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CHANGES IN PHYTOTOXIC COMPONENTS OF SAWDUST BARNYARD MANURE DURING ITS ROTTING PROCESS

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Summary

Changes in known phytotoxic compounds of sawdust barnyard manure during its rotting process were examined to evaluate maturation of the manure. The obtained results were as follows :

1) Phenolic substances known as phytotoxic compounds, such as p-coumaric, ferulic and syringic acids, and coniferaldehyde were most abundant at three months after the initiation of composting, and afterwards decreased. p-Coumaric acid concentratration at nine months decreasd to 1/3 of that at the initial stage, but ferulic acid concentration decreased not to the level at the initial stage.
2) Major long-chain fatty acids, such as octadecenoic and palmitic acids, showed the highest concentration at six months, and then these contents decreased, but the level at nine months was higher than that at the initial stage.
3) Long-chain fatty acids with an odd number of carbons, which were contained considerably at the initial stage, decreased at three months, but afterwards accumulated gradually with progress of the rotting.
4) 3-Hydroxydecanoic acid (Myrmicacin) with phyto- and fungi toxicities was contained moderately during three months, but its concentration at nine months decreased to 1/10 of the initial concentration.

The results would give a significant clue to evaluate the manure maturation, because the manure should be applied to land after the sufficient decrease of phytotoxic substances.

Introduction

The re-utilization of various organic wastes, such as wood industrial and municipal wastes, as organic fertilyzers is necessary throughout the world, not only for treating their organic wastes increasing every years but for improving inexpensively soil productivity.

The woody wastes, sawdust or bark, have been frequently used for deodorization and excessive moisture-regulation of livestock excrement as spreading materials in a loose-barn type of cowshed. After rotting the mixture of sawdust and animal excrement, the mixture, "so-called, sawdust barnyard manure", has been widely applied to the cultivated soil as an organic fertilyzer.

Its application could usually improve the physical and chemical properties of the soil,[2] whereas the application of sawdust barnyard manure with high contents of resistant organic matters such as resin, wax and lignin came to give higher accumulation of humus in the soil,[7] and frequently to inhibit plant growth because of higher concentrations of phytotoxic substances from the sawdust or microbial metabolites.[4),24] Recently, Yoshida[34] reported that the application of unsatisfyingly rotted manure gave intensively crop-growth inhibition caused by phytotoxic substances (phenolics and lipids) from the sawdust.

In the case of composting of straw, physical factors such as earthy odor, dark color, fluffy stru-

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cture, low specific gravity and chemical factor such as the C/N ratio give an obvious indication of compost-maturation. Especially, the decrease of the C/N ratio to 10-20 can be a good indicator of the completion of straw composting.[23] In the sawdust barnyard manure, however, it is difficult to evaluate its maturation by the conventional method usually used for straw composting, because the C/N ratio decreases only to 25-35 even after 9 months and a considerable amount of phytotoxic substances as described above is included.[18] Hitherto, several workers[6,28,31,35] published studies on relationship between changes in major components of the manure and progress in its rotting process. However, as to changes in phytotoxic substances of the manure during its rotting process, no report have been seen.

In this paper, we attempted to estatlish a new method for judging the maturation from changes in phytotoxic substances of the sawdust barnyard manure during its rotting process.

Materials and Methods

Sawdust barnyard manure was prepared by the following procedure in the farm of the Faculty of Agriculture, Kobe University. The manure was the mixture of cow-excrement and sawdust (the mixed sawdust from *Pinus densiflora* and minor other wood), which was used as an auxiliary material for deodorization and moisture -regulation in a loose-barn type of cowshed. The mixture (ratio of cow-excrement and sawdust was approximately 1 : 1) was piled up in woody flame (160×80×80 cm) to ferment. The compost was allowed to stand for one year under mixing once a month. The sample for chemical analyses was air-dried, and then pulverized with a ball mill to pass through a tray with the network base of 0.1 mm. Contents of moisture, ash and total nitrogen were determined by the conventional methods.

Extraction of phytotoxic substances from the sample was carried out by Kuwazuka's phenolics extraction method.[12] As shown in Fig. 1, five grams of the pulverized sample was suspended in 350 ml of mixture of methanol and 0.1 N aqueous sodium hydroxide solution (7 : 3v/v), and allowed to stand for 18 hrs. after stirring for 1 hr. The extract filtered over filter paper was neutralized with a diluted hydrochloric acid solution, and evaporated to 100 ml under reduced pressure. The concentrate was extracted four times with 50 ml of ether, after acidifying to pH 1.0 with hydrochloric acid. The ether extract was further extracted four times with a saturated sodium bicarbonate solution (50ml) to separate strongly acidic fraction from neutral- and weakly acidic fraction. The latter fraction was obtained by evaporating the ether phase dried over angydrous sodium sulfate (Fraction N). The extract with a sodium bicarbonate solution was again acidified to pH 1.0, and extracted three

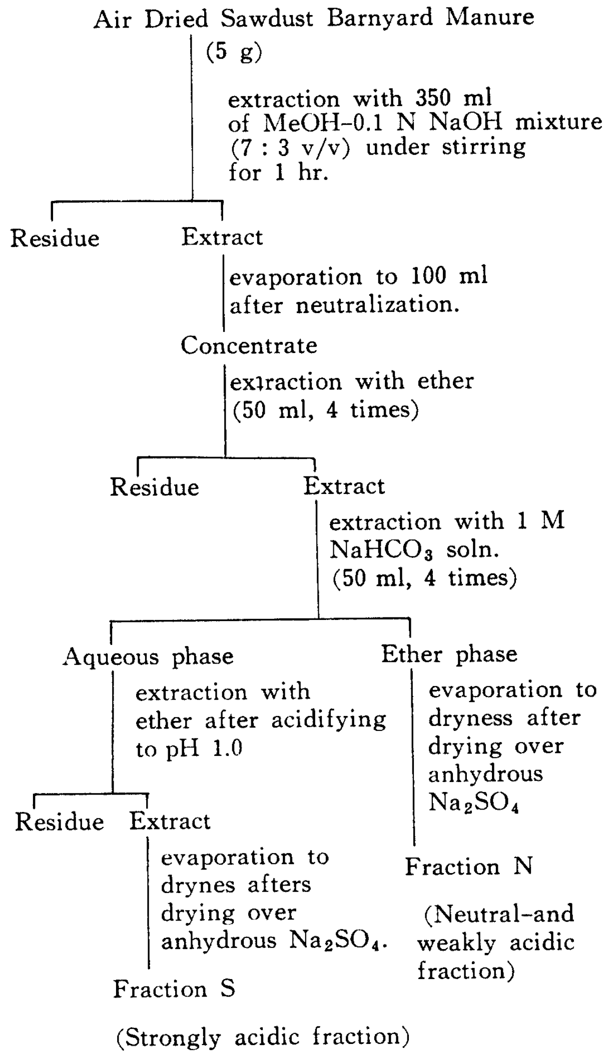

Fig. 1. Scheme of Fractionation of MeOH-0.1 N NaOH Extract from Sawdust Barnyard Manure to Fraction S and Fraction N.

times with ether (100ml). The strongly acidic fraction was obtained by evaporating the ether extract dried over anhydrous sodium sulfate (Fraction S).

Trimethylsilylation of Fraction N and S was carried out with N,O-bis (trimethylsilyl)-acetamide (BSA) as follows ; 10 mg of each fraction was reacted with 0.1 ml of BSA containing 0.2 ml of acetnitril.

Gas liquid chromatography (GC) was made with a 300×0.26 cm glass column packed with 2% silicon OV-1 on 100-200 mesh Shimalite WAW DMS. The oven temperature was programmed from 120° to 290°C at 5°C/min., and the flow rate of nitrogen gas was 50 ml/min. The injector and detector were maintained at 300 °C. The used apparatus was a Shimazu Model 8 A Gas Chromatograph equipped with a flame ionization detector and with a Shimazu Model CR 1 B Chromatopack. p-Coumaric and palmitic acids were determined by the internal standard method, and the other compounds by the area-percent method corrected with molecular weight factors.

Combined gas liquid chromatography-mass spectrometry (GC-MS) analysis was performed with a Hitachi Model RMU-6 MG Gas Chromatograph-Mass Spectrometer using an all glass jet separator as the GC-MS interface. The interface temperature was held at 300°C. The mass spectrometer was operated under the following conditions : ionizing electron energy of 20 eV, ion accelerating voltage of 3.2 kV (m/z max. 1500), and ion source temperature of 200°C.

Results and Discussion

Changes in moisture and temperature of sawdust barnyard manure during its rotting process are shown in Fig. 2. The moisture required for microbial activity was maintained from 67 to 75 % during the rotting process. Increase of temperature in the pile was not observed during three months, and afterwards the temperature slowly increased and came to 59 °C at ten months.

The C/N ratio decreased slowly to 32 during four months, but afterwards the value was almost unvarying.

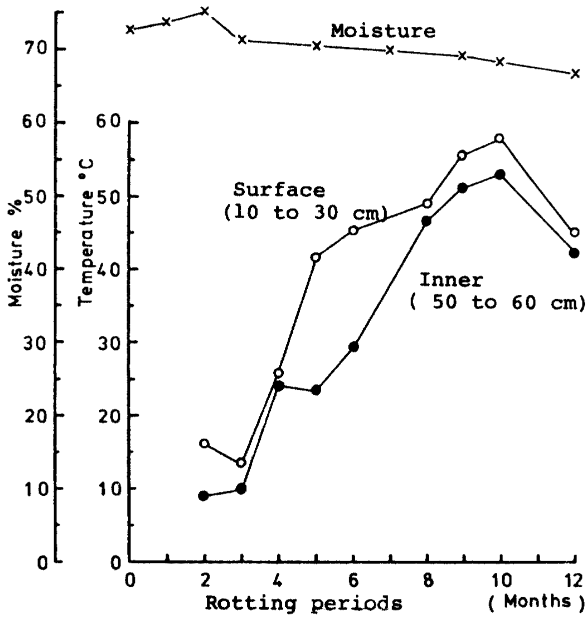

Fig. 2. Changes in Moisture and Temperature of Sawdust Barnyard Manure during Its Rotting Process.

Table 1. Ratio of Organic Matter per Ash of Sawdust Barnyard Manure during Its Rotting Process.

Rotting Period (Months)	Moisture (% of air-dried matter)	Ash (% of dried matter)	Organic matter (% of dried matter)	Organic m./Ash Ratio
initial	10.00	9.31	90.69	9.74
3	8.51	9.79	90.21	9.22
6	8.59	14.25	85.75	6.02
9	12.99	21.88	78.12	3.57
12	15.49	25.02	74.98	3.00

Table 2. Contents of Fraction S and Fraction N from Sawdust Barnyard Manures Rotted for Various Periods.

Rotting Period (Months)	Fraction S		Fraction N	
	mg/g of dried matter	mg/g of org. matter	mg/g of dried matter	mg/g of org. matter
initial	4.04	4.45	9.45	10.42
3	5.09	5.64	8.04	8.91
6	3.72	4.34	8.49	9.90
9	3.20	4.10	6.93	8.87

Changes in the ratio of organic matter to ash of sawdust barnyard manure during its rotting process are shown in Table 1. The ratio decreased from 9.74 to 3.00 during the rotting period over one year. On postulating that the amount of ash is unchangeable throughout the rotting process, decomposition rates of organic matter at 3, 6, 9 and 12 months to that at the initial stage were estimated to be 7%, 40%, 62% and 68%, respectively.

Lipids and phenolic components, which were extractable with the solvent (MeOH-0.1N NaOH, 7 : 3 v/v), were fractionated to ether extractable Fractions S and N. Contents of their fractions are shown in Table 2. The content of Fractions S increased at three months and afterwards decreased slightly. On the contrary, the content of Fraction N decreased slightly at three months and again decreased afterwards.

Gas liquid chromatograms of Fractions S and N as trimethylsilyl derivs. are shown in Figs. 3 and 4. At least, twenty two peaks were detected on the chromatogram of Fraction S, and twenty seven peaks on that of Fraction N.

Identification of lipids and phenolic compounds by GC-MS.

The identification of the phenolic compounds could readily be carried out by GC-MS. Peak Nos. 5, 8, 9, 13, 14, 15 and 18 in Fraction S (Fig. 3), and peak Nos. 4 and 6 in Fraction N (Fig. 4), which were painted with black, were identified as p-hydroxybenzoic, vanillic, cis-p-coumaric, syringic, cis-ferulic, trans-p-coumaric and trans-ferulic acids, and cis- and trans-coniferaldehydes, respectively, by comparison of the obtained GC-MS data.[3),19)]

On the basis of the interpretation of mass fragmentation data shown in Tables 3 and 4, most of major peaks in Fraction N (Fig. 4) could be assigned to homologous members of long-chain fatty acids, and two peaks (peak Nos.6 and 10) in Fraction S (Fig. 3) could be assigned respectively to 3-hydroxydecanoic acid[5)] and azelaic acid.[21)] However, details of the mass spectral characteritics of TMS-derivs. of long-chain fatty acids and the other acids were not available. These compounds were, therefore, confirmed by comparison of mass fragmentation patterns of their peaks with those of authentic C_{12} to C_{20} fatty acids with an even number of carbon atoms, 3-hydroxydecanoic and azelaic acids.

Mass spectral data of peak Nos. 9, 15, 20 and 26 in Fraction N (Table 4) were identical with those of the TMS-derivs. of myristic, palmitic, stearic and arachidic acids, respectively. Their mass fragmentation modes are shown in Fig. 5 ; molecular ion (M^+) with weak intensity, m/z (M-15), 145, 117 and 73 with strong intensities and m/z 132 from McLafferty rearrangement were clearly characterized on their fragmentation patterns. Molecular ions of peak Nos. 3, 11, 17, and 23 in Fraction N were the values, which were respectively 14 less than the molecular weights of myristic, palmitic, stearic and arachidic acids TMS-derivs. These mass fragmentation modes were the same with those shown in Fig. 5. Therfore, these peaks were assigned to TMS-derivs. of tridecanoic, pentadecanoic, heptadecanoic and nonadecanoic acids, respectively. The mass fragmentation modes of peak Nos. 7, 14 and 19 were also similar to those shown in Fig. 5. The molecular weight of peak No 7 was the same with that of myristic acid TMS-deriv., but the retention time of peak No. 7 was shorter that of myristic acid TMS-deriv. and in mass fragmentation pattern of peak No. 7, a characteric

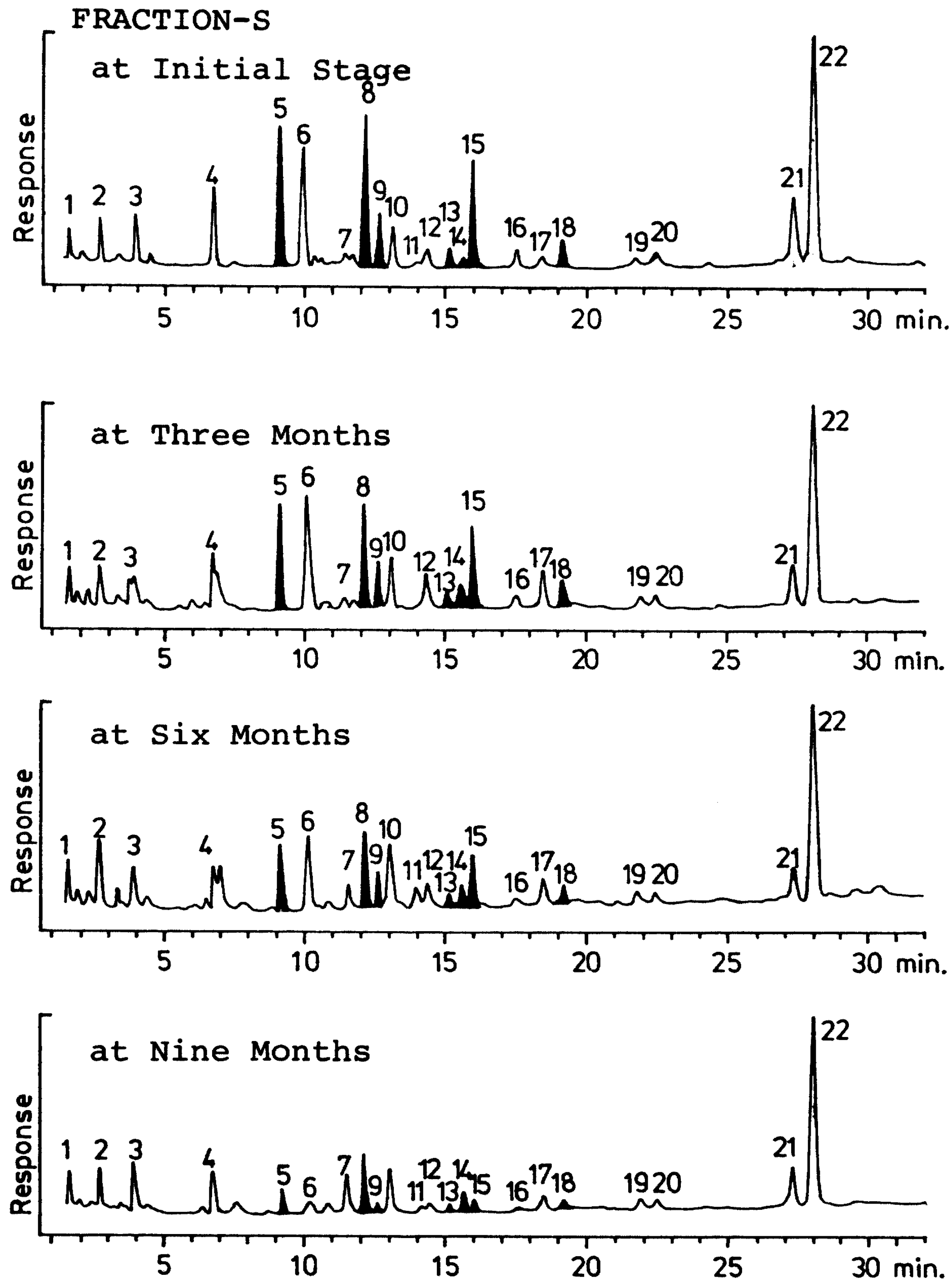

Fig. 3. Gas-Liquid Chromatograms of Fraction S from Sawdust Barnyard Manures Rotted during Various Periods.

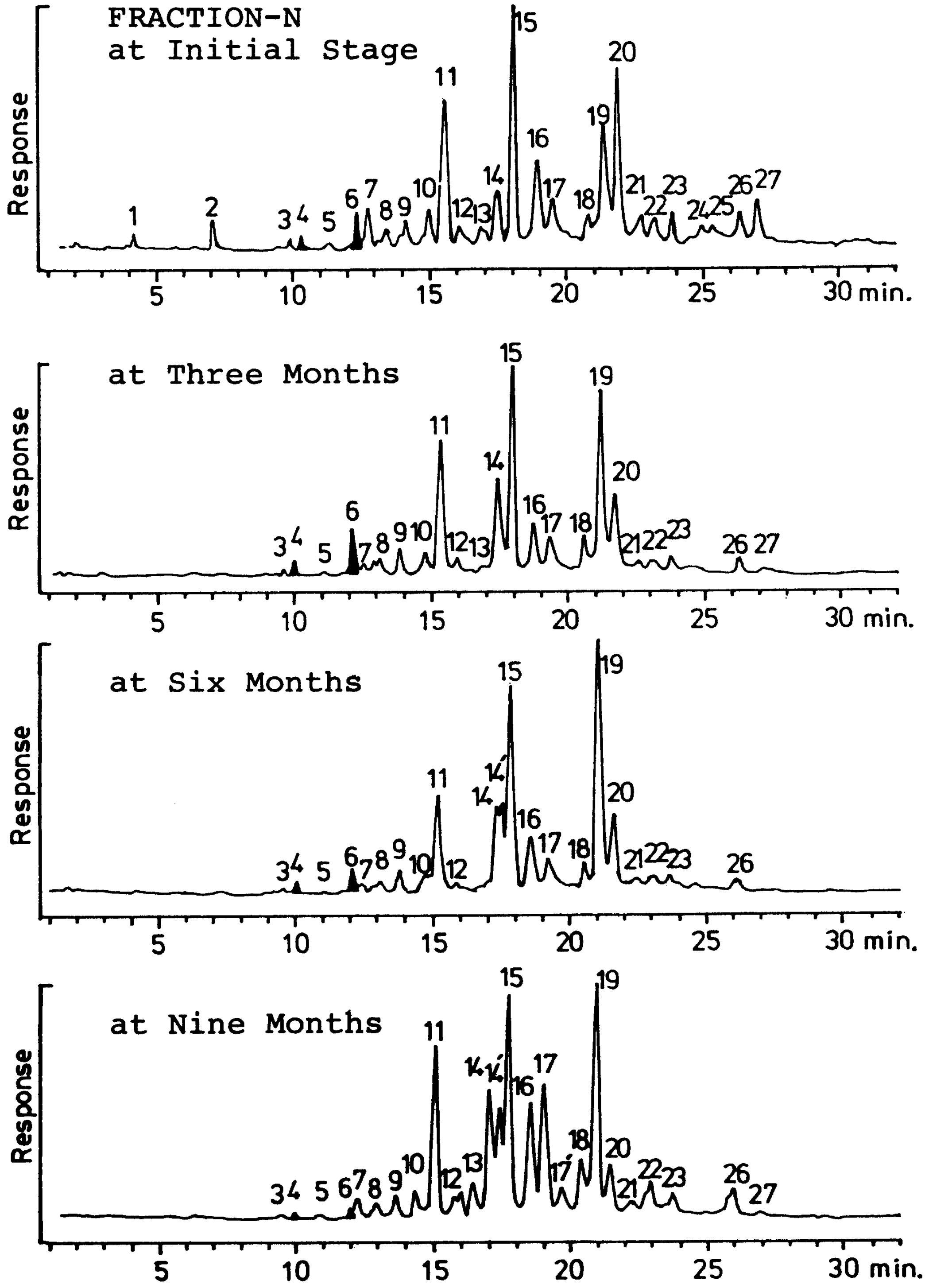

Fig. 4. Gas-Liguid Chromatograms of Fraction N from Sawdust Barnyard Manures Rotted during Various Periods.

Table 3. GC-MS Data of Fraction S from Sawdust Barnyard Manure.

Peak No.	Compound (TMS deriv. of)	Mass Fragment (m/z (Intensity %)
1	Unknown S-1	
2	Unknown S-2	
3	Unknown S-3	
4	Sesquiterpenoic acid	M^+408(24.0), 318(6.0), 307(100.0), 246(5.0), 221(6.5), 190(20.0), 188(8.5), 172(16.2), 147(61.0), 75(16.5), 73(82.8).
5	p-Hydroxybenzoic acid	M^+282(23.2), 267(100.0), 223(25.8), 193(24.0), 73(5.0).
6	3-Hydroxydecanoic acid	M^+332(2.0), 317(8.2), 265(20.0), 259(7.2), 233(59.8), 201(57.0), 192(13.5), 189(30.9), 147(100.0), 129(7.6), 117(27.0), 83(11.0), 73(88.9).
7	Unknown S-4	
8	Vanillic acid	M^+312(78.5), 297(100.0), 282(32.0), 267(41.8), 253(26.2), 223(26.5), 193(6.0), 73(6.5).
9	cis-p-Coumaric acid	M^+308(92.0), 293(100.0), 249(45.0), 219(86.7), 192(8.0), 179(29.8), 147(7.0), 73(13.2).
10	Azelaic acid	M^+332(2.0), 317(100.0), 243(6.5), 217(25.5), 203(35.0), 201(76.2), 152(44.6), 149(42.5), 147(38.2), 129(15.0), 117(38.2), 111(13.0), 97(14.0), 83(17.5), 75(18.0), 73(72.0), 67(8.0), 55(14.0).
11	Unknown S-5	
12	Unknown S-6	
13	Syringic acid	M^+342(91.5), 327(100.0), 312(66.5), 297(35.8), 283(14.2), 259(17.0), 223(8.0), 179(5.0), 73(7.5).
14	cis-Ferulic acid	M^+338(100.0), 323(54.0), 308(48.5), 293(22.4), 279(10.0), 249(34.3), 219(7.5), 179(4.5), 73(7.5).
15	trans-p-Coumaric acid	M^+308(89.5), 293(100.0), 249(38.9), 219(75.2), 192(7.0), 179(18.0), 147(6.0), 73(12.0).
16	Unknown S-7	M^+340(13.0), 325(69.0), 295(31.0), 223(92.5), 147(62.0), 103(37.8), 75(46.2), 73(100.0).
17	Unknown S-8	
18	trans-Ferulic acid	M^+338(100.0), 323(54.0), 308(48.2), 293(23.5), 279(11.5), 249(29.6), 219(8.0), 179(6.0), 147(4.5), 73(6.5).
19	Unknown S-9	M^+454(13.5), 439(15.0), 381(7.0), 339(31.5), 309(56.0), 294(31.4), 278(62.0), 145(62.0), 117(54.0), 75(46.0), 73(100.0).
20	Unknown S-10	M^+500(3.8), 485(3.0), 397(41.5), 383(8.2), 306(28.0), 280(33.0), 223(21.8), 191(44.4), 189(100.0), 145(50.0), 75(32.5), 73(61.0).
21	Unknown S-11	
22	Unknown S-12	

Table 4. GC-MS Data of Fraction N from Sawdust Barnyard Manure.

Peak No.	Compound (TMS deriv. of)	Mass fragments m/z (Intensity %)
1	Unknown N-1	
2	Unknown N-2	
3	Tridecanoic acid	M^+286(1.2), 271(56.8), 181(13.0), 145(26.5), 132(37.0), 131(29.9), 129(21.0), 117(100.0), 87(12.0), 73(82.0).
4	cis-Coniferaldehyde	M^+250(100.0), 235(64.0), 219(26.5), 203(40.2), 192(6.0), 175(6.0), 89(12.0), 73(12.5).
5	Unknown N-3	
6	trans-Coniferaldehyde	M^+250(100.0), 235(63.2), 219(27.0), 203(33.8), 192(6.0), 175(5.5), 89(11.2), 73(16.5).
7	Isomyristic acid	M^+300(1.2), 285(56.0), 257(5.0), 241(3.0), 213(5.0), 201(4.5), 145(26.0), 132(33.0), 131(15.0), 129(12.5), 117(100.0), 87(25.0), 73(62.0).
8	Unknown N-4	
9	Myristic acid	M^+300(1.0), 285(100.0), 201(10.0), 145(37.0), 132(53.0), 131(15.0), 129(26.0), 117(96.2), 87(8.0), 73(69.0).
10	Unknown N-5	
11	Pentadecanoic acid	M^+314(1.0), 299(100.0), 271(6.0), 201(11.0), 145(48.0), 132(58.0), 131(13.0), 129(26.0), 117(96.0), 97(6.0), 83(5.0), 73(64.0).
12	Unknown N-6	
13	Unknown N-7	
14	Hexadecenoic acid	M^+326(1.5), 311(42.2), 267(11.0), 236(6.4), 194(8.0), 185(7.6), 145(38.6), 132(32.2), 131(10.6), 129(28.0), 117(100.0), 96(13.0), 73(62.0).
15	Palmitic acid	M^+328(2.2), 313(96.2), 285(6.0), 233(6.0), 201(10.0), 159(5.8), 145(46.5), 132(63.2), 131(11.0), 129(22.5), 117(100.0), 97(4.0), 83(4.0), 73(60.0).
16	Unknown N-8	
17	Heptadecanoic acid (Margaric acid)	M^+342(1.2), 327(53.2), 299(7.5), 285(6.0), 201(9.8), 189(6.2), 159(5.5), 145(43.6), 132(42.0), 131(20.6), 129(36.0), 117(100.0), 97(27.0), 83(11.6), 73(78.0).
18	Unknown N-9	
19	Octadecenoic acid	M^+354(1.4), 339(76.4), 299(16.0), 264(11.0), 222(16.0), 199(16.8), 185(12.5), 180(8.5), 145(53.0), 132(35.6), 131(14.0), 129(50.0), 117(100.0), 96(26.0), 84(22.0), 81(21.5), 73(54.5).
20	Stearic acid	M^+356(1.2), 341(55.0), 297(5.0), 284(5.5), 201(10.0), 171(7.0), 159(5.0), 145(53.0), 132(36.0), 131(13.5), 129(30.0), 117(100.0), 97(11.6), 83(12.0), 73(65.4).

Table 4.

Peak No.	Compound (TMS deriv. of)	Mass fragments m/z (Intensity %)
21	Unknown N-10	
22	Unknown N-11	
23	Nonadecanoic acid	M^+370(1.1), 355(46.0), 313(10.0), 257(7.7), 201(15.0), 159(8.2), 145(54.2), 132(44.0), 131(20.6), 129(35.0), 117(100.0), 97(17.0), 83(16.0), 73(56.0).
24	Unknown N-12	
25	Unknown N-13	
26	Eicosanoic acid (Arachidic acid)	M^+384(2.0), 369(40.0), 341(6.5), 201(14.5), 159(12.0), 145(48.0), 132(54.5), 131(16.5), 129(26.0), 117(100.0), 97(8.0), 83(12.0), 73(79.0).
27	Unknown N-14	

fragment, m/z (M−43), indicating the presence of $-CH<^{CH_3}_{CH_3}$ group, was detected. This supports that peak No. 7 is isomyristic acid TMS-deriv. Molecular weights of peak Nos. 14 and 19 were the values, which were respectively 2 less than those of palmitic and stearic acids TMS-derivs. These peaks were therefore assigned to TMS-derivs of hexadecenoic and octadecenoic acids, respectively.

Mass spectral data of peak Nos. 6 and 10 in Fraction S were identical with those of 3-hydroxydecanoic and azelaic acids. 3-Hydroxydecanoic acid TMS-deriv. formed some characteritic fragments, m/z 233, 201, (131-1), and 99 from α-cleavage induced by trimethylsilyloxy group on β-carbon, and m/z 147 rearranged from two trimethylsilyloxy groups (A in Fig. 6). Fragmentation of azelaic acid TMS-deriv. showed

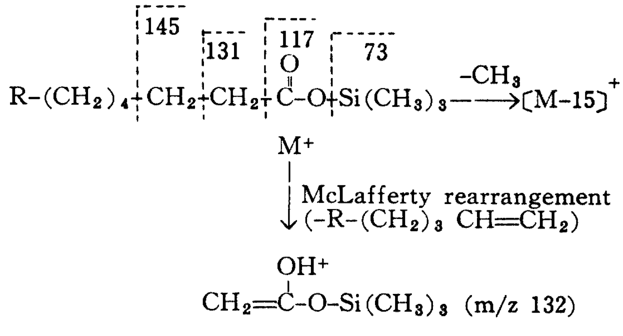

Fig. 5. Mass Fragmentation of Long-chain Fatty Acid Trimethylsilyl Derivatives.

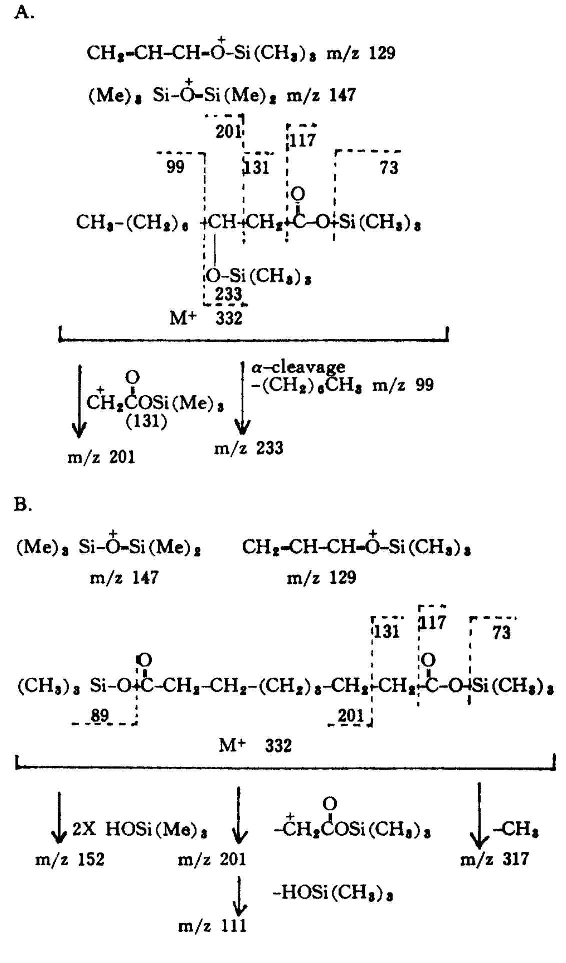

Fig. 6. Mass Fragmentations of 3-Hydroxydecanoic acid and Azelaic acid TMS-Derivatives.

the following characteric fragments ; m/z 201 formed from β-cleavage induced by carbonyl group, m/z 152 [M-2 X HOSi $(Me)_3$] and m/z 111 [201-HOSi $(Me)_3$], in addition to m/z 147, 129, 117 and 73 (B in Fig. 6).

Changes in Phenolic Compounds during the Rotting process.

The identified phenolic compounds were determined by GC method, and these results are shown in Table 5. Changes in the phenolic compounds of the sawdust barnyard manure during its rotting process are shown in Fig. 7. In the case of phenolic compounds including cis- and trans-isomers, their contents were summed up. Contents of p-hydroxybenzoic and vanillic acids decreased with progress of the rotting, and came to about 1/4 and 1/2 of their initial contents. The contents of p-coumaric, ferulic and syringic acids and coniferaldehyde increased, most at three months, and afterwards decreased, but especially, the rates of decrease of ferulic and syringic acids were lower than those of the others. These results indicate the possibilities that the microbial hydrolysis of bound phenolic compounds, such as phenylglycosides and lignohemicellulose, rises at the initial stage without elevation of temperature.

The phytotoxic effects of these phenolic compounds have been examined by workers.[30),32),33)] Recently, Glass[8)] reported that the phenolic acids caused inhibition of ion uptake in roots, and

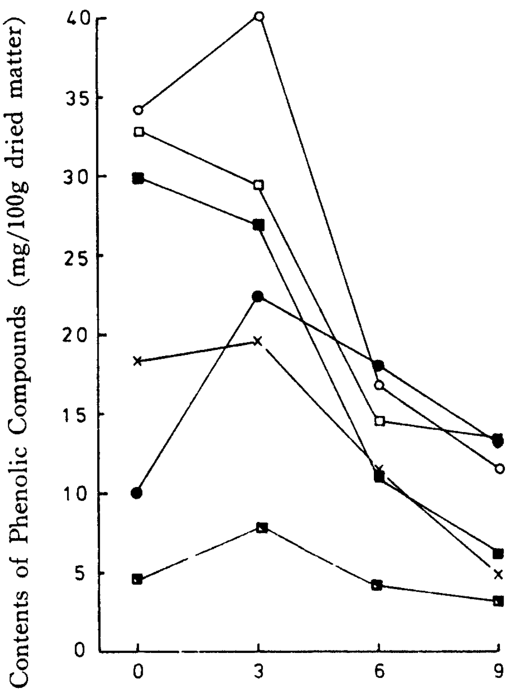

Fig. 7. Changes in Phenolic Components of Sawdust Barnyard Manure during Its Rotting Process.

—○—○— cis-and trans-p-Coumaric acids
—□—□— Vanillic acid
—■—■— p-Hydroxybenzoic acid
—×—×— cis-and trans-Coniferaldehydes
—●—●— cis-and trans-Ferulic acids
—◪—◪— Syringic acid

Table 5. Contents of Phenolic Compounds in Sawdust Barnyard Manures Rotted for Various Periods.

Compound	Content (mg/100 g of Dried Matter)			
	at Initial	at 3 Months	at 6 Months	at 9 Months
p-Hydroxybenzoic acid	29.83	26.65	11.52	6.44
Vanillic acid	32.70	29.53	14.44	13.35
cis-p-Coumaric acid	10.46	13.91	6.48	2.50
trans-p-Coumaric acid	23.50	26.20	12.23	3.93
Syringic acid	4.73	7.76	4.23	3.20
cis-Ferulic acid	2.50	10.51	6.97	6.83
trans-Ferulic acid	7.57	12.00	11.17	6.73
cis-Coniferaldehyde	5.05	5.74	3.91	1.96
trans-Coniferaldehyde	13.23	13.91	7.55	3.01

Stenlid[25] showed that they gave various effects on enzymic oxidation of indole-3-acetic acid. Knosel[11] published that they inhibited growth of fungi in rhizosphere. According to their reports, these phenolic compounds caused an inhibition-effect on plant growth at concentrations of 10^{-5} to 10^{-8} M. This indicates that concentration of total phenolic components in the sawdust barnyard manure is sufficiently high to adversely affect plant- and fungi growths.

Changes in Long-chain Fatty Acids during the Rotting Process.

Contents of the identified fatty acids are shown in Table 6 and changes in their compounds of the sawdust barnyard manure during its rotting process are shown in Fig. 8. Major components of long-chain fatty acids with an even number of carbons exclusive of stearic acid considerably increased with the progress of rotting during 6 months, and afterwards their contents decreased to the slightly higher level than those on the initiation of composting. On the contrary, the long-chain fatty acids with an odd number of carbons slightly decreased at 3 months, and afterwards slowly increased with the progress of the rotting.

In spite of marked decrease of the total organic matters with the progress of the rotting during 6 months, a large increase of octadecenoic and palmitic acids may be caused by increasing numbers of mesophilic microorganisms producing these fatty acids rather than by accumlating more resistant fatty acids or by producing them from sawdust lipids. The decrease of their compounds after 6 months can be associated with the increase of some thermophilic bacteria or fungi having high activity of lipid-decomposition. The slight increase of the fatty acids with an odd number of carbons may be caused by being more resistant than the others. Although it is known that fatty acids with an odd number of carbons are found in significant amounts in the lipids of many plants and some microorganisms,[14] the fatty acids in the sawdust barnyard manure were contained more abundantly than in the used sawdust.

The physiological effects of fatty acids have also been reported by several workers.[10),13),16),]

Table 6. Contents of Long-chain Fatty Acids and Other Some Acids in Sawdust Barnyard Manures Rotted for Various Periods.

Compound	Contents (mg/100g of Dried Matter)			
	at Initial	at 3 Months	at 6 Months	at 9 Months
Tridecanoic acid	2.62	2.61	1.91	1.13
Isomyristic acid	17.06	6.05	6.48	8.95
Myristic acid	15.00	11.57	10.92	7.65
Pentadecanoic acid	61.04	55.70	57.65	66.07
Hexadecenoic acid	34.37	47.53	59.13	32.49
Palmitic acid	60.43	62.04	82.80	65.03
Heptadecanoic acid	35.37	31.79	38.80	60.35
Octadecenoic acid	59.57	90.69	160.54	95.41
Stearic acid	58.74	32.87	32.40	22.93
Nonadecanoic acid	16.57	13.29	18.26	22.60
Sesquiterpenoic acid	17.85	30.47	9.21	10.07
β-Hydroxydecanoic acid (Myrmicacin)	24.96	30.85	11.74	2.87
Azelaic acid	10.46	17.74	12.75	11.58

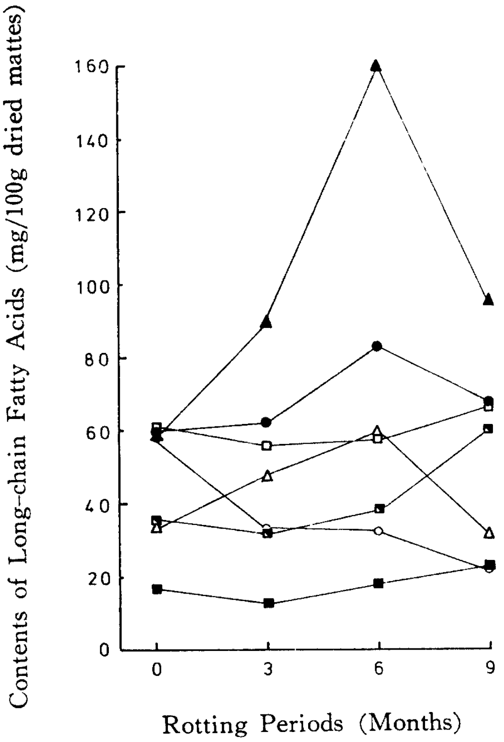

Fig. 8. Changes in Long-chain Fatty Acids of Sawdust Barnyard Manure during Its Rotting Process.
—▲—▲— : Octadecenoic acid
—●—●— : Palmitic acid
—□—□— : Pentadecanoic acid
—△—△— : Hexadecenoic acid
—◪—◪— : Heptadecanoic acid
—○—○— : Stearic acid
—■—■— : Nonadecanoic acid

[22],[29] Takijima[29] examined the effects of a wide range of fatty acids by measuring growth inhibition using a rice seedlings as an index of phytotoxicity. They found that the aliphatic mono-basic acids were more inhibitory than the dibasic and tribasic acids and that the toxicity generally increased with increase of carbon-chain length in the direction, acetic>propionic>hexanoic>octanoic>decanoic acids. Jackson[10] has observed that this progression of increasingtoxicity is accompanied by an increasing affinity of the compounds for lipids, and that exposure of roots to compounds with increasing lipid affinity leads to increasing quantities of ion leakage from roots. A similar response has been observed in the mycellium of ectomycorrhizal fungus.[22] The relationship between lipid affinity and ion leakage naturally leads to the suggestion that the inhibitory effects of the acids with intermediate carbon chain length are due to direct action upon the structure. Evidence for such interactions has been obtained in plant[13] and fungi.[16] In the contrast to octanoic and decanoic acids, which have been known mainly as phytotoxic acids, nonanoic acid is a fungitoxin and it gives a potent inhibition to spore germination.[9] Though it has been reported that elaidic acid (a octadecenoic) adversely affects on bacterial growth,[20] as to the phytotoxic effcts of the long-chain fatty acids identified in this paper, no report has, hitherto, been seen.

However, Lewis[15] found that phytotoxic compounds, such as octanoic, nonanoic and decanoic acids, were produced from long-chain fatty acids by soil fungus *Cunninghamella echinulata*. This suggests that long-chain fatty acids are probably potential phytotoxic substances. On considering from Takijima's report,[29] in which it is described that even rice roots are dramatically inhibited by the fatty acids with intermediate carbon chain length over the range 1×10^{-4} to 4×10^{-4} M, concentrations of the long-chain fatty acids in the sawdust barnyard manure at 6 months are considerably high even though being diluted on its application to land. Therefore, it would be advised that the manure should be applied to land after the decrease of their compounds.

Changes in 3-hydroxydecanoic acid of the sawdust barnyard manure during its rotting

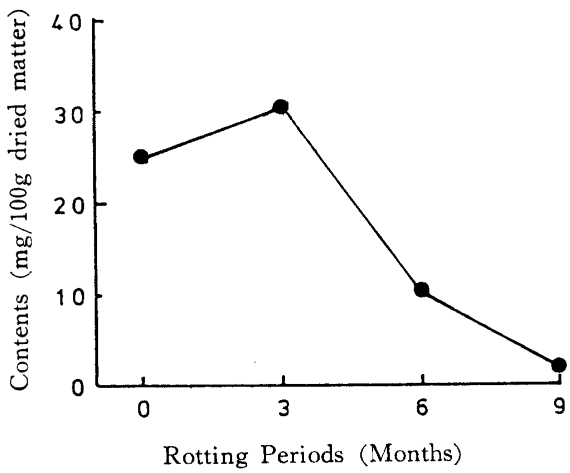

Fig. 9. Changes in 3-Hydroxydecanoic acid of Sawdust Barnyard Manures during Its Rotting Process.

process are shown in Fig. 9. Hydroxyaliphatic acids have been believed to be the compounds responsible for the low fertility of wornout soils [26] and to be one of phytotoxic compounds.[27] Among these acids, 3-hydroxydecanoic acid has a selective fungicidal activity.[1] As shown in Table 6, the content of the compound in the manure was moderately high, but at 9 months decreased to one tenth of the initial content as shown in Fig. 9. In the case of fungicidal test to *Altenaria* and *sp. Botrytis sp.*, 0.015% to 0.03% of 3-hydroxydecanoic acid (Myrmicacin) sufficiently inhibits their germination and growth.[1] The concentration of the compound in the manure over 3 months is in the level of toxic concentration, but reaches the nontoxic level already at 6 months.

The obtained results would give a significant clue to evaluate the manure-maturation, because the manure should be applied to land after the significant decrease of phytotoxic substances in all case.

As to the practical problems relating to effects of its application to cultivated soil on crop growth, some reports[7),34)] have been seen. Futami et. al.[7] and Yoshia[34] have observed the obviously poor growth of crops in the plot with the manure. The formers explained the phenomenon probably to be cause by nitrogen-starvation, and in contrast, the later emphasized that the phenomenon could be caused by an inhibitory effect of phytotoxic substances from sawdust.

Therefore, these evidences of the cause remain to be elucidated by investigating from both viewpoints of microbial activity and change of phytotoxic compound concentration in the cultivated soil with the manure.

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おが屑堆肥の腐熟過程における植物毒性成分の変化

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要　　約

おが屑中には，ワックス，樹脂，リグニン等の難分解性成分含量が高いため，その堆肥化には長期間を要するのみでなく，その堆肥の腐熟度判定にも，種々の問題が残されている。筆者らは，その適切な判定法を確立するための一基礎研究として，おが屑堆肥中の植物毒性成分の堆肥化過程における濃度変化を明らかにすることを試みた。その結果，次のことが明らかになった。

1. P－クマル酸，フェルラ酸，シリンガ酸およびコニフェルアルデヒドのような既知の植物毒性成分の含量は，3カ月目に最も増加し，その後，減少した。P－クマル酸の9カ月後の濃度は，堆肥化開始時の濃度の3分の1に減少したが，フェルラ酸の濃度は9カ月後においても開始時の濃度まで減少しなかった。
2. 偶数炭素数の高級脂肪酸である Octadecenoic acid およびパルミチン酸は6カ月目に最も高濃度になるが，その後，減少した。しかし，9カ月後においても，堆肥化開始時の濃度レベルにまでは減少しなかった。
3. 奇数炭素数の高級脂肪酸である Pentadecanoic acid, Heptadecanoic acid 等は，3カ月目に減少するが，その後，堆肥化の進行に伴って，増加する傾向が見られた。
4. 植物および糸状菌に対する毒性物質として知られている 3–Hydroxydecanoic acid の濃度は，初めの3カ月間ほとんど減少が見られなかったが，その後，減少し，9カ月後には，堆肥化開始時の量の1/10までに減少した。

この種の堆肥を施用する場合には，これら毒性成分ができるだけ減少した時点を確認すべきであり，以上の結果は，おが屑堆肥熟度判定のための一つの基礎資料として役立つものと考えられる。