# VOLATILE PRODUCTS AND LOW-MOLECULAR-WEIGHT PHENOLIC PRODUCTS OF THE ANAEROBIC DECOMPOSITION OF ORGANIC MATTER

K. Tsutsuki

The kinetics of volatile products, including volatile fatty acids, alcohols, aldehydes, ketones, volatile S compounds, and low molecular phenolic acids in submerged soils, is reviewed. Incubation of soils at high temperature reduces the accumulation of volatile fatty acids. The formation of alcohols, aldehydes, ketones, and volatile S compounds was minimal. The kinetics of volatile products in submerged soils was greatly affected by the amounts of easily decomposable organic matter and oxidizing substances such as ferric compounds in soils, and by temperature. Concentrations of phenolic acids in paddy soils are considered low enough to cause no injury even when organic materials are incorporated. However, a locally developed adverse environment around the fragment of decomposing plant residue should be taken into consideration in both the formation of volatile fatty acids and that of phenolic acids.

Although organic manures are assuming increasing importance in rice production, there is little information on the decomposition of organic matter in tropical rice soils. In wetland rice soils, organic matter usually undergoes anaerobic decomposition. Organic manures may cause, besides beneficial effects, adverse effects on crops because of their decomposition products, especially during anaerobic decomposition. Anaerobic decomposition includes anaerobic respiration and fermentation. Through fermentation, pyruvic acid, the key metabolite of carbohydrate metabolism, is transformed into an array of substances that include alcohols, diols, glycerol, aldehydes, ketones, volatile and nonvolatile organic acids, $CO_2$, $H_2$, $CH_4$, and $C_2H_2$ (Ponnamperuma 1972). Most of these products have been identified collectively in anaerobic soils. Some of them are toxic to plants and soil biota, depending on their concentrations. Most of these products are volatile or gaseous.

Besides fermentation products, phenolic substances are also considered potentially harmful substances in soils. Phenolic substances are very abundant in soils. Humic acid and fulvic acid can be regarded as high-molecular-weight phenolic acids because of their high contents of carboxylic and phenolic hydroxyl groups (Tsutsuki and Kuwatsuka 1978). Low-molecular-weight phenolic compounds are present in plant residues, cleaved or microbially formed from humic substances or lignin.

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The International Rice Research Institute, P. O. Box 933, Manila, Philippines.

## VOLATILE FATTY ACIDS

### Kinetics

The formation of volatile fatty acids resulting from the decomposition of organic matter in soil has drawn the attention of researchers for a long time. Takaishi et al (1909) studied the formation of organic acids during the decomposition of organic manure in soils. Their work elucidated the formation of formic acid, acetic acid, butyric acid, and lactic acid during the decomposition processes of oil cake, rice bran, and compost. Acharya (1935a) incubated rice straw with soil and followed the decomposition under anaerobic conditions. He observed that the first phase of the decomposition was the rapid formation of organic acids, followed by the formation of $CH_4$. The decomposition products detected were acetic acid, butyric acid, $CO_2$, $CH_4$, and negligibly small amounts of $H_2$. In his further experiments, Acharya (1935b,c) studied the effects of temperature, pH, and aeration on the course of decomposition. Maximum decomposition of rice straw was observed at 30-35° C at pH 8. Outside this temperature range and above and below pH 8, accumulation of organic acids and retardation of gas formation were observed.

Takijima (1960) detected organic acids in paddy soils in the following order: acetic > butyric > formic > fumaric > propionic > valeric > succinic and lactic. Formation of branched fatty acids such as iso-butyric and iso-valeric acids in paddy soils was detected by Takeda and Furusaka (1975b). Formation of volatile and gaseous products in submerged soils is almost entirely due to facultative and obligate anaerobic bacteria. Pyruvic acid, the final product of glycolysis pathways, is converted to many kinds of organic compounds, depending on the kinds of bacteria and on the environmental conditions during fermentation (Ponnamperuma 1972). Takeda and Furusaka (1970) used anaerobic incubation processes to conduct extensive surveys of bacteria isolated from paddy soil. The number of strict anaerobes was always about 10% of the facultative anaerobes, and the strict anaerobes were mostly clostridia. Among the facultative anaerobes, Enterobacteriaceae predominated during nonsubmerged periods, and *Aeromonas* and coccoid bacteria predominated during flooding (Watanabe and Furusaka 1980).

Acetic acid, which is accumulated in the greatest amount among the organic acids in submerged soil, can be produced by both facultative and obligate anaerobes through many classes of carbohydrate fermentation. It is also a product of proteolytic clostridia (Doelle 1975). Pyruvate is usually transformed via acetyl-CoA and acetylphosphate to acetate. A pathway by which $CO_2$ is converted to acetic acid is also known in some species of clostridia. In the former pathway, one ATP is produced, but no NADH is consumed; in other words, no intermediate is working as an electron donor. Therefore, acetic acid formation is usually accompanied by the formation of other products by which NADH is consumed. Such examples are propionic acid fermentation by *Propionibacterium;* butyric acid fermentation by clostridia; and ethanol, lactate, and succinate formation by mixed acid producers among the Enterobacteriaceae (Doelle 1975). However, acetic acid surpasses other fermentation products in amount in submerged soils. As noted by Neue and Scharpenseel (1983), this may be attributed to the interspecies transfer of $H_2$ liberated from NADH using ferredoxin as an intermediate carrier.

Formic acid is produced concomitantly when acetyl-CoA is produced from pyruvic acid and Coenzyme A by facultative anaerobes belonging to the Enterobacteriaceae, but it is not produced by saccharolytic clostridia (Doelle 1975). Its formation keeps step with acetic acid, but the amount formed is very small (Gotoh and Onikura 1971, Yamane and Sato 1970) probably because formic acid is further decomposed to $H_2$ and $CO_2$, and the $CO_2$ is then used in the synthesis of other products like succinate and $CH_4$ (Doelle 1975).

Butyric acid and priopionic acid are usually produced concomitantly with acetic acid by saccharolytic clostridia and propionibacteria. Strains of *Propionibacterium* increased in a submerged soil after prolonged submergence (Watanabe and Furusaka 1980).

The very small amounts of branched fatty acids such as isobutyric and isovaleric acids in paddy soils are considered products of proteolytic clostridia (Takeda and Furusaka 1975a,b).

Volatile fatty acids are intermediate products that are transformed to $CO_2$ by oxidation while the reduction of $Fe^{+3}$ is going on in submerged soils (Kamura et al 1963). After the reduction of $Fe^{+3}$ ceases, the volatile fatty acids are used by methanogenic bacteria as substrates to produce $CH_4$. In methanogenesis in submerged soils, $CH_4$ is formed preferentially from the methyl group of acetic acid, and the formation of $CH_4$ from $CO_2$ is of less importance (Takai 1970).

In soils with a high content of active Fe, only small amounts of volatile fatty acids accumulate, while the contrary is true in soils with a low content of active Fe (Takai et al 1957). Asami and Takai (1963) also found that the formation of volatile fatty acids and $CH_4$ is repressed remarkably when amorphous $Fe(OH)_3$ is added to the soil and then submerged. There was a highly significant positive correlation between the amounts of $Fe^{+2}$ and $CO_2$ formed within the first 2 weeks of submergence. Moreover, when acetic acid was added to a well-reduced soil, a large amount of $CH_4$ was formed, but when acetic acid and amorphous $Fe(OH)_3$ were added together to the reduced soil, a large amount of $CO_2$ was formed instead of $CH_4$. From these results, Asami and Takai assumed the coupling of the oxidation of acetic acid and the reduction of Fe in submerged soils.

An increase in volatile fatty acid formation because of the addition of fresh organic matter has been reported in many papers, including those cited above (Chandrasekaran and Yoshida 1973, Gotoh and Onikura 1971, Lynch 1978, Yamane and Sato 1970, Rao and Mikkelsen 1977).

Temperature greatly affects the kinetics of volatile fatty acids (Yamane and Sato 1967, Cho and Ponnamperuma 1971). Higher temperature accelerates both production and destruction of volatile fatty acids. However, methanogenesis, which accompanies the destruction of acids, is much more accelerated than the production

**Table 1. Characteristics of soil samples.**

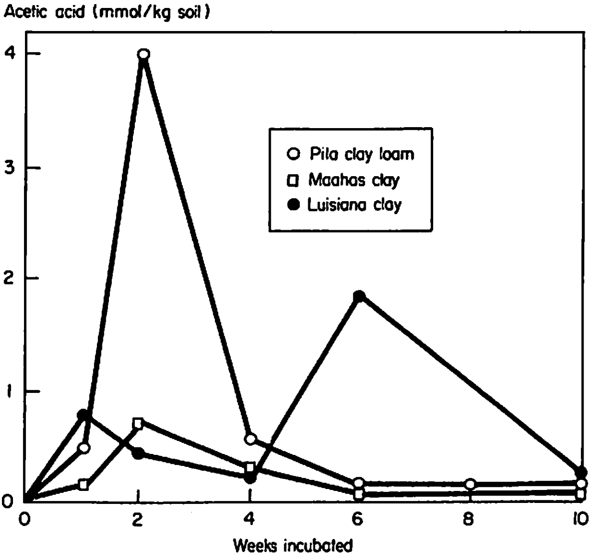
| Soil type | pH | C (%) | Total N (%) | Active Fe (%) |
|---|---|---|---|---|
| Pila clay loam | 7.2 | 2.48 | 0.204 | 0.32 |
| Maahas clay | 6.0 | 1.43 | 0.140 | 1.39 |
| Luisiana clay | 5.4 | 1.56 | 0.117 | 1.89 |

**Table 2. Analyses of organic materials.**

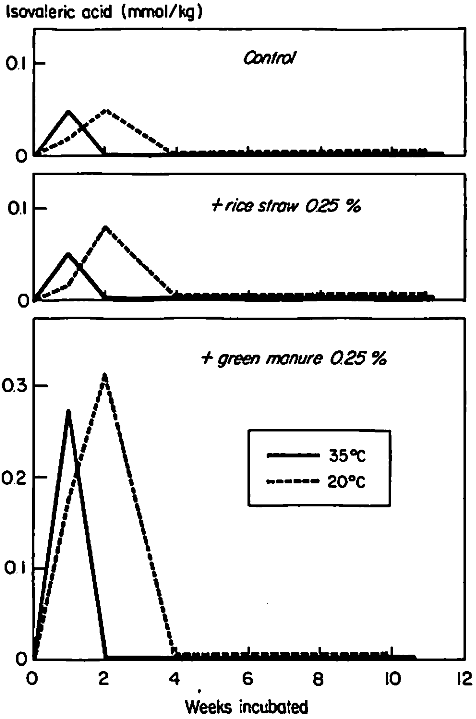
| Material | C (%) | Total N (%) | C:N |
|---|---|---|---|
| Rice straw | 39.6 | 0.56 | 70.5 |
| Rice straw compost | 14.2 | 1.69 | 8.4 |
| Green manure | 45.8 | 3.21 | 14.3 |

of acids at a temperature higher than 25° C (Yamane and Sato 1967). As a result, the accumulation of organic acids is diminished at higher temperatures.

The kinetics of volatile fatty acids was also studied using three soils (Table 1) treated with three kinds of organic materials (Table 2) and incubated anaerobically at 20° and 35° C (Tsutsuki and Ponnamperuma, unpublished). The kinetics of acetic acid in 3 submerged soils treated with rice straw (0.25%) at 20° C is shown in Figure 1. In Pila clay loam, a large amount of acetic acid accumulated within 2 weeks of submergence. A low active Fe content and large amounts of easily decomposable organic matter in the soil were assumed to be the cause of the large accumulation within such a short incubation period. In Maahas clay, organic acid accumulation was low at both temperatures, even when organic matter was added to the soil. A slight accumulation was seen after 2 weeks of submergence. Organic acid accumulation lasted longer in Luisiana clay than in the other soils. When rice straw or green manure was added to this soil at 20° C, acetic acid accumulation showed two peaks — at the first and at the sixth week of incubation. Luisiana clay is a heavy clay and contains a large amount of active Fe. The second peak implies that acetic acid could accumulate after most of the active $Fe^{+3}$ in the soil had been reduced. The acetic acid formed in the first week might have come from strictly anaerobic sites that developed locally in the soil.



1. Kinetics of acetic acid in 3 submerged soils treated with rice straw (0.25%) at 20° C.



2. Effect of organic matter on kinetics of isovaleric acid in submerged Pila clay loam at 20 and 35° C.

In Figure 2, the accumulations of isovaleric acid in Pila soil treated with rice straw and green manure (both 0.25%) are compared with controls at 20° and 35° C. Isovaleric acid accumulation was prominent when green manure was applied to the soil. Addition of rice straw slightly enhanced the accumulation of isovaleric acid. Pila clay loam produced a significantly larger amount of isovaleric acid than the other soils. The accumulation of isovaleric acid was earlier and lower at 35° C than at 20° C. Isovaleric acid is produced by clostridia utilizing amino acids as electron donors and acceptors (Takeda and Furusaka 1975a). Such a metabolism would become active where the content of other electron acceptors like $Fe^{+3}$ compounds is low and proteinous material is abundant. That is why isovaleric acid was produced in large amounts in Pila clay loam (low active Fe content) treated with green manure, which contains a substantial amount of N. Rice straw did not enhance the formation because of its low N content.

### Volatile fatty acid accumulation in fields

Studies on volatile fatty acid accumulation in arable lands have been closely connected with the evaluation of volatile fatty acids as factors in phytotoxicity. As the phytotoxicity of organic acids is discussed in another session, some field data that might be suggestive in solving this problem are cited below.

Yamane and Sato (1970) found that large amounts of acetic acid (13.5 mmol/kg soil) and butyric acid (5.5 mmol/kg soil) were produced when Italian ryegrass

(10.6 t/ha of shoot and 19.3 t/ha of root) was incorporated into rice fields. However, within 2-3 weeks after incorporation, volatile fatty acids almost disappeared.

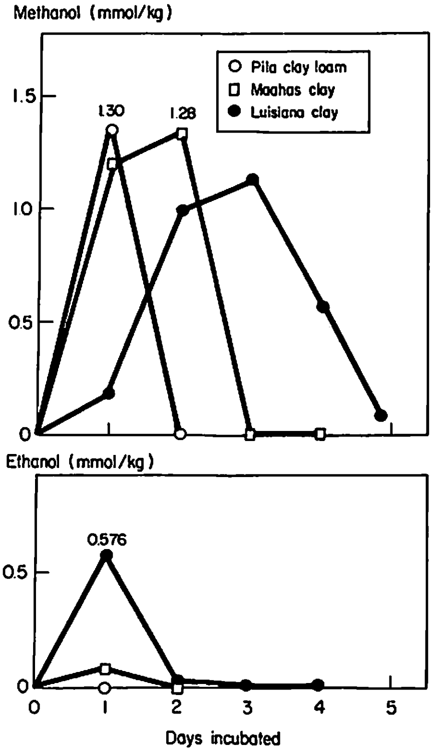
Gotoh and Onikura (1971) conducted a field experiment to examine whether organic acids accumulate to a toxic level in a rice field to which rice straw has been applied. They detected a maximum of 0.31 meq/kg acetic acid (0.91 mM in soil solution) 4 weeks after the incorporation of rice straw (15 t/ha). From the low concentration of volatile fatty acids detected, they concluded that the main factor governing plant growth in the early stages was probably N deficiency, with volatile fatty acids being a minor factor. In warm areas such as Kyushu, where the experiment was conducted, they assumed that volatile fatty acids derived from the decomposing rice straw would probably not cause injury to plants. Though toxicity is considered to be further alleviated in submerged rice soils because of the neutrality of soil pH, Chandrasekaran and Yoshida (1973) suggested that volatile fatty acids can inhibit the growth of rice plants even in a neutral soil because the pH around the rice root is much lower than the pH outside the rhizosphere.

An adverse environment developing in the vicinity of decomposing plant residue was found responsible for the poor establishment of direct-drilled winter oats associated with the presence of unburnt straw in the drill slits (Agricultural Research Council Letcombe Laboratory 1976). The same type of inhibition may occur if rice is direct seeded where applied rice straw or other organic matter has not decomposed well. The distribution of added organic matter in soil is heterogenous, and the concentrations of volatile fatty acids are highest and the pH values lowest at the site of decomposing organic debris itself. It was found that the acetic acid concentration declined exponentially with distance from the straw surface (Agricultural Research Council Letcombe Laboratory 1977).

## ALCOHOL FORMATION

Methanol, ethanol, n-propanol, and n-butanol were detected and quantified in soils treated with leaves of sugarcane and *Crotalaria juncia* by Wang et al (1967a). Isopropanol, iso-butanol, sec-butanol (Adamson et al 1975), and 2,3-butane-diol (Kubota and Furusaka 1981) were detected in soils treated with glucose and incubated anaerobically. Alcohols were found in both submerged and aerobic soils (Wang et al 1967a), but formation and decomposition of alcohols proceeded more slowly in aerobic soils than in submerged soils. Alcohols were not detected in soils that did not receive glucose or other carbonaceous amendments (Adamson et al 1975, Kubota and Furusaka 1981), and they were formed in significant amounts in submerged soils only when 1% green manure (*Glydicidia sepium*) was added; addition of rice straw did not favor the formation of alcohols in submerged soils (Tsutsuki and Ponnamperuma, unpublished).

The kinetics of alcohols in 3 soils at 35° C is shown in Figure 3. The formation of methanol in Pila clay loam, Maahas clay, and Luisiana clay attained a peak 1, 2, and 3 days after submergence, respectively. The order in which peak concentrations were formed seems to reflect how easily each soil is reduced. In Pila clay loam, a reduced state developed rapidly because of the high content of easily decomposable organic matter and the low content of active $Fe^{+3}$, while it developed more slowly in Luisiana



3. Kinetics of alcohols in 3 submerged soils treated with green manure (1%) at 35° C.

clay because of the high content of active Fe. Formation of ethanol was observed only within 2 days of incubation in all those soils. The amount was largest in Luisiana clay, followed by Maahas clay and Pila clay loam in that order. Ethanol formation in Pila clay loam was insignificant at 35° C.

When the soils were incubated at 20° C, methanol and ethanol were formed more slowly, but accumulated in slightly larger amounts than at 35° C. At 20° C, methanol content attained a peak 2, 4, and 6 days after submergence in Pila clay loam, Maahas clay, and Luisiana clay, respectively. The accumulated amounts of methanol at the peaks were between 1.4 and 1.7 mmol/kg soil. Ethanol content attained a peak after 1 day in Pila clay loam and Maahas clay, and after 4 days in Luisiana clay. The accumulated amounts were 0.8-1.1 mM, which were also slightly larger than those at 35° C.

Kinds and amounts of alcohols formed in soils vary with type of soil and with the kind of amended organic matter. Ethanol and butanol were formed in large amounts when glucose was added to soils (Adamson et al 1975, Kubota and Furusaka 1981). When leaves of sugarcane, *Crotalaria*, and *Glydicidia* were added to soils, methanol was formed in the largest amount, followed by ethanol. Butanol formation was favored in soils with higher organic C content or in those amended with *Crotalaria* (Wang et al 1967a).

According to Kubota and Furusaka (1981) methanol, ethanol, and butanol were detected only within the first few days of incubation. The same result was obtained by Tsutsuki and Ponnamperuma (unpublished). However, those alcohols were detected even after 4 or 8 weeks of incubation by Wang et al (1967a).

Kubota and Furusaka (1981) detected 2,3-butane-diol and its percursor acetoin in submerged soils even without glucose, but addition of glucose enhanced and increased the production. They concluded that carbohydrates are metabolized through the 2,3-butane-diol fermentation pathway as well as through volatile fatty acids under anaerobic soil conditions.

## ALDEHYDES AND KETONES

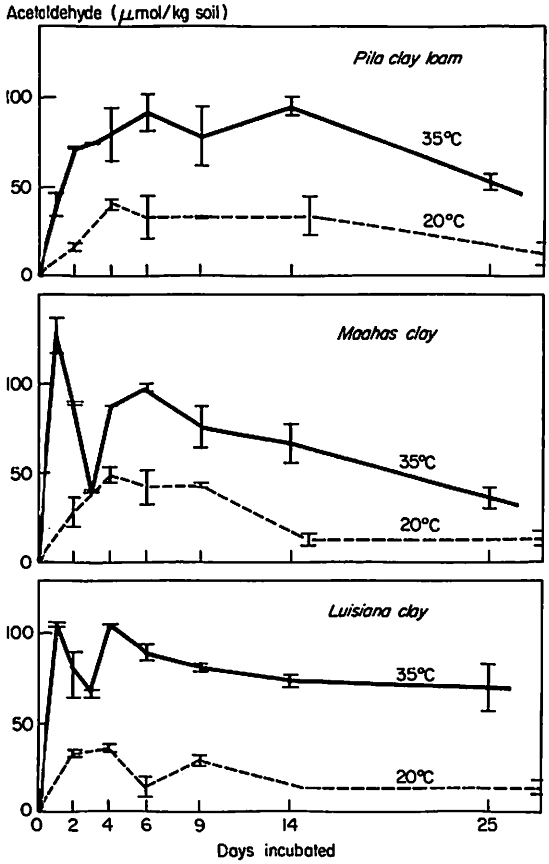
Aldehydes and ketones have been detected in soils under anaerobiosis of glucose (Adamson et al 1975). Acetaldehyde and butyraldehyde were formed in very small amounts representing about 0.01% of the added glucose, while acetone and methylethylketone were formed in larger amounts representing at most 0.4% of the added glucose. In an effort to identify reducing organic substances in the leachate from submerged rice soil, Okazaki et al (1981) detected formaldehyde, acetaldehyde, propionaldehyde, n-butyraldehyde, and n-valeraldehyde at concentrations of 0.03, 0.05, 0.04, 0.01, and 0.01 mmol/kg soil, respectively, from an excessively eluviated wetland rice soil. They considered that these aldehydes play important roles in the dissolution and removal of Fe and Mn from subsurface horizons of excessively eluviated wetland rice soils.

The kinetics of aldehydes was also studied by Tsutsuki and Ponnamperuma (unpublished) in submerged soils amended with green manure (1%) and incubated at 20° C and 35° C (Fig. 4). Formaldehyde, acetaldehyde, and propionaldehyde were detected. The major aldehyde was acetaldehyde, but the amount formed was very low — at most 0.1 mmol at 35° C or 0.05 mmol at 20° C. Because it is an unstable intermediate, the amount of acetaldehyde detected in soil should be regarded as the amount formed within a few days before analysis. The greater amount of acetaldehyde at 35° C than at 20° C may be explained by higher microbial activity at 35° C.

In Maahas clay and Luisiana clay, the formation of acetaldehyde showed two peaks, the first after 1 day and the second after 4-6 days of incubation. After the second peak was attained, acetaldehyde did not disappear rapidly like alcohols, but decreased gradually over 4 weeks. Several percursors are known for acetaldehyde in bacterial metabolism (Doelle 1975). In fermentation, pyruvate, acetyl-CoA, ethanol, and acetate are assumed to be important. Acetaldehyde formed in the first peak might have come from pyruvate, acetyl-CoA, or ethanol derived from the carbohydrates in green manure, and the acetaldehyde formed in the second peak might have come from acetic acid accumulated in the soil. The amounts of formaldehyde and propionaldehyde formed were much lower than the amount of acetaldehyde.

## VOLATILE SULFUR COMPOUNDS

In anaerobic media, the main changes involving S are the reduction of $SO_4^{2-}$ to sulfide and the dissimilation of the amino acids cystine and methionine to $H_2S$,



4. Kinetics of acetaldehyde in 3 submerged soils added with green manure (1%).

thiols, ammonia, and fatty acids (Ponnamperuma 1972). Methylmercaptan has been found in submerged soils (Takai and Asami 1962, Asami and Takai 1963, IRRI 1965). Mercaptans showed concentration peaks within 2 weeks of flooding and then declined (IRRI 1965). Higher temperature enhanced the disappearance of methylmercaptan (Asami and Takai 1963). The addition of green manure and stable manure enhanced the formation of methylmercaptan, while the addition of $Fe_2O_3$ interfered with the accumulation of methylmercaptan (Asami and Takai 1963).

Sensitive gas chromatographic techniques using a flame photometric detector enabled the analysis of trace amounts of volatile S gases (Banwart and Bremner 1974). Though the main change of S in anaerobic soils is the reduction of $SO_4^{2-}$ to sulfide, no trace of $H_2S$ was detected over submerged soils treated with $SO_4^{2-}$ or S-containing organic materials (Banwart and Bremner 1976a,b). However, a considerable amount of $H_2S$ was detected from animal manures decomposed without soil (Banwart and Bremner 1975). Not only gaseous $H_2S$ but also water-soluble $H_2S$ is almost chemically undetectable in submerged soils (IRRI 1965). This is due to their removal as insoluble sulfides, chiefly as FeS, and to their instability in the air phase (Ponnamperuma 1972).

According to Banwart and Bremner (1976a), S volatilized from unamended and $SO_4^{2-}$-treated soils, chiefly in the form of $CH_3SCH_3$ (55-100%), associated with small amounts of COS, $CS_2$, $CH_3SH$, and $CH_3SSCH_3$. The amended $SO_4^{2-}$ was not transformed to volatile S compounds. More S volatilized under submerged soil conditions than under aerobic conditions. However, the amount of S volatilized from unamended and $SO_4^{2-}$-amended soils was very small (0-84 $\mu$g S/kg soil) and did not account for more than 0.05% of the total S in unamended soils.

The release of volatile S compounds from soils treated with S-containing organic materials was also studied by Banwart and Bremner (1976b). Most of the S volatilized from soils treated with sewage sludges was in the form of $CH_3SCH_3$ and $CH_3SSCH_3$, whereas most of the S volatilized from soils treated with animal manures and plant materials was in the form of $CH_3SH$ and $CH_3SCH_3$. Small amounts of COS and $CS_2$ were associated with these compounds. Submerged soil conditions and the addition of S-containing organic materials favored the formation of volatile S compounds. When the application rate of organic matter to the soils was 1%, amounts of S volatilized were 0.27 mg/kg for animal manure-treated soils, 0.38 mg/kg for sewage sludge-treated soils, and 4.36 mg/kg for plant material-treated soils, on the average.

Minami et al (1981) also analyzed volatile S compounds evolved from rice soils treated with rice straw, compost, and cystine (Table 3). In the soil treated with rice straw, $H_2S$ was detected in the greatest amount among the S-containing products by shaking the flask immediately before analysis. Dimethyl sulfide ($CH_3SCH_3$) was the second most abundant S-containing product; $CH_3SH$, $CH_3SSCH_3$, COS, and $CS_2$ were formed in smaller amounts. The addition of compost did not increase the formation of volatile S compounds from soil as compared with the control.

## PHENOLIC ACIDS

As Takijima (1964) suggested, injury of the rice plant in peat soils or in soils amended with fresh organic matter cannot be explained only by volatile fatty acids; some other toxic substance may participate in the injury.

Aromatic organic acids have much higher toxicity than aliphatic acids. Phenolcarboxylic acids exhibited 50% inhibition of the growth of the roots of rice seedlings at concentrations of 0.6-3 mM (Takijima 1960b). Many researchers found the lowest

**Table 3. Sulfur-containing gases evolved from a submerged soil (*Nyuzen*) treated with organic materials (adapted from Minami et al 1981).**

| Compound | Amt (nmol/kg soil) | | | |
|---|---|---|---|---|
| | Control | Rice straw | Compost | Cystine |
| $H_2S$ | 0 | 1700 | 0 | 4720 |
| COS | 17 | 78 | 23 | 566 |
| $CS_2$ | 7 | 4 | 8 | 71 |
| $CH_3SH$ | 17 | 56 | 0 | 145 |
| $CH_3SCH_3$ | 129 | 483 | 129 | 113 |
| $CH_3SSCH_3$ | 53 | 54 | 0 | 74 |

concentrations of phenolic acids causing injury to various plants to range between 0.1 and 0.01 mM (Takijima 1963).

Walters (1917) may have been the first researcher to recognize phenolic acids as the cause of a soil-borne plant disease. He found 22 ppm of p-hydroxybenzoic acid and 1.7 ppm of benzoic acid in the soil from an orange orchard that was badly affected with the citrus disease commonly known as "die back." Later, Winter (1955) studied the occurrence of toxic substances in stubbles to elucidate the cause of poor growth of successively grown crops. His co-worker Borner (1955, 1956) detected p-hydroxybenzoic acid, p-coumaric acid, and ferulic acid in the straw and stubble of rye, wheat, and barley. The quantitative determination of phenolic acids in soils was accomplished by Whitehead (1964), who found p-hydroxybenzoic acid, vanillic acid, p-coumaric acid, and ferulic acid in 4 soils, each at a concentration lower than 0.05 mM. Phenolic acids have been assumed to be growth inhibitors for dryland crops (Wang et al 1967b), the phytotoxic substances associated with the decomposition of plant residues in anaerobic soil (Patrick 1971), and the cause of phytotoxic effects of decomposing rice residues in soil (Chou and Lin 1976).

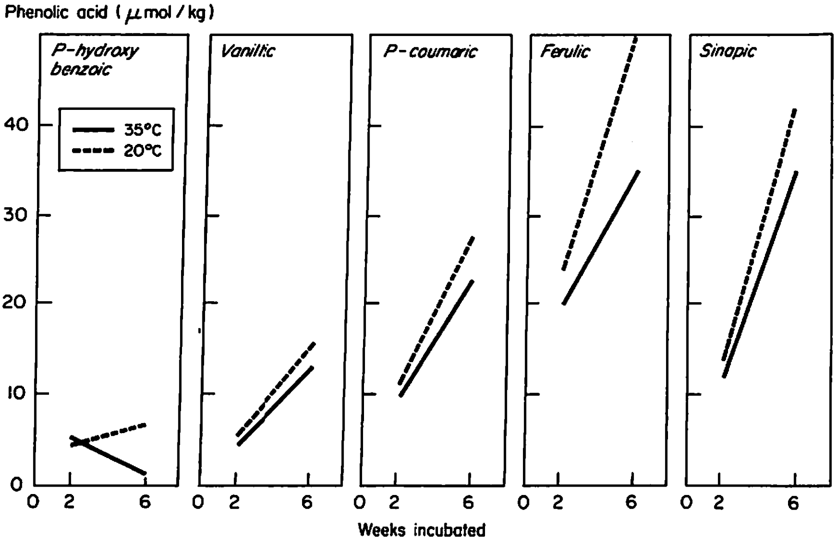
Kuwatsuka and Shindo (1973) determined phenolic acids extractable with a methanol-0.1 *N* NaOH mixture from both fresh rice straw and decomposing rice straw to be p-coumaric, ferulic, p-hydroxybenzoic, and vanillic acids and trace amounts of salicylic, syringic, protocatechunic, $\beta$-resorcylic, caffeic, sinapic, gallic, and gentisic acids. From the results of incubating rice straw at different temperatures under moist and flooded conditions, Shindo and Kuwatsuka (1975a) assumed that phenolic acids, which initially existed in rice straw, were rapidly degraded in the early stages of incubation and subsequently produced again from the lignin component. The pathways from p-coumaric acid to p-hydroxybenzoic acid and from ferulic acid to vanillic acid were deduced from the changes in amounts of these phenolic acids. To alleviate their toxic effects, p-coumaric acid and ferulic acid were also temporarily methylated by soil microbes (Shindo and Kuwatsuka 1975b) and gradually transformed to p-hydroxybenzoic acid and vanillic acid. These latter acids are further transformed via protocatechuic acid to humic substances by polymerization or to aliphatic acids by ring cleavage.

When rice straw was mixed with soil and incubated under moist and flooded conditions, phenolic acids due to rice straw decreased gradually with time. It took nearly 30 days for the amounts of p-coumaric and ferulic acids to decrease to half of their initial levels when the rate of rice straw to soil was 8% (Shindo and Kuwatsuka 1977). After 6-10 weeks of incubation, decreasing curves of p-coumaric and ferulic acids showed shoulders, indicating the formation of these acids in the soil. Phenolic acids decreased more rapidly under moist conditions than under flooded conditions.

Shindo and Kuwatsuka (1978) determined the concentrations of phenolic acids in 9 rice soils in Japan as 10-26 ppm (average 21 ppm); the levels were not affected by rice straw incorporated after the preceding cropping season. It seems that phenolic acids at those levels in rice soils do not affect the growth of either the root or the whole rice plant. Shindo and Kuwatsuka (1976) ascribed the low concentration of phenolic acids in rice soils to leaching. Leaching of phenolic acids in soil profiles is also obvious in the data of Wang et al (1967b).

The kinetics of phenolic acids was investigated by Tsutsuki and Ponnamperuma (unpublished) in 3 submerged soils treated with 0.25% rice straw, rice straw compost, or green manure at 2 temperatures (20° and 35° C). The largest concentrations of phenolic acids ranged between 13.6 μmol/kg soil for p-hydroxybenzoic acid and 74.2 μmol/kg soil for ferulic acid when rice straw was applied. Addition of 0.25% green manure or compost affected the concentration of phenolic acids in soils only a little. Changes in the concentrations of phenolic acids in Luisiana clay after 2 and 6 weeks of submergence at 20° and 35° C are shown in Figure 5. After 2 weeks of anaerobic incubation, the concentration of each phenolic acid was always higher at 20° C. However, the difference due to the incubation temperature was small at this time. The concentration of p-hydroxybenzoic acid increased after 6 weeks of incubation at 20° C, while it decreased with time at 35° C. At 35° C, degradation of p-hydroxybenzoic acid may have been faster than its formation. Concentrations of the other phenolic acids such as vanillic, p-coumaric, ferulic, and sinapic acids increased with increased period of incubation at both temperatures. In Luisiana clay, concentrations of phenolic acids were higher at 20° than at 35° C after 2 and 6 weeks of incubation. However, in Pila clay loam and Maahas clay the concentrations of phenolic acids were higher at 35° after 6 weeks of incubation. Higher temperature might have favored the formation of phenolic acids from rice straw.

The discussions that consider phenolic acids as plant growth inhibitors in soils are usually based upon the concentration of phenolic acids determined by alkaline extraction, and the amounts of water-soluble phenolic acids are very low. The amounts of water-soluble phenolic acids in the soil under permanent pasture at pH 5.8 were equivalent to concentrations in the soil solution ranging from 1.4 μM for p-hydroxybenzoic acid to <10 nM for ferulic acid (Whitehead et al 1981). Amounts up to 2,000 times greater than these were extracted by 2 M NaOH. Kaminsky and Muller (1978) recommend against the use of alkaline soil extraction in the study of



5. Kinetics of phenolic acids in submerged Luisiana clay treated with rice straw (0.25%).

allelopathy, because the use of an alkaline extractant results in the chemical modification of the compound under study, and the compounds that are not solubilized by neutral extractants may not be available to plants. However, localized concentrations of phenolic acids close to fragments of decomposing plant material might be sufficiently high to have some effect. Moreover, Rice (1979) considers that some compounds may influence plant growth at concentrations at which they are completely adsorbed by soil particles and are not extractable with water.

## REFERENCES CITED

Acharya, C. N. 1935a. Studies on the anaerobic decomposition of plant materials. I. The anaerobic decomposition of rice straw (*Oryza sativa*). Biochem. J. 29:528-541.

Acharya, C. N. 1935b. Studies on the anaerobic decomposition of plant materials. II. Some factors influencing the anaerobic decomposition of rice straw. Biochem. J. 29:953-960.

Acharya, C. N. 1935c. Studies on the anaerobic decomposition of plant materials. III. Comparison of the course of decomposition under anaerobic, aerobic, and partially aerobic conditions. Biochem. J. 29:1116-1120.

Adamson, J. A., A. J. Francis, J. M. Duxbury, and M. Alexander. 1975. Formation of volatile organic products in soil under anaerobiosis. I. Metabolism of glucose. Soil Biol. Biochem. 7:45-50.

Agricultural Research Council Letcombe Laboratory. 1976. Annual report for 1975. Wantage, UK. pp. 29-31.

Agricultural Research Council Letcombe Laboratory. 1977. Annual report for 1976. Wantage, UK. 107 p.

Asami, T., and Y. Takai. 1963. Formation of methyl mercaptan in paddy soils (II). Soil Sci. Plant Nutr. 9:65-69.

Banwart, W. L., and J. M. Bremner. 1974. Gas chromatographic identification of sulphur gases in soil atmospheres. Soil Biol. Biochem. 6:113-115.

Banwart, W. L., and J. M. Bremner. 1975. Identification of sulfur gases evolved from animal manures. J. Environ. Qual. 4:363-366.

Banwart, W. L., and J. M. Bremner. 1976a. Volatilization of sulfur from unamended and sulfate treated soils. Soil Biol. Biochem. 8:19-22.

Banwart, W. L., and J. M. Bremner. 1976b. Evolution of volatile compounds from soils treated with sulfur containing organic materials. Soil Biol. Biochem. 8:439-443.

Borner, H. 1955. Phenolic compounds from straw and stubble of wheat, barley, and rye [in German]. Naturwissenshaften 42:583-584.

Borner, H. 1956. Paperchromatographic identification of ferulic acid in the water extracts of straw and stubble of wheat, barley, and rye [in German]. Naturwissenshaften 43:129-130.

Chandrasekaran, S., and T. Yoshida. 1973. Effect of organic acid transformations in submerged soils on growth of the rice plant. Soil Sci. Plant Nutr. 19:39-45.

Cho, D. Y., and F. N. Ponnamperuma. 1971. Influence of soil temperature on the chemical kinetics of flooded soils and the growth of rice. Soil Sci. 112:184-194.

Chou, C. H., and H. J. Lin. 1976. Autointoxication mechanism of *Oryza sativa*. I. Phytotoxic effects of decomposing rice residues in soils. J. Chem. Ecol. 2:353-367.

Doelle, H. W. 1975. Bacterial metabolism. 2nd ed. Academic Press, New York. 738 p.

Gotoh, S., and Y. Onikura. 1971. Organic acids in flooded soil receiving added rice straw and their effect on the growth of rice. Soil Sci. Plant Nutr. 17:1-8.

International Rice Research Institute. 1965. Annual report for 1964. Los Baños, Philippines. 357 p.

Kaminsky, R., and W. H. Muller. 1978. A recommendation against the use of alkaline soil extractions in the study of allelopathy. Plant Soil 49:641-645.

Kamura, T., Y. Takai, and K. Ishikawa. 1963. Microbial reduction mechanisms of ferric iron in paddy soils (Part I). J. Sci. Soil Manure, Jpn. 32:135-138.

Kubota, M., and C. Furusaka. 1981. Formation of 2,3-butane-diol, acetoin, ethanol, and butanol in paddy soils [in Japanese]. J. Sci. Soil Manure, Jpn. 52:149-154.

Kuwatsuka, S., and H. Shindo. 1973. 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. 19:219-227.

Lynch, J. M. 1978. Production and phytotoxicity of acetic acid in anaerobic soils containing plant residues. Soil Biol. Biochem. 10:131-135.

Minami, K., K. Okayama, and S. Fukushi. 1981. Volatile sulfur compounds evolved from paddy soils treated with organic materials [in Japanese]. J. Sci. Soil Manure, Jpn. 52:375-379.

Neue, H. U., and H. W. Scharpenseel. 1983. Gaseous products of the decomposition of organic matter in submerged soils. Pages 315-332 *in* International Rice Research Institute. Organic matter and rice. Los Baños, Laguna, Philippines.

Okazaki, M., H. Wada, and Y. Takai. 1981. Reducing organic substances responsible for removal of Fe (III) and Mn (IV) from subsurface horizon of lowland rice soil. Pages 235-250 *in* Institute of Soil Science, Academia Sinica. Proceedings of symposium on paddy soil. Science Press, Beijing, China.

Patrick, Z. A. 1971. Phytotoxic substances associated with the decomposition in soil of plant residues. Soil Sci. 111:13-18.

Ponnamperuma, F. N. 1972. The chemistry of submerged soils. Adv. Agron. 24:29-96.

Rao, D. N., and D. S. Mikkelsen. 1977. Effect of rice straw additions on production of organic acids in a flooded soil. Plant Soil 47:303-311.

Rice, E. L. 1979. Allelopathy — an update. Bot. Rev. 45:15-109.

Shindo, H., and S. Kuwatsuka. 1975a. Behavior or phenolic substances in the decaying process of plants. I. Changes of phenolic substances in the decaying process of rice straw under various conditions. Soil Sci. Plant Nutr. 21:215-225.

Shindo, H., and S. Kuwatsuka. 1975b. Behavior of phenolic substances in the decaying process of plants. III. Degradation pathway of phenolic acids. Soil Sci. Plant Nutr. 21:227-238.

Shindo, H., and S. Kuwatsuka. 1976. Behavior of phenolic substances in the decaying process of plants. IV. Adsorption and movement of phenolic acids in soils. Soil Sci. Plant Nutr. 22:23-33.

Shindo, H., and S. Kuwatsuka. 1977. Behavior of phenolic substances in the decaying process of plants. VI. Changes in quality and quantity of phenolic substances in the decaying process of rice straw in a soil. Soil Sci. Plant Nutr. 23:319-332.

Shindo, H., and S. Kuwatsuka. 1978. Behavior of phenolic substances in the decaying process of plants. IX. Distribution of phenolic acids in soils of paddy fields and forests. Soil Sci. Plant Nutr. 24:233-243.

Takai, Y. 1970. The mechanism of methane fermentation in flooded paddy soil. Soil Sci. Plant Nutr. 16:238-244.

Takai, Y., T. Koyama, and T. Kamura. 1957. Microbial metabolism of paddy soils. Part III. Effect of iron and organic matter on the reduction process (1). J. Agric. Chem. Soc. Jpn. 31:211-215.

Takai, Y., and T. Asami. 1962. Formation of methyl mercaptan in paddy soils I. Soil Sci. Plant Nutr, 8:132-136.

Takaishi, S., K. Tasaki, and M. Imai. 1909. Formation of organic acids during the decomposition of organic manures [part I, in Japanese]. Nougaku-kai-hou 89:3-8.

Takeda, K., and C. Furusaka. 1970. Studies on the bacteria isolated anaerobically from paddy field soil. Rep. Inst. Agric. Res. Tohoku Univ. 21:1-22.

Takeda, K., and C. Furusaka. 1975a. Studies on the bacteria isolated anaerobically from paddy field soil. III. Production of fatty acids and ammonia by *Clostridium* species. Soil Sci. Plant Nutr. 21:119-127.

Takeda, K., and C. Furusaka. 1975b. Studies on the bacteria isolated anaerobically from paddy field soil. IV. Model experiments on the production of branched fatty acids. Soil Sci. Plant Nutr. 21:119-127.

Takijima, Y. 1960. Metabolism of organic acids in soils and their harmful effects on paddy rice growth (part I). Column chromatography of organic acids using home-made silica gel and its application to soil samples [in Japanese]. J. Sci. Soil Manure, Jpn. 31:435-440.

Takijima, Y. 1963. Studies on behavior of the growth inhibiting substances in paddy soils with special reference to the occurrence of root damage in the peaty paddy fields [in Japanese, English summary]. Bull. Nat. Inst. Agric. Sci. Jpn. Ser. B. 13:117-252.

Takijima, Y. 1964. Studies on organic acids in paddy field soils with reference to their inhibitory effects on the growth of rice plants: 2. Soil Sci. Plant Nutr. 10:212-219.

Tsutsuki, K., and S. Kuwatsuka. 1978. Chemical studies on soil humic acids. II. Composition of oxygen containing functional groups of humic acids. Soil Sci. Plant Nutr. 24:547-560.

Walters, E. H. 1917. The isolation of p-hydroxybenzoic acid from soil. Am. Chem. Soc. J. 39:1178-1784.

Wang, T. S. C., T. K. Yang, and T. T. Chuan. 1967a. Soil alcohols, their dynamics and their effect upon plant growth. Soil Sci. 104:40-45.

Wang, T. S. C., T. K. Yang, and T. Chung. 1967b. Soil phenolic acids as plant growth inhibitors. Soil Sci. 103:239-246.

Watanabe, I., and C. Furusaka. 1980. Microbial ecology of flooded rice soils. Pages 125-168 *in* M. Alexander, ed. Advances in microbial ecology, Vol. 4.

Whitehead, D. C. 1964. Identification of p-hydroxybenzoic, vanillic, p-coumaric and ferulic acids in soils. Nature. (London) 202:417-418.

Whitehead, D. C., H. Dibb, and R. D. Hartley. 1981. Extractant pH and the release of phenolic compounds from soils, plant roots and leaf litter. Soil Biol. Biochem. 13:343-348.

Winter, A. G. 1955. Occurrence and significance of antimicrobial and antiphytotic substances in natural soils [in German]. Zeits. Pflanzenernah, Dung, Bodenk. 69:224-233.

Yamane, I., and K. Sato. 1967. Effect of temperature on the decomposition of organic substances in flooded soil. Soil Sci. Plant Nutr. 13:94-100.

Yamane, I., and K. Sato. 1970. Plant and soil in a lowland rice field added with forage residues. Rep. Inst. Agric. Res. Tahoku Univ. 21:79-101.