

**From:** A. Watanabe, K. Itoh, S. Arai and S. Kuwatsuka: Comparison of the composition of humic acid and fulvic acids prepared by the IHSS method and NAGOYA method. *Soil Sci. Plant Nutri.*, **40**(4), 601-608 (1994).

## MATERIALS AND METHODS

- 1. Samples.** Samples used were obtained from A-horizons of a brown forest soil (Dystric Cambisol, Dando, Aich) and an ando soil (Humic Andosol, Inogashira, Shizuoka) in Japan. Some properties of the soils and the details of the NAGOYA method and IHSS method were described in Kuwatsuka et al. (1992).
- 2. Elemental analysis.** The determination of C, H, N, and total ash contents were requested to the Micro-analysis Laboratory of the School of Agriculture, Nagoya University. S, Fe and Al contents were determined using a fluorescence X-ray analyzer, Kevex Ultratrace 0660. O content was calculated by subtraction of C, H, N, S and ash contents from total weight. SiO<sub>2</sub> content was calculated by subtraction of Fe<sub>2</sub>O<sub>3</sub> and Al<sub>2</sub>O<sub>3</sub> contents from total ash content.
- 3. Determination of functional groups.** Total acidity was determined by the titration method of Martin et al. (1963) as modified by Tsutsuki and Kuwatsuka (1978a). Carboxyl group content was determined by the calcium acetate method of Blom et al. (1957). Total hydroxyl group content was determined by the acetylation method of DeWalt and Glenn (1952) as modified by Tsutsuki and Kuwatsuka (1978a). The difference between the total acidity and the carboxyl group content was regarded as the phenolic hydroxyl group content. The difference between the total hydroxyl group content and the phenolic hydroxyl group content was regarded as the alcoholic hydroxyl group content, respectively. Carbonyl group content was determined by the oximation method of Schnitzer and Riffaldi (1972).
- 4. Determination of hexose, uronic acid and amino acid.** Hexose and uronic acid contents were determined according to the methods of Tsutsuki and Kuwatsuka (1979) based on the colorimetry with anthrone-sulfuric acid (Atake and Seno 1968) and with carbazole-sulfuric acid (Bitter and Muir 1962), respectively. Amino acid content was determined by the TNBS method of Satake and Okuyama (1958) as modified by Tsutsuki and Kuwatsuka (1978b).
- 5. <sup>13</sup>C-NMR spectroscopy.** <sup>13</sup>C-NMR spectra were obtained for the HA and FA prepared by IHSS method as well as the XAD-8 adsorbed fraction of the FA by the NAGOYA method from the Inogashira ando soil. Samples were dissolved in a 0.5 mol NaOD with concentration of 100 mg mL<sup>-1</sup> for HA and 500 mg mL<sup>-1</sup> for FA and XAD-8 adsorbed fraction. The spectra were recorded under the same conditions described in Watanabe and Kuwatsuka (1992), and 22,000-25,000 scans were accumulated. The data for the HA and FA prepared by the NAGOYA method from the same soil was quoted from Watanabe and Kuwatsuka (1992).

Table 1. Elementary composition of HAs and Fas prepared by the NAGOYA method and IHSS method (%).

Humus fr.	Soil	Preparation method	C	H	N	O	S	Total ash	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	SiO <sub>2</sub>
HA	Dando <sup>a</sup>	NAGOYA	54.41	5.57	5.10	34.61	0.31	1.41	0.33	0.16	0.92
		IHSS	53.04	5.25	4.49	36.93	0.29	0.67	0.31	0.10	0.26
	Inogashira <sup>b</sup>	NAGOYA	57.25	4.21	4.17	34.23	0.14	3.42	0.28	0.12	3.02
		IHSS	54.83	4.27	4.01	36.63	0.26	0.49	0.22	0.10	0.17
FA	Dando <sup>a</sup>	NAGOYA	45.57	4.06	1.70	48.09	0.68	2.53	0.47	0.14	1.92
		IHSS	47.57	3.51	0.77	48.14	0.01	1.52	0.73	0.19	0.60
	Inogashira <sup>b</sup>	NAGOYA	43.13	4.52	2.58	49.09	0.67	1.29	0.45	0.11	0.73
		IHSS	43.28	3.47	1.73	51.44	0.08	5.99	0.98	0.16	4.85

The values of C,H,N,O and S are weight % on ash and water free basis. <sup>a</sup>brown forest soil. <sup>b</sup>ando soil.

Table 2. Composition of functional groups in HAs and Fas prepared by the NAGOYA method and IHSS method (me/g).

Humus fr.	Soil	Preparation method	Total acidity	Carboxyl	Total OH	Phenolic OH	Alcoholic OH	Carbonyl
HA	Dando <sup>a</sup>	NAGOYA	5.28	3.22	5.10	2.06	3.04	4.48
		IHSS	5.88	3.70	5.62	2.18	3.43	4.24
	Inogashira <sup>b</sup>	NAGOYA	5.81	3.75	3.45	2.06	1.39	6.33
		IHSS	6.31	4.35	4.43	1.96	2.56	6.05
FA	Dando	NAGOYA	8.64	7.27	3.13	1.37	1.76	5.80
		IHSS	9.08	7.72	0.52	1.36	—	5.83
	Inogashira	NAGOYA	6.66	6.11	5.16	0.55	4.61	4.96
		IHSS	10.88	9.81	0.69	1.07	—	5.44

<sup>a</sup>brown forest soil. <sup>b</sup>ando soil.

Table 3. Contents of hexose, uronic acid and amino acid in HAs and Fas prepared by the NAGOYA method and IHSS method .

Humus fr.	Soil	Preparation method	Hexose (%)	Uronic acid (%)	Amino acid (%)
HA	Dando(brown forest soil)	NAGOYA	3.15	1.10	9.65
		IHSS	4.15	1.40	8.80
	Inogashira (ando soil)	NAGOYA	3.45	1.30	6.50
		IHSS	5.60	1.80	5.60
FA	Dando	NAGOYA	8.20	4.10	4.55
		IHSS	0.25	0.70	2.38
	Inogashira	NAGOYA	18.4	9.90	8.05
		IHSS	1.25	0.70	3.55

Table 4. Composition of carbon species in HAs and Fas prepared by the NAGOYA method and IHSS method .

Humus fr.	Soil	Preparation method	Aliphatic-C (%) (10-60 ppm)	Carbohydrate-C (%) (60-110 ppm)	Aromatic-and olefinic-C (%) (110-165 ppm)	Carbonyl-C (%) (165-216 ppm)
HA	Dando (brown forest soil)	NAGOYA	32.8	19.3	32.8	15.2
		IHSS	30.3	23.4	32.7	13.6
	Inogashira (ando soil)	NAGOYA	18.8	10.9	44.7	25.7
		IHSS	22.5	22.2	36.5	19.0
FA	Dando	NAGOYA	24.2	29.5	17.7	28.8
		IHSS	22.0	18.8	37.0	22.2
	Inogashira	NAGOYA	17.6	38.6	16.9	26.9
		IHSS	26.3	16.3	27.1	30.5