

## CHEMICAL STUDIES ON SOIL HUMIC ACIDS

### VII. pH-Dependent Nature of the Ultraviolet and Visible Absorption Spectra of Humic Acids

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The pH-dependent nature of the ultraviolet and visible absorption spectra of 39 humic acids (HAs) was investigated. Two kinds of difference absorbance,  $\Delta E(\text{pH } 12.4-7)$  and  $\Delta E(\text{pH } 7-3.5)$ , were compared for these HAs and related substances, and their characteristics are discussed. The wavelengths at the peaks of  $\Delta E(\text{pH } 12.4-7)$  were common to Rp type HAs and the milled wood lignin of rice straw, but not to A type HAs. The  $\Delta E(\text{pH } 12.4-7)$  values of HAs at 600 nm had no linear correlation with the phenol contents but these were approximately proportional to the absorbance of HAs at 600 nm. This result suggests that the  $\Delta E(\text{pH } 12.4-7)$  of HAs at 600 nm does not depend on the phenolic content but on the amount of conjugate systems in HAs. The  $\Delta E(\text{pH } 7-3.5)$  of HAs at 285 and 360 nm increased linearly with increasing absorbance at 285 and 360 nm. The chemical structures in HAs responsible for the change in absorbance with change in pH from 3.5 to 7 was presumed to increase with increasing degree of humification.

*Additional Index Words:* bathochromic effect, hyperchromic effect, phenolic hydroxyl group, carboxyl group, lignin.

Our study on the chemical structure of the dark-colored polymers in humic acids (HAs) has been focused on the relationship between the ultraviolet and visible spectra and the chemical structure of HAs. In the preceding study (12), the distribution of  $pK_a$  values of various types of HAs and the dissociation patterns of the dissociative groups in HAs were investigated in relation to the absorbance and the pH values of HA solutions. The aim of this study is to investigate the effect of the phenolic and the carboxyl groups on the ultraviolet and visible spectra of HAs. The difference spectra between pH 10 and 6 of A, B, and Rp type HAs and lignin have already been investigated by KOBAYASHI and FUJISAWA (2) and of the HAs obtained from various rotted plants by SUZUKI and KUMADA (7) both according to the method of GOLDSCHMIDT (1). This report describes the difference absorption spectra caused by the pH change in alkaline and acidic ranges as well as the relationship between the difference absorbance and the degree of humification or the contents of dissociative groups in HAs.

## MATERIALS AND METHODS

1. *Samples. Humic acids (HAs)*: Thirty-nine samples of HAs which have been used in our series of study (3, 8-12) were used. Analytical properties of these samples, such as elementary composition (3), functional group contents (8), distribution of nitrogen (8), acid-hydrolysable constituents (10), and KOH degradation products (11) are available in our previous papers.

*Fulvic acids*: Fr. B<sub>4</sub> and Fr. D<sub>1</sub> which were obtained from the Bh layer of Hichiso podzolic soil were used. The method of preparation and analytical data of these samples were reported previously (11).

*Related substances*: Glucose-HA, hydroquinone-HA, and polymaleic acid used here are those reported in the previous paper (11). Milled wood lignin of rice straw was offered by Prof. N. Terashima of Nagoya University.

2. *Experimental methods*. HA solutions with concentrations ranging between 10 and 60 mg/100 ml were prepared in 0.1% NaOH. Five milliliter portions of each of these solutions were placed in 3 test tubes. To each of the test tubes, 20 ml of 0.1% NaOH, 1/12 M phosphate buffer (K<sub>2</sub>HPO<sub>4</sub> 0.975%-KH<sub>2</sub>PO<sub>4</sub> 0.570%), or 0.5 M glycine buffer (pH 3.0) was added to make the pH value 12.4, 7.0, or 3.5, respectively. The ultraviolet and visible absorption spectra of the solutions and the difference spectra between pH 12.4 and 7.0 as well as between pH 7.0 and 3.5 solutions were measured from 220 to 700 nm with a Hitachi 124 spectrophotometer. The same solution without HA was used as a reference solution. The difference in absorbance ( $\Delta E$ ) between pH 12.4 and 7.0 and between pH 7 and 3.5 are expressed as  $\Delta E(\text{pH } 12.4-7)$  and  $\Delta E(\text{pH } 7-3.5)$ , respectively, hereafter. In the case of FA fractions and polymaleic acid, the pH of the acidic solution was set to 1 by addition of 20 ml 0.1 N HCl, since there was no precipitation at pH 1.

## RESULTS AND DISCUSSION

The  $\Delta E(\text{pH } 12.4-7)$  spectra of the milled wood lignin of rice straw and B and Rp type HAs had peaks around 250, 300, and 360 nm (Fig. 1). The peaks of the  $\Delta E$  spectra of HAs were much broader than those of lignin. Furthermore,  $\Delta E(\text{pH } 12.4-7)$  of the HAs extended to 700 nm while that of the lignin was zero at wavelength longer than 480 nm. According to GOLDSCHMIDT (1), the peak at 300 nm represents the phenolate anion of simple phenol, the peak at 360 nm the phenolic compound in which hydroxyl group is conjugated with carbonyl group through the rings, and the peak at 250 nm both. The  $\Delta E(\text{pH } 12.4-7)$  in the visible range increased and the peaks of the  $\Delta E$  spectra became broader with increasing degree of humification. This may suggest that the conjugate systems in HAs become longer and more complex with humification.

The  $\Delta E(\text{pH } 12.4-7)$  spectra of A type HAs (Fig. 1) showed a high plateau-like absorption band in the visible range with peaks at 280 and 340 nm. The  $\Delta E$  spectra

Table 1.  $\Delta E$ (pH 12.4–7) in the ultraviolet range.

Type	Wavelength (nm)			
	250	300	360	
A	$\bar{x}^a$	22.4	24.4	28.2
	$s^b$	4.3	3.0	2.4
B	$\bar{x}$	35.5	26.8	36.7
	$s$	12.6	3.9	8.4
P <sub>0</sub>	$\bar{x}$	27.1	29.1	29.4
	$s$	7.7	3.1	6.6
Rp(1)	$\bar{x}$	33.8	18.8	35.4
	$s$	12.4	3.3	10.6
Rp(2)	$\bar{x}$	51.0	23.2	41.8
	$s$	7.5	0.7	11.5
MWL of rice straw <sup>c</sup>	90.5 (242 nm)	21.7 (293 nm)	134 (374 nm)	

<sup>a</sup>  $\bar{x}$ : average value for each type. <sup>b</sup>  $s$ : standard deviation. <sup>c</sup>  $E$  values of the MWL of rice straw are obtained at its peak wavelengths.

were quite different from those of Rp type HAs. The  $\Delta E$  spectra of B type HAs had transitional shapes between those of A and Rp type HAs.

Table 1 shows the average and the standard deviation of  $\Delta E$  (pH 12.4–7) at 250, 300, and 360 nm for each type of HAs. The  $\Delta E$  at 250 and 360 nm decreased in the order: Rp(2) > Rp(1)  $\approx$  B > P<sub>0</sub> > A type. Here, Rp(1) type HAs was extracted from the soil samples of mineral layers and Rp(2) type HAs from A<sub>0</sub> layers of forest soils or rotted wood. The  $\Delta E$  of the milled wood lignin of rice straw was 2–3 times larger than the average  $\Delta E$  values of Rp(2) type HAs at 250 and 360 nm. This may suggest that lignin structure occupy about 1/3 to 1/2 of the chemical structure of Rp(2) type HAs which have the lowest degree of humification. The standard deviations of  $\Delta E$  at 250 and 360 nm were large for Rp(1), Rp(2), and B type HAs but small for A type HAs. This may imply that the degree of degradation of the lignin structure varies significantly among B and Rp type HAs but little among A type HAs. It is presumed that the degradation of the lignin structure was almost completed, and complex but similar structures were formed among A type HAs. On the contrary,  $\Delta E$  at 300 nm had no relation to the types of HAs. The  $\Delta E$  at 300 nm is used to determine the content of non-conjugated phenolic OH group in lignin (1) but cannot be used for HAs because the  $\Delta E$  near 300 nm is affected by the other difference absorption bands at 250 and 360 nm.

The  $\Delta E$  of HAs at 250, 300, and 360 nm had no significant correlations with the

total phenolic OH contents though the change in absorbance was caused by the dissociation of phenol groups. The  $\Delta E(\text{pH } 12.4-7)$  of HAs at 600 nm had also no significant correlation with the phenolic OH content. However, the  $\Delta E$  at 600 nm increased with increasing degree of humification unlike the  $\Delta E$  at 250, 300, and 360 nm, and had a very significant linear correlation with the absorbance at 600 nm (Fig. 2). This result may imply that the  $\Delta E$  at 600 nm does not depend on the phenolic OH content but on the amount of the conjugate systems affecting the absorbance at 600 nm because the chemical structure of HAs becomes more unsaturated with increasing absorbance at 600 nm. The  $\Delta E$  values of A type HAs at 600 nm except for one sample (Nagara P) appeared to attain a constant value with increasing absorbance at 600 nm (Fig. 2). The same trend was also observed for the relationship between the carboxyl and carbonyl

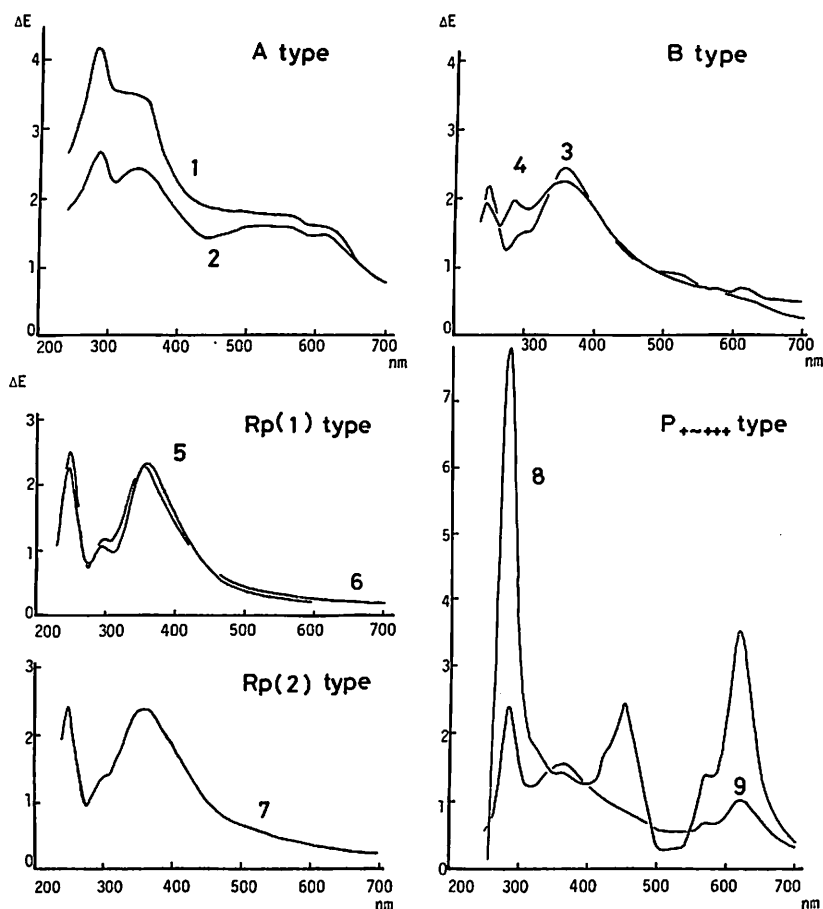
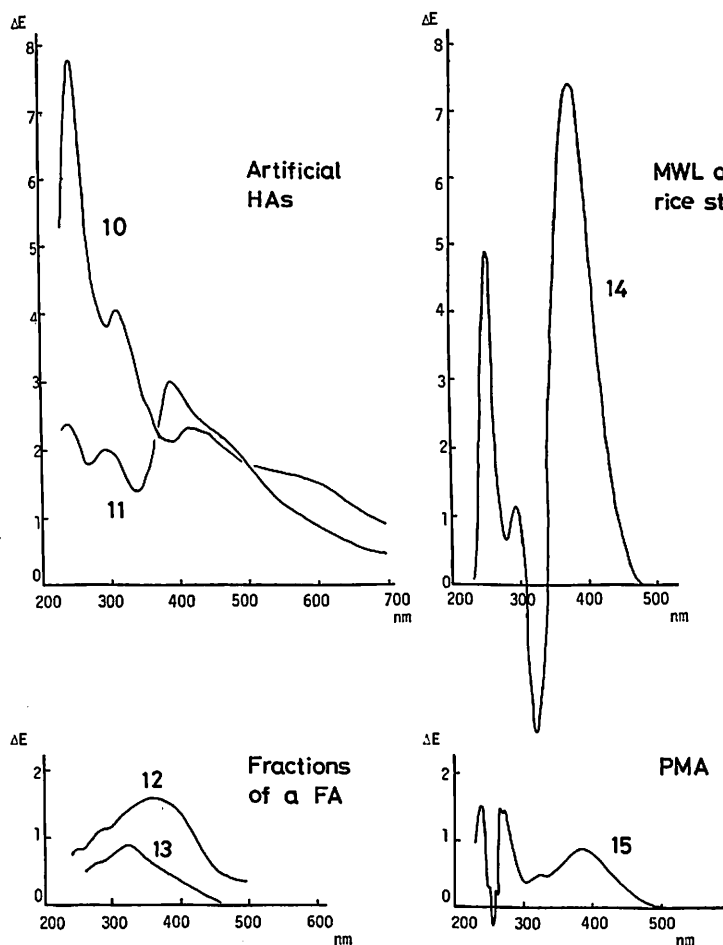


Fig. 1.  $\Delta E(\text{pH } 12.4-7)$  spectra of humic acids and related compounds.

1. Nagara P, 2. Inogashira, 3. Kinshozan F, 4. Kuragari, 5. Gifu, 6. Kinshozan OH,
7. Higashiyama (FH), 8. Tsubame Pg (1/2 scale), 9. Tsubame, 10. hydroquinone-HA,

contents of HAs and the degree of humification ( $\delta$ ). Since the phenolic OH contents of HAs were similar to one another, the phenolic OH contents may limit the  $\Delta E$  at 600 nm for A type HAs which have long conjugate systems.

The peaks at 280, 455, 570, and 620 nm in the  $\Delta E$ (pH 12.4-7) spectra of  $P_{+~++++}$  type HAs are characteristic of dissociated 4,9-dihydroxyperylene-3,10-quinone (DHPQ) (5). These peaks were more prominent in the  $\Delta E$  spectra (Fig. 1) than in the absorption spectra of  $P_{+~++++}$  type HAs in alkaline solution. These peaks due to Pg pigments, were also found in the  $\Delta E$  spectra of the HAs of other types, suggesting that Pg pigments are widely present in various soils.



11. glucose-HA, 12. Fr. D<sub>1</sub> of Hichiso FA, 13. Fr. B<sub>4</sub> of Hichiso FA, 14. milled wood lignin of rice straw, 15. polymaleic acid. Concentrations were fixed to 0.1% organic matter.

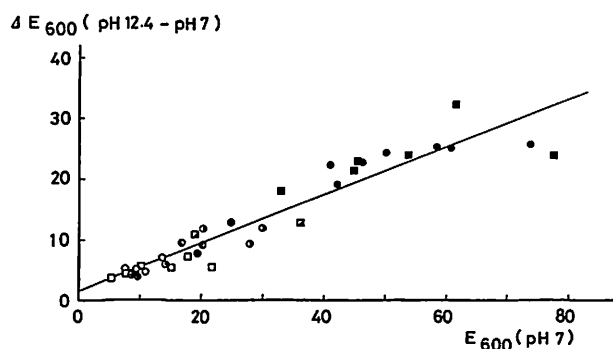


Fig. 2. The relationship between  $\Delta E(\text{pH } 12.4-7)$  and  $E(\text{pH } 7)$  at 600 nm of soil HAs (concentration: carbon 1 g/100 ml).

- , ▨, □ represent A, B, and Rp type HAs, respectively, obtained from calcareous soils.
- represents A type HAs obtained from Kuroboku soils.
- represents B type HAs obtained from forest soils.
- ⊕ represents HAs obtained from paddy soils.
- represents Rp (2) type HAs obtained from A<sub>0</sub> layer of forest soils.

The  $\Delta E$  spectrum of the artificial hydroquinone-HA had peaks at 245, 315, 420 nm and a plateau-like absorption band in the visible range similar to that of A type HAs. This may suggest that the quinone structure contributes to the chemical structure of A type HAs. The glucose-HA had  $\Delta E$  peaks at 240, 295, and 390 nm and considerably high  $\Delta E$  in the visible range, but the shape of the  $\Delta E$  spectra was different from those of soil HAs.

The peaks of the  $\Delta E$  spectra for the two fulvic acid fractions, Fr. B<sub>4</sub> and Fr. D<sub>1</sub>, of the Hichiso podzolic soil were different from each other (Fig. 1). The  $\Delta E$  spectra of both were also different from those of soil HAs or lignin. The  $\Delta E$  spectrum of polymaleic acid which was used as a model compound for fulvic acids, had peaks at 237, 266, and 380 nm, and minimum at 255 nm. It was neither similar to the  $\Delta E$  spectra of fulvic acids nor to humic acids.

The absorbance of HAs in the ultraviolet and visible range decreased with decreasing pH value till about pH 3. At pH lower than 3.5, HAs began to flocculate and the absorbance increased again due to turbidity. The solutions of A type HAs were stable even though the pH was lowered to 2 but those of Rp type HAs began to flocculate at pH higher than 3 (12). This may be because A type HAs contain large amounts of dissociative groups with strong acidity while Rp type HAs contain less.

The  $\Delta E(\text{pH } 7-3.5)$  spectra of soil HAs were characterized by maximum peaks at 280–285 nm and a shoulder at 320 nm (Fig. 3). The  $\Delta E$  at 280 nm increased with increasing degree of humification. The shoulder at 320 nm was hardly noticeable among A type HAs. The  $\Delta E$  spectra of some Rp type HAs such as Gifu and Kinshozan OH had a minimum at 340 nm. Because no symptom of flocculation such as the in-

crease in absorbance was observed at pH higher than 3.5, all the HA solutions used are presumed to be true solutions. Therefore, the  $\Delta E(\text{pH } 7-3.5)$  spectra may represent the change in ultraviolet and visible absorption spectra of true solutions.

The  $\Delta E(\text{pH } 7-3.5)$  spectra of soil HAs are thought to be caused mainly by the dissociation of carboxyl groups in HAs. Generally, however, the dissociation of carboxyl groups has no effect on the ultraviolet spectra of low molecular organic acids. It was reported that HA particles associated, changing their shapes and configurations when the pH of the solution decreased (6). The change in the ultraviolet and visible absorption spectra of HAs is also presumed to be related to these phenomena. The dissociation of dissociative groups may allow the configuration of HA particles to be more free, making the electrons on HA molecules more labile. Polypeptides and DNA are also known to intensify their absorption as their molecules are transformed from a helical to a random coiled configuration or from a two stranded to an uncoiled structure (hyperchromic effect) (4). Special phenolic OH groups which dissociate at pH lower than 7 may also contribute to the  $\Delta E(\text{pH } 7-3.5)$  spectra of HAs. The contribution of such phenolic OH groups with strong acidity, however, may be small, because even the total amounts of phenolic OH groups were less than carboxyl groups for most of the soil HAs (8).

The  $\Delta E(\text{pH } 7-3.5)$  of HAs reached a maximum at 280–285 nm (Fig. 3). The  $\Delta E$  at 285 nm is positively correlated with the  $E(\text{pH } 7)$  at 0.1% level (Fig. 4). Unlike the relationship between the  $\Delta E(\text{pH } 12.4-7)$  and the  $E(\text{pH } 7)$  at 600 nm (Fig. 2) which the regression line ran nearly through the original point, the regression line in Fig. 4 had a relatively large positive value at the intercept on the axis of the  $E(\text{pH } 7)$  at 285 nm. This relationship implies that the  $E(\text{pH } 7)$  at 285 nm can be divided into two parts: one is the absorbance given by the intercept and the other is the residual part of  $E(\text{pH } 7)$  at 285 nm. The absorbance given by the intercept is presumed to be independent of the change in pH from 3.5 to 7. The residual part of the  $E(\text{pH } 7)$  at 285 nm was almost proportional to the  $\Delta E(\text{pH } 7-3.5)$  at 285 nm. This absorbance is presumed to be affected by pH change from 3.5 to 7, and represents the amounts of chemical structures responsible for the pH-dependent absorbance. Because the absorbance at 285 nm of soil HAs generally increase with increasing absorbance at 600 nm which is used to express the degree of humification, the amount of such chemical structure responsible for the pH-dependent absorbance may increase with increasing degree of humification.

A positive linear correlation was also found between  $\Delta E(\text{pH } 7-3.5)$  and  $E(\text{pH } 7)$  at 360 nm (Fig. 5). The distribution of the plots in Fig. 5 had some relationships with the soil sources of HAs as follows: (I) the plots of the HAs obtained from the rendzina-like calcareous soils were placed above or on the regression line except for 3 HAs extracted successively from Jaana soils. (II) All the 4 plots of Rp (2) type HAs were placed above the regression line. (III) Five of the 6 plots of the A type HAs obtained from Kuroboku soils and (IV) all the 5 HAs obtained from paddy soils were placed below the regression line. The distribution of the plots in Fig. 5 seemed to reflect the

carboxyl content of HAs except for Rp(2) type HAs. Comparison between HAs with similar RF values showed that the carboxyl contents of the HAs from the calcareous soils were higher than the HAs of other soils (8). Comparison between HAs having similar RF values again showed that the carboxyl contents of A type HAs obtained from Kuroboku soils and the Rp(1), P<sub>0</sub>, and B type HAs obtained from the paddy soils were lower than those of other HAs. Rp(2) type HAs which were obtained from A<sub>0</sub> layers of forest soils or rotted wood had lower carboxyl contents but higher  $\Delta E(\text{pH } 7-3.5)$  at 360 nm compared with other HAs of similar  $E(\text{pH } 7)$  values. Since Rp type HAs

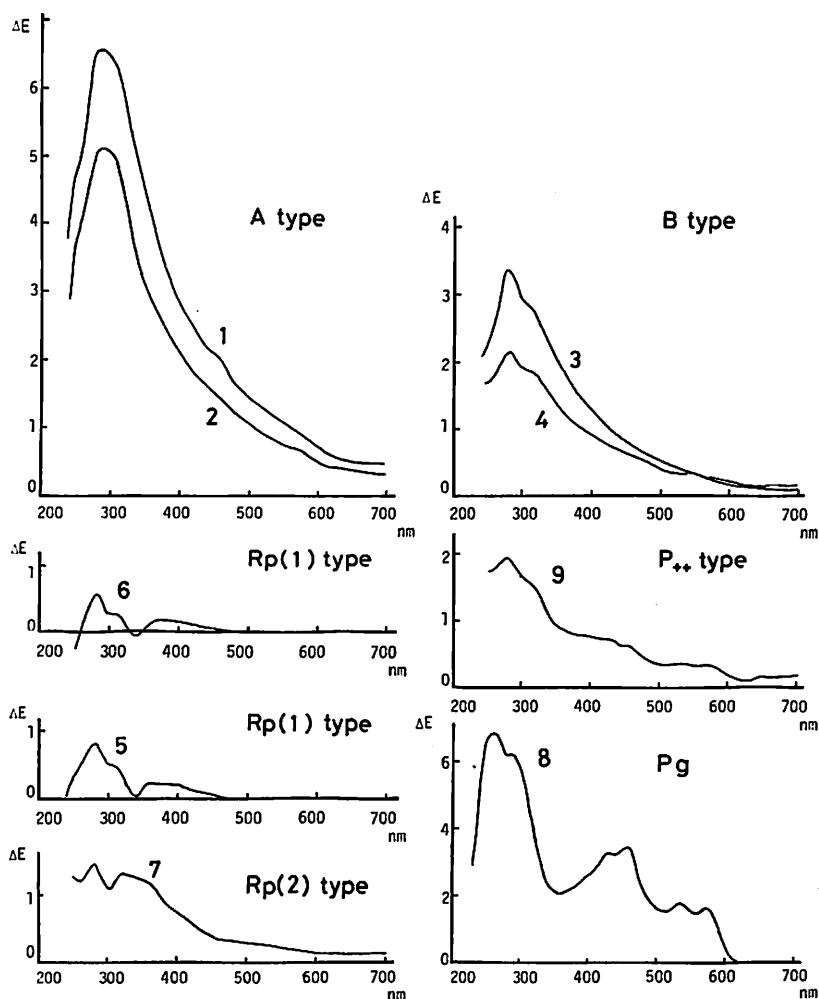


Fig. 3.  $\Delta E(\text{pH } 7-3.5)$  spectra of humic acids and related compounds.

1. Nagara P, 2. Inogashira, 3. Kinshozan F, 4. Kuragari, 5. Gifu, 6. Kinshozan OH, 7. Higashiyama (FH), 8. Tsubame Pg, 9. Tsubame, 10. hydroquinone-HA, 11. glucose-

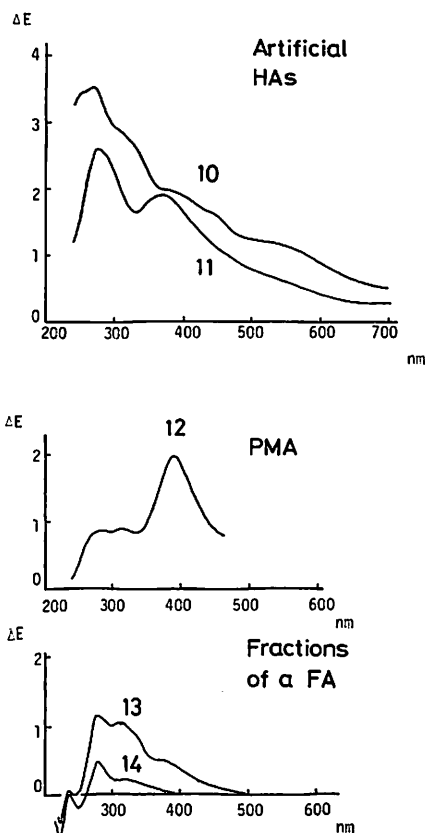


had also high  $\Delta E(\text{pH } 12.4-7)$  at 360 nm due to the phenols conjugated with carbonyl groups (Fig. 1), such phenols may also contribute to the pH-dependency of the absorbance at 360 nm even below pH 7.

The  $\Delta E(\text{pH } 7-3.5)$  spectra of the Pg fraction had peaks at 260, 285, 430, 460, 535, and 570 nm, showing that the change in the absorbance was mainly caused by the dissociation of DHPQ derivatives (Fig. 3). The influence of Pg pigments was also found in the  $\Delta E(\text{pH } 7-3.5)$  spectra of Tsubame, Kuragari, Inogashira, and Nagara PHAs (Fig. 3).

The  $\Delta E(\text{pH } 7-1)$  spectra of the two fulvic acid fractions, Fr. B<sub>4</sub> and Fr. D<sub>1</sub>, had a peak at 280 nm and a peak or a shoulder at 320 nm which is common to the  $\Delta E$  spectra of soil HAs. But the  $\Delta E(\text{pH } 7-1)$  of the fulvic acid fractions was observed only in the ultraviolet range and was very low (Fig. 3).

The  $\Delta E(\text{pH } 7-1)$  spectrum of polymaleic acid also had peaks at 280 and 315 nm,



HA, 12. polymaleic acid, 13. Fr.D<sub>1</sub> of Hichiso FA, 14. Fr.B<sub>4</sub> of Hichiso FA. Concentrations were fixed to 0.1% organic matter.

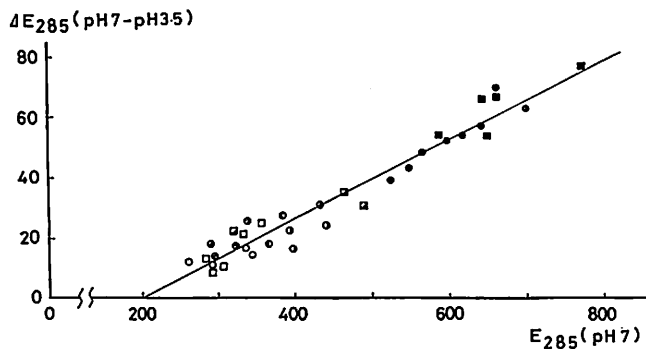


Fig. 4. The relationship between  $\Delta E(\text{pH } 7-3.5)$  and  $E(\text{pH } 7)$  at 285 nm of soil HAs (concentration: carbon 1 g/100 ml). See the legend of Fig. 5 for the symbols.

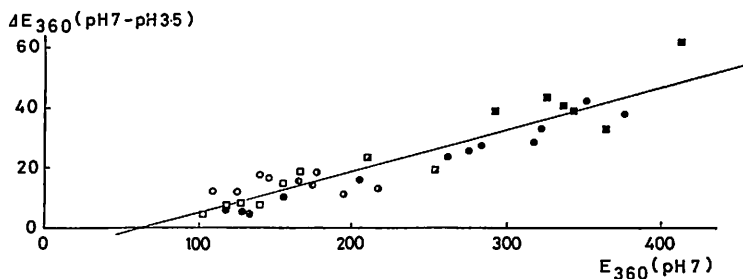


Fig. 5. The relationship between  $\Delta E(\text{pH } 7-3.5)$  and  $E(\text{pH } 7)$  at 360 nm of soil HAs (concentration: carbon 1 g/100 ml).

- , ▨, □ represent A, B, and Rp type HAs, respectively, obtained from calcareous soils.
- represents A type HAs obtained from Kuroboku soils.
- ⊙ represents B type HAs obtained from forest soils.
- ⊕ represents HAs obtained from paddy soils.
- represents Rp(2) type HAs obtained from A<sub>0</sub> layer of forest soils.

and another additional peak at 390 nm which was not found in the  $\Delta E$  spectra of HAs and FAs.

The  $\Delta E(\text{pH } 7-3.5)$  spectrum of the hydroquinone-HA had a peak at 260 nm and shoulders near 320, 390, 450, and 550 nm. The  $\Delta E$  decreased gradually with increasing wavelength. The  $\Delta E$  spectrum resembled those of P<sub>+-+++</sub> type HAs with regard to the high  $\Delta E$  in the visible region. This may be due to the presence of the quinone structure in both hydroquinone-HA and P<sub>+-+++</sub> type HAs. The  $\Delta E(\text{pH } 7-3.5)$  spectrum of the glucose-HA had two peaks at 280 and 370 nm, and did not resemble those of soil HAs and FAs.

The rates of change in absorbance with change in pH from 7 to 12.4 or 3.5 for each type of HAs are summarized in Table 2. The wavelength 600 nm was selected to

Table 2. The rates of change in absorbance with change in pH from 7 to 12.4 or 3.5.

Type	Sample number		$\Delta E(\text{pH } 12.4-7)/E(\text{pH } 7)$			$\Delta E(\text{pH } 7-3.5)/E(\text{pH } 7)$		
			600 nm	360 nm	285 nm	600 nm	360 nm	285 nm
A	13	$\bar{x}$	46.0	14.2	8.59	11.8	10.9	9.12
		$s$	6.8	1.9	1.11	2.2	1.8	0.96
B	6	$\bar{x}$	46.2	18.3	5.53	10.0	8.25	6.05
		$s$	11.3	4.8	1.79	2.3	2.18	1.22
P <sub>0</sub>	4	$\bar{x}$	42.2	18.3	5.20	9.45	8.58	6.73
		$s$	13.4	2.9	1.18	0.44	1.17	1.17
Rp(1)	8	$\bar{x}$	49.9	25.9	4.16	11.4	6.14	5.14
		$s$	10.3	6.5	1.51	5.3	2.36	1.43
Rp(2) <sup>a</sup>	3	$\bar{x}$	50.3	27.5	4.77	14.7	10.9	4.53
		$s$	4.3	1.1	0.91	3.1	1.0	0.57
Mean square ratio			0.60	13.7	16.6	1.47	8.65	21.6
Probability <sup>b</sup>			0.67	$2.2 \times 10^{-8}$	$3.5 \times 10^{-7}$	0.24	$1.0 \times 10^{-4}$	$2.4 \times 10^{-8}$

<sup>a</sup> Yamamomo HA, which was extracted from the rotted wood of *Mirica rubra*, was excluded because its ultraviolet spectrum was different from the spectra of the other Rp type HAs to some extent.

<sup>b</sup> The probability for the null hypothesis was calculated according to the *F*-distribution at degrees of freedom 4, 29.

represent the visible absorption, 360 nm for the peak of  $\Delta E(\text{pH } 12.4-7)$  spectra, and 285 nm for the peak of  $\Delta E(\text{pH } 7-3.5)$  spectra. At 600 nm, no significant differences were found among the average values of both  $\Delta E(\text{pH } 12.4-7)/E(\text{pH } 7)$  and  $\Delta E(\text{pH } 7-3.5)/E(\text{pH } 7)$  by the analysis of variance. This indicates that the rates of contribution of both phenolic and carboxyl groups to the visible absorption of HAs are not affected by the types of HAs. The increasing rate of absorbance at 600 nm with increase in pH from 7 to 12.4 was as high as 50%, which was very high compared with the other rates in Table 2. This shows that phenolic OH groups contribute greatly to the visible absorption and also to the dark-colored polymers in HA structure independently of the types of HAs.

In contrast with the average values of both  $\Delta E(\text{pH } 12.4-7)/E(\text{pH } 7)$  and  $\Delta E(\text{pH } 7-3.5)/E(\text{pH } 7)$  at 600 nm, the values at 285 nm and 360 nm were significantly different among the different types of HAs. However, these rates were smaller compared with the  $\Delta E(\text{pH } 12.4-7)/E(\text{pH } 7)$  values at 600 nm, indicating that phenolic OH and carboxyl groups participate to a smaller extent in the ultraviolet absorption than in the visible absorption.

The order of the  $\Delta E(\text{pH } 12.4-7)/E(\text{pH } 7)$  values was  $\text{Rp}(2) \approx \text{Rp}(1) > \text{P}_0 \approx \text{B} > \text{A}$  type at 360 nm and  $\text{Rp}(2) = \text{Rp}(1) < \text{P}_0 < \text{B} < \text{A}$  type at 285 nm. The trend showing that the rates at 360 nm decrease with humification seems to indicate that the phenolic OH groups conjugating with carbonyl group through the benzene ring decrease in the process of humification. However, the opposite trend was observed for the rates at

285 nm. This may indicate the increase in the phenolic OH groups inherent in dark-colored polymers. Total phenolic OH contents of HA also increased in the order  $Rp(1) < B < A$  type ( $\delta$ ) in conformity with the rates at 285 nm.

The orders of the  $\Delta E(\text{pH } 7-3.5)/E(\text{pH } 7)$  values were  $Rp(2) > Rp(1) < P_0 \rightleftharpoons B < A$  type at 360 nm and  $Rp(2) < Rp(1) < P_0 \rightleftharpoons B < A$  type at 285 nm. The decreasing rates of absorbance with decrease in pH from 7 to 3.5 increased in the process of humification except for the decrease from  $Rp(2)$  to  $Rp(1)$  type at 360 nm. This may correspond to the increase in carboxyl content of HAs during humification ( $\delta$ ). The decrease in the rates at 360 nm from  $Rp(2)$  to  $Rp(1)$  type suggests that part of phenolic substances such as lignin originating from plant materials also participate in  $\Delta E(\text{pH } 7-3.5)$  and such phenolic substances decrease from  $Rp(2)$  to  $Rp(1)$  type.

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