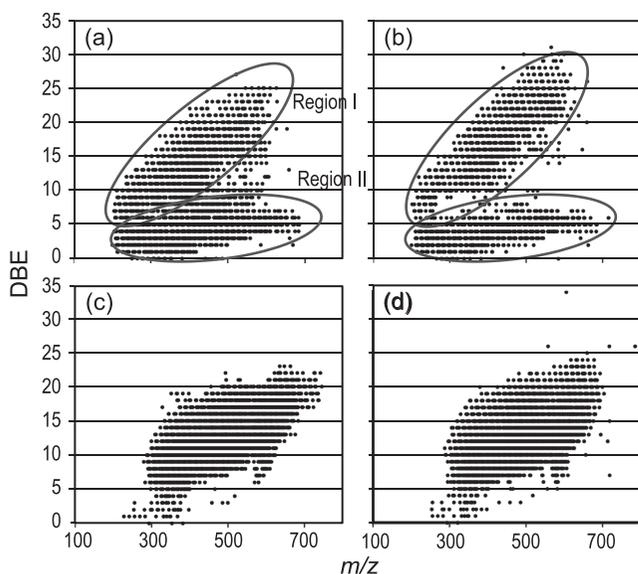
plus O-alkyl C) content was larger in Dando HAs than in Inogashira HAs (Watanabe et al., 2004). Furthermore, this difference agrees with the previous results of thermochemolysis-GC/MS analysis (Watanabe et al., 2004), in which the sum of the yield

of lignin-derived phenols from the Dando HAs was twice of that from the Inogashira HAs, as was generally observed between the soil HAs with high and low degrees of humification (Ikeya et al., 2004).

In the FAs, the components having molecular formulas similar to lignin, condensed hydrocarbons, and tannins were dominant (Fig. 3). Dense plots in the lignin region compared with the HAs, especially Inogashira HAs, corresponded to the larger yields of lignin-derived phenols in the thermochemolysis-GC/MS analysis (Watanabe et al., 2004). The larger number of points having H/C and O/C ratios similar to tannins in the two FAs (as opposed to that in the two HAs) likely contribute to the larger O/C values in the bulk FAs than in the bulk HAs (Table 1 and Fig. 3). The distribution of the plots in the condensed hydrocarbon region differed between the HAs and FAs, i.e., the molecular formulas with lower H/C and O/C values were fewer in the FAs than in the HAs. This difference may be related to the higher solubility of FAs in acidic or neutral solutions.

Figure 4 shows the  $m/z$ -DBE diagrams of the HAs and FAs. The DBE values were in the range of 0–25 and 0–30 in the Dando and Inogashira HAs, respectively, and 0–23 and 0–24 in the Dando and Inogashira FAs, respectively. As such, the HAs contained components having skeletal structures with higher degrees of unsaturation compared to the FAs. DBE values tended to be higher in the higher  $m/z$

components in the FA diagrams. Although a similar trend was also observed in the HA diagrams, the distribution of the plots was grouped into two regions, I and II, having different slopes. Region I of the Inogashira HAs included points with higher DBE than that of the Dando HAs, suggesting the presence of components with higher degrees of condensation in the HAs with a higher degree of humification, as was observed in the other analyses such as  $\text{RuO}_4$  degradation (Ikeya et al., 2007a,b) and XRD profile analysis (Ikeya et al., 2011). The higher DBE values in Inogashira HAs than in Dando HAs correlate with the plots having lower H/C ratios in the condensed hydrocarbon region in Inogashira HAs than in Dando HAs (Fig. 3). Plots in the region II include the components having H/C 1.5–1.9 and O/C 0.1–0.4 and unsaturated aliphatic components with higher H/C values, such as series of mono- and di-carboxylic fatty acids. In the Inogashira FAs diagram, plots with similar  $m/z$  values tended to be spread toward higher DBE than those in the Dando FAs diagram. Thus, the Inogashira FAs may contain the components with higher degrees of condensation than the Dando FAs. This trend agrees with the observation of a peak at around 480 nm in the pH12-7 ionization difference spectra suggesting the occurrence of condensed aromatic components in the Inogashira FAs from the Andosol (Watanabe and Kuwatsuka, 1991).



**Fig 4.**  $m/z$ -DBE diagrams of Dando HAs (a), Inogashira HAs (b), Dando FAs (c), and Inogashira FAs (d).

#### XRD 11 band analysis

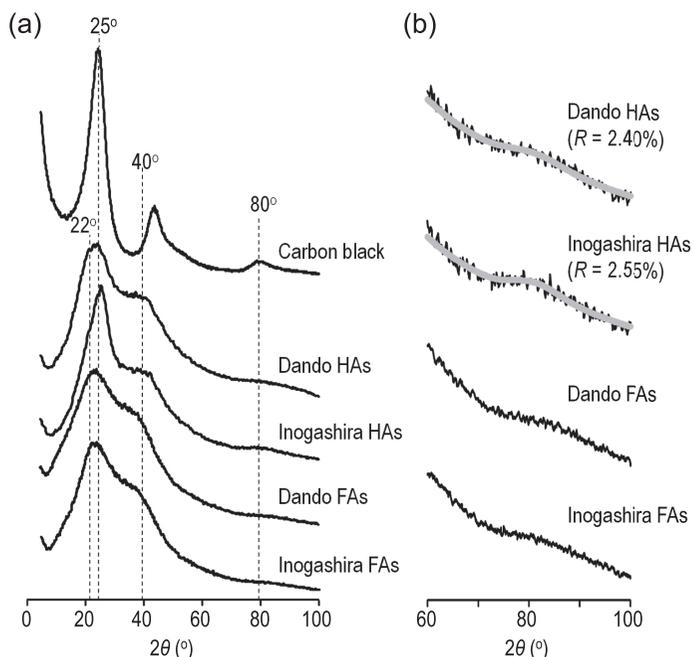
Figure 5 shows the XRD profiles of the HAs and FAs with that of carbon black used previously as a reference (Ikeya et al., 2011). Three peaks at  $2\theta = 24^\circ$  or  $25^\circ$ ,  $40$ – $43^\circ$ , and  $80^\circ$  in the profile of carbon black, designated 002, 10, and 11 bands, are a typical feature of turbostratic structures (Fujimoto, 2003). The 002 band derived from stacked structures of carbon layer planes at  $2\theta = 25^\circ$  was prominently observed in the profile of Inogashira HAs, in which the 10 band is observed as a shoulder and the 11 band was also clearly observed, despite its low intensity. The peak with maximum at  $2\theta = 25^\circ$  in the XRD profiles of Dando HAs and the two FAs was smaller and broader, which may be due to overlapping with the  $\gamma$  band ( $2\theta < 21^\circ$ , Matsui et al., 1984; Schnitzer et al., 1991). However, the occurrence of 10 and 11 bands supported

the inclusion of 002 band in this peak of the Dando HAs.

Table 2 also shows the weight proportion of carbon layer planes and the mean carbon layer plane size in the HAs as well as the relative area of the 11 band in the HAs and FAs. If the peak area of the 11 band in the Inogashira HAs was regarded as 1.00, that in the Dando HAs was 0.58 (Table 2). This result indicates that the content of carbon layer planes in the Dando HAs was equivalent to approximately 60% of that in the Inogashira HAs. The size of carbon layer planes in the Inogashira HAs ranged from 0.48 nm to 1.68 nm, corresponding to model condensed aromatic nuclei (Fujimoto, 2003) consisting of 4–37 rings, and the mean size was 0.88 nm. Half of carbon layer planes in the Dando HAs had the size of 0.72 nm (similar to coronene), and the largest size of carbon layer planes was 1.20 nm (consisting of 19 rings). The mean size, 0.80 nm, was smaller than the Inogashira HAs. Similar results were also observed between the soil HAs with high and low degrees of humification (Ikeya et al., 2011).

In the XRD profiles of the two FAs (Fig. 5), the 10 band in the two FAs were weaker and the 11 band was observed as a shoulder. The 11 band area was 0.15 and 0.17 relative to that in the Inogashira HAs (Table 2). The rationale results of the weight

proportion of the carbon layer plane could not be obtained (not shown) due to the 11 band intensity being too weak. However, in the RuO<sub>4</sub> degradation analysis of the same samples (Ikeya et al., 2007a), sum of the yields of the BPCAs that were derived possibly from condensed aromatic nuclei consisting of 2–7 rings was 20 and 42 mg g<sup>-1</sup> for Dando and



**Fig 5.** XRD profiles (a) and the least-square fitting to theoretical profiles (broad gray line; only for the two HAs) of the 11 band<sup>a</sup> (b) of carbon black reference<sup>b</sup> and JHSS HA and FA standard samples.

<sup>a</sup> Expanded 3 times compared to (a).

<sup>b</sup> Cited from Ikeya et al. (2011).

*R* indicates the R-factor (%). Smaller *R* value indicates the higher accuracy in the fitting (Ikeya et al., 2011).

**Table 2** Weight proportion and mean size of carbon layer planes in humic acids and peak area of 11 band profile indicating carbon layer plane content in humic and fulvic acids.

Sample name	--- Weight proportion of each carbon layer plane (%) ---						Mean carbon layer plane size (nm)	Peak area of 11 band <sup>a</sup> (AU mg <sup>-1</sup> )	Sum of the yields of BPCAs <sup>b</sup> (mg g <sup>-1</sup> )
	0.48 nm (4) <sup>c</sup>	0.72 nm (7)	0.96 nm (14)	1.20 nm (19)	1.44 nm (30)	1.68 nm (37)			
<i>Humic acids</i>									
Dando	13	54	21	12	0	0	0.80	0.58	14
Inogashira	20	31	22	19	7	1	0.88	1.00	35
<i>Fulvic acids</i>									
Dando	-	-	-	-	-	-	-	0.15	20
Inogashira	-	-	-	-	-	-	-	0.17	42

<sup>a</sup> Determined for the 11 band profile by the base line method. Relative to 11 band area of the Inogashira HAs.

<sup>b</sup> Benzenepolycarboxylic acids (BPCAs) yielded from the RuO<sub>4</sub> degradation analysis (Ikeya et al., 2007a).

<sup>c</sup> Number of six-membered rings in model structure corresponding to each carbon layer plane.

Inogashira FAs, respectively, which was similar to that for Dando and Inogashira HAs, 14 and 35 mg g<sup>-1</sup>, respectively (Table 2). As stated above, the FTICR-MS results also indicate the abundance of components with molecular formulas similar to condensed aromatic hydrocarbons in the two FAs. Therefore, it is considered that the FAs contain polynuclear aromatic components with lower degrees of condensation at a similar level to the HAs but poor in larger and/or stacked carbon layer planes that can be detected and evaluated by XRD profile analysis. To prove this, an improvement of the present XRD profile analysis or development of another technique is required.

## Conclusions

In FTICR-MS, molecular formulas with H/C and O/C ratios similar to condensed hydrocarbons were dominant in both the HAs, while those with higher H/C ratios including formulas similar to lignin were more abundant in Dando HAs than in Inogashira HAs. These results correspond to the previous results of both the higher aliphatic C content (<sup>13</sup>C NMR) and lignin-derived phenols (thermochemolysis-GC/MS) in Dando HAs than in Inogashira HAs. In both FAs, molecular formulas having H/C and O/C ratios similar to lignin, condensed hydrocarbons, and tannins were dominant. Comparison of DBE values indicates that the HAs contain components having skeletal structures with higher degrees of unsaturation than the FAs. The larger DBE values were observed in Inogashira HAs with a larger degree of humification than in Dando HAs, which agrees with the previous studies using RuO<sub>4</sub> degradation. XRD profile analysis results also highlight that the Inogashira HAs contain larger amounts of condensed aromatic structures with a higher degree of condensation. However, the size distribution of the carbon layer planes in the FAs could not be estimated, suggesting that their size and content in the FAs were too small to be detected by this method.

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