

CuO-Oxidation Products of Peat as a Key to the Analysis of the Paleo-Environmental Changes in a Wetland

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The peat profile in the Ohnohara wetland in Central Japan is very valuable due to the continuous accumulation of peat since even before the last glacial maximum era. Using this profile, we investigated the environmental and climatic changes which occurred since the last glacial era based on the determination of the contents and composition of phenolic compounds released from peat by CuO oxidation. For describing the changes in the anaerobic/aerobic conditions in the wetland, the phenolic degradation products were grouped into phenolic acids, acetophenones, cinnamic acids, and phenolic aldehydes. The changes in the ratios between cinnamyl, vanillyl, syringyl, and *p*-hydroxyphenyl compounds as well as the ratio of ferulic acid/*p*-coumaric acid corresponded well to the changes in the vegetation type of the wetland. Phenolic compounds released from the peat samples corresponded well to the observations based on palynological studies and to the data relating to post-glacial climatic changes. Therefore, it is suggested that the contents and composition of phenolic compounds of peat could be used as an index of the changes in paleo-environments.

Key Words: CuO oxidation, paleo-environments, peat, phenolic compounds, tephra.

Peat layers provide valuable information about past environmental changes in relation to volcanic ash tephra, pollen, and opal phytoliths, etc. (Tsutsuki et al. 1993) along with the composition of stable organic constituents preserved in the peat layers. For example, the composition of phenolic compounds derived from lignin is very characteristic of a particular plant class and tissue type. Lignin can not be easily decomposed under anaerobic conditions. We analyzed the environmental and climatic changes that took place since the last glacial era based on the determination of the contents and composition of the phenolic acids released from peat by CuO oxidation (Hedges and Ertel 1982). The peat profile in the Ohnohara wetland is very valuable due to the continuous accumulation of peat since even before the last glacial maximum era.

MATERIALS AND METHODS

Soil profile and samples. Figure 1 shows the diagram of the Ohnohara peat profile in Tsukude Village, Minami-Shitara, Aichi Prefecture, Japan (latitude 34°58'N, longitude

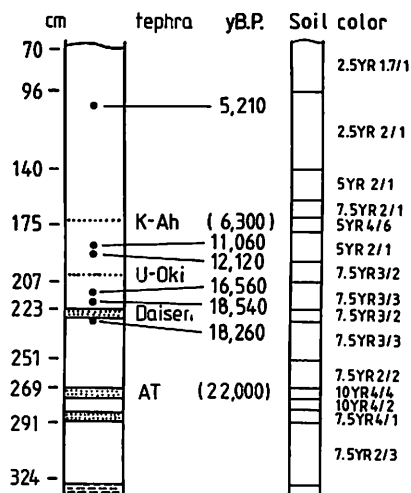


Fig. 1. Diagram of the Ohnohara wetland 86-1 profile.

137°26'E, altitude 530 m). The peat layer was as deep as 324 cm, and the zone from 70 to 324 cm was collected as a monolith-block on January 11, 1986. The sample was divided into ca. 4 cm thick sections for analysis. Description of the profile has been reported by the Tsukude Research Group et al. (1989) and Arai et al. (1988). Arai et al. (1988) also reported the values of soil pH, phosphate absorption coefficient, and degree of humification of humic acids. They examined the effect of tephra on dark colored humic acid formation. Compared with the Shirasu and Hosoda profiles (Tsutsuki et al. 1993), this profile covers the whole period since even before the last glacial maximum era and was sampled in thinner sections.

As shown in Fig. 1, this peat profile is interlaced with several key tephra, including Akahoya (K-Ah: 6,300 yBP), Utsuryo-Oki (U-Oki: 9,300 yBP), Daisen (DMs, DHg, and DSs: 17,000-18,000 yBP), and Aira (AT: 22,000-25,000 yBP) (Tsukude Research Group et al. 1989; Machida and Arai 1992). Radio-carbon date at several depths of the profile were cited from the Tsukude Research Group et al. (1989). Although the age of the deepest peat layer has not been determined in this profile, radio-carbon dating of another peat layer in the same wetland ($32,140 \pm 440$ yBP, Tsutsuki and Kuwatsuka 1992) indicated that peat layer formation in the Ohnohara wetland dated back to at least 32,000 yBP.

Carbon and nitrogen. Peat samples were freeze-dried and crushed with a disk mill (Heiko-TI-100). Each sample (100 mg) was mixed with 4 g cobalt oxide and carbon and nitrogen were analyzed using a CN auto-analyzer (Yanaco-MT500).

CuO-NaOH oxidation. A peat sample equivalent to 10 mg of carbon and 100 mg of a 1:5 mixture of ferrous ammonium sulfate and cupric oxide were mixed and placed in a Pyrex glass test tube (o.d. 9 mm, i.d. 7 mm). One milliliter of a 2 M NaOH solution was added. This tube was cooled in a salted ice bath and sealed in vacuo. Then it was heated at 150°C for 6 h in an oil bath. After heating, the tube was opened and the content was transferred into a 10 mL centrifuge tube with a small amount of water, and subjected to centrifugation for 10 min at 3,000 rpm. The supernatant was collected into a 50 mL glass centrifuge tube with a ground glass stopper. The residue was washed two more times with water. To the combined supernatant, 1 mL of 3 M HCl and 0.100 mg phenoxyacetic acid in 1 mL ethylacetate were added. The acidified solution was shaken with 30 mL of ethylacetate and centrifuged. The separated ethylacetate layer was carefully pipetted out and the extraction

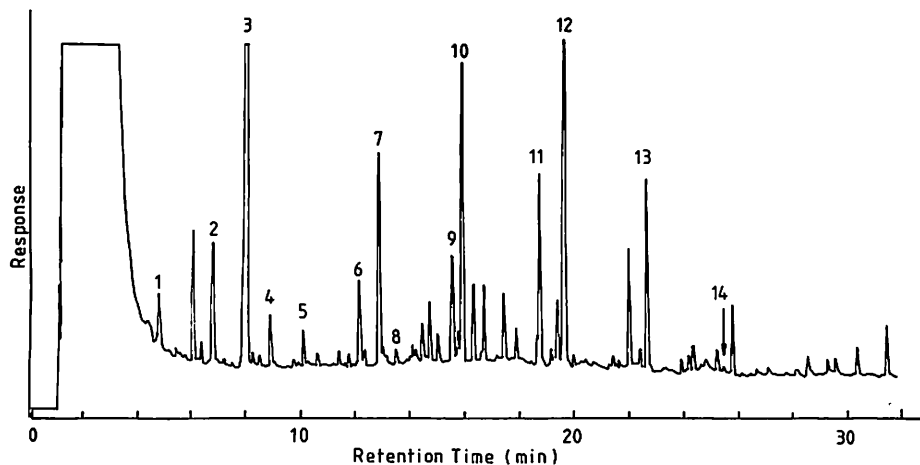


Fig. 2. Gas-capillary chromatogram of CuO-oxidation products derived from 132–136 cm layer sample of the Ohnohara wetland 86-1 profile. 1: benzoic acid; 2: *p*-hydroxybenzaldehyde; 3: phenoxyacetic acid (internal standard); 4: *p*-hydroxyacetophenone; 5: vanillin; 6: acetovanillone; 7: *p*-hydroxybenzoic acid; 8: syringaldehyde; 9: acetosyringone; 10: vanillic acid; 11: syringic acid; 12: *p*-coumaric acid; 13: ferulic acid; 14: sinapic acid.

was repeated two more times in this manner with 20 mL of ethylacetate. The ethylacetate extract was dried with 8 g of anhydrous sodium sulfate and evaporated to dryness using a rotary evaporator. The dried extract was taken into a small test tube with a Teflon-lined screw cap, dried up again, and dried completely in a vacuum desiccator.

Capillary GC analysis of phenolic compounds. To the dried phenolic extract, 0.040 mL of a TRI-SIL/BSA solution (Pierce Co. Ltd.) and 0.060 mL of *N,N*-dimethylformamide were added. The test tubes were tightly capped, and heated at 90°C for 30 min. The mixture was diluted with 0.400 mL of acetonitrile. This solution (1.0×10^{-3} mL) was injected to the capillary GC system under the following conditions. Gas-chromatograph: Hitachi Type 263-50 equipped with a solvent-cut injector (GL Science Co. Ltd.). Capillary column: Dura Bond DB-1 (film thickness: 0.25×10^{-6} m, i.d.: 0.25 mm, length: 15 m, J & W Scientific, California). Column temperature: 100–250°C. Increase rate: 5°C/min. Injector temperature: 280°C. Carrier gas: helium. Recorder data processor: Shimadzu Chromatopac C-R3A. Figure 2 shows one of the chromatograms obtained by this method.

RESULTS AND DISCUSSION

Changes in carbon and nitrogen contents in the profile

The changes in the carbon content, C/N ratio, and the contents of total phenolic compounds are shown in Fig. 3. The carbon content of the soil was relatively high in the layer below 251 cm. These layers were formed during the last glacial era, and the sustained wetland formation suggests that the climate was relatively stable. Then the carbon content decreased due to the interposition of silty material at around 287 cm and Aira tephra (22,000–25,000 yBP) at around 269 cm. The layer between the fall of the Aira tephra and Daisen tephra (17,000–18,000 yBP) corresponded approximately to the last glacial maximum era. In the layer between 251 and 294 cm the carbon content gradually decreased. This part of the layer corresponded to the late glacial era when rapid ice melting occurred. In this

period a large amount of silt and sand flowed into the lowlands due to the increase in snow fall and amount of melting water (Tsukada 1974).

This event also affected the Ohnohara wetland. For example, the clay and silt layers covered the Hosoda peat layers formed during the glacial era or underlay the Shirasu peat layers formed in the post-glacial era (Tsutsuki et al. 1993). The presence of these mineral layers suggests that a large amount of mineral matter flowed into the Ohnohara wetland during the late glacial era. Topographic conditions may have minimized the flow of mineral matter at the site investigated in this study. However, the decrease in the carbon content and the slow rate of peat accumulation in the corresponding layer (204–251 cm) of this profile can be ascribed to this effect.

After the fall of the Utsuryo-Oki tephra (9,300 yBP), the carbon content in the sediment started to increase again. This phenomenon corresponded to the start of the Holocene (post-glacial era). In this period the global temperature increased rapidly and reached maximum values at around 6,000 yBP, when the Akahoya tephra fell. The increase in precipitation also accompanied the increase in temperature (Sakaguchi 1983). The increase in the carbon content of the peat profile may reflect the increase in the grass biomass production and development of the wetland associated with these climatic changes. The carbon content reached a maximum value at a depth of 140 cm and remained almost constant above it, suggesting the resumption of stable wetland conditions. The global temperature started to decrease again after the maximum at around 6,000 yBP (Sakaguchi 1983). Based on radiocarbon dating (Tsukude Research Group et al. 1989), the age at 140 cm should fall between 5,210 and 6,300 yBP.

The ratio of C/N ranged from 24 to 42, and tended to decrease when the organic matter content increased (Fig. 3). Though the C/N ratio decreased in the initial phase of organic matter decomposition, the decomposition of nitrogenous compounds prevailed over that of carbonaceous compounds and resulted in a higher C/N ratio in the subsequent phase of decomposition. The C/N ratios of the soil humic acids also increased along with the increase in the degree of humification (Kuwatsuka et al. 1978).

Total contents of CuO-NaOH degradation products

The change in the yield of total phenolic compounds was similar to that of carbon content, but the fluctuation was more intense. The lignin content may reflect more sensitively the conditions of the wetland. The yield on the organic matter basis (Fig. 3) was constantly high in the layer below 251 cm, which corresponded to the last glacial maximum era. The good preservation of lignin structure suggests a stable and anaerobic wetland condition during this age.

In the layer between 167 and 251 cm, in which three tephtras (Daisen, Utsuryo-Oki, and Akahoya) fell, the contents of phenolic compounds decreased considerably. The content on an organic matter basis also decreased in the layers above 223 cm (fall of Daisen tephra) and below 171 cm (fall of Akahoya tephra). These findings suggest that the wetland was under rather dry conditions which promoted the decomposition of organic matter during this period. In the layer above 140 cm, the contents of the phenolic compounds increased markedly, though considerable fluctuations were still observed. This fact suggests that wetland conditions were resumed although frequent changes in the vegetation as well as in the aerobic/anaerobic conditions of the wetland still occurred.

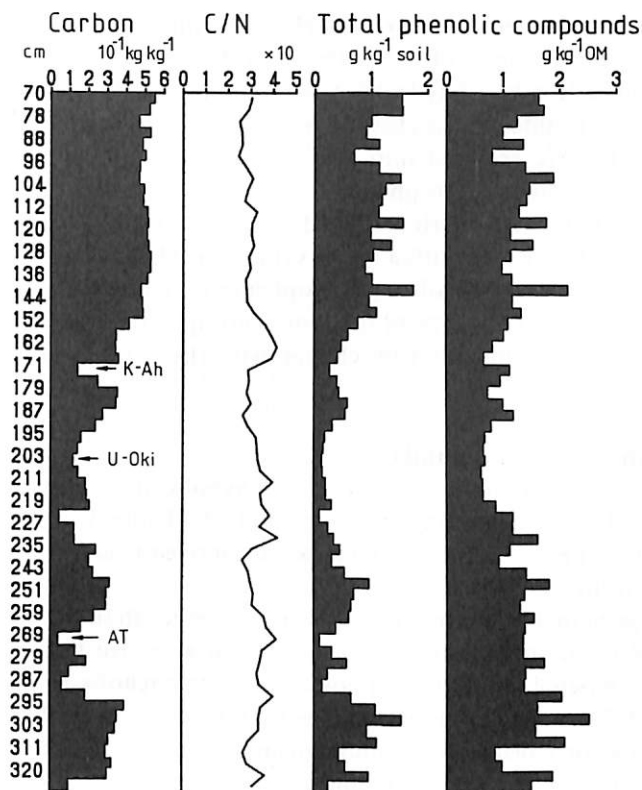


Fig. 3. Carbon content, C/N, and total contents of phenolic compounds derived from CuO oxidation of peat.

Table 1. Principal component analysis of CuO degradation products.

Degradation products	Eigenvector			
	Z1	Z2	Z3	Z4
Benzoic acid	0.229	-0.158	-0.101	0.856 e
<i>p</i> -OH benzoic acid	0.310 a	-0.306	-0.119	-0.100
Vanillic acid	0.342 a	-0.129	-0.117	-0.183
Syringic acid	0.348 a	0.0006	-0.148	-0.178
<i>p</i> -OH acetophenone	0.299 b	-0.287	0.090	0.101
Acetovanillon	0.331 b	-0.264	0.008	-0.169
Acetosyringone	0.327 b	-0.239	-0.008	-0.135
<i>p</i> -OH benzaldehyde	0.252	0.271 d	0.477 d	0.286
Vanillin	0.213	0.211 d	0.603 d	-0.214
Syringaldehyde	0.286	0.305 d	0.211 d	0.030
<i>p</i> -Coumaric acid	0.260	0.442 c	-0.351	-0.070
Ferulic acid	0.223	0.499 c	-0.416	0.050
Eigenvalue	7.616	1.617	0.917	0.664
Proportion	0.635	0.135	0.077	0.055
Cumulative proportion	0.635	0.769	0.846	0.901
Representative groups of compounds	a: phenolic acids b: <i>p</i> -OH acetophenones	c: cinnamic acids d: phenolic aldehydes	d: phenolic aldehydes	e: benzoic acid

Z1, Z2, Z3, Z4: the first-fourth principal components, respectively. a, b, c, d, e: coefficients of the representative groups were marked: they were obviously larger than the other coefficients.

Classification of phenolic compounds released by CuO-NaOH oxidation

The phenolic compounds detected in the digest of CuO-NaOH oxidation consisted of *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, *p*-hydroxyacetophenone, vanillic acid, vanillin, acetovanillone, syringic acid, syringaldehyde, acetosyringone, *p*-coumaric acid, and ferulic acid. These phenolic compounds were grouped into *p*-hydroxyphenyl (*p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, *p*-hydroxyacetophenone), vanillyl (vanillic acid, vanillin, acetovanillone), syringyl (syringic acid, syringaldehyde, acetosyringone) compounds based on the substitution of the benzene ring with a methoxyl group. They were also grouped into phenolic acids, phenolic aldehydes, *p*-hydroxy acetophenones, and cinnamic acids (*p*-coumaric acid and ferulic acid) based on the type of functional group at the C-1 site. Each phenolic compound or group showed a characteristic change with the depth of the profile.

Principal component analysis of phenolic compounds

To facilitate the analysis of the trends in the composition of phenolic degradation products of the peat samples, principal component analysis was applied (Table 1). The original database included the contents (g kg⁻¹) of phenolic compounds derived from the 62 peat layer samples of the Ohnohara profile.

Since the coefficients of the first principal component (Z1) were all larger than 0.2, it was considered that the first principal component could be characterized as a size factor. However, when the coefficients were compared carefully, the coefficients for phenolic acids and *p*-hydroxy acetophenones (0.299-0.348) were obviously larger than those of the other compounds (0.223-0.286). Therefore, the first principal component may also be characterized as a shape factor represented by these groups of compounds.

Among the coefficients of the second principal component (Z2), those of cinnamic compounds were largest (0.442-0.449), followed by those of phenolic aldehydes (0.211-0.305). The values of the other coefficients were all negative or almost zero. Therefore, the second principal component was characterized as a shape factor represented by cinnamic compounds and phenolic aldehydes.

Among the coefficients of the third principal component (Z3), only those of phenolic aldehydes showed large values (0.211-0.603). The values of the other coefficients were almost zero or negative. Therefore, the third principal component was characterized as a shape factor represented by phenolic aldehydes.

The fourth principal component (Z4) was obviously characterized by benzoic acid which exhibited a particularly large coefficient (0.856) compared with the others.

The above four principal components were represented by all the identified compounds, and the cumulative rate of contribution was 90.1%. This value suggested that the degradation products could be grouped into phenolic acids, acetophenones, cinnamic acids, and phenolic aldehydes for analyzing the dynamics of lignin components in a peat profile.

Correlation between phenolic degradation products

Correlation coefficients were also calculated between the organic matter content (represented by the carbon content), total content of phenolic compounds, and relative percentage of the various groups of phenolic compounds (Table 2). Organic matter content represented by the carbon content showed a highly significant positive correlation ($r=0.851$) with the total content of phenolic compounds, presumably because the lignin component is a dominant organic constituent of peat soil.

Table 2. Correlation coefficients between various types of phenolic compounds.

C	Total	Acid%	Acph%	Aldh%	Cinn%	<i>p</i> -OH%	Vanl%	Syr%	
C content	1.000	0.851***	-0.543***	-0.398**	0.017	0.632***	-0.719***	-0.369**	-0.063
Total phenolics	1.000		-0.565***	-0.366**	0.165	0.502***	-0.684***	-0.307	0.112
Phenolic acid %		1.000		0.777***	-0.682***	-0.598***	0.572***	0.403**	0.147
Acetophenone %			1.000		-0.382**	-0.781***	0.538***	0.528***	0.427***
Phenolic aldehyde %				1.000		-0.143	-0.035	0.160	0.130
Cinnamyl compounds %					1.000		-0.706***	-0.732***	-0.439***
<i>p</i> -OH phenols %						1.000		0.281	0.021
Vanillyl phenols %							1.000		-0.015
Syringyl phenols %								1.000	

,* Significantly different from zero at 99 and 99.9 levels, respectively, by *t*-test ($n=62$, d.f.=60). C, carbon content (kg kg^{-1}); Total, total phenolic compounds (g kg^{-1}); Acid%, proportion of phenolic acids to the total phenolic compounds; Acph%, proportion of *p*-OH acetophenones; Aldh%, proportion of phenolic aldehydes; Cinn%, proportion of cinnamic acids; *p*-OH%, proportion of *p*-hydroxyphenyl compounds; Vanl%, proportion of vanillyl phenolic compounds; Syr%, proportion of syringyl phenolic compounds.

Percentages of phenolic acids and *p*-hydroxyphenyl compounds showed significant negative correlations with the total organic matter content ($r = -0.543$ and -0.719 , respectively). They also showed negative correlations with the total content of phenolic compounds ($r = -0.565$ and -0.684 , respectively). These observations suggested that the percentages of phenolic acids and *p*-hydroxyphenyl compounds increased, when organic matter and lignin in peat underwent aerobic decomposition. In contrast, the percentage of cinnamic acids showed a highly significant correlation with the total organic matter content ($r = 0.632$) as well as with the contents of the total phenolic compounds ($r = 0.502$). Since cinnamic acids are mainly derived from non-woody tissues of angiosperm plants (e.g. mainly grasses), the high percentage of cinnamic acids suggests that the grasses were thriving and that the grass lignin was well preserved under anaerobic conditions.

Phenolic acids and acetophenones showed a highly significant correlation ($r = 0.777$) with each other. On the other hand, cinnamic acids and phenolic aldehydes showed highly negative correlations (r : from -0.781 to -0.382) with phenolic acids and acetophenones, presumably because phenolic aldehydes and cinnamyl compounds are derived from the unaltered part of lignin, while phenolic acids and acetophenones originate mainly from partially oxidized lignin.

Phenolic aldehydes and cinnamic acids did not show any significant correlations with each other ($r = -0.143$). Though phenolic aldehydes are the most common building blocks of lignin in any plant, cinnamic acids are only detected in non-woody angiosperm tissues, which may account for the low correlation between these two groups of compounds.

Composition of phenolic acids, acetophenones, aldehydes, and cinnamic acids

Figure 4 illustrates the changes in the contents of phenolic acids, acetophenones, phenolic aldehydes, and cinnamic acids. Phenolic acids and acetophenones showed a similar behavior, while the content of the former was considerably larger than that of the latter. Compared with cinnamic acids and phenolic aldehydes, the contents of phenolic acids and acetophenones did not fluctuate appreciably, because phenolic acids and acetophenones are mostly derived from lignin altered through oxidation, which is the dominant form of lignin in very old peat.

The contents of phenolic aldehydes were high in the layers below the Aira tephra and

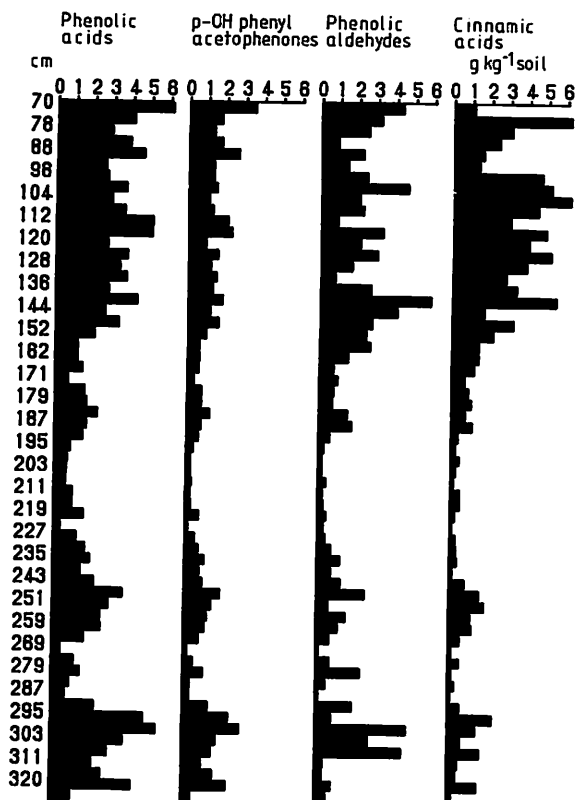


Fig. 4. Changes in contents of phenolic acids, *p*-hydroxyacetophenones, phenolic aldehydes, and cinnamic acids in the peat profile.

those immediately above the Aira tephra, presumably due to the retardation of lignin degradation under stable and anaerobic wetland conditions in the last glacial maximum era. The Hosoda peat profile formed in the same period showed the same characteristics, indicating the presence of stable wetland conditions (Tsutsuki et al. 1993). On the other hand, in the layers between Daisen tephra and the U-Oki tephra (17,000–9,300 yBP), the contents of phenolic aldehydes decreased, suggesting the prevalence of aerobic conditions in the wetland.

The contents of cinnamic acids were considerably lower than those of phenolic aldehydes in the layers formed during the last glacial maximum era (below 251 cm). Although the contents were also very low in the layers formed during the late glacial era (207–251 cm), they increased gradually after the fall of the Akahoya tephra (6,300 yBP) and even exceeded those of phenol aldehydes in the layer above 140 cm. Since cinnamic acids are mainly derived from the lignin of grasses, it is assumed that grasses were thriving in the wetland after ca. 6,000 yBP (above 140 cm). In the peat layer formed during the last glacial maximum era, the contribution of grasses may have been relatively negligible. Palynological studies conducted by Ishida and Nakahori (1987) showed that *Sphagnum* spp. and *Myrica* spp., which are typical high moor plants, thrived in this wetland at this stage.

The contents of cinnamic acids and phenolic aldehydes varied still considerably in the layers above 140 cm. When the contents of phenolic aldehydes and cinnamic acids decreased, those of phenolic acids and acetophenones increased at the same depths. These remarkable changes may be ascribed to climatic changes associated with cooling, drying, or a decrease

in grass production in the wetland.

Composition of *p*-hydroxyphenyl vanillyl, syringyl, cinnamyl group compounds

Grouping of the phenolic degradation products of lignin into vanillyl, syringyl, and cinnamyl compounds is a more common method for assessing the original vegetation type. Vanillyl compounds are derived from both gymnosperm and angiosperm tissues. Syringyl compounds are derived from angiosperm tissues and cinnamic acids are derived from angiosperm non-woody tissues (Hedges and Mann 1979).

The changes in the ratios of cinnamyl/vanillyl compounds (C/V), syringyl/vanillyl compounds (S/V), *p*-hydroxyphenyl/syringyl compounds (H/S), and ferulic acid/*p*-coumaric acid are shown in Fig. 5. The pattern of the changes in the C/V ratio was similar to that of the contents of cinnamic acids (Fig. 5), which were higher in the upper peat layers formed after 6,000 yBP, and lower in the layers formed in the late glacial and last glacial maximum era. These values may also reflect the thriving of grasses in the wetland especially after 6,000 yBP. The low C/V value in the lower part of the profile suggests that grass production was depressed during the last glacial era due to the low temperature.

Compared with the C/V ratio, the S/V ratio did not change appreciably throughout the profile. Syringyl compounds originate from both angiosperm wood components and non-wood ones, while cinnamic compounds originate mainly from non-wood components (Hedges and Mann 1979). This observation suggests that the woody plants contributed sufficiently and constantly to the vegetation of the wetland.

According to the analysis of peatland plants (Tsutsuki et al., unpublished data),

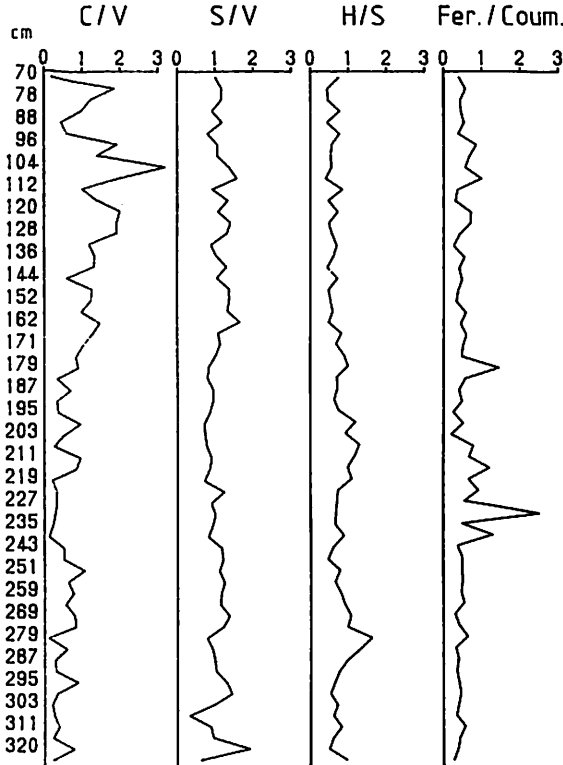


Fig. 5. Changes in the ratios of cinnamyl/vanillyl compounds (C/V), syringyl/vanillyl compounds (S/V), *p*-hydroxyphenyl/syringyl compounds (H/S), and ferulic acid/*p*-coumaric acid in the peat profile.

sphagnum released only *p*-hydroxyphenyl and vanillyl compounds and no syringyl and cinnamyl compounds. On the basis of these data, the ratio of *p*-hydroxyphenyl/syringyl compounds (H/S) was considered to be a good indicator of the prevalence of *Sphagnum* spp. As shown in Fig. 5, this ratio was high during the last glacial maximum and the late glacial era, which suggested that the *Sphagnum* spp. prevailed in the wetland during these periods.

Among the cinnamic acids, *p*-coumaric acid was dominant in grasses, while ferulic acid was dominant in the woody angiosperm plants (Tsutsuki et al., unpublished data). The ratio of ferulic acid/*p*-coumaric acid could become an indicator of the thriving of woody plants. This ratio was high in the layer between 207–240 cm, which corresponded to the late glacial era. Ishida and Nakahori (1987) also indicated the prevalence of *Alnus* spp. in this period.

Based on the above results, it appears that the phenolic compounds released from peat samples by CuO-NaOH oxidation corresponded well to the observations of palynological studies as well as to the data relating to post-glacial climatic changes. The contents and composition of phenolic compounds of peat, therefore, could be used as an index of the changes in paleo-environments.

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