

EVIDENCE OF LIGNIN RESIDUES IN HUMIC ACIDS ISOLATED FROM

VERMICOMPOSTS

C. SAIZ-JIMENEZ, Instituto de Recursos Naturales y Agrobiologia,
C.S.I.C., Apartado 1.052, 41080 Sevilla, Spain

N. SENESI, Instituto di Chimica Agraria, Universita degli Studi,
Via G. Amendola 165/A, 70100 Bari, Italy

J.W. DE LEEUW, Faculty of Chemical Engineering and Material
Sciences, Delft University of Technology, Organic
Geochemistry Unit, de Vries van Heijstplantsoen 2,
2628 RZ Delft, The Netherlands.

ABSTRACT

Three vermicomposts derived from animal manures exposed to the action of the earthworms Eisenia foetida or Lumbricus rubellus were investigated to determine the nature of the humic acid fraction in relation to the potential fertility of the vermicomposts. The isolated humic acid fractions were analysed by pyrolysis-gas chromatography-mass spectrometry. The greater part of the humic acids consisted of lignin and/or lignin residues as revealed by the very prominent and characteristic methoxy- and dimethoxy-phenols pyrolysis products. The chromatograms of the pyrolysates are similar to those of grass lignin. This points to an incomplete degradation or selective preservation of grass lignin during earthworm composting. The results further indicate that neither the quality nor the quantity of the humic acids has changed considerably by vermicomposting.

INTRODUCTION

Vermicomposting is the biological transformation of organic matter present in agricultural, urban and industrial wastes mediated by earthworms feeding on these wastes. This process is supposed to generate a useful material and to ameliorate the severe problems associated with the disposal of large quantities of organic wastes.

Earthworms play an important role in processing organic matter in nature. Darwin (1881) already pointed out that earthworms are essential components of soils because they macerate litter materials thus mixing it with the soil. This phenomenon can be observed by inspection of worm casts which contain more organic matter and more of the smaller particle size fractions than the surrounding soil.

The interest in vermicomposting has increased over the last years due to a shortage of natural organic fertilizers and the

necessity of organic amendment in most of Southern Europe's soils which have a very low carbon content due to climatic conditions and extensive and milenary culturing.

Vermicomposts contain up to 17 per cent alkali-extractable, acid precipitable humic acids (Hervas et al., unpublished data). This paper deals with the chemical characterization of vermicompost humic acids applying analytical pyrolysis. This analytical approach provides data on structural moieties in macromolecular materials and avoids problems encountered in wet chemical degradation methods due to lengthy and tedious procedures.

MATERIALS AND METHODS

Three different types of vermicomposts, commercially available in Spain, were studied. Animal manures, the parent materials, were composted for at least three months with the earthworms Eisenia foetida or Lumbricus rubellus.

The vermicomposts are darkly coloured, show a well decomposed state and a fine texture and are not polluted with metals, plastics, fabrics or glass.

The humic acids were extracted with a cold solution of 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ and NaOH (1:1) under N_2 (Saiz-Jimenez et al., 1979). The elemental compositions and amounts of humic acids obtained are reported in Table 1.

Grass lignin (Bambusa ssp.) was kindly supplied by Dr. O. Faix, Hamburg, F.R. Germany.

One hundred mg of humic acid of sample B (Table 1) was extracted 5 times with 10 ml acetone-water (9:1 v/v) by sonication for 10 min. The extract after evaporation of the solvent weighed 38 mg. The humic acid was subsequently extracted with 10 ml dichloromethane-methanol (2:1) and another 11 mg of extract was thus obtained. The residual amount of humic acid accounted for 51 mg.

Pyrolysis-gas chromatography was performed with a Curie point pyrolysis unit described by van der Meent et al. (1980). The pyrolysis units were mounted in the injection blocks of a Packard Becker 419 gas chromatograph and in a Varian 3700 gas chromatograph of a pyrolysis-gas chromatography-mass spectrometry system. The pyrolysates were separated on a 25 m. fused silica column (i.d. 0.26 mm) coated with DB-1701 (film thickness 0.25 μ m). The GC oven was programmed from 30°C to 290°C at a rate of 3°C/min. Gas chromatography-mass spectrometry was performed with a MAT 44 quadrupole mass spectrometer operated in the EI mode at 80 eV and with a cycle time of 2 sec.

RESULTS AND DISCUSSION

Table 1 shows the yields and elemental composition of the three humic acids isolated from different vermicomposts. The elemental compositions are almost identical and they are similar to those reported for humic acids extracted from soils from widely different climatic zones (Schnitzer, 1977). From these data it is, however, impossible to detect structural similarities and dissimilarities between vermicomposts and soil humic acids.

Over the last years analytical pyrolysis had been demonstrated to be a suitable method for the chemical characterization of high molecular weight substances, providing detailed information of the structural moieties present in the macromolecular assemblages (Nip et al., 1986; Saiz-Jimenez and de Leeuw, 1987 c). Since data of investigations concerning soil humic acids characterization using this method have been reported (Saiz-Jimenez and de Leeuw, 1986 a, 1987 b) a comparison with pyrolysis data of vermicompost humic acids is possible.

Figure 1 shows the chromatogram of the pyrolysis products of humic acid from vermicompost B. Peak numbers refer to the compounds identified and listed in Table 2. All three humic acids generated the same pyrolysis products with no significant quantitative differences. The major pyrolysis products produced

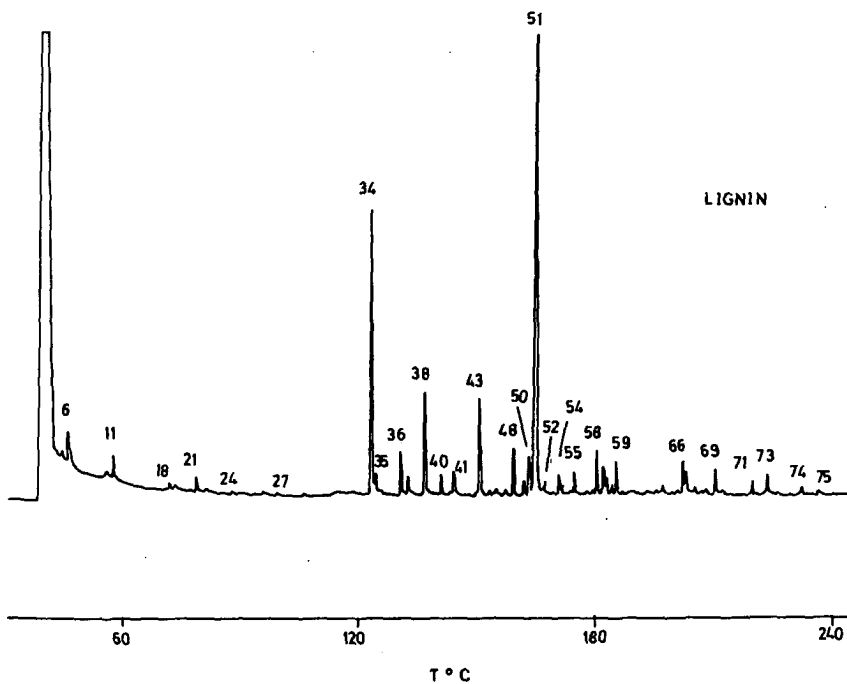
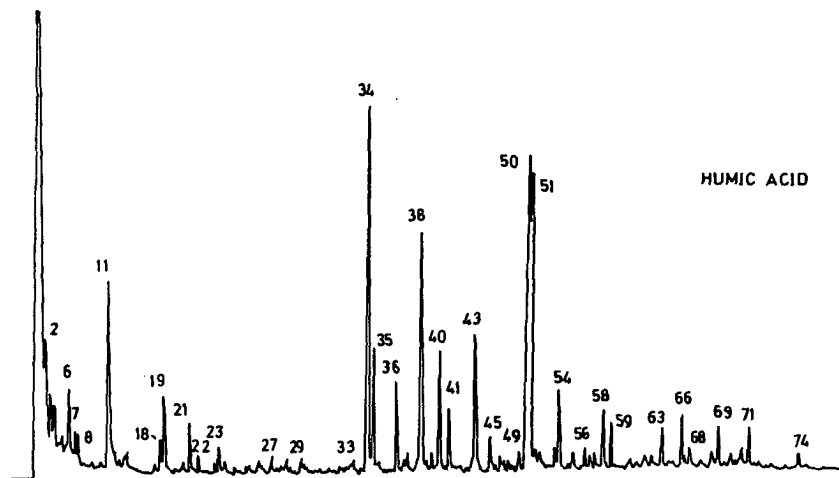


Figure 1. Pyrolysis gas chromatography trace of humic acid from vermicompost B.

Figure 2. Pyrolysis gas chromatography trace of grass lignin (Bambusa spp.)

from these humic acids are highly characteristic of lignins, especially grass lignin (Saiz-Jimenez and de Leeuw, 1986 b). The compounds identified in the pyrolysates of this grass lignin are the same as those in the pyrolysates of the humic acids, phenol (34) and vinylphenol (51) being predominant. This was further substantiated by analysis of Bambusa grass lignin pyrolysed under exactly the same conditions (Figure 2). The major compounds identified in the pyrolysate of this lignin are indeed dominantly present in the pyrolysates of the vermicompost humic acids and the distribution patterns of the pyrolysis products show a high degree of similarity.

The somewhat more abundant presence of quaiacol (35), methylguaiacol (40), vinylguaiacol (50) and 2,6-dimethoxyphenol (54) in the pyrolysates of the humic acids when compared with the relative intensities of these compounds in the grass lignin pyrolysate can be explained easily. Grass lignin contains *p*-hydroxyphenyl units esterified with guaiacyl-syringyl lignin (Higuchi et al., 1967). It is expected that a significant amount of these bonds are cleaved during saponification with cold alkali (Sarkanen and Hergert, 1979) during the extraction and purification process of the humic acids. As a result the *p*-hydroxyphenyl units may end up in other fractions and the relative amount of *p*-hydroxyphenyl pyrolysis products is thus reduced in comparison with guaiacyl and syringyl pyrolysis products. Alternatively, partial hydrolysis of the ester bonds might have taken place already in the digestive tracts of the ruminants.

In addition to the lignin pyrolysis products several nitrogen-containing compounds were identified in low amounts. They are typical protein/peptide pyrolysis products. Also a few furan derivatives, 3-methylpyrocatechol and prist-1-ene were present. Furans are common in pyrolysates of carbohydrates and prist-1-ene is probably a pyrolysis product of tocopherols (Goossens et al., 1984). 3-Methylpyrocatechol has recently been identified as a major pyrolysis product of a fungal melanin (Saiz-Jimenez and de Leeuw, unpublished data). All these

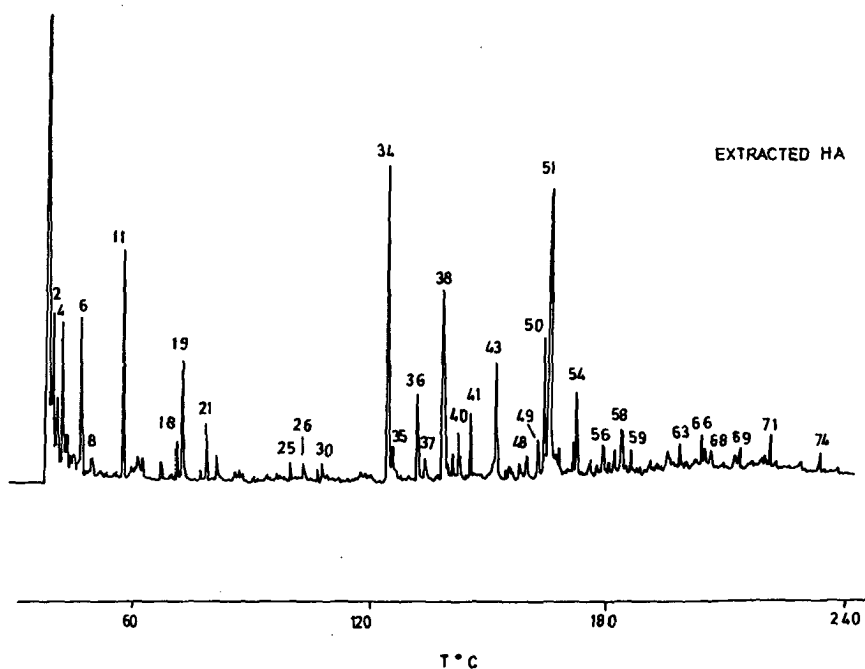
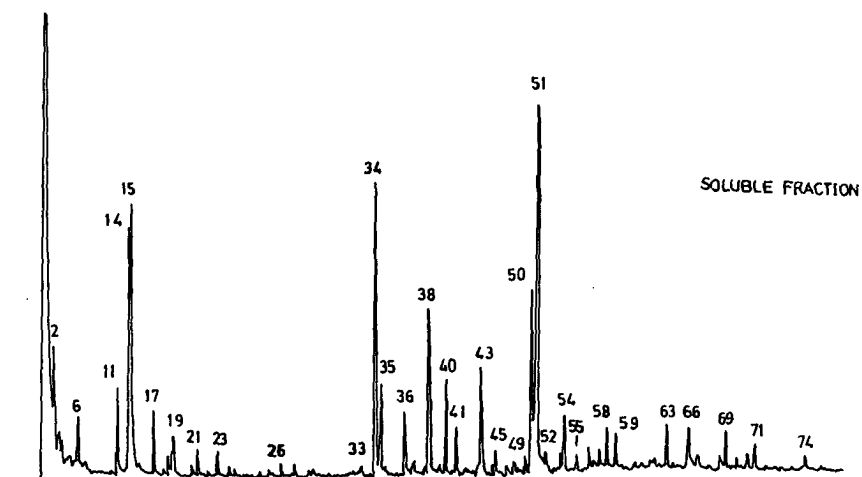


Figure 3. Pyrolysis gas chromatography trace of acetone-water soluble fraction.
 Figure 4. Pyrolysis gas chromatography trace of solvent extracted humic acid.

pyrolysis products point to the presence of minor amounts of proteins, carbohydrates, tocopherols and some microbially synthesized phenols.

To further substantiate the dominant presence of lignins in these humic acid fractions, the vermicompost humic acid isolated from sample B was extracted with acetone-water, a common solvent for lignins (Freundenberg, 1968). The chromatogram of the pyrolysis products of this acetone-water extract is shown in Figure 3. Figure 4 represents the residual humic acid after acetone-water and dichloromethane-methanol extraction. The chromatogram of the pyrolysis products of the dichloromethane-methanol extract (not shown) was similar to that of the acetone-water extract. In summary, the four traces are similar to one another. This probably means that the so-called humic acids from vermicomposts mainly consist of slightly altered grass lignin with only secondary contribution of other origin.

The origin of this lignin seems to be clear. The dietary ingestion of ruminants is based on grasses and other herbaceous plants. They contain lignocellulosic complexes which are not or virtually not metabolized either by the animal or by the microbial gut flora. The predominant bacterial strains in the gut flora of earthworms are facultative anaerobes, from which 73 per cent belong to the genus Vibrio and none of the isolated strains were able to degrade lignin as demonstrated by Marialigeti (1979). Neuhauser et al. (1978) stated that neither Eisenia foetida nor its faecal bacteria are capable to depolymerize lignin and degrade its aromatic constituents. The influence of other bacteria present during the vermicomposting process seems to have been limited as well with respect to lignin degradation.

The vermicompost humic acids cannot be compared with those of soil humic acids previously studied (Saiz-Jimenez and de Leeuw, 1986 a; 1987 a, b). The differences are obvious; upon pyrolysis soil humic acids generate a wide range of components related to polysaccharides, proteins, lignins, and an aliphatic biopolymer, as well as alkylbenzenes, alkyl-naphthalenes, polycyclic aromatic hydrocarbons and lipids. The main pyrolysis products generated

from vermicompost humic acids are almost exclusively derived from lignins as proved above.

From the results presented here it is clear that the potential fertilizing capacity of vermicomposts cannot be predicted by simple determinations such as weight percentages of humic acids. This is caused by the fact that the humic acids are operationally defined in terms of acid-base solubility and not by chemical structural criteria. Therefore, the prediction of the nature and properties of humic acids can only be based on more detailed structural analyses such as pyrolysis in combination with gas chromatography and gas chromatography-mass spectrometry, ^{13}C NMR, FT-IR and, in some cases, by data obtained from chemical degradations.

TABLE 1

Elemental composition of vermicompost humic acids.

<u>sample</u>	<u>origin</u>	<u>%</u>						
		<u>yield</u>	<u>C</u>	<u>H</u>	<u>N</u>	<u>S</u>	<u>O</u>	<u>ash</u>
A	mixed manures	6.0	52.6	5.3	4.7	1.5	35.9	2.7
B	cow manure	17.2	54.3	5.5	4.3	1.3	34.6	2.6
C	animal manure	5.0	55.6	5.6	4.2	1.3	33.3	5.7

TABLE 2

Pyrolysis products of vermicompost humic acids

1	Butyne	39	Benzeneacetonitrile
1	Butene	40	Methylguaiacol
1	Methanethiol	41	o-Xylenol
2	Furan	42	Methylnaphthalene
3	Acrylonitrile	43	p-Xylenol
4	Methylfuran	44	Methylnaphthalene
5	Hexadiene	45	Ethylguaiacol
6	Benzene	46	C ₃ -Alkylphenol
7	Ethylfuran	47	C ₃ -Alkylphenol
8	2,5-Dimethylfuran	48	C ₃ -Alkylphenol
9	Dimethylfuran	49	C ₃ -Alkylphenol
10	C ₇ H ₁₀	50	Vinylguaiacol
11	Toluene	51	Vinylphenol
12	Methylthiophene	52	Eugenol
13	Pyrrrole	53	Dimethylnaphthalene
14	Furfurylalcohol	54	2,6-Dimethoxyphenol + Indole
15	C ₃ -Alkylfuran	55	<u>cis</u> -Isoeugenol
16	Pyridine	56	3-Methylpyrrolcatechol
17	Benzenemethanol	57	Terephthalaldehyde
18	Ethylbenzene	58	<u>trans</u> -Isoeugenol
19	o-Xylene		Methylindole
20	p-Xylene	59	Methyl-2,6-dimethoxyphenol
21	Styrene	60	Vanillin
22	C ₃ -Alkylbenzene	61	C ₃ -Alkylnaphtalene
23	C ₃ -Alkylbenzene	62	Ethyl-2,6-dimethoxyphenol
24	C ₃ -Alkylbenzene	63	Prist-1-ene
25	C ₃ -Alkylbenzene	64	Homovanillin
26	C _{3:1} -Alkylbenzene	65	Acetovanillin
27	C ₃ -Alkylbenzene	66	Vinyl-2,6-dimethoxyphenol
28	C ₃ -Alkylbenzene	67	Guaiacylpropanone
29	Indene	68	Allyl-2,6-dimethoxyphenol
30	Benzonitrile	69	<u>cis</u> -Propene-2,6-dimethoxyphenol
31	C _{4:1} -Alkylbenzene	70	Coniferaldehyde
32	C _{4:1} -Alkylbenzene	71	<u>trans</u> -Propene-2,6-dimethoxyphenol
33	C _{4:1} -Alkylbenzene	72	Biphenol
34	Phenol	73	Syringaldehyde
35	Guaiacol	74	Ethanone-2,6-dimethoxyphenol
36	o-Cresol	75	Propanone-2,6-dimethoxyphenol
37	Naphthalene		
38	p-Cresol		

REFERENCES

- Darwin, C.R. (1881) The formation of vegetable mould through the action of worms with observations on their habits. John Murray, London.
- Freudenberg, K. (1968) The constitution and biosynthesis of lignin. In: K. Freudenberg and A.C. Neish, Constitution and Biosynthesis of Lignin, Springer-Verlag, Berlin, pp. 47-122.
- Goossens, H., de Leeuw, J.W., Schenck, P.A. and Brassell, S.C. (1984) Tocopherols as likely precursors of pristane in ancient sediments and crude oils. *Nature* 312: 440-442.
- Higuchi, T., Ito, Y., Shimada, M. and Kawamura, I. (1967) Chemical properties of milled wood lignin of grasses. *Phytochemistry* 6: 1551-1556.
- Marialigeti, K. (1979) On the community structure of the gut-microbiota of Eisenia lucens (Annelida, Oligochaeta). *Pedobiologia* 19: 213-220.
- Meent, D. van der, Brown, S.C., Philp, R.P. and Simoneit, B.R.T. (1980) Pyrolysis-high resolution gas chromatography and pyrolysis-gas chromatography-mass spectrometry of kerogen and kerogen precursors. *Geochim. Cosmochim. Acta* 44: 99-113.
- Neuhauser, E.F., Hartenstein, R. and Connors, W.J. (1978) Soil invertebrates and the degradation of vanillin, cinnamic acid and lignins. *Soil Biol. Biochem.* 10: 431-435.
- Nip, M., Tegelaar, E.W., de Leeuw, J.W., Schenck, P.A. and Holloway, P.J. (1986) A new non-saponifiable highly aliphatic and resistant biopolymer in plant cuticles. Evidence from pyrolysis and ¹³C-NMR analysis of present-day and fossil plants. *Naturwissenschaften* 73: 579-585.
- Saiz-Jimenez, C. and de Leeuw, J.W. (1986 a) Chemical characterization of soil organic matter fractions by analytical pyrolysis-gas chromatography-mass spectrometry. *J. Anal. Appl. Pyrol.* 9: 99-119.

- Saiz-Jimenez, C. and de Leeuw, J.W. (1986 b) Lignin pyrolysis products: their structures and their significance as biomarkers. *Org. Geochem.* 10: 869-876.
- Saiz-Jimenez, C. and de Leeuw, J.W. (1987 a) Nature of plant components identified in soil humic acids. *Sci. Tot. Environ.* 62: 115-119.
- Saiz-Jimenez, C. and de Leeuw, J.W. (1987 b) Chemical structure of a soil humic acid as revealed by analytical pyrolysis. *J. Anal. Appl. Pyrol.* 11: 367-376.
- Saiz-Jimenez, C., Boon, J.J., Hedges, J.I., Hessels, J.W.C. and de Leeuw, J.W. (1987 c) Chemical characterization of recent and buried woods by analytical pyrolysis. Comparison of pyrolysis data with ^{13}C NMR and wet chemical data. *J. Anal. Appl. Pyrol.* 11: 437-450.
- Sarkanen, K.V. and Hergert, H.L. (1971) Classification and distribution. In: K.V. Sarkanen and C.H. Ludwig (Editors), *Lignins. Occurrence, Formation, Structure and Reactions*. Wiley-Interscience, New York, pp. 43-94.
- Schnitzer, M. (1977) Recent findings on the characterization of humic substances extracted from soils from widely differing climatic zones. In: *Soil Organic Matter Studies*, vol. II, I.A.E.A., Vienna, pp. 117-132.