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BEHAVIOR OF PHENOLIC SUBSTANCES IN THE DECAYING PROCESS OF PLANTS

X. Distribution of Phenolic Acids in Soils of Greenhouses and Fields

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Phenolic compounds in the soils collected from plow layers of greenhouse and field cultures were surveyed quantitatively by gas chromatography. The results are as follows:

1) *p*-Coumaric, ferulic, salicylic, *p*-hydroxybenzoic, vanillic, syringic, and protocatechuic acids were detected in all upland soils, as in the case of paddy and forest soils reported previously. Among these phenolics, *p*-coumaric acid was present in the largest quantity in most of the upland soils up to 29.0 ppm.

2) The total amounts of individual phenolic acids in the upland soils ranged from 9.5 to 62.0 ppm (average 26.0 ppm). Also, the amounts were less than 0.18% (average 0.10%) of the total soil organic matter. The average values in the upland soils were higher than those in the paddy soils, but were lower than those in the forest soils.

3) In upland soils, the concentrations of *p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric, and ferulic acids, which have no chelating ability, were considerably related to the carbon content of soil, but the concentrations of protocatechuic and salicylic acids, which are chelating agents, were not related to that of soil. These relationships agreed with those found in paddy and forest soils.

Additional Index Words: distribution, phenolic acid, greenhouse, field.

In the previous study (*1*), phenolic compounds in soils of paddy fields and forests were surveyed quantitatively by gas chromatography. In that study, it was found that the concentrations of individual phenolic acids in soils and the levels of individual phenolic acids per weight of organic matter were generally higher in the forest soils than in the paddy soils.

Recently, rice straw is applied in large amounts to paddy and upland fields, especially to greenhouse culture, as an important source of organic material for improving soil conditions. Composts of bark and sawdust are also applied to fields and greenhouse culture. However, little is known about the distribution of phenolic compounds in upland soils.

In this study, phenolic compounds in upland soils were analysed quantitatively. The data obtained were also compared with those of paddy and forest soils reported previously (*1*).

MATERIALS AND METHODS

1) Soil samples. Soils were collected from the plow layers of 16 greenhouse and 29 field cultures in Yamaguchi Prefecture in spring, 1978. Undried and air-dried soils were ground and passed through a 2 mm sieve. A brief description of soil samples is given in Table 1.

The soil pH was determined with a glass electrode using a 1 : 2.5 soil/water ratio. The carbon contents in the samples were determined by the method of Tyulin (*2*).

2) Identification and quantitative determination of phenolic compounds. Twenty grams of each moist soil samples was employed for the identification and determination of phenolic compounds. The ether-soluble phenolic compounds were obtained from the acidified solution of the NaOH extracts of soil samples by the method reported previously (*3*). Individual phenolic compounds were identified and determined quantitatively by gas chromatography according to the method reported previously (*4, 5*).

RESULTS AND DISCUSSION

The phenolic compounds in upland soils, *p*-coumaric acid (4-hydroxycinnamic acid), ferulic acid (4-hydroxy-3-methoxycinnamic acid), *p*-hydroxybenzoic acid (4-hydroxybenzoic acid), vanillic acid (4-hydroxy-3-methoxybenzoic acid), syringic acid (3,5-dimethoxy-4-hydroxybenzoic acid), protocatechuic acid (3,4-dihydroxybenzoic acid), and salicylic acid (2-hydroxybenzoic acid), were identified by co-chromatography with authentic compounds. The relative retention times of these phenolic compounds have been reported (*4*).

All upland soils contained the same phenolic acids as those found in the paddy and forest soils (*1*). WHITEHEAD *et al.* (*6, 7*), GUENZI and MCCALLA (*8*), WANG *et al.* (*9, 10*), and MORITA (*11*) reported that *p*-hydroxybenzoic, vanillic, *p*-coumaric, and ferulic acids were contained in the soils of England, America, Taiwan, and Canada. These findings suggest that the 4 phenolic acids mentioned above are widely distributed as major phenolic compounds in soils.

Large amounts of fresh rice straw and rice straw compost were applied into the upland soils. Most of the phenolic acids detected in the upland soils were also the same as those found in the NaOH hydrolysates or KOH degradation products of milled wood lignin of rice straw (*12, 13*), and in both cases, *p*-coumaric acid was found in the largest quantity among the phenolic acids. Furthermore, our previous paper (*5*) showed that free phenolic acids contained in rice straw were rapidly degraded during the decaying process. From these findings, it was presumed that rice straw lignin was an important source of phenolic acids in the upland soils.

Table 1. Upland soil samples used.

Soil No.	Sampling site[a]	pH (H_2O)	Carbon content (%)	Crop[b]
1[c]	Ozu, Iwakuni	5.8	0.76	Tomato
2[c]	Ozu, Iwakuni	7.7	0.84	Tomato
3[c]	Misho, Iwakuni	5.0	0.72	Cabbage
4[c]	Misho, Iwakuni	6.6	1.16	Cabbage
5[c]	Misho, Iwakuni	7.1	1.19	Chinese cabbage
6[c]	Misho, Iwakuni	6.5	1.81	Chinese cabbage
7[c]	Misho, Iwakuni	6.2	1.30	Radish
8[c]	Misho, Iwakuni	4.9	1.48	Radish
9[d]	Minamikouchi, Iwakuni	5.3	2.07	Cucumber
10[d]	Minamikouchi, Iwakuni	6.0	1.31	Cucumber
11[d]	Kawashimo, Iwakuni	5.9	2.95	Strawberry
12[d]	Hirata, Iwakuni	6.7	1.63	Strawberry
13[c]	Kawashimo, Iwakuni	6.0	1.24	Eggplant
14[c]	Kawashimo, Iwakuni	6.6	1.23	Eggplant
15[d]	Imoji, Hofu	6.5	3.67	Strawberry
16[d]	Daidoshigeshi, Hofu	4.5	0.90	Eggplant
17[d]	Imoji, Hofu	5.7	1.73	Tomato
18[d]	Imoji, Hofu	6.2	1.81	Tomato
19[d]	Hanagi, Hofu	6.4	2.60	Cucumber
20[d]	Hanagi, Hofu	6.1	2.76	Cucumber
21[c]	Migitakojima, Hofu	5.8	0.98	Radish
22[c]	Daidodanhigashi, Hofu	6.0	1.08	Chinese cabbage
23[c]	Migitakojima, Hofu	5.4	1.02	Chinese cabbage
24[c]	Migitakojima, Hofu	5.9	1.21	Cabbage
25[c]	Daidodanhigashi, Hofu	5.9	1.00	Onion
26[c]	Tsubaki, Hagi	5.3	2.31	Cucumber
27[c]	Tsubaki, Hagi	5.0	1.54	Cucumber
28[c]	Tsubaki, Hagi	6.5	2.05	Tomato
29[c]	Tsubaki, Hagi	6.0	2.13	Tomato
30[c]	Tsubaki, Hagi	7.0	1.50	Strawberry
31[c]	Tsubaki, Hagi	6.2	1.61	Strawberry
32[c]	Tsubaki, Hagi	6.2	1.97	Eggplant
33[c]	Tsubaki, Hagi	5.3	1.82	Eggplant
34[c]	Kawanaka, Shimonoseki	5.5	2.01	Radish
35[c]	Kawanaka, Shimonoseki	6.2	1.34	Radish
36[c]	Kawanaka, Shimonoseki	5.8	0.74	Radish
37[d]	Hirata, Shimonoseki	5.5	2.45	Tomato
38[d]	Kawanaka, Shimonoseki	6.6	1.05	Tomato
39[d]	Utsui, Shimonoseki	6.4	2.88	Strawberry
40[d]	Utsui, Shimonoseki	6.0	3.08	Strawberry
41[d]	Kawanaka, Shimonoseki	5.8	0.80	Cucumber
42[d]	Kawanaka, Shimonoseki	6.1	1.70	Cucumber
43[c]	Nishiyama, Shimonoseki	6.4	0.79	Cabbage
44[c]	Nishiyama, Shimonoseki	5.9	1.43	Cabbage
45[c]	Kawanaka, Shimonoseki	4.9	0.67	Green soybean

[a] Soil samples were collected from the plow layers of greenhouse and field cultures in Yamaguchi Prefecture. [b] Tomato=*Lycopersicum esculentum* MILL. Cabbage=*Brassica oleracea* L. Chinese cabbage=*Brassica campestris* L. Radish=*Raphanus sativus* L. Cucumber=*Cucumis sativus* L. Strawberry=*Fragaria grandiflora* EHRH. Eggplant=*Solanum melongena* L. Onion=*Allium cepa* L. Green soybean=*Glycine max* MERR. [c] Field. [d] Greenhouse.

Table 2. Concentrations of individual phenolic acids in upland soils.

Soil No.	Salicylic acid	*p*-Hydroxy-benzoic acid	Vanillic acid	Protocate-chuic acid	Syringic acid	*p*-Coumaric acid	Ferulic acid	Total
				ppm[a] (%)[b]				
1	2.6(15.5)	1.1 (6.5)	2.0(11.9)	<0.3 (—)	<0.3 (—)	11.1(66.1)	<0.3 (—)	16.8(100)
2	<0.3 (—)	1.0 (9.4)	<0.3 (—)	<0.3 (—)	<0.3 (—)	9.6(90.6)	<0.3 (—)	10.6(100)
3	<0.3 (—)	1.0(10.5)	<0.3 (—)	<0.3 (—)	<0.3 (—)	8.5(89.5)	<0.3 (—)	9.5(100)
4	3.4(18.5)	1.5 (8.2)	2.4(13.0)	<0.3 (—)	<0.3 (—)	11.1(60.3)	<0.3 (—)	18.4(100)
5	<0.3 (—)	1.3 (7.6)	4.5(26.5)	<0.3 (—)	2.2(12.9)	9.0(52.9)	<0.3 (—)	17.0(100)
6	<0.3 (—)	2.3(13.5)	2.2(12.9)	<0.3 (—)	<0.3 (—)	12.6(73.7)	<0.3 (—)	17.1(100)
7	4.3(20.7)	2.2(10.6)	2.5(12.0)	2.9(13.9)	2.7(13.0)	6.2(29.8)	<0.3 (—)	20.8(100)
8	<0.3 (—)	3.2(22.4)	1.3 (9.1)	1.1 (7.7)	1.9(13.3)	3.2(22.4)	3.6(25.2)	14.3(100)
9	<0.3 (—)	1.4 (9.5)	1.3 (8.8)	1.1 (7.5)	1.8(12.2)	5.6(38.1)	3.5(23.8)	14.7(100)
10	<0.3 (—)	2.7(11.4)	4.1(17.3)	1.1 (4.6)	2.4(10.1)	9.4(39.7)	4.0(16.9)	23.7(100)
11	<0.3 (—)	2.8 (9.6)	2.9 (9.9)	1.1 (3.8)	3.3(11.3)	14.2(48.5)	5.0(17.1)	29.3(100)
12	<0.3 (—)	1.7 (9.4)	1.4 (7.8)	1.1 (6.1)	2.0(11.1)	8.0(44.4)	3.8(21.1)	18.0(100)
13	<0.3 (—)	1.4 (8.9)	1.0 (6.4)	1.0 (6.4)	1.9(12.1)	7.0(44.6)	3.4(21.7)	15.7(100)
14	<0.3 (—)	2.1(14.5)	1.0 (6.9)	1.1 (7.6)	1.7(11.7)	5.2(35.9)	3.4(23.4)	14.5(100)
15	14.1(22.7)	4.8 (7.7)	6.1 (9.8)	<0.3 (—)	3.8 (6.1)	27.2(43.9)	6.0(9.7)	62.0(100)
16	<0.3 (—)	1.8(17.0)	2.2(20.8)	<0.3 (—)	<0.3 (—)	6.6(62.3)	<0.3 (—)	10.6(100)
17	4.6(22.4)	2.8(13.7)	3.0(14.6)	<0.3 (—)	<0.3 (—)	6.5(31.7)	3.6(17.6)	20.5(100)
18	5.1(17.4)	3.5(11.9)	3.5(11.9)	<0.3 (—)	2.8 (9.6)	10.2(34.8)	4.2(14.3)	29.3(100)
19	13.4(22.3)	5.0 (8.3)	4.9 (8.2)	<0.3 (—)	3.3 (5.5)	28.2(47.0)	5.2 (8.7)	60.0(100)
20	9.7(17.3)	5.9(10.5)	5.0 (8.9)	<0.3 (—)	2.6 (4.6)	25.1(44.7)	7.9(14.1)	56.2(100)
21	9.9(34.1)	1.8 (6.2)	1.7 (5.9)	<0.3 (—)	1.8 (6.2)	9.5(32.8)	4.3(14.8)	29.0(100)
22	3.2(12.4)	2.0 (7.7)	1.8 (6.9)	<0.3 (—)	2.0 (7.7)	11.4(44.0)	5.5(21.2)	25.9(100)

23	3.4 (17.7)	3.6 (18.8)	2.0 (10.4)	<0.3 (—)	2.2 (11.5)	3.4 (17.7)	4.6 (24.0)	19.2 (100)
24	3.2 (14.4)	1.7 (7.7)	1.7 (7.7)	<0.3 (—)	1.8 (8.1)	9.4 (42.3)	4.4 (19.8)	22.2 (100)
25	4.5 (19.8)	1.7 (7.5)	2.0 (8.8)	<0.3 (—)	1.9 (8.4)	8.6 (37.9)	4.0 (17.6)	22.7 (100)
26	4.7 (9.7)	4.5 (9.3)	3.6 (7.4)	<0.3 (—)	2.9 (6.0)	23.4 (48.2)	9.4 (19.4)	48.5 (100)
27	4.6 (14.6)	4.4 (14.0)	3.4 (10.8)	<0.3 (—)	2.6 (8.3)	10.1 (32.2)	6.3 (20.1)	31.4 (100)
28	4.7 (14.9)	3.2 (10.2)	2.0 (6.3)	<0.3 (—)	1.7 (5.4)	14.4 (45.7)	5.5 (17.5)	31.5 (100)
29	3.8 (14.3)	2.0 (7.5)	1.5 (5.6)	<0.3 (—)	<0.3 (—)	14.3 (53.8)	5.0 (18.8)	26.6 (100)
30	4.8 (29.6)	2.1 (13.0)	1.5 (9.3)	<0.3 (—)	<0.3 (—)	7.8 (48.1)	<0.3 (—)	16.2 (100)
31	4.9 (18.9)	2.3 (8.9)	1.6 (6.2)	<0.3 (—)	<0.3 (—)	11.9 (45.9)	5.2 (20.1)	25.9 (100)
32	4.6 (18.0)	2.2 (8.6)	1.8 (7.0)	<0.3 (—)	1.7 (6.6)	11.3 (44.1)	4.0 (15.6)	25.6 (100)
33	5.8 (18.8)	3.7 (12.0)	2.9 (9.4)	<0.3 (—)	2.0 (6.5)	16.4 (53.2)	<0.3 (—)	30.8 (100)
34	3.3 (11.1)	3.1 (10.5)	2.8 (9.5)	<0.3 (—)	3.0 (10.1)	12.4 (41.9)	5.0 (16.9)	29.6 (100)
35	3.9 (14.8)	3.0 (11.4)	2.6 (9.9)	<0.3 (—)	2.7 (10.3)	10.3 (39.2)	3.8 (14.4)	26.3 (100)
36	3.8 (20.2)	2.1 (11.2)	2.5 (13.3)	2.9 (15.4)	<0.3 (—)	7.5 (39.9)	<0.3 (—)	18.8 (100)
37	4.9 (9.9)	3.2 (6.5)	4.0 (8.1)	2.8 (5.6)	3.6 (7.3)	22.6 (45.6)	8.5 (17.1)	49.6 (100)
38	3.2 (17.8)	2.7 (15.0)	2.8 (15.6)	<0.3 (—)	3.0 (16.7)	6.3 (35.0)	<0.3 (—)	18.0 (100)
39	0.8 (1.7)	4.3 (8.9)	3.4 (7.1)	<0.3 (—)	3.3 (6.8)	29.0 (60.2)	7.4 (15.4)	48.2 (100)
40	5.4 (11.5)	3.8 (8.1)	4.1 (8.8)	2.9 (6.2)	3.8 (8.1)	19.1 (40.8)	7.7 (16.5)	46.8 (100)
41	3.8 (17.2)	2.2 (10.0)	3.1 (14.0)	2.8 (12.7)	2.8 (12.7)	7.4 (33.5)	<0.3 (—)	22.1 (100)
42	3.7 (13.8)	2.2 (8.2)	3.0 (11.2)	2.8 (10.4)	3.0 (11.2)	7.9 (29.5)	4.2 (15.7)	26.8 (100)
43	3.0 (20.7)	2.6 (17.9)	2.1 (14.5)	<0.3 (—)	<0.3 (—)	6.8 (46.9)	<0.3 (—)	14.5 (100)
44	0.7 (4.4)	1.8 (11.3)	1.8 (11.3)	<0.3 (—)	1.7 (10.7)	6.6 (41.5)	3.3 (20.8)	15.9 (100)
45	0.3 (1.5)	3.8 (18.6)	1.7 (8.3)	1.0 (4.9)	1.7 (8.3)	7.9 (38.7)	4.0 (19.6)	20.4 (100)
Average	3.5 (13.5)	2.7 (10.4)	2.5 (9.6)	0.6 (2.3)	1.8 (6.9)	11.6 (44.6)	3.5 (13.5)	26.0 (100)

[a] Oven-dried basis. [b] Values in parentheses indicate per cent of individual phenolic acids based on total amounts of phenolic acids.

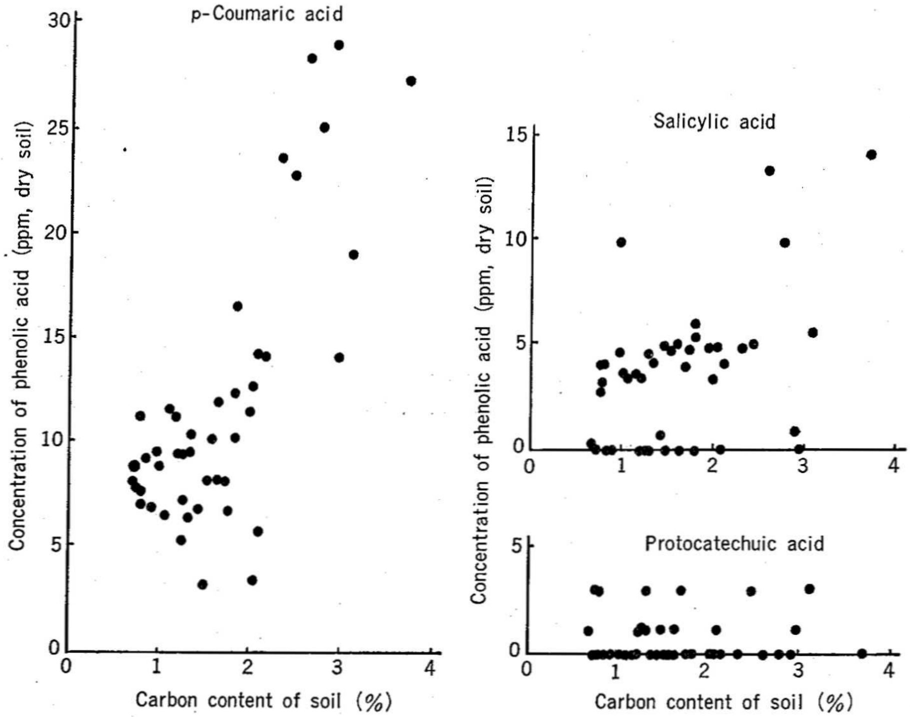
p-Coumaric acid
Salicylic acid
Protocatechuic acid
Concentration of phenolic acid (ppm, dry soil)
Carbon content of soil (%)
Carbon content of soil (%)

Fig. 1. (1)

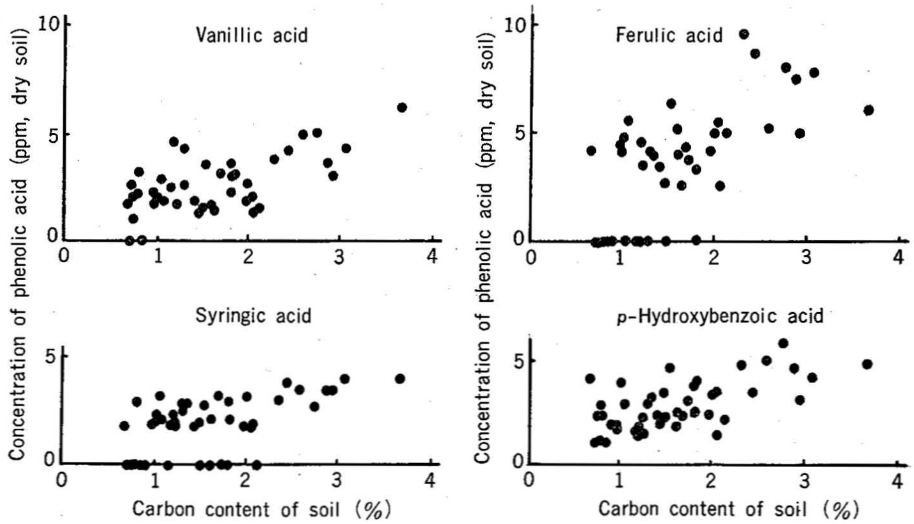
Vanillic acid
Ferulic acid
Syringic acid
p-Hydroxybenzoic acid
Concentration of phenolic acid (ppm, dry soil)
Concentration of phenolic acid (ppm, dry soil)
Carbon content of soil (%)
Carbon content of soil (%)

Fig. 1. (2)

In the hydrolysates of the milled wood lignin of rice straw, trace amounts of gallic, caffeic, and sinapic acids were detected in addition to the phenolic acids mentioned above (*12*). However, these phenolic acids were not detected in the upland soil samples, possibly because only small amounts of these polyphenolic acids are contained in the rice plant and they are decomposed more rapidly than the other phenolic acids. Although rice leaves contain small amounts of flavonoids (*14*), phloroglucinol and resorcinol, which are known to be the degradation products of flavonoids (*14*), were not detected in the upland soils. These polyphenols may also be decomposed rapidly.

The amounts of individual phenolic acids in the upland soils ranged from less than the detectable limit (0.3 ppm) to 29.0 ppm as shown in Table 2. In many soils, the amounts decreased in the order of *p*-coumaric > ferulic ≒ salicylic > *p*-hydroxybenzoic > vanillic > syringic > protocatechuic acids. The total amounts of phenolic

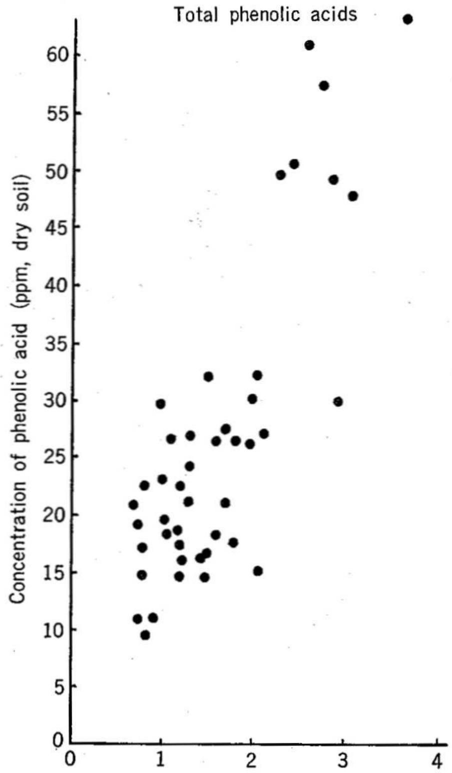

Fig. 1. (3)

Fig. 1. Relationship between concentrations of individual phenolic acids and carbon content of soil. *r*: correlation coefficient (calculated as 0 ppm for values less than 0.3 ppm). *p*-Coumaric acid, $r=0.785$; ferulic acid, $r=0.668$; vanillic acid, $r=0.618$; *p*-hydroxybenzoic acid, $r=0.640$; syringic acid, $r=0.558$; salicylic acid, $r=0.458$; protocatechuic acid, $r=0.033$; total phenolic acid, $r=0.810$.

acids ranged from 9.5 to 62.0 ppm (average 26.0 ppm). As in the case of paddy and forest soils reported previously (*1*), in most upland soils, *p*-coumaric acid was found in the largest quantity among the phenolic acids. In most upland, paddy, and forest soils, the amount exceeded 35, 20, and 25% of the total amounts of phenolic acids in these soils respectively.

The amounts of individual and total phenolic acids in the upland soils were less than 0.09% (average 0.01%) and 0.18% (average 0.10%) respectively of the total soil organic matter which was calculated by multiplying carbon content of soil by 1.72.

According to our previous paper (*1*), the average concentrations of the total phenolic acids in paddy and forest soils were 21.3 and 210.3 ppm, respectively. Also, the average levels of the phenolic acids per weight of soil organic matter were 0.06 and 0.12%, respectively. The values in the upland soils used in this study were higher than those in the paddy soils, although phenolic acids are degraded more rapidly under upland, than under flooded, conditions (*3, 5*). This may be because many phenolic acids are easily leached with water (*15, 16*) in paddy fields. On the other hand, the leaching of phenolics may not be extensive in upland soils where transportation of water is much lower compared with paddy fields. The average concentrations of the total phenolic acids in the upland soils and their average levels per weight of organic matter were lower than those in the forest soils. This may be due mainly to 2 reasons; first, the amount of phenolic substances in decayed leaves of trees is generally higher than that in decayed straw (*17*), second, phenolic acids are degraded more rapidly at higher temperatures (*5*).

WANG *et al.* (*10*) quantitatively determined *p*-coumaric, *p*-hydroxybenzoic, vanillic, and ferulic acids in 23 upland soils of Taiwan by paper chromatography, and found that the average summed amounts of these phenolic acids in soils and their average levels per weight of organic matter were 3.6 ppm and 0.03%, respectively. These values were lower than those detected in the upland soils of this study. This may be due to the fact that the levels of phenolic acids in the leaves and roots of sugarcane (*9*) were much lower compared with the amount in rice straw (*3*) and that both analytical methods were different.

The relationship between the concentration of each phenolic acid and the carbon contents of the upland soils is shown in Fig. 1. The concentrations of *p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric, and ferulic acids, which have no chelating ability, were generally higher in soils containing larger amounts of carbon, and the correlation coefficients of these phenolic acids with carbon content were considerably high. This may be because larger amounts of these phenolic acids are adsorbed in soils containing larger amounts of organic matter (*15*). On the other hand, no such relationship was observed for protocatechuic and salicylic acids which have chelating ability, and the correlation coefficients were low. These acids are strongly adsorbed by clay minerals, forming chelate complexes with soil metals (*16*). Therefore, these acids may be present regardless of the organic matter content of the soil.

As reported previously (*1*), in the paddy and forest soils, the concentrations of those phenolic acids with no chelating ability were also considerably related to the carbon content of soil. Accordingly, it is considered that the organic matter content of the soil is an important factor in determining the concentrations of these phenolic acids in various soils.

The concentrations of various phenolic acids in the upland soils were not related to soil pH.

Because soil temperature is generally higher in the greenhouse than in the field and that phenolic acids are more rapidly degraded at higher temperatures, it was supposed that the concentrations of phenolic acids in soils were higher in the field than in the greenhouse. However, the comparison of the concentrations of phenolic acids between the type of culture (Tables 1 and 2) revealed that the average concentrations of total phenolic acids was markedly higher in greenhouse soils than in field soils and that the average levels of phenolic acids in soil organic matter was almost the same for greenhouse and field soils. These results may be explained by the fact that the amounts of organic materials such as rice straw which is applied into the soils are markedly larger in greenhouse soils (about 2 tons/10 a/year) than in field soils (about 0.5 ton/10 a/year) and the carbon content is also larger in greenhouse soils.

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