Chapter 4

EFFECTS OF SOIL PROPERTIES, ADDED ORGANIC MATERIALS AND TEMPERATURES ON THE KINETICS OF PHENOLIC ACIDS IN SUBMERGED SOILS

SUMMARY

The kinetics of phenolic acids were investigated in 3 submerged soils treated with 0.25% rice straw, compost and green manure and incubated for 2 and 6 weeks at 20°C and 35°C. Ferulic acid, p-coumaric acid, sinapic acid, vanillic acid and p-hydroxybenzoic acid were detected in submerged soils. By adding rice straw, concentrations of phenolic acids except p-hydroxybenzoic acid increased with increased period of incubation at both temperatures. p-Hydroxybenzoic acid was more unstable and showed a different kinetics. Addition of 0.25% green manure or compost did not affect the concentration of phenolic acids in soils significantly at both temperatures. The level of total phenolic acids was between 8 ~ 14 ppm in soils except the levels in rice straw treated soils (23 ~ 36 ppm) after 6 weeks of incubation. These results suggest that most part of phenolic acids in soils are due to indigenous soil organic matter and phenolic acids at this level may not be toxic to plants. Phenolic acid formation from added rice straw was favored at higher temperature in alkaline and neutrals soils, but at lower temperature in a acidic soil.

INTRODUCTION

Aromatic organic acids have much higher toxicity than aliphatic acids as confirmed by many researchers (Takijima, 1963).

Phenolic acids have been assumed to be growth inhibitors for dryland crops (Wang, et al., 1967) and for rice due to the decomposing rice residues in soil (Chou and Lin, 1976). Kinetics of phenolic acids in the decomposing rice straw were studied by Kuwatsuka, et al. (1977). The levels of phenolic acids in fields were also determined by Shindo and Kuwatsuka (1978). In this study, effects of soil properties, added organic materials and temperatures on the kinetics of phenolic acids in submerged soils were investigated by incubation. Previous study showed that the amount of organic reducing substances had peaks of formation at 2 and 6 weeks of incubation. Whether phenolic acids were formed in large amounts at this time or not was one of the questions to be answered here.

MATERIALS AND METHODS

Incubation

Three soils (Pila, Maahas and Luisiana) with different properties (Table 1) were used. Twenty-five gram of soil sample was weighed in a 125 ml conical flask, and a ground sample (passed through 2 mm sieve) of compost, rice straw or green manure (Griricidia sepium) was added at the rate of 0.25% to the soil. No organic matter was added for the control treatment. After the soil and organic matter were mixed well, 50 ml of distilled water was added. Submerged soil sample was then degassed under reduced pressure. The flask was sealed by parafilm in the flush of nitrogen gas, and incubated at 20°C and 35°C for 2 weeks and 6 weeks. Chemical compositions of added organic materials are given in Chapter 8.

Extraction

After incubation, the content of the incubation flask was transferred into a 250 ml conical flask with 100 ml of 0.75 N NaOH. The flask was stoppered tightly and shaken for 60 min. After shaking, 7.5 g of Na_2SO_4 anhydride was added to the soil-extractant mixture, and the mixture was shaken for another 15 min. It was then centrifuged at 7000 rpm and the supernatant was further filtered through a dry Whatman No. 42 filter paper. One hundred ml portion of the filtered extract was taken into a 250 ml conical flask and acidified by 3.2 ml of conc. H_2SO_4. Fifty ml of CH_2Cl_2 and 25 μl of 0.2% p-chlorobenzoic acid methanol solution (as an internal standard) were added to the acidified extract and stirred by magnetic stirrer for 1 hour with the flask stoppered tightly. Upper aqueous layer was decanted off after leaving the mixed sample for some minutes. Emulsified layer was transferred to a centrifuge tube and centrifuged at 7000 ppm for 5 minutes. A forty ml portion of clear CH_2Cl_2 layer was carefully transferred to a 50 ml glass beaker and it was left in a fumehood overnight to evaporate CH_2Cl_2. The dried residue was transferred into a 3 ml Reacti Vial by using small volume of CH_2Cl_2 as solvent. The solvent was evaporated again under a stream of nitrogen gas. Fifty μl of silylation reagent, N, O-bis-trimethylsilyl-acetamide, was added to the dried residue and the vial was stoppered tightly with teflon-silicon rubber septum. It was then heated on a hot plate at 100°C for 30 min. After cooling, 2 μl of this concentrate was injected to a gas-chromatograph.

Gas-chromatography

A Varian aerograph model 1868 equipped with a FID detector was used. This instrument features on-column injection and direct connection of the column to the detector. A glass column (6 ft x 6 mm O.D. x 2 mm I. D.) paked with 2% Silicon OV-17 on Uniport HP (60/80 mesh) was used. Nitrogen was used as carrier gas at a flow rate of 50 ml/min. Flow rates of hydrogen and air were 30 ml and 300 ml/min, respectively. The temperature of injector and detector was maintained at 300°C. The column temperature was programmed to increase from 100°C to 200°C at a rate of 10°C/min for 10 min, then to 250°C at a rate of 2.5°C/min for the following 20 min. After reaching 250°C the temperature was kept constant.

RESULTS AND DISCUSSION

Kinds of phenolic acids and kinetics of p-hydroxybenzoic acid

A gas chromatogram of phenolic acid analysis is given in Fig. 16. Following phenolic acids were detected: ferulic acid, p-coumaric acid, p-hydroxybenzoic acid, vanillic acid and sinapic acid. Levels decreased in this order in Pila and Maahas soil. In Luisiana soil, sinapic acid was most abundant among the phenolic acids. One peak which appeared after the peak of sinapic acid is not identified. Its amount was almost same or larger than that of sinapic acid. In most cases, the level of each phenolic acid was less than 5 mg/kg soil unless rice straw was added.

Kinetics of p-hydroxybenzoic acid was different from that of other phenolic acids. Addition of organic matter (rice straw, green manure, and compost) to soils even decreased the concentration of p-hydroxybenzoic acid after 2 weeks of incubation. It decreased in the order of control > rice straw treated soil > greenmanure treated soil > compost treated soil. Difference due to incubation temperature was not significant after 2 weeks of incubation.

When soils were incubated at 20°C, rice straw treatment caused significant increase and compost treatment caused slight increase in p-hydroxybenzoic acid after 6 weeks. In the control and green manure treated soil, p-hydroxybenzoic acid decreased with time at 20°C.

On the other hand, when soils were incubated at 35°C, p-hydroxybenzoic acid decreased with time in all treatments. The decrease was largest in the control and least in the rice straw treated soils. Therefore, the concentration of p-hydroxybenzoic acid was highest in the rice straw treated soils after 6 weeks at 20°C and 35°C.

In this experiment, the level of added organic matter (0.25%) was much lower than that of native organic matter in soils (Pila 4.3%, Maahas 2.5%, and Luisiana 2.7%). Most part of p-hydroxybenzoic acid probably came from indigenous soil organic matter at least in the beginning of incubation. Added organic matter seemed to stimulate the degradation of p-hydroxybenzoic acid except rice straw at 20°C.

Kinetics of p-coumaric, ferulic, vanillic and sinapic acid

Kinetics of p-coumaric acid, ferulic acid, vanillic acid, and sinapic acid were similar to one another (Tables 6, 7 and 8). Compared with the control, addition of rice straw to soils slightly increased the concentration of these acids after 2 weeks and markedly after 6 weeks of incubation at 20°C and 35°C. In the early period of incubation most part of these phenolic acids probably came from soil organic matter and significant amount of phenolic acids were released from added rice straw after 6 weeks. Formed amounts of phenolic acids were higher at 35°C than at 20°C in Pila clay loam and Maahas clay, while they were slightly higher at 20°C in Luisiana clay. Higher temperature may enhance the formation of these phenolic acids from rice straw and these phenolic acids are probably not so easily decomposable as p-hydroxybenzoic acid at 35°C. Addition of compost also slightly increased the concentration of these phenolic acids after 2 weeks of incubation. However, significant difference was not observed in the levels of these phenolic acids between the control and compost treated soils after 6 weeks.

Addition of green manure showed a trend to depress phenolic acid formation. Concentrations of these phenolic acids were lowest in soils added with green manure after 6 weeks of incubation. Altnough lignin content in green manure was almost same as that in rice straw, phenolic acids which came from green manure itself were very low. Decomposition of phenolic acids may be enhanced by the co-existence of green manure as a nitrogen source, or the formation of phenolic acids may be repressed under extremely reduced soi condition caused by green manure.

In Luisiana clay, sinapic acid was the most abundant phenolic acid (Table 3). In other soils it was less than the concentration of p-hydroxybenzoic acid or vanillic acid. The concentration of sinapic acid in soils increased remarkably after 6 weeks when rice straw was added to soils.

However, the increase in sinapic acid concentration due to rice straw addition was also largest in Luisiana clay. Abundance of sinapic acid may be due to a specific nature of the humus of Luisiana clay. The concentrations of vanillic acid and the unknown product whose peak appeared after sinapic acid in the gas-chromatogram were also high in Luisiana clay.

Total phenolic acids

Sum of concentrations of p-hydroxybenzoic, vanillic, p-coumaric, ferulic, sinapic acids and the unknown compound was calculated in each table. This is regarded as total phenolic acids in this study. Unknown compound which was calibrated by the standard curve for sinapic acid was included in the sum because its behavior was similar to sinapic acid and likely to belong phenolic acids. In spite of slight differences in the composition of phenolic acids, total phenolic acids content in the 3 soils after 2 weeks of incubation were between 8 - 14 ppm regardless of treatments. After 6 weeks, remarkable increase of total phenolic acids was observed only in rice straw treated soils compared with that after 2 weeks. In other treatments, difference between the total phenolic acid contents after 2 and 6 weeks was insignificant in all the soils. These results suggests that most part of phenolic acids came from indigenous soil organic matter and contribution of added organic materials at 0.25% level was little except rice straw after 6 weeks of incubation.

Kinetics of water soluble organic reducing substance was studied in the previous paper (Chapter 3). Organic reducing substance had a peak after 2 weeks of incubation at 35°C and after 4-6 weeks at 20°C.

It is unlikely that free phenolic acids contribute to the peaks of organic reducing substance, because the peak concentrations of organic reducing substance (20-30 ppm) were higher than that of total phenolic acids (about 10 ppm) and the solubility of phenolic acids in neutral and acidic solutions is very low. In addition, total phenolic acids concentration increased only by rice straw treatment although organic reducing substance increased by green manure and compost as well as by rice straw.

In this study, it was found that rice straw incorporation at 0.25% level (5 t/ha) increased the phenolic acid concentration 2-3 times as high as original level (about 10 ppm) after 6 weeks of incubation. Toxicity of phenolic acids at this level is unlikely because of their low solubility. Even if water soluble organic reducing substance cause any injury to plants, it may not be due to free phenolic acids but to their combined forms or other compounds. The discussions which consider phenolic acids as plant growth inhibitors are usually based upon the concentration of phenolic acids determined by alkaline extraction, but the amounts of water soluble phenolic acids are very low. The amounts of water soluble phenolic acids in the soil solution of a permanent pasture soil at pH 5.8 ranged from $< 10 \times 10^{-9}$ M for ferulic acid to 1.4×10^{-6} M for p-hydroxybenzoic acid, while amounts up to 2000 times greater than these were extracted by 2 N NaOH (Whitehead, 1981). Kaminsky and Müller (1978) recommends against the use of alkaline soil extraction in the study of allelopathy. However, because the effect of localized high concentrations of phenolic acids close to fragments of decomposing plant material is unknown, the possibility of injury by phenolic acids can not be neglected completely.

LITERATURE CITED

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Table 6. Effects of added organic materials and temperatures on the concentration of phenolic acids in submerged Pila clay loam.

Treatment		p-Hydroxy-benzoic	Vanillic	p-Coumaric	Ferulic	Sinapic	Unknown	Total
		mg/kg soil						
At 20°C								
control	2 wks	1.25	0.70	2.15	4.36	0.20	1.38	10.0
	6 wks	0.23	1.03	3.86	8.02	0.15	1.01	14.3
+ rice straw	2 wks	0.79	0.63	2.82	4.76	0.57	2.00	11.6
	6 wks	1.12	1.20	6.19	8.67	2.19	5.00	24.4
+ green manure	2 wks	0.58	0.61	2.53	3.76	0.30	1.18	9.0
	6 wks	0.54	0.59	2.72	4.43	0.43	1.08	9.8
+ compost	2 wks	0.49	0.70	2.63	4.65	0.68	1.66	10.8
	6 wks	0.57	0.74	2.96	3.99	0.64	1.29	10.2
At 35°C								
control	2 wks	1.18	0.68	2.58	4.25	0.45	1.23	10.4
	6 wks	0.23	0.53	2.20	3.34	0.15	0.48	6.9
+ rice straw	2 wks	0.94	0.57	2.31	3.45	0.48	1.42	9.2
	6 wks	0.64	1.59	6.35	8.56	2.56	5.54	25.2
+ green manure	2 wks	0.65	0.67	2.38	3.72	0.55	1.46	9.4
	6 wks	0.42	0.74	3.03	4.70	0.50	1.57	11.0
+ compost	2 wks	0.41	0.52	2.09	3.40	0.55	0.97	7.9
	6 wks	0.25	0.85	3.32	4.49	0.69	1.54	11.1

Table 7. Effects of added organic materials and temperatures on the concentration of phenolic acids in submerged Maahas clay.

Treatment		p-Hydroxy-benzoic	Vanillic	p-Coumaric	Ferulic	Sinapic	Unknown	Total
		------------ mg/kg soil ------------						
At 20°C								
control	2 wks	1.20	0.71	2.00	4.46	0.25	0.52	9.14
	6 wks	0.34	0.98	3.88	8.76	0.53	0.84	15.3
rice straw	2 wks	0.78	0.46	2.47	5.61	0.31	0.41	10.0
	6 wks	1.88	1.19	6.63	7.99	1.41	3.86	23.0
green manure	2 wks	0.66	0.72	2.60	5.34	0.39	0.84	10.6
	6 wks	0.37	0.54	2.04	4.74	0.26	0.44	8.39
compost	2 wks	0.37	0.63	2.29	4.67	0.57	0.79	9.32
	6 wks	0.45	0.68	2.48	4.23	0.46	0.67	8.97
At 35°C control	2 wks	1.29	0.43	2.08	4.27	0.19	0.47	8.73
	6 wks	0.16	0.50	2.95	5.97	0.09	0.14	9.81
rice straw	2 wks	0.81	0.43	2.10	4.59	0.32	0.64	8.89
	6 wks	0.45	1.90	8.13	14.4	3.22	5.79	33.9
green manure	2 wks	0.60	0.61	2.50	5.08	0.46	0.67	9.92
	6 wks	0.17	0.48	1.81	3.92	0.26	0.49	7.13
compost	2 wks	0.38	0.53	2.01	4.57	0.73	0.76	8.98
	6 wks	0.20	0.70	2.75	5.13	0.53	0.80	10.1

Table 8. Effects of added organic materials and temperatures on the concentration of phenolic acids in submerged Luisiana clay.

Treatment		p-Hydroxy-benzoic	Vanillic	p-Coumaric	Ferulic	Sinapic	Unknown	Total
		mg/kg soil						
At 20°C								
control	2 wks	0.77	1.22	1.24	2.28	2.74	2.88	11.1
	6 wks	0.18	1.47	2.02	3.54	3.45	3.70	14.4
rice straw	2 wks	0.66	0.91	1.84	4.61	2.95	3.43	14.4
	6 wks	0.93	2.59	4.63	9.79	9.36	8.67	36.0
green manure	2 wks	0.46	0.96	1.78	2.88	3.27	3.42	12.8
	6 wks	0.33	1.16	1.55	2.74	2.68	2.84	11.3
compost	2 wks	0.21	1.21	2.01	2.13	2.86	3.31	11.7
	6 wks	0.19	1.24	1.69	2.49	3.22	3.21	12.0
At 35°C								
control	2 wks	0.73	0.92	1.12	1.93	2.50	2.62	9.82
	6 wks	0.13	0.88	1.15	1.80	2.09	2.19	8.24
rice straw	2 wks	0.72	0.75	1.61	3.87	2.72	2.90	12.6
	6 wks	0.16	2.19	3.73	6.70	7.84	6.93	27.6
green manure	2 wks	0.34	1.10	1.68	2.54	3.30	3.42	12.4
	6 wks	0.12	1.05	1.40	2.49	2.73	3.03	10.8
compost	2 wks	0.20	1.07	1.88	1.99	2.97	3.24	11.4
	6 wks	0.11	1.40	1.79	2.74	4.03	4.07	14.1

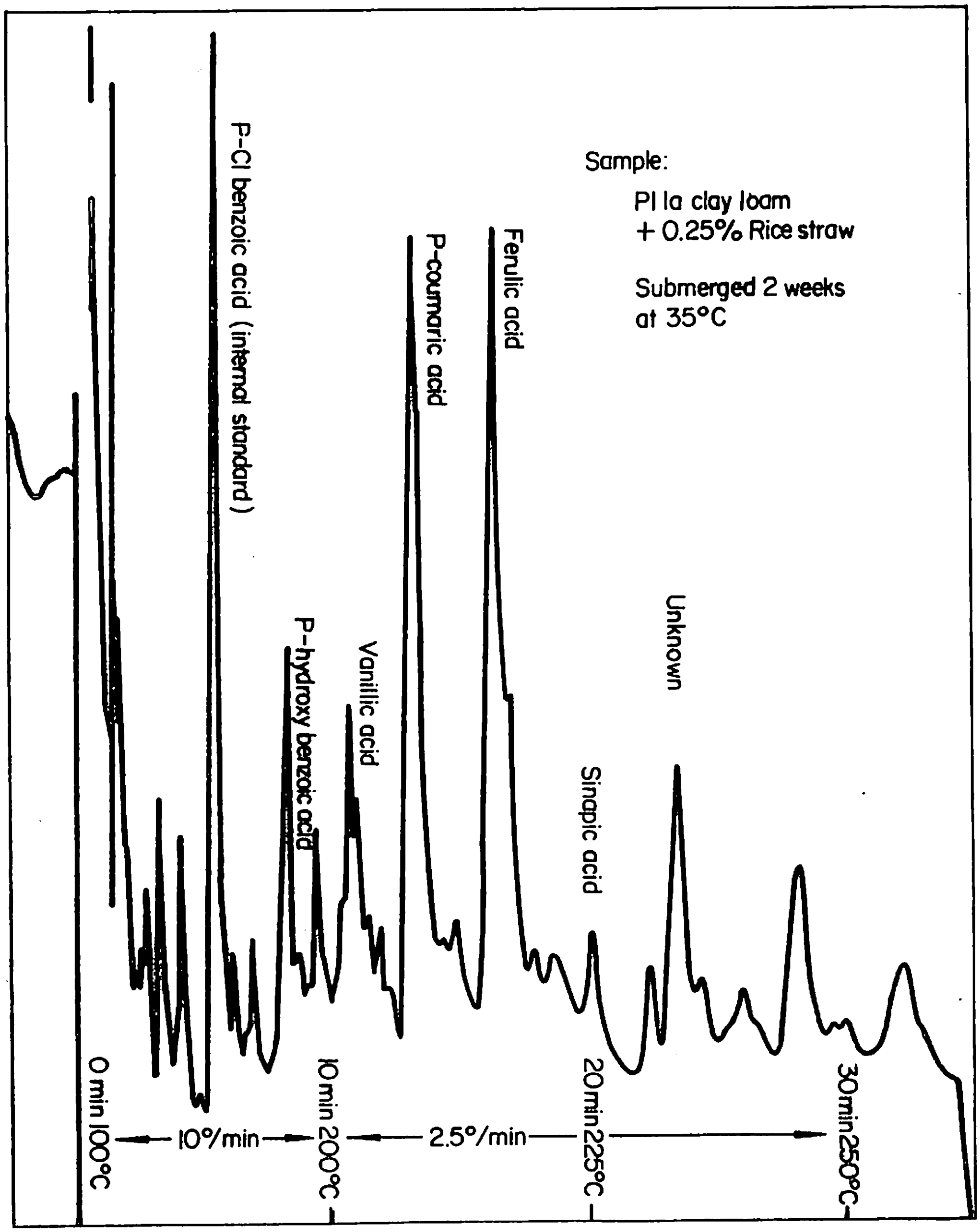

Fig. 16. Gas-chromatogram of phenolic acid analysis (cf. text for conditions)