

DEGRADATION AND STABILIZATION OF HUMUS IN BURIED VOLCANIC ASH SOILS

I. Humus Composition, Molecular Size Distribution of Humic Acids, and Sugar Composition of Soils

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Changes in the characteristics of the humus of buried volcanic ash soils were investigated in relation to the soil age attaining up to 30,000 YBP. With increasing soil age, the ratios of humin C/total C and $\text{Na}_4\text{P}_2\text{O}_7$ -extractable C/NaOH-extractable C increased. The initial decrease of the ratio of sugar C/total C in the recent layers (< 10,000 YBP) was followed by the increase in this ratio in the buried layers older than 10,000 YBP. These phenomena suggested that the humus and polysaccharides of buried volcanic ash soils are stabilized in bound forms with mineral components. The treatment of the $\text{Na}_4\text{P}_2\text{O}_7$ -extracted residue with NaBH_4 solubilized the HA exhibiting an intense visible absorption of Pg. The molecular size of HA increased in the recent layers with age, but partially decreased in the buried layers. It is inferred from these results that the decomposition of the easily decomposable organic components and the formation of humic substances took place in the recent layers, while the decomposition of unprotected humic substances proceeded in the buried layers.

Key Words: humus composition, molecular size distribution, soil age, sugar composition.

SCHARPENSEEL *et al.* (1986) and TSUTSUKI *et al.* (1988) have investigated the stabilization of the humus in Mollisols by ^{14}C -dating and the humus composition analysis of particle size fractions of the soils. On the basis of these studies the humus with high ^{14}C -ages and HA with a higher degree of humification were associated with medium sized and coarse clay complexes. The mechanism of humus-stabilization in other types of soils should also be investigated. Andosols, which are one of the most widely distributed soils in Japan, accumulate larger amounts of humus than Mollisols where the mechanism of the stabilization of the humus is assumed to be different from that in Mollisols. In this and the following papers, the characteristics of the humus in the buried volcanic ash profile which dates back to *ca.* 30,000 years before present (YBP) were investigated for the purpose of clarifying the mechanism of degradation and stabilization of the humus in Andosols. The characteristics of the humus were investigated with regard to the composition of humic acid (HA), fulvic acid (FA), and humin, C/N ratios of soils and humin fractions as well as the degree of humification

and the molecular size distribution of HAs, and the sugar composition of the H_2SO_4 -hydrolysates of soils.

MATERIALS AND METHODS

1. *Soil samples.* An array of soil samples were collected from the archaeological excavation site at Ashitaka-Onoue in Numazu City, Shizuoka Prefecture, Japan. The soil profile holds several key layers with known radio-carbon ages (Ashitaka Loam Research Group 1970; KATO *et al.* 1986) as illustrated in Fig. 1. Recent humic layers (<10,000 YBP, depth <1.5 m) and the deeper "upper loam" layers (10,000–30,000 YBP, depth <7 m) were collected from two different profiles located several meters apart, because the recent layers were removed at the excavation site. The soil samples thus obtained were marked by the letters A, B, C, D, and figures 1–21, respectively. Six layers with high organic matter contents and with known ages (A, D, 4, 8, 15, 20) were selected for the detailed analysis of the humus.

2. *Preparation of the humin fraction.* Soils were extracted with 0.1 N NaOH at room temperature for 48 h under intermittent shaking. The ratio of the soil carbon (g) to the volume of extractant (ml) was 1 : 300. The residue of extraction (humin fraction) was washed with water and freeze-dried. This humin fraction is assumed to contain the combined-form humic and fulvic acids. Therefore, the term "humin fraction" is not used in its strict meaning here.

3. *Determinations of C and N contents of soils and humin fractions.* The C and N contents of the soils and humin fractions were determined with a CN analyzer (Yanaco MT 500) using cobalt oxide as an oxidizing agent.

4. *Analysis of humus composition.* In the selected samples, the humus composition was analyzed by a more detailed method using smaller amounts of samples. Soil samples containing *ca.* 100 mg of carbon were weighed and extracted successively with 0.1 N NaOH and 0.1 N $Na_4P_2O_7$ in a steam bath according to the method of KUMADA *et al.* (1967). Each extraction was repeated three times (30+20+20 ml). To the residue, 100 mg of $NaBH_4$ and 30 ml of 0.1 M $Na_4P_2O_7$ were added, left to stand overnight, centrifuged and washed two more times with $Na_4P_2O_7$. Extracted solutions were acidified to pH 1 with sulfuric acid and filtered (Toyo filter paper No. 5B) to separate HA and FA. The HA (after dissolution in 0.1 N NaOH) and FA were made up to fixed volumes. Carbon contents of these solutions were determined by 0.1 N $KMnO_4$ titration. Ultraviolet and visible absorption spectra of HA solution in 0.1 N NaOH were measured in the range of 230–700 nm. The following parameters were calculated for estimating the degree of humification:

$$RF = (E_{600} / \text{Cons. } KMnO_4) \times 1,000,$$

and

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

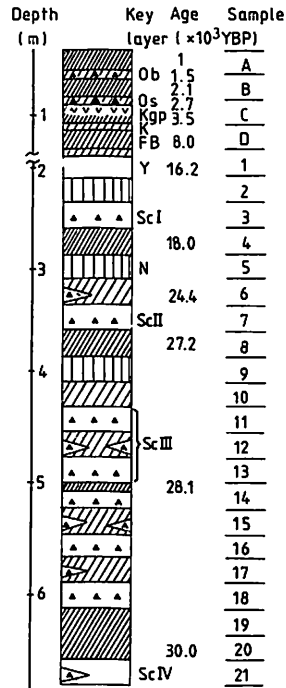


Fig. 1. Diagram of Ashitaka-Onoue buried volcanic ash soil profile. Ages of soils were cited from KATO *et al.* (1986). Names of key layers are as follows: Ob, Ohbuchi scoria; Os, Ohsawa scoria; Kgp, Kawagodaira pumice; K, Kuri-iro bed; FB, Fuji black soil; Y, Yasumiba bed; ScI, the first scoriaceous bed; N, Nise loam bed; ScII-IV, the second-fourth scoriaceous beds.

where E_{600} and E_{400} are the extinction coefficients at 600 and 400 nm and *Cons.* KMnO_4 is the amount (ml) of 0.1 N KMnO_4 by 30 ml of the HA solution, respectively.

5. *Permeation chromatography of HA on CPG10-170.* Humic acid extracted with 0.1 N NaOH was precipitated with dil. HCl from the solution. The precipitate was collected and redissolved in 0.05 M Tris-phosphate buffer (pH 7.5). A 0.2 ml portion of this solution was eluted on a CPG10-170 column (glass, 100 cm \times 8.2 mm i.d.) with the same buffer. Detailed procedures have been described elsewhere (TSUTSUKI and KUWATSUKA, 1984).

6. *Sugar composition.* Soils were soaked in 26 N H_2SO_4 at room temperature for 16 h and then refluxed with 1 N H_2SO_4 for 5 h. After the addition of myoinositol as an internal standard, the alditols of sugars were prepared by the method of OADES *et al.* (1970) and trifluoroacetylated with 0.2 ml TFAA+0.2 ml ethylacetate at room temperature.

Conditions of gas-liquid chromatography were as follows: gas-chromatograph—Hitachi 023 with a FID detector, column—Silicone OV-105 2% on Uniport HP 80/

100 mesh packed in a glass column 2 m×3 mm i.d., temperature—130°C constant during the analysis and 220°C for 30 min to regenerate the column after every analysis, carrier gas—N₂ 50 ml/min.

RESULTS AND DISCUSSION

1. Changes in soil total C and humin C contents and C/N ratios with age

Figure 2 shows the distribution of total C and humin C in the profile. Total C content in the recent humic layers (A-D) decreased from 12 to 4-6% with age. Humin C accounted for *ca.* 60-70% of the total C in the uppermost horizons, while the ratio increased with age. In layers below Layer C, the total C content ranged from 2-5%, of which humin C accounted for *ca.* 80-90%. The humin fraction, as discussed here, contains the combined form humus due to the mild separation method, and the increase in this fraction can be attributed, therefore, to the increase in the combined form as shown in the results of humus composition analysis (Table 1). The results indicated that soil organic matter was stabilized in buried volcanic ash layers in the form of humin including the combined form humus and remained stable for as long as *ca.* 30,000 years.

C/N ratios of soils and humin fractions (Fig. 2) increased with age in the profile. The increase was relatively small and the C/N ratios were relatively constant (*ca.* 30)

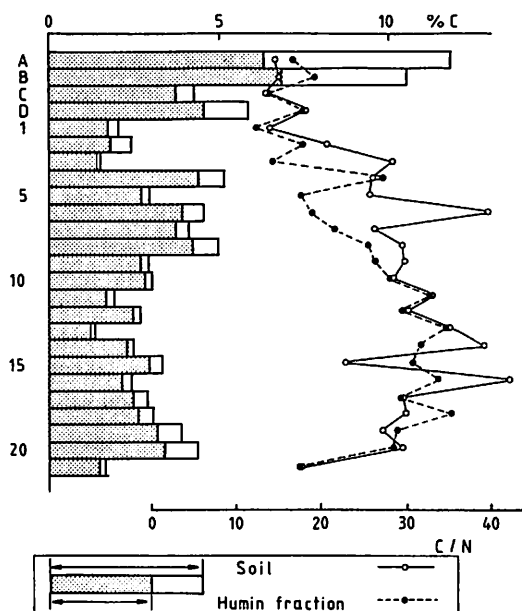


Fig. 2. Total carbon and humin form carbon contents (bar graphs) and C/N ratios of soils (solid line) and humin fractions (broken line) of the buried volcanic ash profile.

among the layers below Layer 8 (>27,200 YBP). The C/N ratios of the humin fractions were lower than the soil C/N of Layers 1-9 (16,200-27,200 YBP). The presence of a lower C/N in the humin fraction is interesting, because the C/N of humin was always higher than the C/N of soil in a peat soil profile (unpublished data). The small difference between the soil C/N and humin C/N in layers below Layer 10 is due to the very high percentage (*ca.* 90%) of humin C in soil organic C.

2. Composition of humus extracted with different solvents

Table 1 shows the yield of HA and FA extracted successively with three different solvents. In the uppermost layer A, the amount of FA exceeded that of HA and the amounts of HA and FA extracted with $\text{Na}_4\text{P}_2\text{O}_7$ were *ca.* 1/10 of those extracted with NaOH. In layers lower than D, the percentage of HA and FA in bound forms increased with age. The same trend was also reported for another buried volcanic ash profile by YOSHIDA *et al.* (1978). The amounts of HA and FA solubilized by the NaBH_4 reduction were low, but their percentages increased with age. The increase in the percentages of $\text{Na}_4\text{P}_2\text{O}_7$ -extractable humus with age indicates that the complex formation with soil minerals is an important mechanism of humus stabilization in the volcanic ash soils. This may be due to the change in the structure of humic substances which increases their affinity to soil minerals. Though the amount of humus solubilized by the reduction with NaBH_4 was low, absorption maxima of HAs in this fraction were observed at 615, 570, 450, and 430 (inflection) nm, which were characteristic of the Pg pigment (Fig. 3). This observation suggests that Pg (green fraction found in HAs by SATO and KUMADA (1976)) is preserved in a stable manner in volcanic ash soils by forming a complex with ferric compounds.

Table 2 shows the changes in the values of *RF* and $\Delta \log K$ of HAs extracted successively. Regardless of extractability, the value of *RF* of HAs increased remarkably from Layer A to Layer D. In the buried horizons, the value of *RF* of NaOH- and

Table 1. Amounts of HA and FA extracted successively with three different extractants from the surface and buried layers of Ashitaka-Onoue volcanic ash soil profiles (mg C/g soil).^a

Layer	NaOH-extract		$\text{Na}_4\text{P}_2\text{O}_7$ -extract		NaBH_4 -extract		Total C
	HA	FA	HA	FA	HA	FA	
A	33.2	44.6	2.93	4.36	0.58	0.58	169.0
D	16.6	13.2	3.77	5.59	0.44	0.57	79.5
4	15.9	14.3	3.40	4.10	0.42	0.76	68.7
8	13.0	6.12	7.88	4.94	0.59	0.99	61.5
15	3.12	2.39	4.66	4.36	0.15	0.31	42.3
20	10.2	8.19	6.16	5.36	0.45	0.80	54.8

^aRate of conversion from the amount of 0.1 N KMnO_4 consumed (ml) to the amount of carbon (mg): HA 0.45 mg C/ml, FA 0.40 mg C/ml. Total C content was determined by dichromate oxidation (WALKLEY 1946).

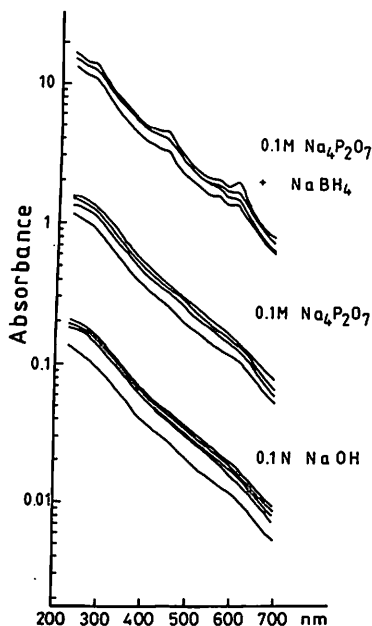


Fig. 3. Ultraviolet and visible absorption spectra of HAs extracted successively from humic layers of the buried volcanic ash soil profile.

Table 2. RF and $\Delta \log K$ of HAs of Ashitaka-Onoue volcanic ash soil profile.

Layer	NaOH-extract		Na ₄ P ₂ O ₇ -extract		NaBH ₄ -extract	
	$\Delta \log K$	RF	$\Delta \log K$	RF	$\Delta \log K$	RF
A	0.527	95.8	0.486	123	0.435	143
D	0.525	144	0.490	149	0.430	187
4	0.534	138	0.487	167	0.402	201
8	0.531	160	0.501	163	0.430	187
15	0.551	132	0.534	154	0.481	158
20	0.527	152	0.504	171	0.422	176

Na₄P₂O₇-extractable HAs did not increase remarkably with age. On the other hand, the $\Delta \log K$ values of HA was already low even in the uppermost layer (A), but did not show remarkable changes with age. Lower $\Delta \log K$ implies a higher ratio of E_{600}/E_{400} , and the development of the conjugate system responsible for the visible absorption of HAs at the same time. This finding suggests that the highly conjugated chromophore of HA was already formed during the period when each buried layer was at the surface of the earth and changed little after the burial. RF of HA extracted with NaBH₄ showed maximum values in Layer 4 (18,000 YBP), which reflects the high Pg content

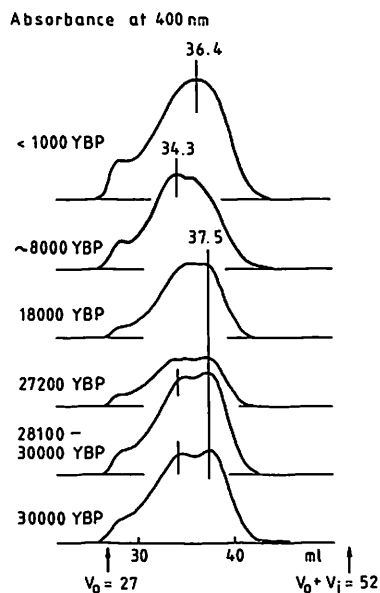


Fig. 4. Permeation chromatogram of HAs extracted from humic layers of the buried volcanic ash soil profile on CPG-10 (pore size 170 Å).

in this layer, and the correlation between the Pg formation and the cold climate was inferred, because the age around 18,000 YBP corresponded to the last glaciation maximum (MÖRNER 1984).

RF values were larger in the order of NaBH_4 -extract $>$ $\text{Na}_4\text{P}_2\text{O}_7$ -extract $>$ NaOH -extract, while the $\Delta \log K$ values were lower in the order of NaBH_4 -extract $<$ $\text{Na}_4\text{P}_2\text{O}_7$ -extract $<$ NaOH -extract. These trends indicate that the HA which was present in more stable organo-mineral complexes exhibited also a higher degree of humification.

3. Changes in the molecular size distribution of HAs with age

DANNEBERG (1977) and TSUTSUKI and KUWATSUKA (1984) used permeation chromatography on controlled pore glass as a new technique to monitor the molecular size distribution of HAs and FAs. Figure 4 shows the change with age in the molecular size distribution of HAs extracted with NaOH . Except for the very small void volume fraction, the permeation chromatogram of the HA of Layer A showed only one peak near 36.4 ml. For the HA of Layer D, a new peak appeared near 34.3 ml, indicating the increase in molecular size. This observation suggests that the decomposition of the easily decomposable low molecular size fraction took place in the recent layers. The peak or shoulder near 34.3 ml was common in all the HAs of buried layers. However, a new peak appeared near 37.5 ml in the HAs of Layers 4, 8, 15, and 20. The

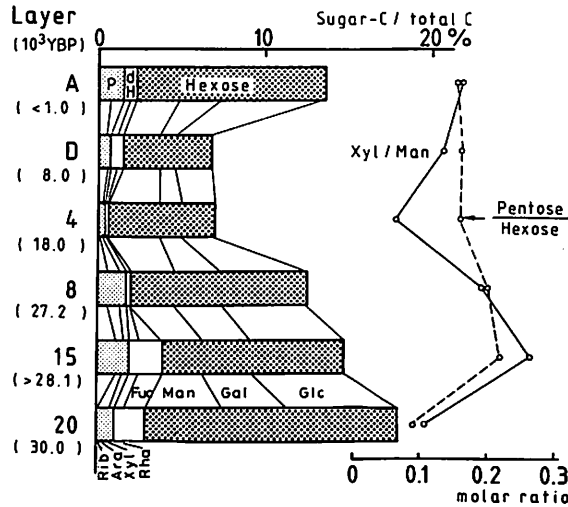


Fig. 5. Sugar composition in H_2SO_4 hydrolysates of humic soils in the buried volcanic ash soil profile. Rib, ribose; Ara, arabinose; Xyl, xylose; Rha, rhamnose; Fuc, fucose; Man, mannose; Gal, galactose; Glic, glucose; P, pentose; dH, deoxyhexose.

appearance of this low molecular size component suggests the decomposition of once-formed humic substances with age.

4. Changes in the sugar composition with age

Figure 5 shows the changes in the composition and yields of sugars from selected humic layers. Hexoses were dominant (>70%) and pentoses and deoxyhexoses were minor components in the hydrolysates. Among the hexoses, the amount of mannose and galactose was almost the same as or larger than that of glucose. These considerably high contents of mannose and galactose are typical features of the sugar composition in volcanic ash soils (MURAYAMA 1980). From Layers A to D, the ratio of sugar C to total soil C decreased, indicating the decomposition of relatively easily decomposable polysaccharides in the recent layers. In contrast, the ratio increased steadily with age in layers below D, implying that some part of the soil polysaccharides is stabilized remarkably and becomes less decomposable than the other soil organic components in volcanic ash soils with age. Stabilization of the soil polysaccharides in allophanic soils has been reported and the complex formation with mineral components is considered to be the main cause (MARTIN 1971; MURAYAMA 1980).

It is also possible that these sugar contents reflect the amounts originally accumulated in the layer when the layer was at the surface. Without any protection mechanism, however, these easily decomposable polysaccharide constituents could not have been preserved during such a long period.

The low values of the xylose/mannose and pentose/hexose ratios are also illustrated in Fig. 5. In paddy soils and upland soils, high xylose/mannose ratios (>1) have been reported, and pentose/hexose ratios of many arable soils are also high (>0.3) (MURAYAMA 1977, 1981; KANKE 1987). In contrast, the ratios in buried volcanic ash soils were very low (<0.25) throughout the profile, which suggests that it is unlikely that the polysaccharides in these soils originate from plant. After reaching minimum values in Layer 4 (18,000 YBP), the amounts of pentoses and deoxyhexoses as well as the ratio of xylose/mannose increased again with age and reached a peak in Layer 15 ($>28,100$ YBP). The results obtained suggest that some part of the pentoses and deoxyhexoses (presumably of microbial origin) was spared from the process of decomposition in buried volcanic ash soils, although these sugars are easily decomposable in top soils.

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