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CHARACTERIZATION OF WASTES FROM OLIVE AND SUGARBEET PROCESSING INDUSTRIES AND EFFECTS OF THEIR APPLICATION UPON THE ORGANIC FRACTION OF AGRICULTURAL SOILS

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The lipidic fraction compositions of both concentrated vinasses, a by-product of the sugar industry, and a compost made basically from olive oil vegetation waters (alpechin) were studied. The alpechin lipids are composed mainly by series of *n*-alkanes and lineal and branched fatty acids, whereas the major lipids in vinasses were *n*-alkanes, *n*-alkanols and acetals. Concentrations and composition of lipids in both materials do not seem of concern.

Preliminary results on the effects of the application of both materials over two years on the organic status of an agricultural soil are also reported. No significant changes were observed in total organic carbon and contents in humic fractions and lipids before and after the applications. However, analysis by GC-MS of the lipid compounds present in bound forms in the subsoil layer revealed that some hydrophobic components were accumulated in the soil following the waste applications.

KEY WORDS: Vinasses, olive vegetation water, lipids, soils, agricultural wastes.

INTRODUCTION

Land application is recognized as an alternative method for ultimate disposal of liquid and solid wastes, and at the same time as a means of maintaining or increasing soil fertility. As a widely extended practice it has become today a topic of major interest for soil scientists, agronomists and environmentalists.

In recent decades, the demand for organic fertilizers in South Spain has increased, while their availability, in particular farmyard manures and other natural organic substrates, has decreased. For this reason, a number of organic wastes, such as urban refuse, sewage sludges and by-products from agricultural product treatments have been introduced as conditioners and low-grade fertilizers.

This is the case of olive mill effluents, the so-called 'alpechin', and vinasses, a by-product of the sugar industry, which are generated in great amounts and constitute a major environmental problem due to their extremely high BOD. In the past they were dumped into rivers and streambeds causing major ecological damage, but more recently are commonly disposed of in ponds and lagoons where they become concentrated during the hot summer periods. Several alternative uses for the resulting sludges, including anaerobic treatment for energy production or the utilization of

their nutritional properties have also been proposed^{1,2}. Their use as fertilizers, either directly or after composting, has also been suggested and carried out in practice, but so far the effects of their application on soil properties has not been systematically investigated.

In this context, a long-term study on the characteristics and fate of a commercial compost made from alpechin and concentrated vinasses is being carried out. Previous studies have shown that the application of these products do not affect *in vitro* seed germination and biomass production in greenhouse experiments^{3,4}.

The main purpose of this work is to obtain a more complete knowledge about these materials by studying the chemical characteristics of their organic matter, one of the more important factors regarding their potential agronomic value. Investigations have been focussed on their lipidic fractions, which contain hydrophobic constituents, which can be considered non-stabilized in comparison with other polymeric material detected in the olive vegetation water⁵.

Since lipid accumulation following the addition of fresh organic matter to soil may have significant effects upon some important soil properties, as reported for sewage sludge applications^{6,7}, investigations were made to determine whether the repeated application of the alpechin-compost and the vinasses could affect the status of the soil organic matter as occurs with other wastes^{8,9}. Our approach involved the isolation and characterization of lipids present in the soil—both free or adsorbed to the organo-mineral soil complex—before and after two years of applications.

MATERIAL AND METHODS

The origin and chemical characteristics of the commercial Alpechin-compost (A) and the concentrated vinasse (V) have been described elsewhere^{3,4,10}. Range of values for a number of analytical characteristics of both wastes are given in Table 1.

The wastes were applied as a function of their N contents at rates of 15 Tm Ha⁻¹ (A) and 3 Tm Ha⁻¹ (V) over the last two years to field plots located at the Aljarafe farm (Coria del Rio, Sevilla) on a Xerofluvent (1.5% C). Plots with no treatment and application of 350 Kg Ha⁻¹ of a 15-15-15 NPK mineral fertilizer were included for comparison. The plots were established in December 1988 and cropped in 1989 and 1990 with maize and barley. Waste applications were made each time one month prior to planting. The plots were laid out in a randomized complete block design with three replications.

Waste samples were lyophilized and then ground to obtain homogeneous, powdered (<2 mm) samples. To bring the concentrated vinasse, a syrupy liquid, to dryness it was necessary to dilute it several times and dialyze through cellophane to reduce its high ash content.

For the study of the lipidic fraction samples of V and A were Soxhlet extracted with 3:1 light petroleum:ethyl acetate mixture during 24 hours. Total extracts (1.8% V and 2.1–3.3% A) were chromatographed on neutral alumina (grade 1) and eluted successively with *n*-hexane and methylene chloride. The latter fractions were also methylated with diazomethane prior to the GC-MS study.

Table 1 Chemical characteristics of the wastes (*)

	Concentrated vinasse	Compost-alpechin
pH (H ₂ O)	4.6 – 5.3	7.4– 8.1
density (g/cc)	1.16– 1.35	—
dry matter %	33.0 –63.0	72.5–86.9
organic matter %	17.0 –39.8	14.5–33.8
E.C. (1:5) (mS/cm)	29.0 –29.5	2.9– 7.4
CaCO ₃ %	9.8 – 1.2	17.6–20.2
C/N	6.0 – 7.5	11.4–18.9
N total %	2.0 – 3.6	0.5– 1.5
P (P205) %	0.02– 0.04	0.1– 0.8
K (K20) %	2.1 – 4.6	1.2– 2.5
Ca %	0.1 – 0.5	4.9–12.2
Mg %	0.3 – 1.0	0.5– 0.8
Na %	1.4 – 3.0	0.1– 0.3
Cl %	1.8 – 2.2	0.1– 0.2
Fe ppm (mg/Kg)	15 –25	8100–10925
Cu ppm	5 –10	27–50
Mn ppm	16 –20	154–338
Zn ppm	26 –29	38–67
Cd, Ni, Pb, Cr, Hg ppm	< 5	< 5

* Range of values from various applications (References 3, 4, 10)

The procedures for the extraction of “humic fractions” and the determinations of sugars and polyphenols have been previously reported^{11–13}.

FT-IR and ¹³C-NMR spectra of the wastes were recorded as described in detail elsewhere¹⁴.

Soil samples were taken at two depths (0–25 and 30–45 cm), air-dried, ground and sieved through a 10 mesh screen prior to analysis. Both free and bound lipids were extracted from soils before and after the waste applications following the procedure outlined by Moucawi *et al.*¹⁵. Total extracts were separated into *n*-hexane and methylene chloride fractions prior to the GC and GC-MS analysis. A simplified schematic representation of the lipid isolation procedures is shown in Figure 1.

GC and GC-MS analysis were performed on Hewlett Packard instruments (5890 and 5988A) using a fused silica OV-101 capillary column (25 m × 0.2 mm i.d.). Samples were injected in the splitless mode, with the oven temperature programmed from 50°C (1 min) to 100°C at a rate of 32°C min⁻¹ and then from 100°C to 270°C at a rate of 6°C min⁻¹ with 15 min final hold. Nitrogen (GC) and helium (GC-MS) at a flow rate of 1 ml min⁻¹ were used as carrier gas. Mass spectra were measured at 70 eV ionizing energy.

The recognition of each compound was achieved using mass fragmentography (by key ion monitoring for characteristic peaks of different series) and low resolution mass spectrometry.

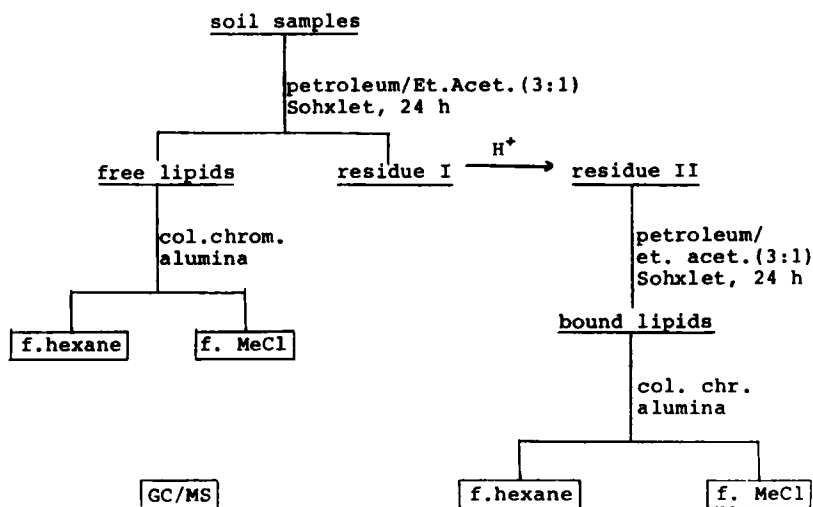


Figure 1 Isolation procedure for free and bound soil lipids.

RESULTS AND DISCUSSION

FTIR and high resolution ^{13}C -NMR spectra of the samples are shown in Figure 2.

As would be expected for complex molecular systems they are poorly resolved and show only broad absorption bands, with exception of three sharp bands in the region $700\text{--}1000\text{ cm}^{-1}$ in the spectrum of the Alpechin compost. These bands are typical of $(\text{CH}_2)_n$ in long chains (700 cm^{-1}) and cycloalkyl and cycloalkenyl derivatives (825 and 1000 cm^{-1}) and are indicative of the probable presence of complex mixtures of fats¹⁶ in the A-sample. Both spectra show broad OH absorption bands near 3400 cm^{-1} , and bands due in part to aromatic structures ($1600\text{--}1620\text{ cm}^{-1}$) and alkyl vibrations ($2920, 1420\text{ cm}^{-1}$). One striking difference between both spectra is the intensity of the band at 1720 cm^{-1} for carboxylic groups, which is well resolved in the V spectrum and negligible in the A-spectrum.

The NMR spectra reveals some structural features that were only vaguely defined in the IR spectra. They are usually analysed quantitatively by subdividing them into four major regions: aliphatic carbons in the range from 5 to 46 ppm, carbons of carbohydrate origin (resonances of O and N-substituted carbons) from 46 to 110 ppm, $\text{sp}^2\text{ C}$ atoms from aromatic rings or olefins between 110–160 ppm, and carbonyl-/carboxyl-groups in the range from 160 to 200 ppm. The relative concentrations of the four classes of carbons, as obtained from the integration of the spectra, are given on each spectrum in Figure 2. The structures of carbohydrate origin (49%) predominate in the V sample, while the most prominent area in the A is that of the aliphatic carbons (69%). In agreement with the IR data, resonances for carboxylic C atoms at about 173–176 ppm are only present in the spectrum of V.

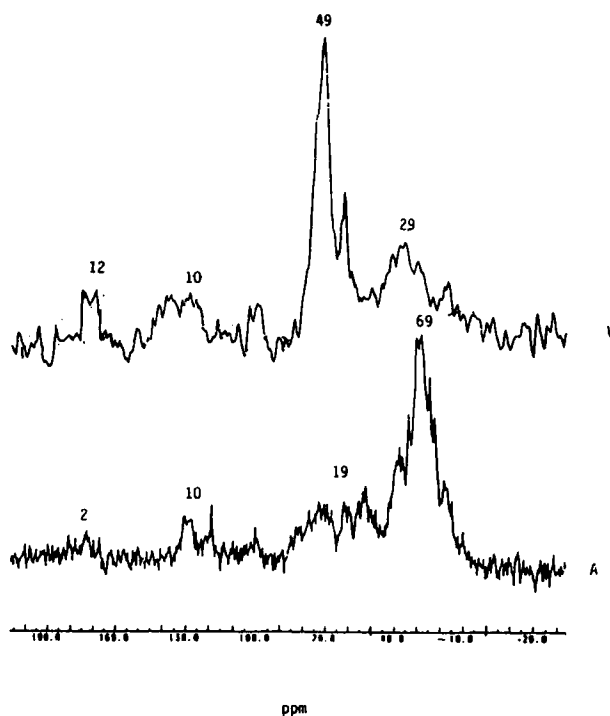
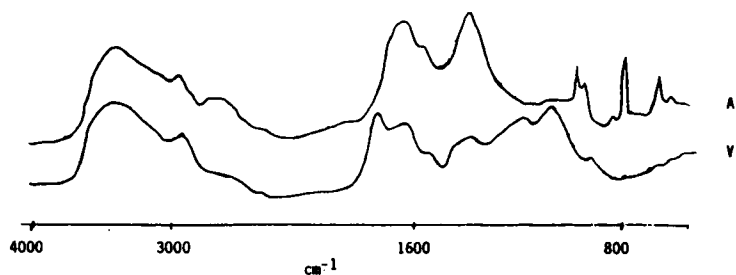


Figure 2 IR and ¹³C-NMR spectra of the vinasse (V) and alpechin-compost (A) samples.

Table 2 shows the range of values found for the quantitative distribution of organic fractions in the samples. The concentrations of sugar and phenolic compounds seem to be significant in samples V and A, respectively. Both wastes contain also relatively high amounts of humic fractions and lipids. In fact, from 2.1 to 3.3% of the total weight of the A sample and 1.8% of the V sample consisted of lipids, as they were extracted by the light petroleum:ethyl acetate solvent mixture. These values represent approximately 4.5% (V) and 12.5% (A) of the total organic carbon present in the whole materials.

Table 2 Organic composition of the wastes

	V	A
oxidizable OM %	14.9–18.0	17.5–23.8
humic acids (%)	2.3– 3.6	2.1– 2.8
fulvic acids %	7.5– 9.0	6.0– 7.3
red. sugars %	2.0– 2.8	0.1– 0.2
total sugars %	6.9– 7.3	0.2– 0.6
total polyphenols %	0.7– 0.8	3.5– 8.5
lipids	1.6– 1.9	2.1– 3.3
n-alkanes	+ C12–C20	+ + + C14–C32
n-alkanols	+ + C12–C24	+ C18–C22
acetals	+ +	–
n-fatty acids (FA)	–	+ + C16–C18
methyl branched FA	–	+ + + C14–C26

+, + +, + + +: low, medium and high abundance.

The qualitative composition of these lipids is very complex, as revealed by the total ion chromatograms (TIC) of the hexane and methylene chloride fractions of the sample extracts (Figure 3).

The identities and relative amounts of the different series of compounds detected in each extract are included in Table 2. The lipidic fraction of V consisted mainly of *n*-alkanes, *n*-alkanols and acetals, whereas the A sample is dominated by series of *n*-alkanes and linear and branched fatty acids.

The acetals are products of the reaction between aldehydes and alkanols, and showed typical mass spectra with extremely high abundance of the base peaks at *m/e* 73.

The *n*-alkanes in sample A showed a bimodal distribution with maxima at C₁₆ and C₂₇, indicative of microbial and plant origins¹⁷. In the vinasse lipids the alkanes exhibited a modal distribution with maximum at C₁₆.

Free and esterified *n*-C₁₆, *n*-C₁₈ and *n*-C_{18:1} fatty acids were the most important components in A. The former were detected in the non methylated methylene chloride fraction. Their presence is an indication of the incomplete removal of olive oil during the production process, since they are the major lipids present in olive oil.

In the hexane fraction of the A extract molecular sulfur was found, arising probably from the agricultural by-products (grapeseed residues, cotton wastes, branches and twigs pruned from olive trees, etc) mixed with alpechin before composting.

Several dialkyl phthalates (di-*n*-butyl-, di-*n*-octyl- and di-2-ethylhexyl-), well known as ubiquitous pollutants¹⁸, were present in both wastes in relatively high amounts. Metabolites of biocides of hexadecadien-ol and triazine type were also tentatively identified in the methylene chloride fraction of the vinasse extract¹⁹.

There were no peaks resulting from polynuclear aromatic compounds or polychlorinated biphenyls, although in the soil samples traces of methyl and methoxy fluorene were observed.

Table 3 shows quantitative data on TOC and different organic fractions in the soil before and after two years of wastes application. Although some significant

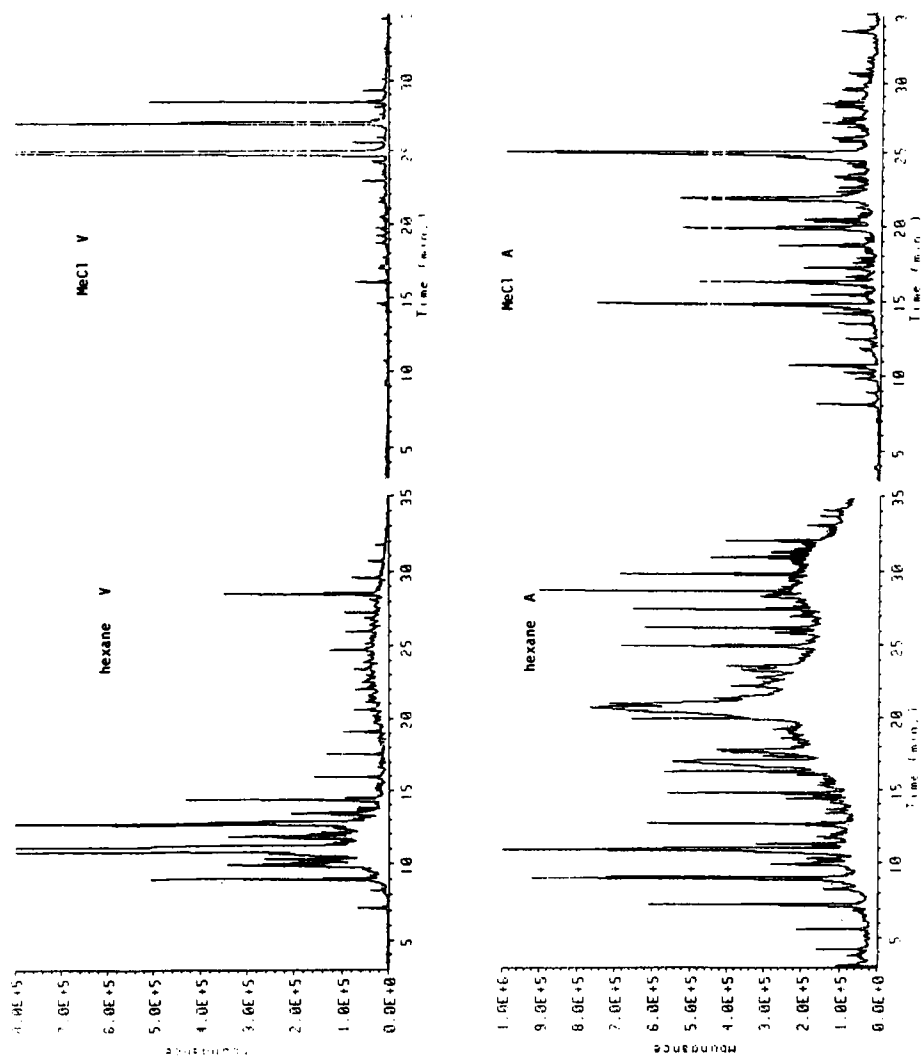


Figure 3 Total ion chromatograms of the hexane and methylene chloride (MeCl) fractions of the vinasse (V) and alpechin-compost (A) extracts.

Table 3 Distribution in soil of humic fractions and lipids after two years of wastes application (4 treatments) (*)

	0–25 cm			30–45 cm		
	No treat.	V	A	No treat.	V	A
TOC (%)	1.57 a	1.58 a	1.69 b	1.00 a	1.08 a	1.13 a
HA (%)	0.64 b	0.39 a	0.52 ab	0.39 b	0.52 c	0.31 a
FA (%)	0.18 a	0.17 a	0.19 a	0.10 a	0.17 b	0.10 a
Lipids (**)	6.0 a	19.0 b	21.0 b	4.0 a	15.0 b	18.0 c

(*) Values followed by different letters in the same row differ significantly ($P < 0.05$, test of Tukey).

(**) Lipids (free + bound, mg/100 g).

statistical differences in soil organic carbon or contents in humic fractions occurs in plots, they are not likely to have any agronomic value.

Although the percentage of lipids extracted from the original soil was very low (0.045–0.06%), falling into the lower limits of the wide range of values reported for soils of various geographical origin^{20–22}, the application of the wastes has led to only slight increases of lipids in the soil. In fact, this could be expected, since taking into account the maximum contents of total lipids in the samples V and A, the successive applications add only low amounts of lipids ($< 1000 \text{ mg kg}^{-1}$) to the soil upper layer. In addition, it is possible that the soil humic acids (not studied here) could retain lipidic compounds in its structure, as previously found for humic acids extracted from municipal refuse amended soils²³.

The soil lipid compositions were rather similar before and after the treatments. However, disposal brought noticeable changes in the composition of the bound lipids in the subsoil layer, as is observed in the TIC of Figure 4.

After two years, no definite conclusions can yet be drawn on the possible accumulation of hydrophobic compounds in the treated soils. As is well known, the fate of chemicals in soils depends on several physical, chemical and biological processes, which determine their possible dissipation or accumulation. On the other hand, the low increase in the lipid levels and the qualitative differences observed cannot be related to the disposal only. Rather it can result from new microbially synthesized lipids²⁴ due to the increase of the soil microorganism populations after the applications. In addition, the 'crop effect', i.e. the restitution of lipid materials by plant remains, must also contribute to the changes observed.

At the end of the planned long-term experiment to study the effects on physical and biochemical soil properties and plant production, the fate of the hydrophobic components of vinasses and alpechin-compost will be further investigated.

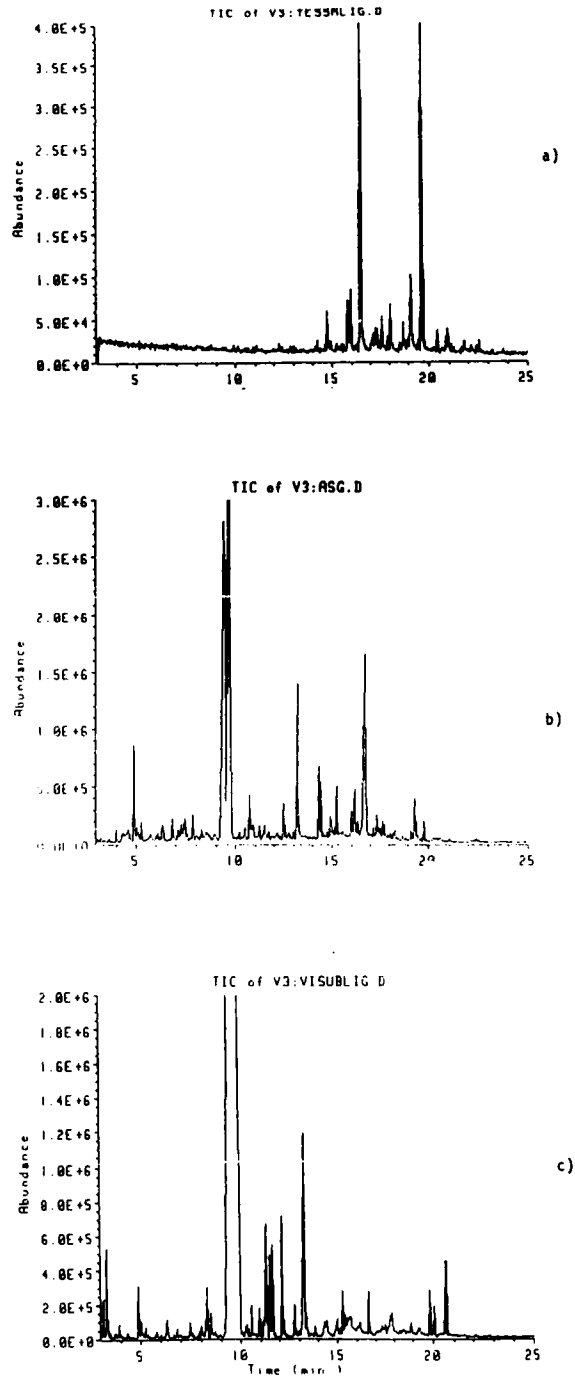


Figure 4 Total ion chromatograms of the hexane fractions of bound lipids in the subsoil layer, before (a) and after the applications of vinasse (b) and alpechin-compost (c).

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