BEHAVIOR OF PHENOLIC SUBSTANCES IN THE DECAYING PROCESS OF PLANT MATERIALS

Shozo KUWATSUKA, Haruo SHINDO and Kiyoshi TSUTSUKI

Faculty of Agriculture, Nagoya University,
Nagoya, Japan

A REPRINT FROM

PROCEEDINGS OF THE INTERNATIONAL SEMINAR ON
SOIL ENVIRONMENT AND FERTILITY MANAGEMENT IN INTENSIVE AGRICULTURE

TOKYO-JAPAN, 1977

BEHAVIOR OF PHENOLIC SUBSTANCES IN THE DECAYING PROCESS OF PLANT MATERIALS

Shozo KUWATSUKA, Haruo SHINDO and Kiyoshi TSUTSUKI

Faculty of Agriculture, Nagoya University, Nagoya, Japan

ABSTRACT

Phenolic substances in soil play important roles in humus formation, and some of them also influence the growth of plants. Amounts of total phenolic substances and individual phenolic acids in rice straw, ladino clover, fallen leaves of red oak, and bark and wood of several species of tree were determined. The changes in quality and amount of phenolic substances in the decaying process of these plant materials were also compared under various conditions in the laborator. Amounts of phenolic substances decreased more rapidly at higher temperature and under moist, but not flooded, conditions. The change in the level of phenolics was mainly due to the level in fulvic acid fraction, but not in humic acid fraction. In the case of barks amounts of water soluble phenolics decreased rapidly, compared with alkali-methanol extractable phenolics. Individual phenolic acids in fresh material were degraded rapidly in the early stage of the decaying process, but these acids were produced from lignin-like materials in the decaying process. The degradation pathways of these phenolic acids were proposed. The origin and fate of phenolics in the decaying process of plant materials are also discussed.

Phenolic substances are widely distributed in various plant materials, including leaf, straw and root of rice and wheat, vegetables, and bark and wood, and their decayed residues. Some of the phenolic compounds of plant origin such as p-coumaric acid, p-hydroxybenzoic acid, p-hydroxybenzaldehyde, juglone, and some isoflavones, influence the growth of plants. Also, phenolic substances in soil derived from plant residues play important roles in humus formation. Molecules of humic acids have fair amounts of phenolic moieties in their chemical structure.

Recently, rice straw has been plowed in large amounts into paddy and also upland fields, especially for green-house croppings, as a source of organic material mainly for improving soil conditions. Compost of bark and sawdust are also applied to the fields and green-houses. Phenolic substances in these materials may influence the growth of crops. The elucidation of the mechanism of formation and degradation of humus from and to phenolic compounds is also of importance for understanding their influence in plant growth.

From these points of view, fate and behavior of phenolic substances, especially phenolic acids, in the decaying process of plant materials such as rice straw, and bark and saw dust, as well as in soil were studied.

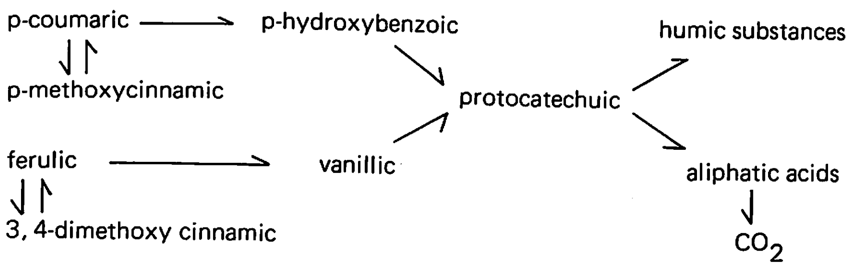
DEGRADATION OF PHENOLIC SUBSTANCES

In a series of studies in laboratory conditions (1-7), qualitative and quantitative changes of the total phenolic substances and individual phenolic acids in the decaying process of rice straw, ladino clover, and fallen leaves of red oak, under wet and flooded conditions were studied.

Ether-extractable low-molecular-weight phenolic compounds were detected in rice straw and its decayed products, which included p-coumaric, ferulic, p-hydroxbenzoic and vanillic acids, and trace amounts of salicylic, syringic, protocatechuic, β-resorcylic, caffeic, sinapic, gallic and gentisic acids. p-Coumatic and ferulic acids were predominant (1).

From the results of incubating rice straw at different temperatures under wet and flooded conditions, it is assumed that phenolic compounds which initially existed in rice straw are rapidly degraded in the early stage of incubation and subsequently produced again from high molecular compounds such as lignin. In this process, the pathways from p-coumaric acid to p-hydroxybenzoic acid and from ferulic acid to vanillic acid were indicated by the changes in the amounts of these compounds (2).

Microbial degradation of individual phenolic acids in culture media revealed the following pathways (3):



p-Coumaric acid and ferulic acid were reversibly methylated to a large extent temporarily, to alleviate poisonous effects of these acids, and gradually degraded to p-hydroxybenzoic acid and vanillic acid by soil microorganisms. These latter acids were further degraded rapidly via protocatechuic acid to humic substances by polymerization or to aliphatic acids by ring cleavage.

The total amount of phenolic substances was much higher in red oak fallen leaves than in rice straw and ladino clover. The changes in total amounts of phenolic substances in the decaying process of these plants were mainly due to the changes in the levels of phenolics in fulvic acid fraction, and especially due to the levels of lipophilic phenolics in the case of rice straw. Rice straw contained extremely large amounts of p-coumaric and ferulic acids compared to the other plant materials, but the amounts decreased rapidly to similar levels as those in the other materials. The changes in amounts of phenolics were largely influenced by temperature, moisture, pH, and redox condition, but not greatly by C/N ratio and the presence or absence of soil.

The qualitative and quantitative changes of phenolic substances in the decaying process of rice straw in soil were also studied (Fig. 1). One eighth weight of rice straw was added into a paddy soil sample and incubated at 30° C under moist and flooded soil conditions. Total phenolic substances and individual phenolic acids decreased more rapidly in the decaying process under moist conditions than under flooded contitions. The phenolic contents were not attributed to the soil itself, but mainly to the straw added to it. The decrease in the total level of phenolics in the earlier stage of the decaying process was mainly due to the decrease in fulvic acid fraction, especially in ether-extractable phenolics, and in the later stage, was mainly due to the decrease in humic acid fraction, especially in butanol-extractable fraction. The degradation patterns of individual phenolic

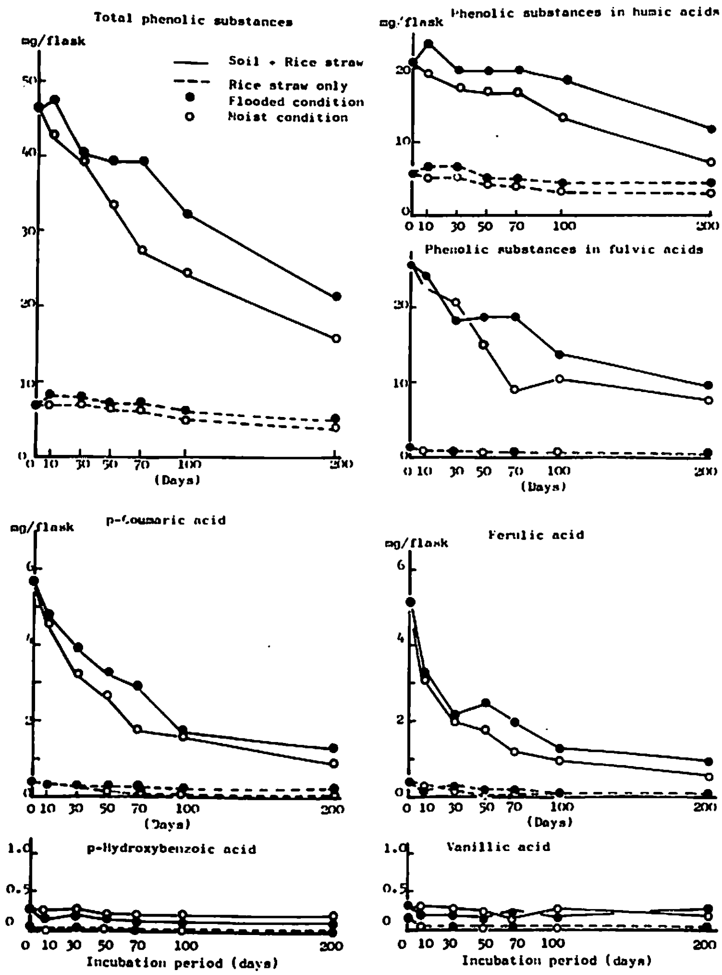


Fig. 1. Changes in amounts of phenolic substances in the decaying process of rice straw in soil. Each flask contains 25 g of soil mixed with or without 2 g of crushed rice straw, and incubated at 30° C for designated periods.

acids in the decaying process of rice straw added to soil were the same as those of straw which decayed without soil. Phenolic substances in the soil were mostly contained in the humic acid fractions, and decreased very slightly.

The phenolic substances, especially phenolic acids, remained at comparatively high levels even after 200 days of incubation at 30° C in soil added with rice straw compared to soil without rice straw. The paddy soil contained small amounts of these phenolic acids even though the soil was collected from a paddy field in which a large amount of rice straw compost had been applied in the previous year before the collection. This probably means that, in paddy fields, phenolic acids are not only degraded, but also are leached out to some extent by percolating wate. A large part of monophenolic acids in rice straw or its decayed products was also found to be only slightly adsorbed by soil particles and easily eluted with water (4).

DISTRIBUTION OF PHENOLIC ACIDS IN SOILS

Phenolic compounds in 6 paddy soils and 10 brown forest soils in Japan were determined by gas chromatography. Seven phenolic acids, p-coumatric, ferulic, vanillic, p-hydrobenzoic, syringic, protocatechuic, and salycylic acids, were found in all soils examined. In most soils, p-coumaric acid was the largest in amount among these acids. Gallic acid, caffeic acid, sinapic acid, catechol, phloroglucinol, p-hydroxybenzaldehyde, and vanillin were not detected.

The concentration of total and individual phenolic acids in paddy soils and the forest soils are shown in Table 1. The total concentration in the paddy soils ranged from 10 to 26 ppm (average 21 ppm). The levels of phenolic compounds in paddy soils were not affected by the surface application of rice straw in winter before rice planting. The levels of total and individual phenolic acids in paddy soils were very much lower than those in H horizones of forest soils, in which the total concentration ranged from 34 to 307 ppm (average 210 ppm). The amounts of these acids per carbon content of paddy soils were also less than half the quantities found in forest soils. From the results of inhibition experiments for rice seedling by these phenolic acids, it is presumed that phenolic acids, at those levels in paddy soils, do not influence the growth of either the root or the whole rice plant. As phenolic acids (except protocatechuic acid) were adsorbed only slightly by soil particles (4), the low levels of concentration of phenolics in paddy fields may be caused by the washing of soil by irrigation water.

On the other hand, as shown in Table 1, the levels of phenolic acids in forest soils were much higher than in paddy soils. The amounts, per carbon content, were also higher than those in paddy soils, although phenolic acids were degraded more easily under aerobic conditions than anaerobic conditions such as in the case of flooded paddy fields described above. In forest soils surveyed, the amounts of phenolic acids decreased consistently with the depth of horizon, but the ratio of phenolic acid to carbon content of soil showed an opposite relation.

PHENOLIC SUBSTANCES IN WOOD AND BARK

Recently, composts made from waste wood and bark are often applied to farm lands. Sometimes these composts happen to be harmful to the growth of crops. One of the possible causes is the effect of phenolic substances contained in these composts. Accordingly, the levels of phenolic substances were determined with water extracts from fresh wood and bark of various trees, several composts made from waste wood, and waste bark heaped up from0-5 years. The effect of water extracts of those materials on the growth of crops were also tested.

These organic materials were crushed and extracted with water and with alkaline methanol (7 volumes of methnol was mixed with 3 volumes of 0.1N NaOH). The levels of phenolic substances in the extracts were determined colorimetrically with Folin-Ciocalteu's reagent using Phenol as a standard. Because this procedure may result different values according to the kinds of phenols, the obtained values are approximate. A hundred m.e./kg corresponds to about 1 % of Phenol.

Fifteen wood samples consisting of 12 species of trees and 14 bark samples of 14 trees were tested (Table 2). In general, larger amounts of phenolic substances were extracted from bark than from wood, and with alkaline methanol than with water as extractants. Some wood and bark of the Fagaceae family contained fairly high levels of phenolic substances. For some of the wood and bark of this species, the water extract contained more phenolic substances than alkaline methanol extract. This result is thought to be accounted.for by the oxidative polymerization of tannins in the alkaline medium.

Table 1. Amounts of phenolic acids in paddy and forest soils

(ppm)

Phenolic acid	Paddy soils (9 samples)			Forest soils* (11 samples)		
	min.	max.	ave.	min.	max.	ave.
p-coumaric	3.1	6.9	5.5	12.0	188	69.2
ferulic	0.5	5.0	3.7	7.2	110	32.1
vanillic	3.0	3.7	3.4	1.4	45.3	24.2
p-hydroxybenzoic	3.0	3.6	3.3	4.0	161	36.4
syringic	<0.5	3.5	2.4	1.4	14.0	17.0
salicyric	< 0.5	2.1	1.5	7.8	93.9	24.4
protocatechuric	< 0.5	1.9	1.5	<0.5	13.8	9.0
Total	10.4	25.8	21.3	34.1	632	210

* 8 samples from A layer, 2 from H-A layer, and 1 from A-B layer.

Table 2. Levels of phenolic substances * in Woods, Barks, and thier composts

Sample	Number of samples	Phenolic levels (m.e./ka) in						B/A (%)
		Alkaline MeOH extracts			Water extracts			
		min.	max.	ave.(A)	min.	max.	ave.(B)	
Woods	15	16.0	131	55.6	8.7	97.5	33.4	60.1
Barks	14	38.0	355	202	10.3	558	144	71.5
Composts	9	13.4	169	41.4	1.5	15.1	4.8	16.4

* Determined colorimetrically using Phenol as a standard.
100 m.e./kg corresponds to about 1 % of phenols.

Table 3. Levels of phenolic substances in heaped barks

(dry and ash free basis)

Period of heaping (years)	Phenolic levels (m.e./ka) in		B/A (%)
	Alkaline MeOH extracts (A)	Water extracts (B)	
0	754	310	41.1
2	625	212	32.5
3	351	54	15.3
5	284	29	10.2

See footnote of Table 2.

Quantities of phenolic substances extracted from waste wood composts sold in a market were fairly low compared with the values for fresh wood and bark. Particularly, the amount of water-extractable substances was significantly low.

The levels of phenolic substances extracted from the bark of tropical wood heaped up outdoors for 0-5 years were consistently high. But relatively speaking they tended to decrease with the period of heaping (Table 3). Especially the quantity of water-extractable phenolic substances decreased dramatically to one tenth of the initial level after 5 years.

The water extract solutions of these heaped barks (sample to extractant ratio (w/v) was 1:6 and 1:100) were tested for the effects on the growth of seedlings of Chinese cabbage (*Brassica campestris* L.) and cucumber (*Cucumis sativus* L.). Water extracts from the samples with 0-2 years of heaping inhibited the growth of both crops, and especially the growth of roots. These solutions were estimated to contain 200-300 ppm (1:100 extracts) or 3000-4000 ppm (1:6) of phenolic substances, and such levels are high enough to cause inhibition of growth, because many phenolic compounds are known to show inhibition at a concentration of more than 100-1000 ppm.

Though many explanation may be given to account for the inhibition caused by the organic matter applied practically to farm lands, the levels of phenolic substances contained in fresh bark and wood are high enough to be harmful to crops, especially when applied in large amounts. However, the water soluble phenolic substances, which affect the plant growth, decrease rapidly to low levels with the decaying process of the original materials. Accordingly, the waste wood composts should undergo sufficient decay before application to soils for crop cultivation.

REFERENCES

1) KUWATSUKA, S. and SHINDO, H., Behavior of phenolic substances in the decaying process of plants (I). Identification and quantitative determination of phenolic acids in rice straw and its decayed product by gas chromatography. Soil Sci. Plant Nutr., Vol. 19, 219-227 (1973).
2) SHINDO, H. and KUWATSUKA, S., ibid (II). Changes of phenolic substances in the decaying process of rice straw under various conditions. ibid, Vol. 21, 215-225 (1975).
3) SHINDO, H. and KUWATSUKA, S., ibid (III). Degradation pathway of phenolic acids. ibid, Vol. 21, 227-238 (1975).
4) SHINDO, H. and KUWATSUKA, S., ibid (IV). Adsorption and movement of phenolic acids in soils. ibid, Vol. 22, 23-33 (1976).
5) SHINDO, H. and KUWATSUKA, S., ibid (V). Elution of heavy metals with phenolic acids from soil. ibid, in press.
6) SHINDO, H. and KUWATSUKA, S., ibid (VI). Changes in quality and quantity of phenolic substances in the decaying process of rice straw in a soil. ibid, in press.
7) SHINDO, H. and KUWATSUKA, S. ibid (VII). Characteristics of phenolic substances in the humic acids of decayed rice straw and compost supplied field soil. ibid, in press.

ERRATA

Page	Line	For	Read
733	Fig. 1 legend	Rice straw only	Soil alone
734	3	p-coumatric	p-coumaric
734	11	in H horizones of	in
734	12	307 ppm	632 ppm
734	14 fb	from 0-5 years	for 0-5 years
734	3 fb	species	family
735	Table 1 (column 1)	salicyric	salicylic
735	Table 1 (column 1)	protocatechuric	protocatechuic
735	Table 2 (line 1)	m.e./ka	m.e./kg
735	Table 3 (line 1)	m.e./ka	m.e./kg