

## DEGRADATION AND STABILIZATION OF THE HUMUS IN BURIED HUMIC ANDO SOILS.

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### SUMMARY

The change in the characteristics of the humus of buried humic ando soils was investigated with regard to the soil age attaining up to 30,000YBP. The decomposition of the easily decomposable organic components and the formation of humic substances occurred concomitantly in the recent layers, while the decomposition of unprotected humic substances proceeded in the buried layers. Humus compositions also differed according to particle sizes. It was considered that the formation of highly humified humic substances occurred first on the surface of clay particles but they were stabilized in larger aggregates thereafter. The sand particle size fraction was found to be the most important fraction in holding and stabilizing the humus in buried humic ando soils.

### INTRODUCTION

Ando soil, which is one of the most widely distributed soils in Japan, accumulates larger amounts of humus than Mollisols and the mechanism of the stabilization of the humus is assumed to differ from that in Mollisols (refs. 1-2). In this paper, the characteristics of the humus in the buried ando soil 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 ando soils. Since the humus composition in the soil is not homogeneous and the dynamics of the humus in each organo-mineral complex differ even according to the size of soil particles, changes with age of several important characteristics of the humus in buried humic ando soils were also investigated with reference to the size of soil particles.

### MATERIALS AND METHODS

#### Soil Samples

An array of soil samples were collected from the archeological excavation site at Ashitaka-Onoue in Numazu city, Shizuoka prefecture, Japan. The soil profile held several key layers with known radio-carbon ages (refs. 3-4). Recent humic layers (<10,000YBP, depth <1.5m) and the underlying "upper loam" layers (10,000-30,000YBP, depth <7m) were collected from two adjacent profiles located several meters apart. Obtained soil samples were marked A, B, C, D, and 1 - 21, respectively. Six layers with high organic matter contents and with

known ages (A, D, 4, 8, 15, 20) were subjected to particle size fractionation and to detailed characterization of the humus. Ages of these layers were: A, <1000YBP; D,  $8.0 \times 10^3$ YBP; 4,  $18.0 \times 10^3$ YBP; 8,  $27.2 \times 10^3$ YBP; 15,  $28.1 \times 10^3$ YBP; and 20,  $30.0 \times 10^3$ YBP.

Particle size (p.s.) fractionation of organo-mineral complexes in soils.

Organo-mineral complexes in soils were fractionated according to their particle sizes which were equivalent to coarse sand (>0.2mm), fine sand (0.2mm-0.02mm), silt (20 $\mu$ m-2 $\mu$ m), coarse clay (2 $\mu$ m-0.63 $\mu$ m), medium clay (0.63 $\mu$ m-0.2 $\mu$ m), and fine clay (<0.2 $\mu$ m) by the method of Tsutsuki et al. (ref. 2). Soils were dispersed by treating the soil suspension (pH8.5) with ultrasonics. Clay fractions were separated by centrifugation, silt fraction by the pipet method, and the remaining sand fraction was further divided into fine and coarse sand fractions by sieving.

Measurements of carbon contents of soil samples and p.s. fractions.

Carbon contents of untreated soil samples were measured with a CN analyzer (Yanaco MT 500) using cobalt oxide as an oxidizing agent. The C contents of the p.s. fractions were determined by the Walkley-Black method (ref. 5) in its 1/5 scale due to low sample amounts.

Analysis of humus composition.

This was carried out according to the Nagoya method of humus composition analysis (ref. 6). The authors reduced further the extracted residue with NaBH<sub>4</sub> and determined the solubilized humus by this treatment. For p.s. fractions, humus composition analysis was carried out on a reduced scale.

Permeation chromatography of HA on CPG10-170.

Humic acid extracted with 0.1N NaOH was precipitated from solution with 1N HCl. The precipitate was collected and redissolved in 0.05M Tris-phosphate buffer (pH 7.5). A 0.2ml portion of this solution was eluted on a CPG10-170 column (glass, 100cm x 8.2mm i.d. ) with the same buffer. Detailed procedures are given in Tsutsuki and Kuwatsuka (ref. 7).

Sugar composition.

Soils were soaked in 26N H<sub>2</sub>SO<sub>4</sub> at room temperature for 16h and then refluxed with 1N H<sub>2</sub>SO<sub>4</sub> for 5h. After adding myo-inositol as an internal standard, alditols of sugars were prepared by the method of Oades et al. (ref. 8) and trifluoroacetylated with 0.2ml TFAA + 0.2ml ethylacetate at room temperature, then analysed by GC with a FID detector.

RESULTS AND DISCUSSION

The change in soil total C and humin C contents and C/N ratios with age.

Fig. 1 shows the change in total C and humin C in the profile. The total C content in the recent humic layers (A-D) decreased from 12% to 4-6% with age. The humin C constituted ca. 60-70% of the total carbon in the uppermost hori-

zons, while the percentage increased with age. In layers lower than Layer C, the total C content ranged from 2 to 5%, of which the humin C constituted ca. 80-90%. The results indicated that soil organic matter in buried and soils is stabilized in the form of humin and can be preserved stable for as long as ca. 30,000 years.

C/N ratios of soils and humin fractions (Fig. 1) increased steeply with age until Layer 8 (>27,200YBP) and took similar and constant values (ca. 30) among the lower layers. C/N ratios of humin fractions were lower than those of soils in upper layers (16,200-27,200YBP).

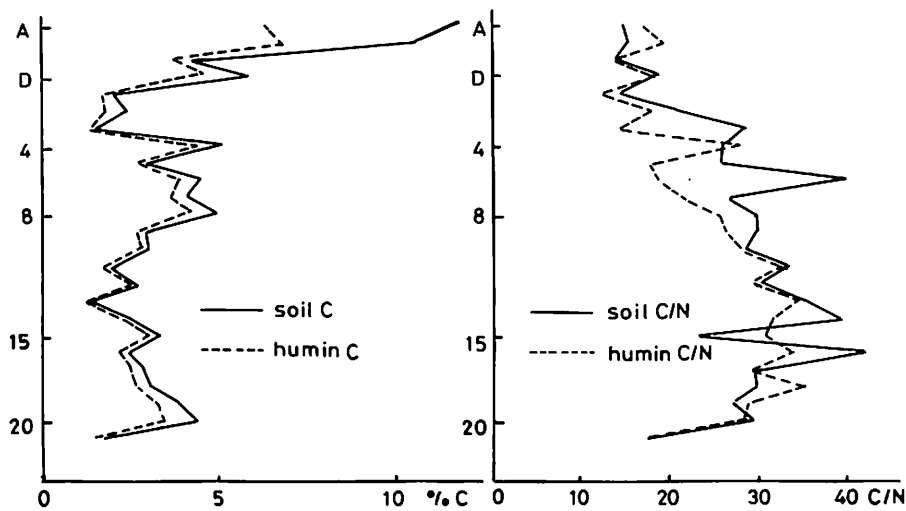


Fig. 1. The change with age in soil total C and humin C as well as in soil C/N and humin C/N of the buried humic and soils.

#### Composition of the humus with different extractability.

Table 1 shows the yields 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, HA and FA extracted with  $\text{Na}_4\text{P}_2\text{O}_7$  increased their percentages with age. The same trend was also reported for another buried volcanic ash profile (ref. 9). HA and FA solubilized by the  $\text{NaBH}_4$  reduction were low in amount, but their percentages increased with age. This trend indicates that complex formation with soil minerals is the major mechanism of humus stabilization in ando soils. Though the amount of humus solubilized by the reduction with  $\text{NaBH}_4$  was low, absorption maxima of HAs in this fraction were observed at 615, 570, 450, and 430(inflexion) nm, which

were characteristic of the Pg pigment (Fig. 2, ref. 6). This suggests that the Pg (green fraction found in HAs is preserved stably in ando soils by forming a complex with ferric compounds.

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 profile.

Layer	Soil $^{14}\text{C}$ -age	NaOH-extract		$\text{Na}_4\text{P}_2\text{O}_7$ -extract		$\text{NaBH}_4$ -extract	
		HA	FA	HA	FA	HA	FA
	(YBP)	-----		mgC/g soil*	-----		
A	$<1.0 \times 10^3$	33.2	44.6	2.93	4.36	0.58	0.58
D	$8.0 \times 10^3$	16.6	13.2	3.77	5.59	0.44	0.57
4	$18.0 \times 10^3$	15.9	14.3	3.40	4.10	0.42	0.76
8	$27.2 \times 10^3$	13.0	6.12	7.88	4.94	0.59	0.99
15	$28.1 \times 10^3$	3.12	2.39	4.66	4.36	0.15	0.31
20	$30.0 \times 10^3$	10.2	8.19	6.16	5.36	0.45	0.80

\* The rate of conversion from the consumption (ml) of 0.1 N  $\text{KMnO}_4$  to the amount of carbon (mg): HA 0.45 mgC/ml, FA 0.40 mgC/ml.

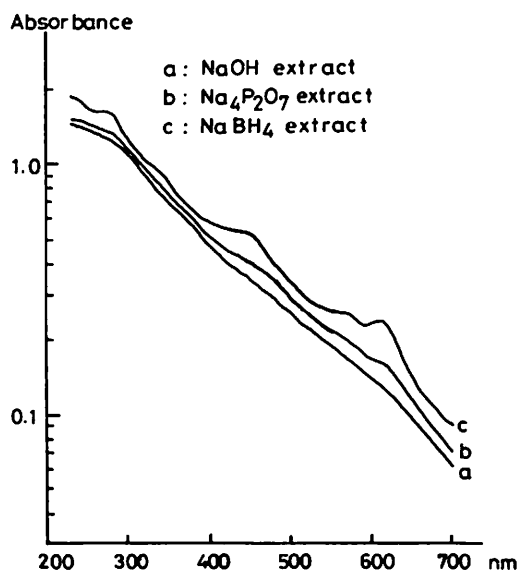


Fig. 2. The uv-visible absorption spectra of HAs extracted successively with 0.1N NaOH, 0.1M sodium pyrophosphate, and 1% sodium borohydride from a buried humic ando soil (Layer 4).

Yields of different p.s. fractions and the distribution of carbon among them.

Yields of organo-mineral complexes in different particle sizes are shown

Table 2. Yields of organo-mineral complexes in different particle size fractions obtained from Ashitaka-Onoue buried volcanic ash soils (% of unfractionated soil weight).

Layer	Coarse sand	Fine sand	Silt	Coarse clay	Medium clay	Fine clay	Total
A	8.76	39.2	40.3	3.44	0.73	0.09	92.6
D	70.0	14.6	1.84	0.12	0.01	0.01	86.7
4	72.0	17.3	4.33	0.26	0.03	0.00	93.9
8	69.6	12.7	5.62	0.22	0.11	0.05	88.2
15	52.4	20.8	11.1	0.75	0.33	0.07	85.4
20	68.6	17.4	3.92	0.23	0.03	0.10	90.3

Table 3. Distribution of organic carbon among different p.s. fractions (% of the total carbon in the unfractionated soil).

Layer	Coarse sand	Fine sand	Silt	Coarse clay	Medium clay + Fine clay + Loss
A	5.60	34.3	40.0	4.5	15.6
D	66.2	14.6	1.8	0.16	17.2
4	72.3	17.6	4.6	0.34	5.2
8	77.5	14.3	5.6	0.27	2.3
15	62.6	23.1	7.3	0.69	6.3
20	74.5	17.9	3.9	0.29	3.4

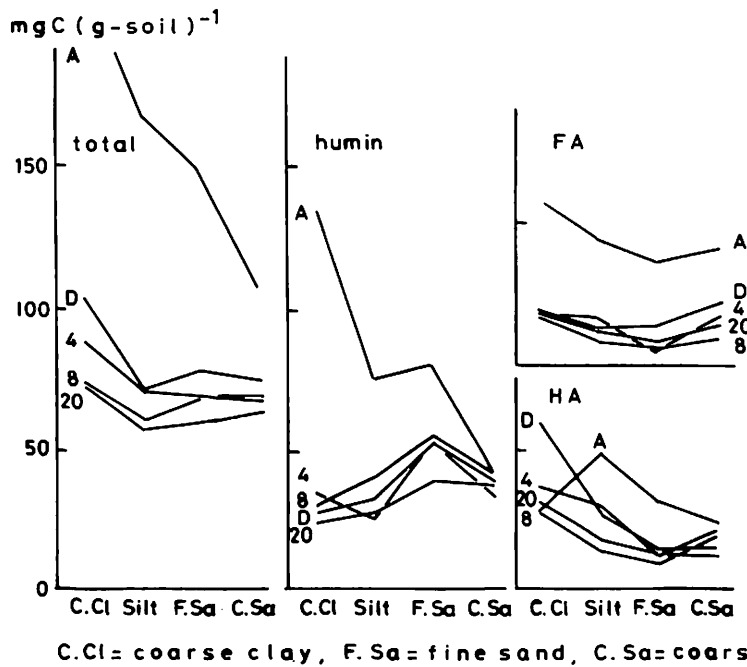


Fig. 3. The relationship between C contents in different humus forms and particle sizes of organo-mineral complexes obtained from buried humic and soils of different ages.

in Table 2. In all buried humic soils examined, the yield of particle size fraction was largest in the coarse sand fraction, and decreased with decreasing particle size. This was partly due to the predominance of scoriaceous particles in soils and also to the compression of soil particles in buried layers. In the uppermost layer (A), to the contrary, predominant fractions were fine sand and silt fractions.

Table 3 shows the distribution of carbon among different organo-mineral p.s. fractions. In the uppermost layer (A), more than one half of the total carbon was distributed into silt and smaller p.s. fractions. However, in buried layers, carbon in these finer p.s. fractions had very low contribution to the total carbon and most of the carbon (>90%) was found in the sand size fraction. The amount of carbon distributed in coarse and fine sand fractions were almost the same between the buried layers. These results suggest that the carbon contained in finer p.s. fractions was decomposed preferentially with age.

#### Humus composition in different p.s. fractions.

The distributions of total organic C, humin C, HA-C, and FA-C among different p.s. fractions were very different between the surface soil and the buried soils (Fig. 3). Carbon contents in fine clay and medium clay fractions were not determined because their yields were very low. (1) Total organic C contents of p.s. fractions increased remarkably with decreasing particle size, especially in the surface soil. In buried soils, the total organic C contents were highest in the coarse clay fraction but they were lower and almost at the same level in other fractions. (2) Though humin C content was also highest in the coarse clay fraction in the surface soil, it was highest in the fine sand fraction in the buried soil. (3) In the surface soil, HA-C was highest in the silt fraction, while in buried soils it was highest in the coarse clay fraction and decreased with increasing particle size. (4) FA-C contents were high in the surface soil but they were very low in all p.s. fractions of buried soils. FA-C contents were relatively higher in the two opposite size fractions (coarse clay and coarse sand).

The above results indicated that coarse clay and fine sand fractions obtained very different humus compositions after the long burial time (>10,000 yr). The HA-C was held preferentially by the coarse clay fraction, while the humin C were held by the fine sand fraction in humic ando soils. At the beginning of soil formation in ando soils, organic matter was stabilized in the clay fractions due to their larger adsorption capacity, while it was transformed to larger aggregates in the form of humin with age.

#### The degree of humification of HAs from different p.s. fractions.

RF value is the absorbance at 600nm of HA, divided by the consumed amount (ml) of 0.1N  $\text{KMnO}_4$  by 30 ml of HA solution, and multiplied by 1000 (ref. 6).

RF value, which serves as an indicator of the degree of humification of HA, changed differently with age in different p.s. fractions (Fig. 4-a). In the uppermost layer (A), RF was highest in the coarse clay fraction and lowest in the fine sand fraction. In buried layers, however, RF of coarse clay HA decreased to the lowest among all the p.s. fractions, and that of fine sand HA increased steadily with age, and reached the highest value among p.s. fractions at 27,200YBP. RF value of coarse sand HA was similar to that of fine sand HA, and that of silt HA was between coarse clay HA and sand HAs. The change in RF value with age showed that highly humified HA was formed first on the surface of clay particle but stabilized in larger particles thereafter, while HA remaining in the clay fraction was decomposed with age in buried layers. Values of  $\Delta \log K (= \log E_{400}/E_{600})$  were similar among HAs of different p.s. fractions and soils with different ages.

The change in  $C_h/C_f$  ratios of different p. s. fractions with age.

The change in  $C_h/C_f$  of different organo mineral p.s. fractions with age is shown in Fig. 4-b. In all p.s. fractions  $C_h/C_f$  increased once and after attaining the peak decreased again with age. The age at the peak of  $C_h/C_f$ , however, differed among different p.s. fractions. It attained the peak at 8,000 YBP in the coarse clay and silt fractions, at 18,000 YBP in the fine sand fraction, and at 27,200 YBP in the coarse sand fraction. In short, it took a longer time for the  $C_h/C_f$  of coarser fractions to attain peaks.

In the early stage of humus formation, FA is considered to consist mainly of slightly decayed bio-polymers and is decomposed more readily than HA, which causes the increase in  $C_h/C_f$ . After easily decomposable constituents in FA were exhausted, however, the main constituents of FA would become the decomposition products of HA and humin, which leads to the decrease in  $C_h/C_f$  with age. The

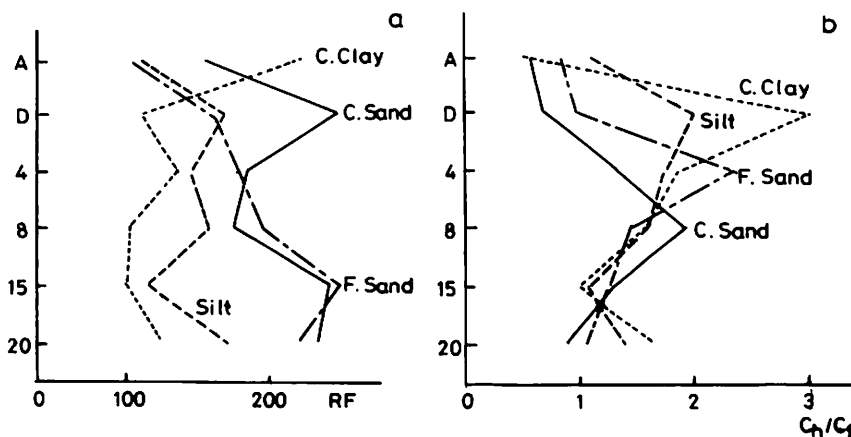


Fig. 4. The change with age in (a)RF values of HAs and (b) $C_h/C_f$  ratios of different particle size fractions obtained from buried humic and soils.

rate of such dynamics differed with the size of soil particle, and the humus composition of finer particles changed more quickly probably due to their larger surface area.

The change in the molecular size distribution of HAs with age.

Permeation chromatography on the controlled pore glass has been used as a novel technique monitoring the molecular size distribution of HAs and FAs (refs. 7, 10). Fig. 5-a 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 36.4ml. For the HA of Layer D, a new peak appeared near 34.3ml, indicating the increase in molecular size. This was also accompanied by the increase in the degree of humification. This suggests that the decomposition of the easily decomposable low molecular weight fraction as well as the formation of high molecular weight humic substances took place in the earlier period of humification. The peak or shoulder near 34.3ml was common in all the HAs of buried layers. However, a new peak appeared near 37.5ml in HAs of Layers 4, 8, 15 and 20. This appearance of a low molecular size component suggested the degradation of once-formed humic substances with age.

The change in sugar composition with age.

Fig. 5-b shows the change in the sugar composition of selected humic layers. Hexoses were dominant (>70%) and pentoses and deoxyhexoses were minor

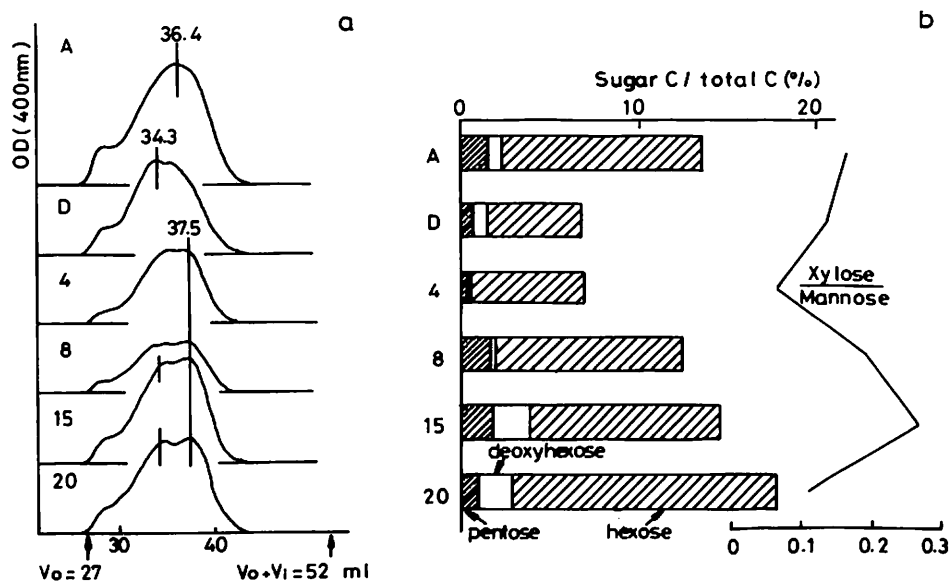


Fig. 5. The change with age in (a)molecular size distribution of HAs and (b)sugar composition in sulfuric acid hydrolysate of different particle size fractions obtained from buried humic and soils.



components in the hydrolysates. Among hexoses, amounts of mannose and galactose were 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 (ref. 11). From Layer 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 lower than D. This implies that some parts of soil polysaccharides are stabilized remarkably and become hardly decomposable in the buried ando soils. Stabilization of soil polysaccharides in allophanic soils has been reported and complex formation with mineral components is considered to be the dominant cause (refs. 11-12). The low value of xylose/mannose is also illustrated in Fig. 5-b. In paddy soils and upland soils, high xylose/mannose ratios ( $>1$ ) have been reported (ref. 13). In contrast, the ratios in buried humic ando soils were very low ( $<0.25$ ) throughout the profile, which suggested that polysaccharides in these soils are mostly of non-plant origin.

#### How humus is stabilized in the buried humic ando soils.

With respect to the relationship between the  $^{14}\text{C}$  age of organic matter and the particle size of soil, the oldest fraction was the silt fraction (ref. 14) or coarse clay fraction (ref. 15) or medium and coarse clay fractions in two Mollisols (ref. 2). From the viewpoint of the distribution of organic carbon and the degree of humification of HAs, silt and coarse clay fractions are known to contribute most to the stabilization of organic matter in various kinds of soils (refs. 2 and 16). On the other hand, it has been confirmed that the biomass is accumulated in silt size aggregates in calcareous soils (ref. 17). The biomass accumulated in the silt fraction undergoes degradation, and is considered to be stabilized on the coarse clay and medium clay particles, which have larger surface area.

In this paper, however, fine sand and coarse sand fractions in buried humic ando soils were the most important fractions in stabilizing humic substances. It is considered that the existence of large amounts of humic substances and active Al and Fe ions as well as the compression under the large pressure have promoted the stabilization of organic matter in larger aggregates in buried humic layers. It is also reported that large aggregates will be stabilized if sufficient quantities of mucilage or humic materials associated with metal ions are spread uniformly through the soil (ref. 18).

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## REFERENCES

- 1 H.W.Scharpenseel, K. Tsutsuki, P. Becker-Heidmann, and J. Freytag, Untersuchungen zur Kohlenstoffdynamik und Bioturbation von Mollisolten, *Z. Pflanzenernaehr., Bodenkund.*, 149 (1986) 582-597.
- 2 K. Tsutsuki, C. Suzuki, S. Kuwatsuka, P. Becker-Heidman, H.W. Scharpenseel, Investigation on the stabilization of the humus in mollisols, *Z. Pflanzenernaehr. Bodenk.*, 151 (1988) 87-90.
- 3 Ashitaka loam research group,  $^{14}\text{C}$ -ages of the buried humic soils in the upper loam of Ashitaka volcanic ash profile., *Chikyu Kagaku*, 24 (1970) 73-75 (in Japanese)
- 4 Y. Kato, T. Sase, S. Sakai, and N. Kanazawa, Age estimation of the buried humic soils in volcanic ash profile from plant opals. (II) Estimation from degree of corrosion on the surface of plant opal, *The quarternary research*, 25 (1986) 105-111 (in Japanese with English summary)
- 5 A. Walkley, A critical examination of a rapid method for determining organic carbon in soils--effect of variation in digestion conditions and of inorganic soil constituents. *Soil Sci.*, 63 (1946) 251-263.
- 6 K. Kumada, *Chemistry of soil organic matter*, Japan Scientific Societies Press/ Elsevier, Tokyo, 1987.
- 7 K. Tsutsuki, and S. Kuwatsuka, Molecular size distribution of humic acids as affected by the ionic strength and the degree of humification, *Soil Sci. Plant Nutr.*, 30 (1984) 151-162.
- 8 J.M. Oades, M.A. Kirkman, and G.H. Wagner, The use of gas-liquid chromatography for the determination of sugars extracted from soils by sulfuric acid, *Soil Sci. Soc. Amer. Proc.*, 34 (1970) 230-235.
- 9 M. Yoshida, K. Sakagami, R. Hamada, and T. Kurobe, Humus composition of buried humic horizon. Studies on the properties of organic matter in buried humic horizon derived from volcanic ash. I., *Soil Sci. Plant Nutr.*, 24 (1978) 277-287.
- 10 O.H. Danneberg, Chromatography of humic substances on controlled pore glass, in: *Soil Organic Matter Studies*, Vol. II, International Atomic Energy Agency, Vienna, 1977, p.221-228.
- 11 S. Murayama, The monosaccharide composition of polysaccharides in ando soils. *J. Soil Sci.*, 31 (1980) 481-490.
- 12 J.P. Martin, Decomposition and binding action of polysaccharides in soil. *Soil Biol. Biochem.*, 3 (1971) 33-41.
- 13 S. Murayama, Saccharides in some Japanese paddy soils. *Soil Sci. Plant Nutr.*, 23 (1977) 479-489.
- 14 H.W. Scharpenseel, Radiocarbon dating of soils - problems, troubles, hopes, in: D.H. Yaalon (Ed.), *Paleopedology - Origin, Nature and Dating of Paleosols*, Israel University Press, Jerusalem, 1971, pp. 77-88.
- 15 E.A. Paul, Dynamics of organic matter in soils. *Plant and Soil*, 76 (1984) 275-285.
- 16 L.W. Turchenek, and J.M. Oades, Fractionation of organo-mineral complexes by sedimentation and density techniques, *Geoderma*, 21 (1979) 311-343.
- 17 M. Ahmed, and J.M. Oades, Distribution of organic matter and adenosine triphosphate after fractionation of soils by physical procedures, *Soil Biol. Biochem.*, 16 (1984) 465-470.
- 18 J.M. Oades, Soil organic matter and structural stability: mechanisms and implications for management, *Plant and Soil*, 76 (1984) 319-337.