

CHEMICAL STUDIES ON SOIL HUMIC ACIDS

I. Elementary Composition of Humic Acids

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Relationships between elementary compositions and types of humic acids or the degrees of humification were studied statistically using 39 humic acids prepared from various types of soils. Mean values of C%, H%, N%, O%, H/C, N/C, and O/C of the different types of humic acids (A, B, Rp(1), Rp(2), and Po) were compared. The elementary compositions of various types of humic acids were proven by variance analyses to be significantly distinct. Linear regression analyses of C%, H%, N%, O%, H/C, N/C, or O/C on *RF* or $\Delta\log K$ values were also carried out on 35 humic acids excluding the P_{~+++} type humic acids. The linear associations were found to be significant between C%-*RF* (5% level), H%-*RF*, H%- $\Delta\log K$, H/C-*RF* and H/C- $\Delta\log K$ (0.1% level), N/C-*RF* (0.1% level), N%-*RF* (1% level), N%- $\Delta\log K$ and N/C- $\Delta\log K$ (5% level), and O%-*RF* (1% level), while no significant relationship was found with regard to C%- $\Delta\log K$, O%- $\Delta\log K$ (both 5% level) and O/C-*RF* and O/C- $\Delta\log K$ (10% level). Carbon and oxygen contents of humic acids may be apt to reflect the different conditions of soils. The deeper the visible light absorption of humic acids and so the higher the degrees of humification, the lower the hydrogen contents of humic acids were. Though nitrogen content showed a trend similar to the trend of hydrogen content against *RF* values as a whole, the nitrogen content of less humified humic acids (Rp type) varied from very low to very high values, suggesting the enrichment of nitrogen into humic acid molecules in the early stage of humification. In the H/C versus O/C diagram, humic acids occupied an area reserved for the oxydation products of lignins. The area was fairly wide and "J"-shaped, that is, in the early to the middle stage of humification humic acids were arranged in the direction of dehydrogenation or demethanation, and in the later stage they were arranged in the direction of dehydration. *Additional Index Words:* degree of humification, elementary composition, humic acid, linear regression analysis.

Humic acids, which account for about one third of soil humus, are dark colored, amorphous, and high molecular materials. Though many studies on the chemical nature of humic acids have been accomplished since the early 18th century (2, 3, 5, 13), little is known about their chemical structures and compositions because they are very complicated, diverse and high molecular.

KUMADA and his co-workers (5, 6, 9) defined the degrees of humification and classified humic acids according to their visible light absorption. Many properties of the humic acids were related to the degree of humification or type of humic acid.

The purpose of this study is to get information on the chemical properties of

Table 1. Elementary composi-

Type	No.	Samples ^a	Origins ^b	Soil types ^c	Symbols ^d	$\Delta \log K$	RF	
A	2	Temmondai	1	K	●	.527	144	
	3	Nagara P	1	R	■	.555	140	
	4	Inogashira	1	K	●	.496	138	
	5	Jaana P	2	R	■	.478	132	
	6	Inogashira	2	K	●	.485	115	
	7	Kinshozan P	1	R	■	.543	115	
	8	Shitara Black	1	K	●	.547	114	
	9	Nagara F	1	R	■	.620	102	
	10	Fujinomiya	3	K	●	.511	97	
	12	Kinshozan P	3	R	■	.565	92	
	13	Komagahara	3	K	●	.542	89	
	14	Nagara OH	1	R	■	.667	89	
	15	Fujinomiya	2	K	●	.541	82	
	B	16	Shitara brown	1	K	⊙	.605	76
		17	Jaana F	2	R	▣	.619	65
18		Kuragari	1	B	⊙	.634	61	
19		Kinshozan F	1	R	▣	.731	60	
20		Anjo black	1	P/K	⊕	.642	57	
21		Higashiyama (A)	1	B	⊙	.664	47	
Rp (1)	28	Ishimakisan F	2	R	□	.729	37	
	29	Sanage	1	P	⊕	.711	32	
	31	Jaana OH	2	R	□	.714	30	
	32	Kinshozan OH	1	R	□	.851	29	
	34	Ishimakisan OH	2	R	□	.804	22	
	35	Gifu	1	P	⊕	.853	22	
	36	Anjo	1	P	⊕	.835	21	
	39	Kinshozan OH	3	R	□	.897	16	
Rp (2)	30	Higashiyama (F-H)	1	Ao	○	.776	31	
	33	Higashiyama (L)	1	Ao	○	.809	23	
	37	Yamamomo	2	R.W.	○	.968	21	
	38	Kisokoma (F)	1	Ao	○	.806	20	
Po	23	Ishimakisan P	2	R	▣	.656	43	
	24	Kuragari	2	B	⊙	.678	43	
	26	Onobaru	3	K	⊙	.678	39	
	27	Sanage	2	P	⊕	.657	38	
P+++	1	Tsubame P _g	4	H.b.	⊙	.272	202	
P+	11	Tsubame	4	H.b.	⊙	.469	94	
P++	22	Tsubame	1	H.b.	⊙	.513	69	
P++	25	Shishi	1	A	⊙	.506	42	

^a P, F, and OH denote the extractants for successive extraction, *i.e.* P 0.1 M sodium pyrophosphate, F 0.1 M sodium fluoride, OH 0.1 N sodium hydroxide. (L), (F), (F-H), and (A) denote the horizons of forest soil. ^b Origins: 1, Humic acid used for the study by KUMADA and SHIROYA (10); 2, Humic acid by KUMADA and KAWAMURA (8); 3, Humic acid prepared by authors; 4, Humic acid by SATO

tions of soil humic acids.

Per cent on moisture and ash free basis					Atomic ratio $\times 100$		
Ash	C	H	N	O	H/C	N/C	O/C
0.51	59.3	3.1	3.1	34.6	62	4.5	44
4.61	56.1	2.5	2.3	39.1	53	3.6	52
0.85	58.9	3.7	3.3	34.0	75	4.9	43
0.34	60.4	3.7	2.4	33.5	72	3.4	42
1.35	60.2	3.1	2.2	34.5	61	3.2	43
trace	57.3	3.8	2.6	36.3	79	3.8	48
3.88	58.7	3.4	3.4	34.5	69	4.9	44
1.62	56.6	3.5	4.0	36.0	73	6.0	48
1.25	55.6	4.1	3.7	36.6	88	5.8	49
1.59	55.1	3.5	2.6	38.8	76	4.0	52
2.08	54.8	3.8	3.1	38.3	82	4.9	52
3.86	56.2	3.9	4.6	35.4	83	7.0	47
0.84	57.2	4.9	3.1	34.2	102	4.6	45
13.9	56.1	5.1	4.4	34.5	108	6.9	46
3.45	56.8	4.6	4.7	34.0	96	7.0	45
4.87	57.9	4.9	4.3	33.0	100	6.2	43
1.46	57.1	4.5	3.9	34.6	93	5.9	46
6.81	56.4	4.9	4.2	34.5	103	6.4	46
1.59	56.5	4.9	3.3	35.4	103	4.9	47
2.56	57.6	5.2	3.7	33.5	107	5.6	49
trace	56.1	5.6	5.8	32.5	119	8.8	44
12.0	53.0	5.9	6.9	34.1	133	11.2	48
6.72	55.2	5.4	3.3	36.0	116	5.0	49
3.34	56.4	5.9	4.5	33.3	125	6.8	44
0.33	54.6	5.7	5.9	33.8	123	9.3	47
trace	55.0	5.4	5.3	34.4	116	8.3	47
4.04	53.7	6.2	6.2	33.9	137	9.9	47
1.43	56.3	5.2	3.4	35.1	110	5.1	47
0.87	59.1	5.7	3.3	32.0	115	4.7	41
1.54	58.5	5.0	1.1	35.4	101	1.6	45
trace	59.0	6.0	6.0	29.0	120	8.8	37
3.25	58.9	6.0	3.3	31.8	120	4.9	41
2.11	58.6	7.1	3.7	30.5	145	5.5	39
1.10	54.5	5.0	4.7	35.8	108	7.5	48
1.63	58.1	5.9	4.6	31.4	121	6.8	41
8.8	57.3	2.5	1.3	38.9	51	2.0	51
6.4	56.3	4.1	2.8	36.8	88	4.3	49
3.4	56.9	5.3	3.7	34.1	111	5.7	45
4.7	58.3	6.6	4.1	31.1	135	6.0	40

and KUMADA (12). ^c Soil types: K, Kuroboku (Volcanic ash soil); R, Rendsina like soil; B, Brown forest soil; P, Paddy soil on alluvial land; P/K, Paddy soil on Kuroboku; Ao, Ao horizon of forest soil; R.W., Rotted wood; H.b., Humic burried soil; A, Alpine grassland soil. ^d Symbols: These symbols are used in the figures.

humic acids and, furthermore, to contribute to the elucidation of their chemical structures and compositions, using many humic acids with different degrees of humification prepared from different types of soils.

In this report, elementary composition was investigated as a fundamental property of humic acids. Thirty-nine samples of humic acids were employed. The relationship between elementary composition and visible light absorption was investigated statistically.

MATERIALS AND METHODS

1) *Humic acids used.* The origins of humic acids used in this study are indicated in Table 1. Of the 39 samples, 34 samples were those which were used in the studies by KUMADA and KAWAMURA (8), KUMADA and SHIROYA (10), and SATO and KUMADA (12). The other 5 samples were prepared additionally for this study. Most of the humic acid samples were extracted and purified according to the method of KUMADA and KAWAMURA (7), but the samples used by KUMADA and SHIROYA (10) were extracted at a solvent/carbon-content ratio of 100 : 1 instead of 300 : 1 for other samples. The humic acid prepared additionally for this study were extracted at room temperature for 24 hr instead of 100°C for 30 min. Tsubame Pg (No. 1) is a green fraction separated from the alkaline solution of Tsubame humic acid (No. 11) by Sephadex G-50 (12). Some pairs of samples used in this study were those which were extracted by different methods from soils collected at the same place, e.g., Inogashira (No. 4, 6), Kinshozan (No. 7, 19, 32 and 12, 39), Fujinomiya (No. 10, 15), Kuragari (No. 18, 24), Sanage (No. 27, 29), and Tsubame (No. 11, 22). Though they were prepared from soils obtained from the same place, differences in the extracting methods and the dates of soil sampling made their elementary compositions and visible light absorptions pretty different. So these pairs of samples were treated as different samples.

2) *Ultimate analysis.* Carbon, hydrogen and nitrogen, and ash contents were determined after the samples were dried over P₂O₅ in a vacuum desiccator and after the weights of samples reached constant values. Oxygen content was calculated by subtracting the weights of ash, carbon, hydrogen, and nitrogen from the total weight. Sulfur content was neglected because it is negligibly small as compared to oxygen content (13).

3) *Definitions of the degrees of humification and the classification of humic acid based on visible light absorption.* The degree of humification is expressed by values of *RF* and $\Delta\log K$ according to the definition by KUMADA *et al.* (9). These values are calculated by the following formula.

$$RF = K_{600}/C \times 1,000$$

$$\Delta\log K = \log (K_{400}/K_{600})$$

where $K_{600}(K_{400})$ is the optical density at 600 (400) nm of the humic acid dissolved in

0.1 N NaOH solution, and C is the volume of 0.1 N KMnO_4 consumed by 30 ml of the humic acid solution whose optical density was measured.

Classification of humic acids into each type: According to the definition by KUMADA *et al.* (9), humic acids are classified into A, B, Rp, and P type. In addition to this, Rp type humic acids are further divided into two groups, one of which consists of humic acids from A layer soils and plowed layer soils, while the other consists of humic acids from Ao layer humus and rotted wood. These are named Rp(1) and Rp(2), respectively. P type humic acids are also divided into Po type humic acids, which do not show the Pg absorption, and $P_{+\sim++++}$ type humic acids, which show the distinct Pg absorption.

RESULTS AND DISCUSSION

The elementary composition of humic acids used are listed in Table 1. They were expressed, as usual, in terms of weight per cent and also by the atomic ratios of H/C, N/C, and O/C in order to clarify the stoichiometric relationships among these elements.

1) Elementary composition and types of humic acids

In Table 1, humic acids were divided into A, B, Rp(1), Rp(2), Po, and $P_{+\sim++++}$ types. Elementary compositions of A, B, Rp(1), Rp(2) and Po types of humic acid were compared. $P_{+\sim++++}$ type humic acids were excluded from the statistical comparison, because $P_{+\sim++++}$ type humic acids are differentiated from other types of humic acids on a different basis such as the presence of Pg, while the other types are classified according to the degree of humification.

The mean values of C%, H%, N%, O%, H/C, N/C, and O/C of humic acids belonging to each type are listed in Table 2. Mean values showed sequences given

Table 2. The difference in elementary composition between the types of humic acids.

	Average value within each type						Mean square ratio	Level of significance
	A	B	Rp(1)	Rp(2)	Po	Total		
C %	57.4	56.8	55.2	58.2	57.5	56.9	3.39	*
H %	3.61	3.81	5.66	5.47	5.99	4.77	7.80	***
N %	3.11	4.13	5.20	3.45	4.10	3.91	4.48	**
O %	35.8	34.3	33.9	32.9	32.4	34.4	4.17	**
100×H/C	75	101	122	112	124	100	30.3	***
100×N/C	4.66	6.22	8.11	5.05	6.18	5.93	5.86	**
100×O/C	46.9	45.5	46.9	42.5	42.3	45.6	2.90	*

Critical values of mean square ratio corresponding to different levels of significance are 2.69 for 5%, 4.02 for 1%, and 6.12 for 0.1% level.

*, **, and *** indicate significant at 5%, 1%, and 0.1% levels of significance respectively.

below, and the levels of significance of the relationships between elementary composition and type of humic acids were tested by variance analysis.

C%; $Rp(1) < B < A = Po < Rp(2)$; significant at 5% level

H% and H/C; $A < B < Rp(2) < Rp(1) < Po$; significant at 0.1% level

N% and N/C; $A < Rp(2) < Po < B < Rp(1)$; significant at 1% level

O%; $Po < Rp(2) < Rp(1) < B < A$; significant at 1% level

O/C; $Po < Rp(2) < B < Rp(1) < A$; significant at 5% level

Rp(2) and Po type humic acids showed higher carbon and hydrogen contents and lower nitrogen and oxygen contents and their elementary compositions seemed to resemble those of lignins. It can be concluded from these tests that the elementary compositions of humic acids are significantly distinct among the types of humic acids.

2) Elementary composition and degrees of humification (RF , $\Delta \log K$)

The results of linear regression analyses between C%, H%, N%, O%, H/C, N/C, or O/C and RF or $\Delta \log K$ were listed in Table 3. Linear regression analysis was carried out on 35 humic acids but not on $P_{+ \sim + + +}$ type humic acids for the same reason mentioned above. The significance of the linear association was tested by the correlation coefficient r . Critical values of correlation coefficients for different

Table 3. Correlation coefficients between elementary compositions and RF or $\Delta \log K$ of soil humic acids. ($n=35$)

Y	X	r	Levels of significance
C %	RF	0.354	*
C %	$\Delta \log K$	-0.314	N.S.
H %	RF	-0.883	***
H %	$\Delta \log K$	0.686	***
H/C	RF	-0.896	***
H/C	$\Delta \log K$	0.706	***
N %	RF	-0.494	**
N %	$\Delta \log K$	0.382	*
N/C	RF	-0.526	***
N/C	$\Delta \log K$	0.389	*
O %	RF	0.459	**
O %	$\Delta \log K$	-0.301	N.S.
O/C	RF	0.146	N.S.
O/C	$\Delta \log K$	0.068	N.S.

Critical values of the correlation coefficient for different levels of significance are 0.279 for 10%, 0.331 for 5%, 0.430 for 1%, and 0.533 for 0.1% level.

*, **, and *** indicate significant at 5%, 1%, and 0.1% levels of significance respectively. N.S. = Not significant.

degrees of significance with 33 degrees of freedom were quoted from the values in Table 7 of Statistical Tables (1).

As shown in Table 3, the linear association between carbon contents (%) and RF values of humic acids proved to be significant at 5% level, while that between carbon contents and $\Delta \log K$ values proved to be not significant at 5% level. So, the tendency of carbon content to increase with the increase in the degree of humification was not very significant. In Fig. 1, carbon contents were plotted against RF values. Though $C\%$ values of humic acids were distributed within a relatively narrow range (53–61%), they were scattered about the regression line considerably. Unlike the results obtained here, SHIROYA and KUMADA (14) showed that the carbon content of each fraction obtained from a humic acid by the fractional alcohol precipitation method increases with the increase in RF values and with the decrease in $\Delta \log K$ values. These results indicate that the carbon content of humic acids may increase during humification on one hand, while, on the other hand, the carbon content may be so apt to reflect the differences in soil conditions because the level of significance of linear association is lowered when many humic acids from different soils are compared together.

Linear associations between hydrogen content and the degree of humification were all proved significant at 0.1% level (Table 3). The atomic ratio H/C showed slightly more significant linear associations with the degree of humification than $H\%$, and RF showed more significant linear associations with hydrogen content than $\Delta \log K$. As shown in Fig. 2, the inclination of H/C ratios to decrease with humification was quite clear. Linear and sequential relationships between hydrogen content and

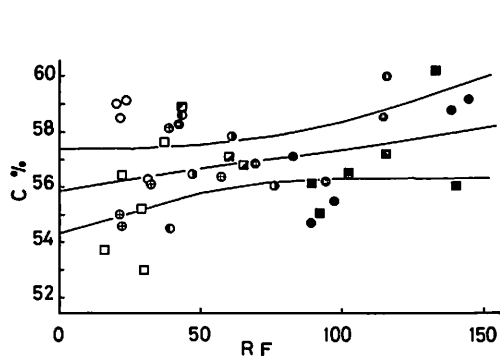


Fig. 1.

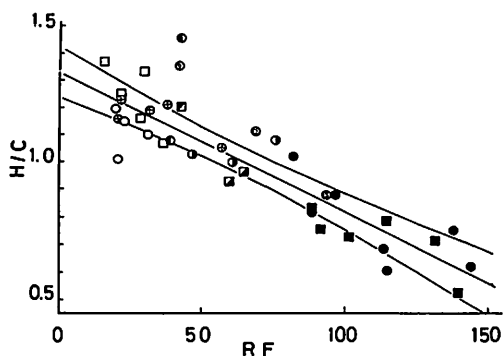


Fig. 2.

Fig. 1. $C\%$ versus RF diagram with a regression line and confidence limit (99%) of the estimate of $C\%$.
 $(C\%) = 1.60 \times 10^{-2} \times (RF) + 55.9$, $r = 0.354^*$.

Fig. 2. Atomic H/C ratio versus RF diagram with a regression line and confidence limit (99%) of the estimate of H/C .
 $(H/C) = -5.15 \times 10^{-3} \times (RF) + 1.33$, $r = -0.896^{***}$.

the degree of humification indicate that the hydrogen content largely contribute to the visible light absorption of humic acids and also suggest that the manner by which humic acids lose hydrogen is independent on the soil conditions or the types of humic acids.

Linear associations between the nitrogen content and the degree of humification were also proven significant, but the levels of significance differed among the pairs, *i.e.* 0.1% for N/C-*RF*, 1% for N%-*RF*, and 5% for N%- $\Delta\log K$ and N/C- $\Delta\log K$. The atomic ratio N/C showed more significant linear associations with the degree of humification than N%, and *RF* showed more significant linear associations with the nitrogen content than $\Delta\log K$.

In Fig. 3, N/C values were plotted against *RF* values. The N/C values of humic acids with low *RF* values were scattered considerably about the regression line, and the N/C values of humic acids with high *RF* values were distributed close to the regression line and decreased as *RF* values increased. SUZUKI and KUMADA (15) showed that the nitrogen contents of Rp type humic acids extracted from rotted plants of several species fluctuated considerably according to their species and degrees of decomposition. SHIROYA and KUMADA (14) showed that the nitrogen content of each fraction of a humic acid fractionated with alcohol increased steeply with the increase in *RF* at an early stage, but they decreased gradually when *RF* values increased to a greater extent. These results seem to suggest that the process of humus formation in soil consists of two processes with regard to the change in nitrogen content, first, the sources of soil humus such as lignin are transformed into humic substances

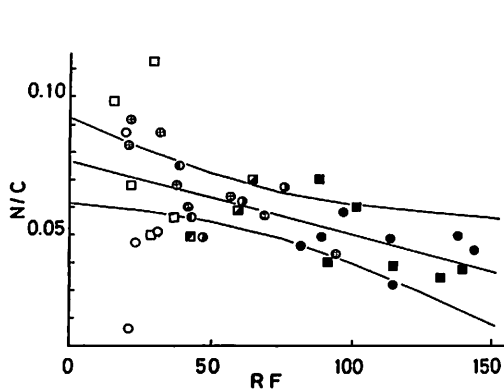


Fig. 3.

Fig. 3. Atomic N/C ratio versus *RF* diagram with a regression line and confidence limit (99%) of the estimate of N/C.

$$(N/C) = -2.66 \times 10^{-4} \times (RF) + 7.66 \times 10^{-2}, r = -0.526^{***}.$$

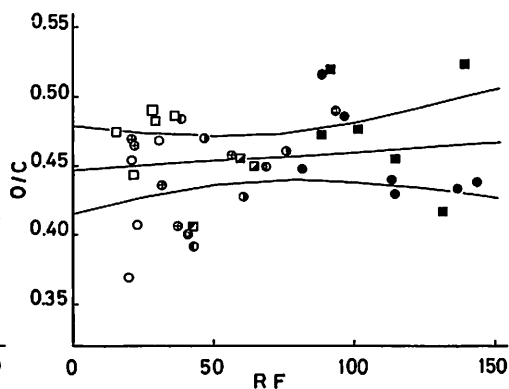


Fig. 4.

Fig. 4. Atomic O/C ratio versus *RF* diagram with a regression line and confidence limit (99%) of the estimate of O/C.

$$(O/C) = 1.28 \times 10^{-4} \times (RF) + 0.447, r = 0.146 \text{ N.S.}$$

rich in nitrogen, and later nitrogen is released from humic substances during humification. Furthermore, though it was indicated by FLAIG *et al.* (2) that nitrogen in the form of hetero-rings contributes to the visible spectra of humic acids, this type of nitrogen may not account for an important part of total nitrogen, because nitrogen content in humic acids decreased as *RF* values (optical density at 600 nm) increased.

Linear associations between oxygen content and the degree of humification were proven to be not significant except O%-*RF* which was significant at 1% level. The atomic ratio is a more essential expression of the stoichiometric relationships between elements, and the linear association of O/C with the degree of humification proved to be not significant even at the 10% level. In Fig. 4, O/C values of humic acids were plotted against *RF* values. Humic acids of Rp(2) type had higher O/C values, and some humic acids of A type had lower O/C values. SHIROYA and KUMADA (14) indicated that the oxygen content of each fraction, fractionated from a humic acid with alcohol, at first gradually increased and then decreased as their *RF* values increased. The result of SHIROYA and KUMADA and also that obtained in this study (Fig. 4) show that oxygen content seems to behave in a similar manner with regard to the degree of humification. The relationships between oxygen content and the degree of humification of humic acids shall be investigated further in a forthcoming paper by analyzing oxygen-containing functional groups.

3) Comparison of C, H, O composition among humic acids, fulvic acids, coals, and several kinds of plant constituents by H/C versus O/C diagram

SHIROYA and KUMADA (14) used H/C versus O/C diagram for comparison of the elementary compositions of humic acids and coals. The relationships between a coal band and plant constituents with regard to the elementary composition was investigated and diagrammed by VAN KREVELEN (4) and MURATA (11). In Fig. 5, figures from the papers of VAN KREVELEN (4) and MURATA (11) were quoted together, in order to analyse how humic acids and fulvic acids are distributed in their diagrams. In addition to the data on humic acids used in this study, the elementary compositions of 12 Rp type humic acids reported by SUZUKI and KUMADA (15) and of 7 humic acids and 7 fulvic acids reported by KONONOVA (3) were quoted and plotted in Fig. 5.

A coal band given by MURATA (11) was fairly broader than that given by VAN KREVELEN (4). This was due to the difference in the species of coal between the Tertiary period coal in Japan and the Carboniferous period coal in Europe. MURATA (11) indicated that coal bands differed depending on the species of coal, so that a coal band becomes broad when many coal bands are graphed together. The area of distribution of lignite given by VAN KREVELEN (4) was also rather smaller than that given by MURATA (11). The lignite of VAN KREVELEN might not have included various kinds of lignites. Though the area of lignin distribution also differed between the two authors, the C, H, O compositions of lignins shown by FLAIG *et al.* (2) are placed in the middle area of those given by VAN KREVELEN (4) and MURATA (11). So the area of lignin distribution must be a wider one including both areas.

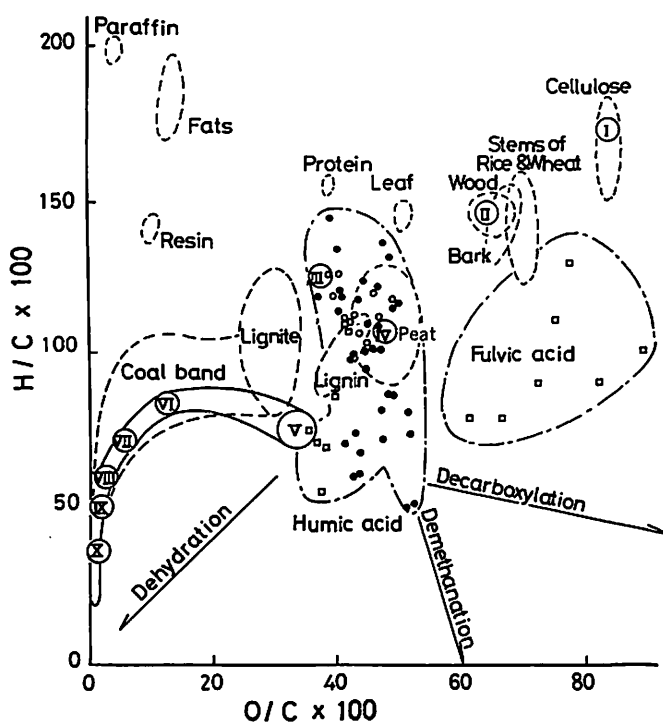


Fig. 5. Atomic H/C versus O/C diagram.

I, Cellulose; II, Wood; III, Lignine; IV, Peat; V, Brown coal (Lignite); VI, Low rank bituminous coal; VII, Medium rank bituminous coal; VIII, High rank bituminous coal; IX, Semi-anthracite; X, Anthracite (by VAN KREVELEN (4)).

Areas enclosed by broken line were quoted from the diagram by MURATA (11). ●, humic acids used in this study; O, humic acids by SUZUKI and KUMADA (15); □, humic and fulvic acids by KONONOVA (4).

Humic acids occupied a considerably broad "J"-shaped area, with H/C ratios ranging from 1.5 to 0.5 and with O/C ratios from 0.35 to 0.55. C, H, O compositions of humic acids were most similar to those of lignins among the plant constituents, and humic acids were placed as oxidation products of lignins in Fig. 5. On the other hand, lignites were placed in an area reserved for the decarboxylation products of lignins. As shown here, humification and coalification seemed to proceed in reverse directions during the early stages. Fulvic acids occupied a very wide area on the right side of humic acids and were indicated to be oxygen rich compounds. Some fulvic acids may be transformed to humic acids releasing oxygen. And the C, H, O compositions of some fulvic acids were similar to those of fresh plant constituents such as carbohydrates, stems of rice and wheat, and woods.

VAN KREVELEN (4) represented the principal reactions such as decarboxylation, demethanation, dehydration, dehydrogenation, hydrogenation and oxidation by

straight lines on an H/C versus O/C diagram. In the early to the middle stage of humification, plots are arranged in the direction of dehydrogenation or demethanation which may indicate that aliphatic parts are split off from humic acid molecules as humification proceeds. However, in the later stage of humification, plots were likely to be arranged in the direction of dehydration, indicating that a dehydrative condensation reaction may be taking place in humic acids of high degrees of humification.

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