

Solid-phase micro-extraction (SPME) in the early detection of potentially active volatile compounds from organic wastes used for the management of soil-borne pathogens

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ABSTRACT

The complex molecular assemblages were analysed in the soil gas phase after applying pine forest wastes (PFW) or sugarbeet vinasses (SBV) for soil-borne crop pests management. For this purpose, solid-phase micro-extraction (SPME) and gas chromatography (GC) were used coupled with mass spectrometry (MS). The organic wastes were applied either to Calcic Entisol or Haplic Arenosol moistened at field capacity and soil was covered with polyethylene sheet for 28–30 days to retain the volatiles. The PFW-treated soil mainly released volatile terpene hydrocarbons (*trans*-caryophyllene, β -myrcene and *p*-cymene), with α -humulene and ethylbenzotriazole prevailing in the untreated soil. After SBV application mainly alkyl compounds and alkylbenzenes were released, whereas cyclohexanone, limonene, butanone, acetic acid, camphor and benzaldehyde occurred in the untreated soil. Compound assemblages also depended on the increasing water saturation in terms of soil depth, with sulphur compounds prevailing in deep horizons. Our results showed that SPME can be directly applied to soils to provide valuable information on volatile products from organic amendments.

Keywords: Biodisinfestation, biofumigation, compost, forest waste, gas phase, pine litter, sugarbeet vinasses.

INTRODUCTION

Gas chromatography coupled with mass spectrometry (GC-MS) is the most frequently used technique in the analysis of complex mixtures of organic volatile compounds. However, this requires their previous isolation from the non-volatile components of the sample. On the other hand, solid phase microextraction (SPME) is a simple, rapid and non-expensive technique which allows the fractionation of the volatiles released from liquid and solid samples. ^[1] Such a technique, which has been mainly used in the analysis of food and in the monitoring of environmental pollution, ^[2,3] could also be useful in other areas, such as agricultural chemistry. However, to date, its application in the analysis of natural volatile products in the soil gas phase has been very scarce, and usually directed towards the determination of pollutants. ^[4] Volatile organic compounds have recently been analyzed by SPME in marine sediments ^[5] and in waste disposal landfills. ^[6]

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Biofumigation or biodisinfestation^[7-11] has been proposed as a valid alternative to the use of agrochemicals for the control of plant pests.^[12-14] In particular, pest management based on the release of volatile compounds from organic wastes has been successful for several pathogenic organisms.^[15] Nevertheless, no extensive research has been developed to unravel the chemical nature of the volatile compounds responsible for the beneficial effects of biodisinfestation practices. Yulianti et al.,^[16] in experiments on the simultaneous use of a polyethylene sheet on soil (solarization or biosolarization) to retain the volatiles, highlighted the importance of sulphur compounds released from decaying Cruciferae and Compositae plants for controlling some soil pathogens. Biosolarization usually leads to a considerable enhancement of the organic matter biocidal effect on the soil pathogens, through increased topsoil temperature and concentration of bioactive compounds. Also, this practice may also lead to remarkable changes in the prevalence patterns within the different trophic groups of the soil macro- and microfauna.^[17]

On the other hand, the molecular assemblages with a bearing on the success of the biodisinfestation process have shown to be to a large extent dependant on the organic source used in each case.^[14] Although the effect of some compounds such as isothiocyanates, glucosinolates and nitriles has been highlighted,^[12, 18-22] no systematic studies have been carried out on the determination of other volatile compounds released after the addition of organic materials to the soil. It must also be taken into account the need to assess the potential of organic materials used for soil biodisinfestation as soil and water pollutants, as well as the possibility of using them to improve soil fertility or for the control of soil-borne pathogens. Assuming the above considerations, this paper focuses on the identification of the compounds occurring in the gas phase after soil amendment with pine forest wastes (PFW) and sugarbeet (*Beta vulgaris* L.) vinasses (SBV). The results of both experiments in laboratory conditions are presented evaluating SPME followed by GC-MS for the detection of soil volatiles.

MATERIAL AND METHODS

Experimental Design

Organic materials were selected mainly based on their large and concentrated production in time and space, which poses a problem on their non-hazardous disposal and raises the interest of finding further environmentally-friendly uses for them. In urban green areas (mainly public gardens) of the Mediterranean areas, PFW is common organic waste, whereas sugarbeet vinasses (SBV) are a common liquid by-product from the Spanish sugar industry.

Both experiments were carried out in laboratory conditions. The first experiment was carried out on Calcic Entisol from Játiva, Alicante (Eastern Spain), a shallow, calcic, poorly developed soil without definite horizons, and of a coarse texture, with a pH of 7.8, and an organic matter content of $17 \text{ g} \cdot \text{kg}^{-1}$. The added organic material was PFW (*Pinus halepensis* Mill.) from municipal gardens at Jávea (Alicante, Spain), consisting of chopped ($\pm 1 \text{ cm}$) needles (83%) and thin branches (17%), with dry matter content of 50.6% (Table 1). The PFW were mixed at 20 and 40 g per kg soil, corresponding to an input equivalent to 50 and 100 Mg PFW·ha⁻¹, in polyethylene bags containing 0.5 kg soil each. After soil moistening at field capacity, polyethylene bags were completely sealed and kept at $30 \pm 2^\circ\text{C}$, in darkness, for 28 days. A control treatment without the application of organic matter was included (PFW control). There were four replications of each treatment.

Table 1. Characterization of pine forest wastes (PFW) and sugarbeet vinasses (SBV)

	PFW	SBV
Organic matter (g·kg ⁻¹)	965	284
Conductivity ^a (μS·cm ⁻¹)	1673	14620
N (g·kg ⁻¹)	14	29
Ca (mg·kg ⁻¹)	5634	2510
Cu (mg·kg ⁻¹)	8.5	7.2
Fe (mg·kg ⁻¹)	388	19
K (mg·kg ⁻¹)	4963	3400
Mg (mg·kg ⁻¹)	1267	6708
Mn (mg·kg ⁻¹)	7	18
Na (mg·kg ⁻¹)	67	22000
P (mg·kg ⁻¹)	1467	2985
Pb (mg·kg ⁻¹)	<5	<5
Zn (mg·kg ⁻¹)	227	27.5

^a 1:10 w:v (soil:water)

The second experiment was carried out on Haplic Arenosol (Jumilla, Murcia, in the Spanish South-East), a characteristic soil in the area of SBV production, with a sandy-loam upper horizon (85% sand and 6-7% clay), 10% carbonates, pH 7.9, and an organic matter content of 2.5 g · kg⁻¹. The SBV (50% dry matter, Table 1), from the Castile-La Mancha region, was applied at a dose of 187 g per kg soil, corresponding to an input equivalent to 25 Mg SBV·ha⁻¹. The experiment was performed in methacrylate cylinders (15 cm diameter × 1 m high) filled with 36 kg soil. The cylinders were sealed with a polypropylene cover and kept at 24 ± 2°C for 30 days. A control treatment without an organic matter application was included (SBV control). There were four replicates (columns) of each treatment. Since the inherent problem of liquid wastes to behave as a leachate when used as a soil amendment, the experiment was specifically designed to check the vertical distribution of the organic matter in terms of soil depth. In consequence, samples were collected from different depths in the metacrylate columns, i.e. 20 cm (sample No. 1), 40 cm (sample No. 2), 60 cm (sample No. 3) and 80 cm (sample No. 4). The SBV gas phase composition was also analysed and also labelled as SBV organic waste.

Solid-Phase Micro-Extraction Analyses

Preliminary experiments were carried out in the PFW experiment with up to five replicates. The same five major components appeared in all the runs, but the relative (%) standard deviation of their concentrations was in the range 30.4 - 47.0 for such compounds. The results led us to focus our study in the SPME potential assessment of qualitative fingerprinting of the volatiles present in the gas phase for the soil samples processed under the same conditions. The chromatogram profile obtained by SPME fractionation could then be related to changes in the soil microfauna. A quantitative analysis was not intended, the relative abundance being marked by one to three asterisks.

At the end of the biodisinfestation experiment (28 days for PFW and 30 days for SBV) each replicate was homogenized by mixing the soil thoroughly. Approximately 1 g soil was

collected from each replicate, introduced into 4 mL glass vials and adding 1 mL of Milli-Q® of water. Soil samples were homogenized by stirring, and vials were sealed with predrilled septa. For headspace sampling, a manual SPME holder equipped with an 85- μm Carboxen/polydimethylsiloxane StableFlex fibre (both from Supelco Co. Bellefonte, PA). This fibre was chosen on the basis of the wide range of polarity for the volatile compounds occurring in the compost gas phase (ranging from between acetic acid and other short-chain alkanolic acids to hydrocarbons) observed in preliminary experiments.^[23] In similar previous experiments with this fibre, the equilibrium time and temperature were reached at values similar to the optimum (maximum amount of volatiles extracted and minimum artifact formation) for other organic substrates, such as food products. The fibre was conditioned at the manufacturer's recommended temperature before the analysis, then the vials were kept at 60 °C for 15 min (equilibrium time) before exposing the fibre to the soil headspace (injector temperature = 250 °C) for 30 min. Under the above conditions the qualitative chromatographic patterns were the same, and the quantitative differences in peak areas were reasonably reproducible.

Gas Chromatographic-Mass Spectrometric Analyses

The GC-MS analyses were performed on an Agilent 6890 (Palo Alto, CA, USA) gas chromatograph coupled with an Agilent 5973 quadrupole mass detector. The SPME fibre was desorbed at 250 °C for 2 min in the splitless mode and chromatographic separation was carried out on a 50 m \times 0.25 mm \times 0.25 μm polyethyleneglycol capillary column (Supelcowax-10, Supelco®, Bellefonte, PA). The oven temperature was programmed from 40 °C (2 min) to 190 °C (30 min) at 4 °C min⁻¹. Helium at 1 mL min⁻¹ was used as a carrier gas. The mass spectra were recorded in electron ionization (EI) mode at 70 eV, by scanning the 35–450 m/z range. Interface and source temperature were 280 and 230 °C, respectively. Compounds identifications were based on comparisons of the spectra obtained with those of the NIST and Wiley mass spectral libraries^[24,25] and were confirmed, when possible, using programmed temperature Kovats indices determined from the retention times of an *n*-alkane mixture analyzed under identical conditions. Available standard compounds were also used for further confirmation.

RESULTS AND DISCUSSION

Tables 2 and 3 list the volatile organic compounds identified by SPME followed by GC-MS in both experiments. Unidentified compounds represented 15% of the chromatographic area. The repeated determinations systematically showed the same compounds, but their relative abundance varied around 30%. This systematic variation between repetitions suggested the transformation of the results into standardized indices indicated with \times , $\times\times$, and $\times\times\times$. Therefore, the results presented for each compound are not significantly ($P < 0.05$) affected by the extent of the variation between repetitions of the same sample.

Table 2. Tentative identification of organic volatiles released from soils treated with pine forest waste (PFW)

MW	Compound	Dose (PFW·kg soil ⁻¹)		Control	Pine forest waste
		20 g	40 g		
72	Butanone	×	×		
111	Trimethylcyclohexanone			×	
126	Dimethylheptene			×	
128	Dimethylheptane			×	
134	<i>p</i> -cymene	xxx	xxx		xxx
136	α -pinene	xx	×	×	xx
136	Dimethyloctadiene	xx	×	×	×
136	α -terpinene	×			×
136	Dimethyloctatriene	xx			×
136	β -myrcene	xxx	xx	×	xx
136	Limonene	×	xx		×
136	α -terpinolene	xxx	×	×	xx
38	Methylbutylidene-cyclopentane	xxx	xx		xx
140	Dimethyloctene	×	×		
147	Ethylbenzotriazole	×	×	xx	
204	<i>Trans</i> -caryophyllene	xxx	xxx	×	xxx
204	α -humulene	xx	xxx	xxx	xx

Semiquantitative data of peak areas calculated as relative abundances are shown as standardized indices: ×= 0–33%, xx= 34–66%, and xxx= 67–100%.

In the gas phase of the untreated soil of the PFW experiment more than 50 alkanes were detected, the main compounds identified being branched alkanes and alkenes with between 11 and 15 carbon atoms and ethylbenzotriazole (Table 2). These compounds could have an anthropogenic origin, e.g. fuel combustion residues from vehicles or agricultural machinery.^[26] Some mono- and sesquiterpenes were also present, but only in low amounts. On the other hand, in the treated soil gas phase, mono- and sesquiterpenes appeared in higher concentrations, *trans*-caryophyllene, β -myrcene, *p*-cymene, α -humulene and α -terpinolene being the most important compounds identified. Branched alkanes and ethylbenzotriazole were present in relatively low amounts. Since terpenes are systematic components of pine needles,^[27–32] there were expected to occur in PFW-amended soils. However, the relatively high amount of α -humulene detected both in treated and non-treated soils suggests an origin other than pine needles. As some authors have mentioned this compound being present in a wide range of plants such as hop,^[33, 34] maize,^[35] pasture,^[36] and several Mediterranean spices,^[37] their occurrence in soils is expected to be frequent.

Table 3. Tentative identification of organic volatiles released from soils treated with sugarbeet vinasses (SBV)

MW	Compound	Sample No.				Control	Sugarbeet vinasses
		1 20 cm	2 40 cm	3 60 cm	4 80 cm		
58	Propanone						×
60	Acetic acid	xx			xx	×	xxx
62	Dimethylsulfide			xx			×
72	Butanone		×	×	xxx	×	×
74	Propanoic acid				xx		
76	Carbon sulfide			xx			
82	Methylfuran		×				×
83	Methylbutanenitrile						×
84	Methylbutanal			×			×
84	Dichlorometane			×			
86	Methylbutanal			×			×
86	Dihydro-2-(3 <i>H</i>) furanone	xx					
86	Terpene compound		×	×			
88	Methylpropanoic acid				×		
88	Butanoic acid						xx
90	Butanediol						xx
92	Methylbenzene (toluene)	xxx	×				
94	Phenol				×		
94	Dimethyldisulfide			xx			
98	Cyclohexanone	xx				xxx	
100	Cyclohexanol	xx					
103	Nitrobutane		xx	×			
103	Benzonitrile		×				
104	Styrene	xx					
106	Dimethylbenzenes (xylenes)	xx					
106	Ethylbenzene	×					
106	Benzaldehyde	xx	xxx	xxx	×	×	
108	Dimethylpyrazine (I)						×
108	Dimethylpyrazine (II)						×
108	4-methylphenol				xx		
122	Ethylmethylpyrazine (I)						×
122	Ethylmethylpyrazine (II)						×
122b	Terpene compound		×	×			
122	Chlorobutanoic acid						×
126	Dimethyltrisulfide			×			
130	Ethylhexanol	xx					
136	Limonene	×				xx	
152	Camphor			×		×	

Roman numbers indicate different isomers. Semiquantitative data of peak areas calculated as relative abundances are shown as standardized indices: ×= 0–33%, xx= 34–66%, and xxx= 67–100%.

Limonene, butanone, acetic acid and camphor were the major compounds present in the headspace of the untreated sample of the SBV experiment (Table 3). In the case of treated soils, methylbenzene (toluene), dimethylbenzene (xylene), cyclohexanol and styrene showed the highest relative amounts. Cyclohexanone and benzaldehyde were major compounds in both SBV-treated and untreated soils. The high relative abundance of limonene in the control soil could be attributed to a contribution of the original vegetation at the soil collection site.^[38] In SBV-treated samples other compounds appear in higher amounts and the relative presence of limonene appears to be smaller.

It was observed that the gas phase composition of the SBV-treated samples to a large extent depended on the sampling depth. At the 20-cm level (Fig. 1A) the major volatile molecules were toluene, xylenes, styrene and cyclohexanol, whereas the most frequent compounds at 40 cm depth were nitrobutane and benzaldehyde, and compounds such as dimethylsulfide, dimethydisulphide and dimethytrisulfide were mainly present at 60 cm (Fig. 1B). The occurrence of sulphur compounds was not unexpected; a series of sulphur-containing compounds have been detected by SPME in previous studies on composts mainly from sewage sludges.^[39] At a depth of 80 cm the main compounds detected were 2-butanone, acetic and propionic acids. The large influence observed on the molecular composition of volatile compounds in terms of sampling depth could be interpreted as a result of the effect of water saturated zones, which coincides with anaerobic activity.

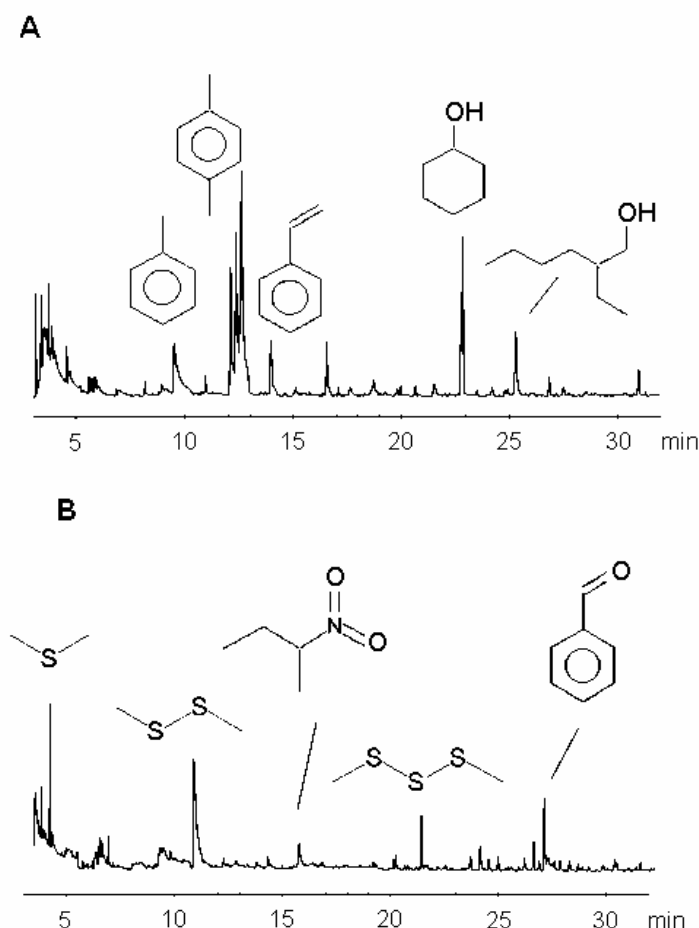


Figure 1. Current profiles of total ion from the atmosphere of soils treated with sugarbeet vinasses under a polyethylene sheet. **A.** At 20 cm soil depth: alkylbenzenes and aliphatic alcohols. **B.** At 60 cm soil depth: aromatic aldehydes, sulphur- and nitrogen compounds.

The observed differences in the volatiles detected in each control soil indicate that the organic matter formerly present in the soil is an important factor in the headspace composition. Local vegetation and microbial metabolism, through their contribution to the formation of the soil organic matter, are largely responsible of the original volatile composition. This fact must be taken into account when studying the soil gas phase composition after amendment with organic materials. Otherwise, it would be difficult to explain which of the detected compounds are released by the organic amendments during their decomposition process in the soil, and which volatiles represent the contribution of the pre-existent soil organic matter.

Dimethyloctadiene, occurring only in the PFW experiment (Table 2), is a volatile microbial product described in soils and ascribed to the metabolism of basidiomycetes.^[40] Terpene compounds have been also typically related to plant defence mechanisms against pests and pathogens.^[35, 41] Nitriles (methylbutanenitrile), which have been previously reported as volatiles produced during biodisinfestation practices,^[22] were only found in SBV. In particular, potentially harmful products such as alkylbenzenes, including styrene as well as chlorinated compounds, are frequent volatile organic molecules from composts.^[42]

CONCLUSION

Solid Phase Microextraction, combined with GC-MS, is a simple and fast fractionation technique useful for the identification of volatile compounds in soils. This technique was highly responsive to the nature and transformations of organic inputs applied to the soil. Characterization of soil samples from its volatile compounds composition is a useful approach to study the behaviour of organic amendments in the course of their decomposition in the soil. Also it could be used to analyze the possible relationships of the released volatiles in the control of pathogenic organisms in agricultural soils. A high number of compounds (more than thirty in the SBV experiment) have tentatively been identified in the soil gas phase. However, since SPME recovery is strongly dependant both on the extracted compound and on the sample matrix, its application for the quantitative characterization of soils from their volatile composition needs to be further studied. In particular, SPME has shown large differences in soil volatiles depending on the type of organic wastes applied and on the soil depth. Consequently, the determination of soil volatiles using SPME followed by GC-MS has a great potential to provide capital information on the chemical factors associated to the soil biologic activity in agro-ecosystems.

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