# DECOMPOSITION OF $^{14}C$-LABELED LIGNINS, MODEL HUMIC ACID POLYMERS, AND FUNGAL MELANINS IN ALLOPHANIC SOILS

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**Summary**—During 1 yr, $CO_2$-C losses from 7 agricultural soils containing 0.5–1.5% organic C ranged from 70 to 243 mg 100 $g^{-1}$ while losses from three allophanic soils containing 4.9–8.9% organic C varied from 92 to 191 mg. Losses as $^{14}CO_2$ from ring-labeled model and cornstalk lignins averaged about 30% from the agricultural soils compared to about 11% for the allophanic soils. Losses of 2-side chain lignin carbons were about the same as for the ring carbons. Carbon losses from 1-side chain and methoxyl C varied from 42 to 59% in the normal soils while losses from the allophanic soils were a third to a half these values. From 6 to 9% of protein, cysteine, lysine, and glucosamine carbons linked into model humic acid polymers were lost during 1 yr in the allophanic soils compared with 13–24% from the normal soils. Comparable losses from two fungal melanins were 7–15% for the normal soils and 2–4% for the allophanic soils.

## INTRODUCTION

Zunino *et al.* (1982b) compared the biodegradation of a variety of readily-available organic substrates in normal agricultural soils of Chile and California with allophanic soils of southern Chile, and a sandy soil to which an allophanic concentrate was added. During 4 months C losses from readily-biodegradable plant and microbial polysaccharides ranged from 70 to 78% in the normal soils. The presence or additions of allophane reduced losses by 36–67% indicating a marked stabilization effect of this clay. Similar effects were noted with protein and plant and microbial residues. Carbon losses from catechol and ferulic acid were more related to the reactivity of the phenols, the soil pH and the organic matter content of the soil, than to the presence or absence of allophane. Our objective was to compare the amount of degradation over 1 yr of a variety of more resistant residues and polymers in the allophanic and normal soils, and to compare the loss of $CO_2$ from both types of nonamended soils.

## METHODS

The synthesis of specifically $^{14}C$-labeled ferulic and coumaric acids, used for labeling the cornstalk lignins, and of coumaryl and coniferyl alcohols for synthesizing the model lignins were described by Haider (1966) and Haider *et al.* (1977). The model lignins were prepared according to the method of Freudenberg and coworkers (Freudenberg, 1968; Harkin, 1973) using purified horseradish peroxidase (1232 units $mg^{-1}$) obtained from United States Nutritional Biochemicals Inc. To label the lignin of corn plants (*Zea mays*), 1 ml of solution containing 1.5 mg labeled ferulic or coumaric acid was injected into the base of young corn stalks with a syringe with the piston removed. The liquid was taken up by the plants within 2–3 days. This procedure was repeated 3 times during a 2-week period. The plants were allowed to grow for another 3 weeks. The tops were extracted three times with 80% boiling ethanol to remove low molecular weight material, and dried (Martin and Haider, 1977).

To prepare the $^{14}C$-labeled fungal melanins, *Hendersonula toruloidea* and *Aspergillus glaucus* cultures were grown in a base mineral medium containing 4.5 g $NaNO_3$, 1.0 g $K_2HPO_4$, 1.0 g $KH_2PO_4$, 0.3 g KCl, and 0.5 g $MgSO_4 \cdot 7H_2O$ $l^{-1}$ and 30 g $l^{-1}$ of uniformly $^{14}C$-labeled glucose. The medium, in 150 ml portions, was placed in 920 ml prescription bottles and sterilized at 120°C for 30 min but the glucose was sterilized separately and added to the sterile media before inoculation. Each flask was inoculated with 1 ml of spore suspension from a 5 to 10 day fungus culture on potato–dextrose agar. The bottles were incubated on their flat sides to form a large surface area and a thin layer of medium. All flasks were incubated until the medium became dark brown to black due to accumulation of melanin. It generally took 5–6 weeks for *H. toruloidea* and 3 months for *A. glaucus* before recovery of the melanins (Linhares and

Table 1. Loss of total C as $CO_2$ during incubation of some soils of Chile and California for 1 yr (Cumulative $CO_2$-C mg 100 $g^{-1}$ oven-dry soil)

| Soil[1] | Organic C (%) | Weeks of incubation 1 | 4 | 8 | 12 | 28 | 52 |
|---|---|---|---|---|---|---|---|
| | | California | | | | | |
| Steinbeck loam | 1.5 | 15 | 35 | 61 | 89 | 154 | 216 |
| Fallbrook S. L. | 0.9 | 11 | 9 | 57 | 70 | 114 | 143 |
| Coachella S. L. | 0.6 | 3 | 14 | 26 | 39 | 62 | 88 |
| Holtville S. C. L. | 0.5 | 3 | 11 | 25 | 37 | 59 | 85 |
| Greenfield S. L. | 1.2 | 16 | 34 | 59 | 88 | 152 | 218 |
| | | Chile | | | | | |
| Normal agricultural soils | | | | | | | |
| Pillan loam | 1.5 | 11 | 28 | 49 | 66 | 162 | 243 |
| Lo Aguirre S. L. | 0.6 | 2 | 7 | 14 | 18 | 56 | 70 |
| Allophanic soils | | | | | | | |
| Puerto Octay topsoil | 8.9 | 23 | 51 | 69 | 82 | 142 | 191 |
| Puerto Octay subsoil | 4.9 | 9 | 18 | 28 | 32 | 74 | 92 |
| Corte Alto topsoil | 8.1 | 14 | 32 | 47 | 56 | 101 | 144 |

[1] S = sandy; C = clay; L = loam.

Martin, 1978). The *H. toruloidea* melanin is a phenolic polymer with linked peptides. The *A. glaucus* melanin contains anthraquinones as well as phenols.

An adaptation of the peroxidase method for synthesizing the model lignins was used to prepare the humic acid polymers (Martin and Haider, 1980). The reaction mixture for each polymer consisted of 1.5 mM each of 2,3-, 2,6- and 3,4-dihydroxytoluene, catechol, orcinol, phloroglucinol, pyrogallol, resorcinol, and 2,4-dihydroxybenzoic, 3,5-dihydroxybenzoic, 2,3,4-trihydroxybenzoic, 2,4,6-trihydroxybenzoic, caffeic, ferulic, gallic, protocatechuic, vanillic, and *p*-hydroxycinnamic acids plus 16 mg of purified peroxidase from horseradish and 1 g of $^{14}C$-labeled protein, cysteine, lysine, or glucosamine. The labeled amino acids and glucosamine were purchased commercially. *Chlorella pyrenoidosa* protein was prepared according to the method of Verma *et al.* (1975).

The model phenolic polymers and the fungal melanins are similar to soil humic acids with respect to elemental composition, high exchange acidity, phenols released upon Na-amalgam reductive degradation, organic fragments released by pyrolysis, oxidative degradation, and resistance to biodegradation (Luther and Lipke, 1980; Martin and Haider, 1971; Martin *et al.*, 1974; Meuzelaar *et al.*, 1977; Mathur and Schnitzer, 1978; Schnitzer *et al.*, 1973).

The soils used, the incubation procedures, and all methods of analysis were as described by Zunino *et al.* (1982b). The fresh soils collected from the field were air-dried, sieved (<2 mm), and weighed in 100 g portions into 250 ml Erlenmeyer flasks. The lignins, fungal melanins, and model humic acids were added at 1000 $\mu g\ g^{-1}$, and thoroughly mixed with the soil. The total $^{14}C$-labeled activity added to each flask varied from about 100,000 to 300,000 dis $min^{-1}$ 100 $g^{-1}$ of soil. The mixtures were adjusted to a moisture content of 60% of the water holding capacity (33 kPa or −1/3 bar). The flasks were connected to a closed system and aerated with a constant stream of air which was humidified and freed from $CO_2$ by passage through 2 M KOH solution. Flask temperatures were maintained at 22°C ± 2. All treatments, including controls, were duplicated.

## RESULTS AND DISCUSSION

During the first 8 weeks of incubation C losses as $CO_2$ from the allophanic top soils with C contents of 8.1 and 8.9% averaged slightly higher than from three normal agricultural soils with C contents of 1.2–1.5% (Table 1). After 1 yr C losses from the allophanic top soils averaged 167 mg while that from the three normal soils was 226 mg 100 $g^{-1}$ dry soil. At 1 yr C losses from the four normal agricultural soils with C contents of 0.5–0.9% ranged from 70 to 143 mg compared to 92 mg 100 $g^{-1}$ for the allophanic subsoil with a C content of 4.9%. These observations indicate that the humus in the allophanic soils is more resistant than that in the normal agricultural soils, and is in agreement with the observations of Broadbent *et al.* (1964) and of Munevar and Wollum (1977). The relatively higher rate of $CO_2$ evolution from the allophanic soils during the early stages of incubation was probably related to a greater quantity of microbial cells killed during the drying process before the soils were moistened and incubated.

The model lignins were relatively resistant to decomposition in all soils but throughout the entire incubation 2-side chain and ring-C losses averaged about 3 times as great from the normal agricultural soils compared to the allophanic soils (Table 2). After 1 yr, 20–28% of these carbons had evolved as $CO_2$ from the agricultural soils while only about 10% loss occurred from the allophanic topsoil and about 6% from the subsoil. The losses of —$OCH_3$ lignin carbon as $CO_2$ were much higher, 42–59% from the normal soils and 29–46% from the allophanic soils. It appears that even though ring and 2-side chain carbons of the lignin or altered lignin molecules were stabilized to a marked degree the soil organisms were still able to metabolize the —$OCH_3$ carbons of the polymers.

The lignin fraction of cornstalks decomposed a little faster during the 1 yr than the model lignins. The C loss from the 2 side chain and ring carbons varied from 26 to 38% in the normal agricultural soils. Loss values for the —$OCH_3$—C were higher and varied from 42 to 59%; values similar to those noted by Martin and Haider (1979) for California soils. Also the losses from the acid Steinbeck soil were usually a

Table 2. Decomposition of specific model lignin and cornstalk lignin carbons in some normal agricultural soils of Chile and California and in an allophanic topsoil and subsoil from Chile

| Label | Soil | Percentage of $^{14}C$ evolved as $CO_2$ | | | | | |
|---|---|---|---|---|---|---|---|
| | | 4 | 28 | 52 | 4 | 28 | 52[1] |
| | | Model lignin | | | Cornstalk lignin | | |
| 2-$^{14}C$ | Steinbeck loam | 3 | 16 | 26 | 8 | 25 | 33 |
| | Greenfield s. loam | 5 | 19 | 28 | 7 | 28 | 33 |
| | Pillan loam | 4 | 13 | 20 | 12 | 30 | 38 |
| | Puerto Octay topsoil | 1 | 5 | 9 | 7 | 20 | 27 |
| | Puerto Octay subsoil | <1 | 3 | 5 | 1 | 8 | 14 |
| $O^{14}CH_3$ | Steinbeck loam | 6 | 31 | 42 | 10 | 32 | 42 |
| | Greenfield s. loam | 12 | 46 | 56 | 20 | 49 | 58 |
| | Pillan loam | 25 | 50 | 59 | 16 | 47 | 59 |
| | Puerto Octay topsoil | 13 | 38 | 46 | 2 | 9 | 19 |
| | Puerto Octay subsoil | 1 | 17 | 29 | 1 | 8 | 17 |
| Ring-$^{14}C$ | Steinbeck loam | 3 | 16 | 26 | 7 | 20 | 26 |
| | Greenfield s. loam | 6 | 20 | 27 | 7 | 30 | 36 |
| | Pillan loam | 6 | 17 | 24 | 7 | 26 | 37 |
| | Puerto Octay topsoil | 1 | 6 | 11 | 1 | 7 | 14 |
| | Puerto Octay subsoil | <1 | 3 | 6 | 1 | 8 | 15 |

[1] Weeks.

little less than from the neutral soils. The cornstalk lignin carbon losses from the allophanic soils were reduced by about 23–61% compared with normal soils. The greatest reduction occurred in the allophanic subsoil.

The decomposition of protein, cysteine, lysine, and glucosamine units linked into model humic acid polymers is summarized in Table 3. Even in the normal agricultural soils these units were highly resistant to degradation. From 13 to 25% of the C was released over the 1 yr period. These values compare with 26–38% losses from the ring and 2 side chain carbons of lignin. The C losses from the model polymers in the allophanic soils were greatly reduced. Only 6–10%

Table 3. Decomposition of $^{14}C$-labeled protein, cysteine, lysine, and glucosamine linked into model humic acid polymers in some normal agricultural soils of Chile and California and in two allophanic soils of southern Chile

| Soil | Percentage of $^{14}C$ evolved as $CO_2$ | | | | | |
|---|---|---|---|---|---|---|
| | 4 | 8 | 12 | 26 | 39 | 52[1] |
| | U-$^{14}C$-labeled protein | | | | | |
| Steinbeck loam | 5 | 7 | 8 | 10 | 12 | 13 |
| Fallbrook sandy loam | 6 | 7 | 8 | 10 | 12 | 14 |
| Greenfield sandy loam | 6 | 9 | 11 | 13 | 14 | 15 |
| Coachella sandy loam | 8 | 10 | 12 | 15 | 18 | 20 |
| Holtvile sandy clay loam | 8 | 10 | 12 | 15 | 17 | 19 |
| Pillan loam | 4 | 8 | 14 | 15 | 17 | 20 |
| Puerto Octay topsoil | 1 | 1 | 4 | 7 | 9 | 10 |
| Corte Alto topsoil | 1 | 1 | 4 | 7 | 9 | 10 |
| | 3-$^{14}C$-labeled cysteine | | | | | |
| Steinbeck loam | 8 | 12 | 14 | 16 | 18 | 19 |
| Greenfield sandy loam | 7 | 10 | 12 | 14 | 15 | 16 |
| Pillan loam | 4 | 6 | 8 | 14 | 16 | 18 |
| Puerto Octay topsoil | <1 | 1 | 2 | 4 | 5 | 6 |
| Corte Alto topsoil | <1 | 1 | 2 | 5 | 6 | 7 |
| | U-$^{14}C$-labeled lysine | | | | | |
| Greenfield sandy loam | 8 | 11 | 13 | 17 | 20 | 22 |
| Pillan loam | 8 | 10 | 12 | 18 | 22 | 24 |
| Puerto Octay topsoil | 1 | 2 | 3 | 6 | 8 | 9 |
| Corte Alto topsoil | 1 | 2 | 3 | 5 | 6 | 7 |
| | U-$^{14}C$-labeled glucosamine | | | | | |
| Greenfield sandy loam | 9 | 13 | 15 | 17 | 19 | 20 |
| Pillan loam | 8 | 9 | 11 | 16 | 20 | 23 |
| Puerto Octay topsoil | 1 | 3 | 4 | 5 | 8 | 9 |
| Corte Alto topsoil | 1 | 3 | 4 | 5 | 8 | 9 |

[1] Weeks.

Table 4. Decomposition of U$^{14}$C-labeled melanins from *A. glaucus* and *H. toruloidea* in normal agricultural soils of Chile and California and in two allophanic top soils of Chile

| Soil | Percentage of $^{14}$C evolved as $CO_2$ | | | |
|---|---|---|---|---|
| | 4 | 12 | 26 | 52[1] |
| | *A. glaucus* melanin | | | |
| Steinbeck loam | 3 | 5 | 6 | 8 |
| Fallbrook sandy loam | 3 | 5 | 7 | 9 |
| Greenfield sandy loam | 2 | 4 | 5 | 9 |
| Coachella sandy loam | 3 | 9 | 10 | 14 |
| Holtvile sandy clay loam | 7 | 11 | 13 | 15 |
| Pillan loam | 1 | 4 | 6 | 9 |
| Puerto Octay topsoil | <1 | <1 | 1 | 3 |
| Corte Alto topsoil | <1 | <1 | 1 | 2 |
| | *H. toruloidea* melanin | | | |
| Steinbeck loam | 2 | 3 | 4 | 7 |
| Greenfield sandy loam | 3 | 5 | 6 | 9 |
| Pillan loam | 2 | 5 | 8 | 10 |
| Puerto Octay topsoil | <1 | 1 | 2 | 4 |
| Corte Alto topsoil | <1 | 1 | 3 | 4 |

[1] Weeks.

losses were observed which represents an average reduction of over 50% compared to normal agricultural soils.

When amino acids and proteins are applied in the free state to soils over 80% of the C is evolved as $CO_2$ in 1–3 months (Verma *et al.*, 1975). Although they are highly resistant to biodegradation when linked into model humic polymers they still are degraded a little faster than the aromatic units in the polymers (Martin *et al.*, 1972; Martin and Haider, 1979).

The two fungus humic acid-type melanins were also highly resistant to decomposition (Table 4). In two desert soils, Coachella sandy loam and Holtville sandy clay loam, only 14 and 15% of the applied C respectively, was evolved as $CO_2$. In the other agricultural soils the losses were still less, 7–9%. In the two allophanic top soils the melanins were extremely resistant to biodegradation. Only 2–4% of the melanin carbons were lost as $CO_2$ which represent about a 50–75% reduction from the Steinbeck, Fallbrook, Greenfield, and Pillan soils, and a greater than 70% reduction compared with the losses from the two California desert soils.

These tests involving a 1 yr incubation indicate that, in general, more resistant-type organic polymers are stabilized to even a greater degree in allophanic soils than are more readily available organic substrates (Zunino *et al.*, 1982b). The results are in agreement with the suggestion of Broadbent *et al.* (1964) that the more resistant products formed during the latter periods of humification are stabilized to the greatest extent in allophanic soils. The stabilization, as with other soils (Jenkinson, 1971; Martin *et al.*, 1978), is not related to the humus content as the addition of allophanic material in which the humus was destroyed with $H_2O_2$ reduced biodegradability of the substrates as shown by Zunino *et al.* (1982b). Also the chemical nature of humus of normal soils and allophanic soils are similar (Griffith and Schnitzer, 1975a).

Wada and Aomine (1975) reported that the mean residence time of humus in volcanic ash derived soils commonly ranged from 2000 to 5000 yr. The stability of the humus, and the decomposition tests summarized, indicate that the allophanic clay interacts very strongly with active groups on the organic colloids, possibly through metal ions, to form resistant complexes as concluded by Griffith and Schnitzer (1975b) and others. These results also confirm the hypothesis that allophanic soils in Chile, in their undisturbed state, represent mature ecosystems (Zunino *et al.*, 1982a).

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