

## Articles

## Production and Several Properties of Humic Acids during Decomposition Process of Charred Plant Materials in the Presence of H<sub>2</sub>O<sub>2</sub>

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### Abstract

In order to obtain more information on the role of charred plant materials in the formation of Type A humic acids in Ando soils, we investigated variations in the yields and degrees of darkening of humic acids during the decomposition process of charred plant materials at 30°C in the absence and presence of different concentrations (0.05–2.5M) of H<sub>2</sub>O<sub>2</sub>. Furthermore, the physicochemical and spectroscopic properties of the humic acids obtained from the H<sub>2</sub>O<sub>2</sub>-treated charred plant residues and from an Ando soil sample were compared. The higher concentrations of H<sub>2</sub>O<sub>2</sub> added and longer reaction periods resulted in the higher yields and higher degrees of darkening of humic acids. The degree of darkening, elementary composition, UV, visible, IR, and <sup>13</sup>C-NMR spectra, and X-ray diffraction pattern of the humic acid obtained from the H<sub>2</sub>O<sub>2</sub>-treated residues were similar to those of a Type A soil humic acid. It was assumed that charred plants produced during the burning of vegetation could be an important source of Type A humic acids in Japanese Ando soils.

### Introduction

Japan is a typical volcanic country and Ando soils (referred to as Kurobokudo or volcanic ash soils) developed in deposits of materials such as volcanic ash are widely distributed. These soils often display a thick black or brownish black A horizon with a high humus content which may reach 300 g kg<sup>-1</sup> soil (MAFJG, 1964). It has been considered that grassland plants such as Susuki (*Eulalia*, *Miscanthus sinensis*) are the major source responsible for the abundance of humus and that burning is necessary to maintain a grassland for a long time, since forest is the climax vegetation under the meteorological conditions prevailing in Japan (Kawamura, 1950; MAFJG, 1964; Yamane, 1973; Shoji, 1984).

An important feature of humus in Ando soils is the predominance of Type A humic acids with a high

degree of darkening (Kumada, 1977, 1981, 1987; Arai et al. 1986). The physicochemical and spectroscopic properties of Type A humic acids have been well documented over the past years (Kumada, 1977, 1981, 1987; Tsutsuki and Kuwatsuka, 1984). Furthermore, on the mechanisms of the formation of Type A humic acids, several hypotheses referred to as 1) transformation theory of humic acids from Type Rp, via Type B (or P<sub>0</sub>), to Type A, 2) oxidative polycondensation theory of polyphenols, and 3) charring theory of plants and soil organic matter, have been proposed by a pioneer study by Kumada (1977, 1981, 1987) and subsequent studies on the synthesis of humic acids from phenolic compounds (Shindo and Huang, 1984; Shindo et al., 1990; Shindo, 1992 a, b) and on the nature and properties of the humic acids isolated from charred plants (Shindo et al., 1986 a, b, c; Shindo, 1991) by Shindo and his co-workers.

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However, the decision of the major mechanism remained to be studied.

Thus, Shindo and his co-workers newly began a series of studies on the role of charred plants, which were produced during the burning process of vegetation by human activity and wildfires, in the formation of Type A humic acids in Ando soils. The results obtained are outlined as follows: 1) charred grassland plants not only contained originally the humic acids belonging to Type A, but also could produce large amounts of corresponding acids after oxidative degradation with a hot  $\text{HNO}_3$  (Shindo and Honma, 1998, 2001), 2) the physicochemical and spectroscopic properties of the humic acids obtained from charred grassland plants before and after the  $\text{HNO}_3$  treatment were similar to those of Type A humic acids in Ando soils (Shindo et al., 1999; Shindo and Honma, 2001), 3) charred plant fragments were widely distributed in Japanese Ando soils containing Type A humic acids, and a significant positive correlation was found between the carbon contents of charred plant fragments and the total carbon contents in soils (Honma and Shindo, 1997; Shindo and Honma, 2001; Shindo et al., 2003). From these findings, we have assumed that charred plants merit close attention as an important source of Type A humic acids in Japanese Ando soils.

The weathering and degradation of charred plant materials to humic acids in terrestrial conditions may be a time-consuming and slow oxidative process under the influence of oxygen and moisture. Accordingly, some kind of model experiment - experimental pedology - may provide an effective approach to clarify the fate and behavior of charred plants in soil ecosystem. The objective of this study was, therefore, to obtain more information on the role of charred plant materials in the formation of Type A humic acids in Ando soils from the standpoint of experimental pedology. We investigated the conversion of charred plant materials to humic acids in the presence of  $\text{H}_2\text{O}_2$  and compared the physicochemical and spectroscopic properties of the humic acid obtained from the  $\text{H}_2\text{O}_2$ -treated charred plant residues with those of a Type A soil humic acid.

## Materials and Methods

### *Charred plant materials produced by burning*

Fire was set in a grassland (N  $34^\circ 13'$ , E  $131^\circ 18'$ ) in the Akiyoshi plateau, Mine, Yamaguchi, Japan, in February, 1999. The dominant vegetation of this

grassland was dense Susuki (*Miscanthus sinensis*) and Kenezasa (*Pleiblastus pubescens*) plants. Fire ran quickly on the soil surface, and charred plants, together with half-charred plants, were scattered all over the area. Only charred grassland plants were collected, pulverized in an agate motor, and then passed through a 70 mesh sieve (PM).

### *Conversion of charred plant materials to humic acids during incubation in the absence and presence of $\text{H}_2\text{O}_2$*

A 1 g aliquot of the PM was suspended in 50 mL of deionized water (water) or different concentrations of  $\text{H}_2\text{O}_2$  (0.05, 0.1, 0.5, 1.0, and 2.5 M). The flasks (100 mL) containing suspensions were covered softly with an aluminum foil and placed in an incubator at  $30^\circ\text{C}$  up to a maximum period of 4 weeks with shaking once a week. Incubation was carried out in duplicate. The lost water by the evaporation was supplemented every two weeks. At the end of the designated reaction periods (1, 2, and 4 weeks), the charred plant residues in the flasks were collected on a suction filter (Toyo Roshi No. 4A), washed with 40 mL of water three times, dried at  $90^\circ\text{C}$  for 24h, and then used for the determination of total carbon content and the extraction of humic acid. The extraction and analysis of optical properties of humic acid were conducted according to the method described by Kumada et al. (1967). Organic matter was extracted successively with 0.1 M NaOH and 0.1 M  $\text{Na}_4\text{P}_2\text{O}_7$  at  $100^\circ\text{C}$  for 30 min. After the NaOH-soluble organic matter was removed, the  $\text{Na}_4\text{P}_2\text{O}_7$ -soluble organic matter was newly extracted. The NaOH and  $\text{Na}_4\text{P}_2\text{O}_7$  extracts were separated into humic and fulvic acids by the addition of conc.  $\text{H}_2\text{SO}_4$ . The precipitate, humic acid, was washed successively with  $\text{H}_2\text{SO}_4$  (1:100) and water, and then dissolved in 0.01 M NaOH. The absorption spectrum of the humic acid solution in the region from 230 to 700 nm was immediately measured using a spectrophotometer JASCO Ubest-50. The amounts of carbon in the humic acids, PM, and its degradation products were determined by the  $\text{KMnO}_4$  oxidation method. In this study, 1 mL of 0.02 M  $\text{KMnO}_4$  consumed was calculated as corresponding to 0.48 mg carbon (Ikeya and Watanabe, 2003). The type of humic acid was determined using  $\Delta\log K$  and  $RF$  values, where the  $\Delta\log K$  value is the logarithm of the ratio of the absorbance of humic acid at 400 nm to that at 600 nm; the  $RF$  value represents the absorbance of humic acid

at 600 nm multiplied by 1,000, and then divided by the number of mL of 0.02 M  $\text{KMnO}_4$  consumed by 30 mL of humic acid solution.

#### Preparation of humic acid

The flasks (5 L) containing 50 g of the PM and 2.5 L of 2.5 M  $\text{H}_2\text{O}_2$  were incubated at 30°C as described above. At the end of the reaction period of 2 weeks, the charred plant residues were collected by centrifugation (10,000 g for 10 min) and filtration, washed thoroughly with water, and then dried at about 75°C (DP).

Humic acid was extracted according to the method described in the previous papers (Shindo et al., 1986 a, c). The flasks (5 L) containing 30 g of the DP and 4.5 L of 0.1 M NaOH were placed in an incubator at 30°C for 48 h with intermittent shaking. Following the separation of the very dark-colored supernatant from the residues by centrifugation and filtration, the alkaline extract was acidified with HCl (1:1) to pH < 2.0 and allowed to stand overnight at room temperature. The resulting humic acid precipitate was redissolved in 0.1 M NaOH, reprecipitated with dilute HCl, recentrifuged, and then dialyzed (seamless cellulose tubing, Sankou Junyaku Co.) against water. Finally, the humic acid (DP-HA) was freeze-dried to determine several properties. For reference, a soil humic acid (S-HA) was also prepared by the same method (but without  $\text{H}_2\text{O}_2$  treatment) from the A horizon of an Ando soil (N 35° 17' 45", E 133° 35' 12") in the Forest Experimental Station, Tottori University, Hiruzen, Okayama, Japan.

#### Analyses of humic acid

**Elementary analysis:** DP-HA and S-HA were heated at 550°C for 6h to determine ash content. Carbon, hydrogen, and nitrogen contents were determined with a Perkin-Elmer 240-C elemental analyzer. Oxygen content was calculated by subtracting the weights of carbon, hydrogen, nitrogen, and ash from the total weight.

**UV and visible absorption spectra:** Ten mg of the humic acid sample was dissolved in 200 mL of 0.01 M NaOH solution, and the absorption spectrum in the region from 230 to 700 nm was immediately measured on a spectrophotometer JASCO Ubest-50.

**$\Delta\log K$  and RF values (degree of darkening) :** These values were determined according to the method described earlier.

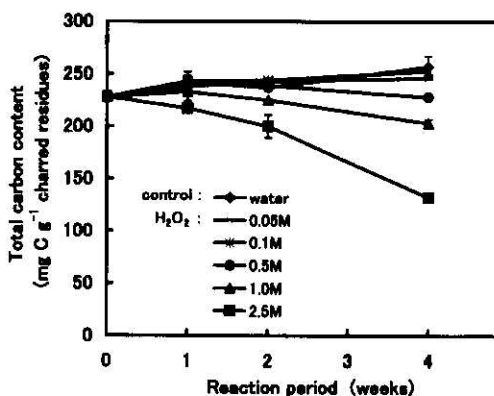
**IR absorption spectrum:** The IR spectra of DP-HA

and S-HA were measured according to the method described elsewhere (Shindo et al., 1986 a), using a Shimadzu FTIR-8600PC Fourier-transform infrared spectrophotometer.

**$^{13}\text{C}$ -NMR spectrum:** The  $^{13}\text{C}$ -NMR spectra of DP-HA and S-HA were obtained at a  $^{13}\text{C}$  resonance frequency of 75.45 MHz on a JEOL JNM-alpha 300 solid NMR system using solid-state crosspolarization magic-angle spinning (CP-MAS) and total suppression of sidebands (TOSS) techniques for eliminating spinning sidebands (Dixon, 1982). The TOSS method may give more reliable spectra, although the sensitivity is not maximized. However, the data obtained by the TOSS method are not comparable with those obtained by the CP-MAS method. The other operating conditions in this study were as follows: CP contact time, 5 ms; recycle delay time, 10 s; accumulation, 8,000-10,000.

To obtain information on the structural changes of charred plant materials during incubation in the presence of  $\text{H}_2\text{O}_2$ , the  $^{13}\text{C}$ -NMR (TOSS) analysis was also conducted for the PM and DP.

**X-ray diffraction:** The diffraction was measured by the powder method using a Rigaku RINT 2200V X-ray diffractometer with Cu radiation and a graphite monochromator. The operating conditions were as follows: scanning angles, 5-50° ( $2\theta$ ); scanning rate, 2° min<sup>-1</sup>.



**Figure 1.** Changes in total carbon content of the charred plant residues during the incubation of charred plant materials at 30°C in the absence and presence of 0.05-2.5 M  $\text{H}_2\text{O}_2$ . The datum given for the reaction period of 0 week indicates that analyzed before incubation. Vertical bars represent standard error (n=2).

## Results and Discussion

### Changes in total carbon content of charred plant residues during incubation

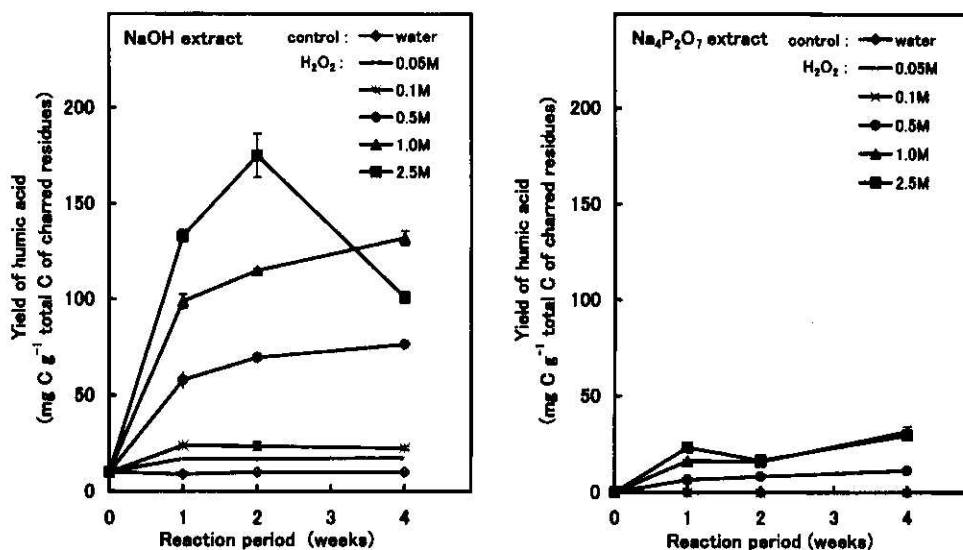
Figure 1 shows the changes in total carbon content of charred plant residues ( $\text{mg g}^{-1}$  oven-charred plant residues) during the incubation of charred plant materials at  $30^\circ\text{C}$  in the absence and presence of  $\text{H}_2\text{O}_2$ . Before incubation (reaction period of 0 week), the total carbon content was 230 mg. Throughout the reaction period, the carbon content ranged from 130 to 260 mg in all the systems studied. The presence of 1.0 and 2.5 M  $\text{H}_2\text{O}_2$  decreased the carbon content to 200 and 130 mg, respectively, at the end of the reaction period of 4 weeks. On the other hand, in the control (absence of  $\text{H}_2\text{O}_2$ , water alone) system and the 0.05, 0.1, and 0.5 M  $\text{H}_2\text{O}_2$  systems, the carbon content kept almost constant or tended to increase, although  $\text{H}_2\text{O}_2$  decomposes organic components in the charred plant materials. The degree of increase was remarkable in the control system. This may be due to the decrease in the weight of charred plant residues during washing, since water-soluble cations such as  $\text{K}^+$  are lost.

### Conversion of charred plant materials to humic acids during incubation

In the 2.5 M  $\text{H}_2\text{O}_2$  system (the yield of alkali-soluble humic acids is the highest among the

systems as described later), pH value decreased from 8.7 before incubation to 7.5 at the end of the reaction period of 0.5 h, 6.3 at 3 days, 5.0 at 1 week, and 3.7 at 2 weeks, respectively, and the reaction solution was yellow. On the other hand, in the control system (the yield of alkali-soluble humic acids is the lowest as described later), pH value was about 8.4 even after the 2-week reaction, and the reaction solution was colorless. These findings indicate that even if humic acids were dissolved into the reaction solution, their amounts were very small. Furthermore, it was very difficult to extract directly humic acids from the systems due to the influence of  $\text{H}_2\text{O}_2$  present. Therefore, in this study, to investigate the effect of  $\text{H}_2\text{O}_2$  on the conversion of the charred plant materials to humic acids, the amounts of alkali-soluble humic acids in the oven-dried charred plant residues were determined.

Figure 2 shows the changes in the yields of NaOH-soluble humic acids ( $\text{mg C g}^{-1}$  total C of oven-dried charred residues) during the incubation of charred plant materials. Before incubation, the yield of humic acid was 10 mg. The presence of  $\text{H}_2\text{O}_2$  increased the yields of humic acids to various degrees, depending on the concentration of  $\text{H}_2\text{O}_2$  added. In the presence of 2.5 M  $\text{H}_2\text{O}_2$ , the yield ranged from 10 to 180 mg throughout the reaction period, and showed a peak at the end of the reaction period of 2 weeks (the reason will be discussed later). In the 0.5 and 1.0 M



**Figure 2.** Changes in the yields of NaOH-soluble and  $\text{Na}_4\text{P}_2\text{O}_7$ -soluble humic acids in the charred plant residues during the incubation of charred plant materials at  $30^\circ\text{C}$  in the absence and presence of 0.05-2.5 M  $\text{H}_2\text{O}_2$ . The data given for the reaction period of 0 week indicate those analyzed before incubation. Vertical bars represent standard error ( $n=2$ ).

H<sub>2</sub>O<sub>2</sub> systems, the yields ranged from 10 to 130 mg, increased rapidly at the early stages and then increased slowly, and the yield in the latter system exceeded that in the former system at any given reaction period. On the other hand, in the 0.05 and 0.1 M H<sub>2</sub>O<sub>2</sub> systems, the yields varied from 10 to 24 mg, and the yield in the latter system exceeded that in the former system throughout the reaction period. In the control system, the yield was always about 10 mg.

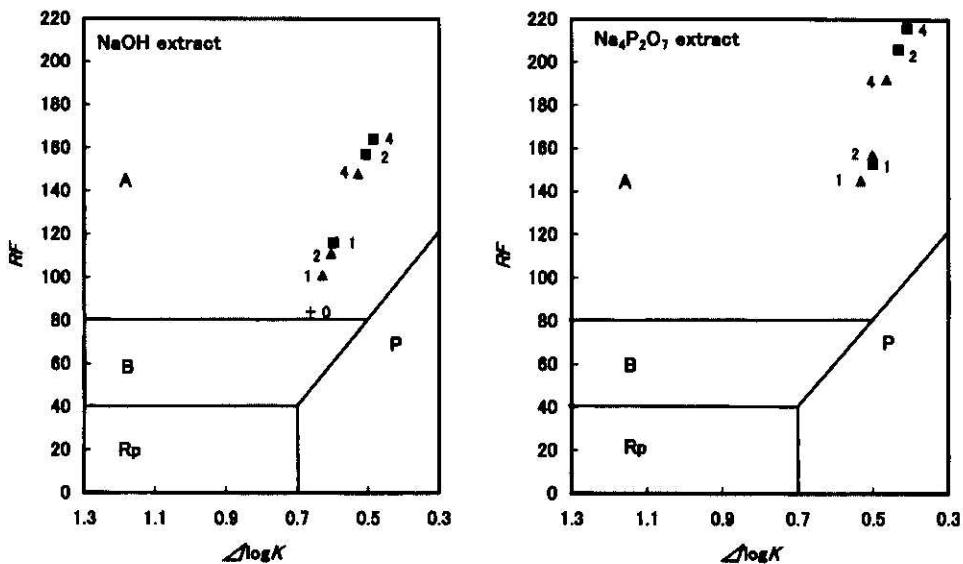
A comparison of Figs. 1 and 2 revealed that in the systems with higher concentrations of H<sub>2</sub>O<sub>2</sub>, the ratios of increase in the yields of humic acids were much larger than those of decrease in total carbon contents of the charred residues. This finding clearly shows that larger amounts of NaOH-soluble humic acids were newly produced from the charred plant materials in the presence of higher concentrations of H<sub>2</sub>O<sub>2</sub>.

Figure 2 also shows the changes in the yields of Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>-soluble humic acids (mg C g<sup>-1</sup> total C of oven-dried charred residues) during the incubation of charred plant materials. In the control system and the 0.05 and 0.1 M H<sub>2</sub>O<sub>2</sub> systems, the amounts of humic acids were negligible. In the 0.5, 1.0, and 2.5 M H<sub>2</sub>O<sub>2</sub> systems, the yields increased and ranged from 7 to 32

mg after only 1-week reaction, indicating that Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>-soluble humic acids were produced rapidly in these systems. The yield was higher in the 1.0 and 2.5 M H<sub>2</sub>O<sub>2</sub> systems than in the 0.5 M H<sub>2</sub>O<sub>2</sub> system throughout the reaction period.

Kumada et al. (1967) proposed to classify soil humic acids into Types A, B, P, and Rp, based on their  $\Delta\log K$  and  $RF$  values. The degree of darkening of humic acids becomes higher as  $\Delta\log K$  value decreases and  $RF$  value increases. In this study, all the humic acids belonged to Type A. Several values of  $\Delta\log K$  and  $RF$  of NaOH-soluble and Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>-soluble humic acids obtained before and after incubation of charred plant materials were illustrated in the diagram proposed by Kumada et al. (1967) (Fig. 3). The higher concentrations of H<sub>2</sub>O<sub>2</sub> and longer reaction periods resulted in the formation of NaOH-soluble and Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>-soluble humic acids with higher degrees of darkening. Furthermore, the degrees of darkening of Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>-soluble humic acids were higher than those of NaOH-soluble humic acids (Fig. 3), although the reverse was true for the yields of humic acids (Fig. 2).

In the 2.5 M H<sub>2</sub>O<sub>2</sub> system, the degree of darkening of NaOH-soluble humic acids substantially increased



**Figure 3.**  $RF$ - $\Delta\log K$  diagram of several humic acids obtained before and after incubation of charred plant materials at 30°C in the presence of H<sub>2</sub>O<sub>2</sub>. The value of  $\Delta\log K$  is the logarithm of the ratio of the absorbance of humic acid at 400 nm to that at 600 nm. The value of  $RF$  represents the absorbance of humic acid at 600 nm multiplied by 1,000, and then divided by the number of mL of 0.02 M KMnO<sub>4</sub> consumed by 30 mL of humic acid solution. These analyzed values are the means of duplicate. +: charred plant materials before incubation; ▲: 1.0 M H<sub>2</sub>O<sub>2</sub> system; ■: 2.5 M H<sub>2</sub>O<sub>2</sub> system. Numbers indicate the reaction period (week). The datum given for the reaction period of 0 week indicates that analyzed before incubation.

throughout the reaction period (Fig. 3), although the yield of the humic acids showed a peak at the end of 2 weeks (Fig. 2). This finding suggests that both production and degradation of humic acids with different degrees of darkening occurred during the incubation of charred plant materials in the presence of  $H_2O_2$ . In the 2.5 M  $H_2O_2$  system, it was assumed that the production of humic acids with a high degree of darkening occurred to a larger extent than the degradation of humic acids with a low degree of darkening at the early stages of the reaction period, and the degradation of the latter acid exceeded the production of the former acids at the later stages.

#### Characteristics of humic acid produced from charred plant materials

Table 1 shows the  $\Delta \log K$  and  $RF$  values and elementary composition of the DP-HA and S-HA. The  $\Delta \log K$  and  $RF$  values of the DP-HA resembled those of the S-HA, and these values corresponded to all the criteria for Type A humic acids (Fig. 3). Type A soil humic acids generally display higher carbon and oxygen contents and lower hydrogen and nitrogen contents than other types of humic acids (Kumada, 1987). The elementary composition of the DP-HA indicated fair agreement with that of the S-HA. These analytical data of the DP-HA fell within the ranges of those of Type A humic acids in Japanese soils (Kuwatsuka et al., 1978).

The UV and visible absorption spectra of the DP-HA and S-HA are shown in Fig. 4. The shapes of their absorption spectra in the UV and visible regions were mostly similar to each other and to those of Type A soil humic acids (Kumada et al., 1967; Kumada, 1977, 1981, 1987).

The IR absorption spectrum of the DP-HA resembled that of the S-HA, with O-H stretching vibration ( $3,400\text{ cm}^{-1}$ ), aromatic C-H stretching vibration ( $3,020\text{ cm}^{-1}$ ), aliphatic C-H stretching vibration ( $2,620\text{ cm}^{-1}$ ), C=O stretching vibration of COOH and ketones ( $1,720\text{ cm}^{-1}$ ), aromatic C=C stretching vibration and H-bonded C=O of quinones (broad absorp-

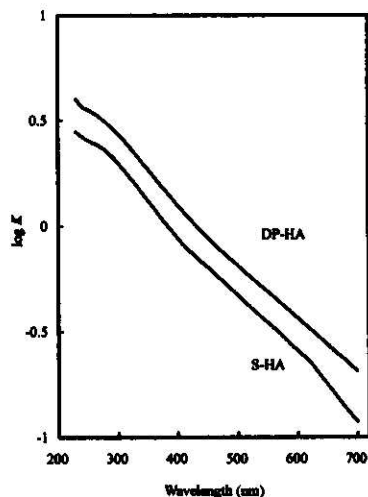


Figure 4. UV and visible absorption spectra of DP-HA and S-HA. See Table 1 for DP-HA and S-HA.

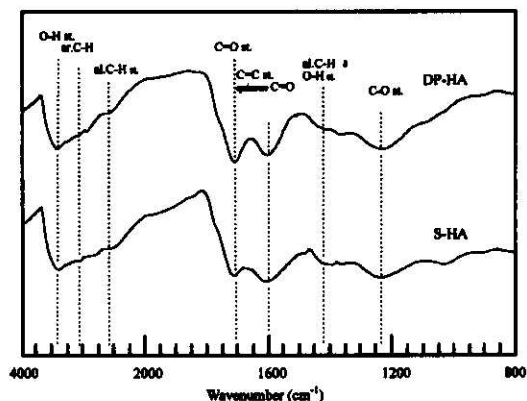


Figure 5. IR absorption spectra of DP-HA and S-HA. See Table 1 for DP-HA and S-HA.

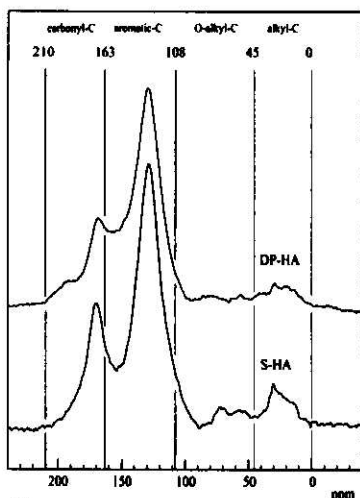
ance near  $1600\text{ cm}^{-1}$ ), aliphatic C-H bending vibration and O-H stretching vibration ( $1,450\text{ cm}^{-1}$ ), and C-O stretching vibration (ca.  $1,200\text{ cm}^{-1}$ ) (Fig. 5) (Stevenson 1982).

The  $^{13}\text{C}$ -NMR spectra (measured by the TOSS method) of the DP-HA and S-HA were similar to each other (Fig. 6). The carbon species were divided

Table 1. Degree of darkening and elementary composition of the humic acids obtained from the charred grassland plants after  $H_2O_2$  treatment (DP-HA) and from the A horizon of Ando soil (S-HA).

Humic acid	Degree of darkening		Elementary composition ( $\text{mg g}^{-1}$ )*			
	$\Delta \log K$	$RF$	C	H	N	O
DP-HA	0.534	143	616	32.2	16.2	336
S-HA	0.533	141	599	29.5	26.2	345

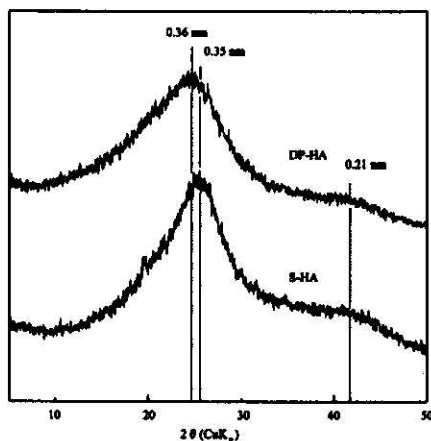
\*Moisture and ash free basis.



**Figure 6.**  $^{13}\text{C}$ -NMR (TOSS) spectra of DP-HA and S-HA. See Table 1 for DP-HA and S-HA.

into 4 groups (Hiradate et al., 2003), namely, alkyl-C (0-45 ppm), O-alkyl-C (45-108 ppm), aromatic-C (108-163 ppm), and carbonyl-C (163-210 ppm), and their relative contents in the DP-HA and S-HA calculated from the  $^{13}\text{C}$ -NMR spectra. As shown in Table 2, the contents of aromatic-C in the DP-HA and S-HA, ranging from 64 to 68%, were the highest among the carbon species. The contents of carbonyl-C, alkyl-C, and O-alkyl-C ranged from 6.6 to 21% in the DP-HA and S-HA.

X-ray diffraction can give valuable information on the three-dimensional structures of humic acids. The X-ray diffraction patterns of Type A soil humic acids are characterized by the presence of two bands at  $d = 0.35$  and  $0.21$  nm, corresponding to the (002)- and (10)-bands of graphite, respectively (Matsui et al., 1984; Kumada, 1987; Schnitzer et al., 1991), unlike those of other types of humic acids. The patterns of



**Figure 7.** X-ray diffraction patterns of DP-HA and S-HA. See Table 1 for DP-HA and S-HA

the DP-HA and S-HA were similar to each other, although the (002)-band of the DP-HA was shifted to  $0.36$  nm (Fig. 7), presumably due to the loosely stacked structures.

Schnitzer and Wright (1960) reported that  $\text{HNO}_3$  treatments of soil organic matter are able to cause oxidative degradation such as depolymerization and carboxylation by the fission of polycyclic aromatic structures and saturated rings as well as the cleavage of an ester linkage. In this study, the mechanisms of the oxidation of charred plant materials by  $\text{H}_2\text{O}_2$  are uncertain. However, all the degree of darkening, elementary composition, UV, visible, IR, and  $^{13}\text{C}$ -NMR spectra, and X-ray diffraction pattern of the DP-HA in this study were similar to those of the humic acid obtained from the charred plant residues after the hot  $\text{HNO}_3$  treatment in a previous paper (Shindo and Honma, 2001). The comparison of

**Table 2.** Relative contents (%) of individual C species to total C of the DP-HA and S-HA.

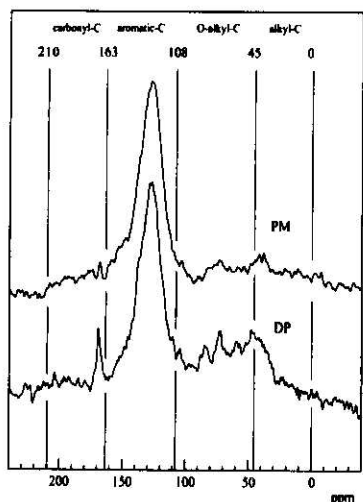
Humic acid*	Alkyl-C	O-alkyl-C	Aromatic-C	Carbonyl-C
DP-HA	6.9	6.6	67.5	18.9
S-HA	8.3	7.1	63.5	21.1

\*See Table 1 for DP-HA and S-HA.

**Table 3.** Relative contents (%) of individual C species to total C of the PM and DP.

Sample*	Alkyl-C	O-alkyl-C	Aromatic-C	Carbonyl-C
PM	14.4	20.2	54.3	11.1
DP	10.9	28.4	51.6	9.1

\*PM and DP stand for original charred plant materials and their degradation products in the presence of  $2.5$  M  $\text{H}_2\text{O}_2$ , respectively.



**Figure 8.**  $^{13}\text{C}$ -NMR (TOSS) spectra of PM and DP. PM and DP stand for original charred plant materials and their degradation products in the presence of 2.5 M  $\text{H}_2\text{O}_2$ , respectively.

$^{13}\text{C}$ -NMR spectrum of the PM with that of the DP indicated that after the 2.5 M  $\text{H}_2\text{O}_2$  treatment, the relative contents of alkyl-C, aromatic-C, and carbonyl-C decreased, while the relative contents of O-alkyl-C with the peak at 70-80 ppm (e.g. -CH(OH) structures) increased (Fig. 8 and Table 3). Furthermore, the yield of NaOH-soluble humic acids with graphite-like structures increased remarkably after the treatment (Figs. 2 and 7). These findings suggest that the  $\text{H}_2\text{O}_2$  treatment in this study involved depolymerization by the fission of polycyclic aromatic structures during the incubation of charred plant materials. The peak at 170 ppm in the DP was assigned to COOH-C, since the pH value in the 2.5 M  $\text{H}_2\text{O}_2$  system became strongly acid with the elapse of reaction period (Fig. 8).

## Conclusions

The findings obtained in this study indicate that the charred plant materials not only contained originally the humic acids belonging to Type A, but also could produce large amounts of corresponding acids during oxidative degradation in the presence of dilute  $\text{H}_2\text{O}_2$ . Furthermore, the physicochemical and spectroscopic properties of the humic acid formed were similar to those of Type A humic acid in an Ando soil, as in the case of  $\text{HNO}_3$  treatment in a previous paper (Shindo and Honma 2001). Charred plant fragments

were widely distributed in Japanese Ando soils containing Type A humic acids, and a significant positive correlation was found between the carbon contents of charred plant fragments and the total carbon contents in soils (Honma and Shindo, 1997; Shindo and Honma, 2001; Shindo et al., 2003). Based on these findings, it was assumed that charred plants produced during the burning of vegetation by human activity and wildfires could be an important source of Type A humic acids in Japanese Ando soils.

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