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Fate in the soil of an oil additive of plant origin

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Abstract: The methyl ester of oleic acid, a plant oil derivative, can be used as an additive oil for pesticides. We compared the biodegradability in soil of this oil with that of a mineral oil by means of laboratory experiments using lysimeters of 70 cm height × 20 cm diameter. The migration in soil of the oils and of the metabolites of the plant ester over 120 days was examined by gas chromatography and liquid chromatography. The plant oil and its metabolites were completely degraded within 60 days, whereas degradation of the mineral oil required 90 days. The molecules did not migrate far into the soil and therefore presented no risk of contaminating groundwater.

Keywords: additive oil; plant origin; migration; environmental impact; soil

1 INTRODUCTION

One of the causes of soil and water pollution is the annual application of almost 2.5 million tonnes of pesticides to crops worldwide. Additives can be added to pesticides to improve their performance, in particular to decrease the amount of product required whilst minimising the effects on the surrounding flora and fauna. Considerable research effort is currently devoted to the development of new additives that are both biodegradable and non-polluting for humans and the environment, and to improve the efficiency of pesticides formulations. Several different types of additive may be used: surfactants, humectants, solvents or oils. The present work focuses on oils.

Most of the additive oils currently used in pesticides are derived from petroleum. However, these are gradually being replaced by a new generation of additive oils of plant origin, which potentially have better environmental qualities. In this work, we have compared the environmental characteristics of an oil derived from oilseed rape (the methyl ester of oleic acid) and a mineral oil by comparing their behaviour in soil. These two oils are sold as additives for pesticides and we studied their fate when used at the recommended doses so as to ensure that our experimental conditions were as close as possible to real application conditions.

As for all other chemical molecules, the fate of these oils in the soil depends on the biodegradation and migration of the parent compound and any degradation products. We cannot conclude that a component is not a potential risk for the environment just because it has disappeared. We must also study the potential effects of the metabolites generated during biodegradation. One important factor in evaluation of the environmental effects of a compound in the soil is the distribution in soil of the product over time: if the product migrates too quickly in drainage water it may contaminate the radose zone and groundwater. Conversely, if the component remains in the soil for long enough, it may be better degraded by micro-organisms.

We used lysimeters to follow the migration and progressive biodegradation of the oils by soil microorganisms over time. We also identified the metabolites formed during the biodegradation of the vegetable oil.

2 MATERIALS AND METHODS

2.1 Soil and additive oils

The soil used came from the Toulouse region in southwestern France. It is an alluvial clay and the plot of land studied had not been worked or been treated with pesticides for at least five years. Soil characteristics are: pH, 6.3; clay content, 19%; cation exchange capacity, 5.9 meq $100\,g^{-1}$; organic matter content, 1.6%.

The plant oil studied was the methyl ester of oleic acid, (methyl oleate; $C_{19}H_{36}O_2$). This compound is the main ingredient (90%) of ACTIROB B®, a phytosanitary additive distributed by Novance. The recommended rate for this additive is 2 litres ha^{-1}. Methyl oleate (99%, Sigma) was applied as an emulsion in water containing 50 g $litre^{-1}$ R508 (sorbitan ester; Novance) as emulsifier.

The mineral additive was an isoparaffin oil with

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6–36 C carbon chain length, already formulated with an emulsifier. The detailed chemical structure and trade name of this produce cannot be given. The recommended rate of use in pesticidal applications is 3 litres ha^{-1}.

2.2 Lysimeter study

Of the various types of lysimeter available,[1–6] it was decided to use those 70 cm high × 20 cm diameter.[7] Lysimeter samples were collected from the site by coring, which provided undisturbed columns of soil representative of the soil profiles of the sample site. The lysimeters were then set up at the Institut de Mécaniques des Fluides at Toulouse (IMFT) and equipped with an automatic watering system to reproduce precisely the rainfall measured at the site. Their hydrodynamic behaviour was checked with a conservative tracer, potassium bromide. Lysimeters with defective behaviour (retardation of Br ion loss exceeded by 20%) were discarded. The others were placed on metal supports such that the water percolating through the soil column could be collected from the bottom of the column.

The temperature and soil moisture at various depths in the soil column were measured by sensors throughout the experiment.

The experiments were duplicated in successive years, during the spring of 1997 and 1998. Experiments were carried out in the spring to simulate the use of the oils as additives for herbicides used following germination of the maize crop.

2.3 Spatio-temporal monitoring

At the start of the experiment, additives were applied at their recommended rates: 2 litres ha^{-1} for methyl oleate, equivalent to 5.5 mg in 10 ml of water for the surface of the lysimeter; and 3 litres ha^{-1} for the mineral oil, equivalent to 9 mg in 10 ml of water. The dispersions were sprayed uniformly over the entire surface of the columns.

For studies of the kinetics of the plant oil and its metabolites, samples were taken after 3, 5, 7, 12, 14, 21, 28, 35, 42, 60, 90 and 120 days. For the mineral oil, samples were taken after 7, 14, 28, 60 and 120 days. At each time point, one soil column was analysed for each additive. The lysimeters were cut at various depths into slices 2.5, 5 or 10 cm thick (0–2.5, 2.5–5, 5–7.5, 7.5–10, 10–15, 15–20, 20–25, 25–30, 30–40, 40–50, 50–60 and 60–70 cm). The various soil slices were treated separately immediately after cutting or after storage at −18 °C in a polyethylene bag. The soil was sieved (mesh size = 2 mm) and a 100-g sample taken according to the standard NF X31-100.[8]

The organic products (original oils and metabolites) were extracted from the soil samples with ethyl alcohol. Previous studies had demonstrated that ethyl alcohol was the most appropriate solvent for the plant oil, its metabolites and for mineral oil. The extraction technique was optimal using 200 ml of solvent per 100 g of soil for 6 h. Yields of extraction in these conditions were: 97% for the plant oil, between 95 and 98% for metabolites and about 95% for the mineral oil. The solvent was evaporated and the samples analysed by gas or liquid chromatography.

2.4 Chromatography

Gas-phase analyses were carried out with a Varian 3800 chromatograph equipped with an FFAP-CB capillary column for the plant oil and its metabolites, and a CPsil 5 column for the mineral oil. Oleic acid was used as an internal standard for quantitative analysis. The samples were analysed at 22 (±2) °C and injected in triplicate.

Liquid-phase analyses were carried with a Spectra-Physics chromatograph equipped with a P 1500 pump, a C18 Spherisorb column and a Milton Roy 4 detector for differential refractometry. The eluent was 0.3% acetic acid in acetonitrile and the internal standard was oleyl alcohol. The samples were analysed at 22 (±2) °C and injected in triplicate.

3 RESULTS

3.1 Soil water content

The moisture tension of the soil in the lysimeters was monitored by gravimetric analysis (standard NF ISO 11 465)[9] and gave moisture content profiles varying by a minimum of 15% at the surface and a maximum of 25% at a depth of 70 cm. These water conditions are not limiting for micro-organisms because they correspond to 50–60% of water retention capacity regardless of depth and are therefore very far from the values for moisture at saturation.[10]

3.2 Soil temperature

Temperature was monitored continuously throughout the experiment, and was found to change in the same manner at all depths: regardless of the depth, the temperature increased 22 °C at the beginning of the afternoon to, before decreasing to reach 19 °C at the end of the day, as at the surface of the soil in the middle of the field. These variations were due to an effect of the sides of the lysimeter.

3.3 Soil pH

The pH of the soil tested was between 6 and 7, which is optimal for the plant growth. The pH increased with depth due to the high concentrations of clay and exchangeable bases[11,12] originating from soil minerals (feldspar: K, Ca and Na, and smectite: Ca) and from various fertilisers; leaching results in the downward migration of bases, which in turn increases pH. The pH is about 6.2 at the surface, and reaches 6.5 at a depth of 70 cm (Fig 1). However, this type of profile, with pH values close to neutral, provides conditions favourable for the development of the micro-organisms potentially involved in biodegradation.[13,14] During the first 28 days of the experiment, the pH increased slightly. This increase was probably linked to the simultaneous presence in the uppermost 20 cm

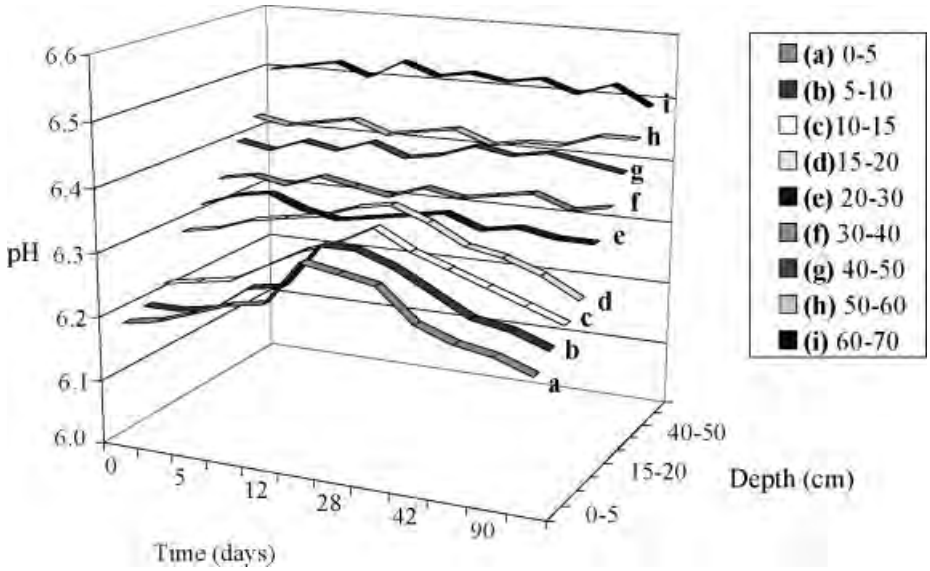

Figure 1. Spatio-temporal changes in the pH of the soil in the lysimeter.

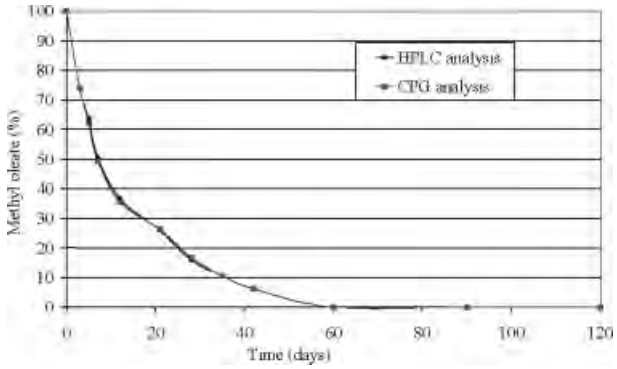

Figure 2. Overall degradation of methyl oleate in the lysimeter at the recommended rate (2 litres ha^{-1}).

of soil of micro-organisms, additive oils, their metabolites and dissolved carbon dioxide resulting from degradation.

3.4 Degradation of methyl oleate in the soil

The degradation kinetics of methyl oleate under the experimental conditions used were determined by measuring the amount of oil remaining in the soil as a function of time by both gas and liquid-phase chromatography. Similar results were obtained with the two techniques, validating the data obtained.

Figure 2 shows the change in the total amount of methyl oleate, expressed as a percentage of the initial, in the entire lysimeter profile with time, and Fig 3 shows the changes in the actual amount of methyl oleate over time and space. Totally degradation had occurred after 60 days. The half-life of a product (the time required to reduce the amount of a substance by half) is a parameter commonly used to describe its persistence in the environment,[15] and for methyl oleate was determined as 7 days; during this time, it migrated by only 15 cm (Fig 3).

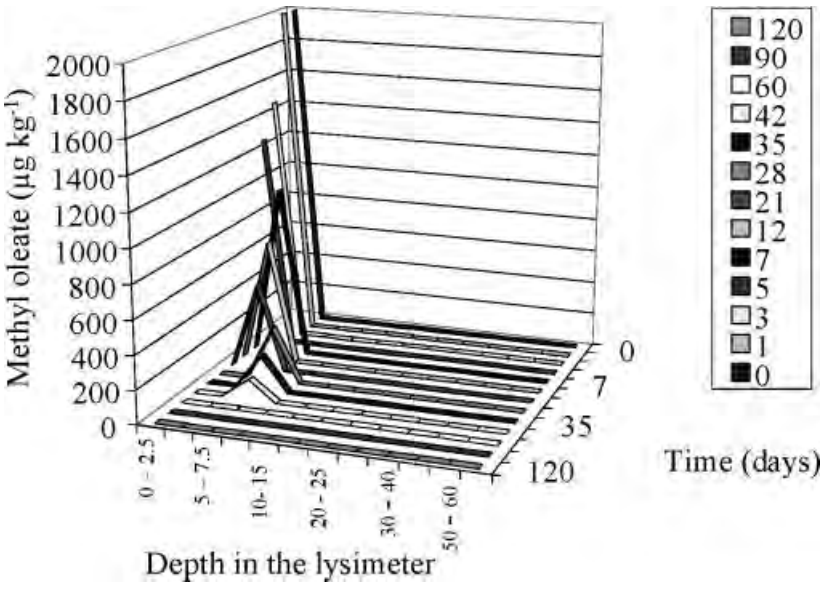

Figure 3. Changes in the concentration of methyl oleate over space and time.

Fatty acid	*Formula*	*log K_{ow}* [a]	*Maximum depth (cm)*
Oleic acid	$CH_3—(CH_2)_7—CH=CH—(CH_2)_7—COOH$	—	15
Heptadecanoic acid	$CH_3—(CH_2)_{15}—COOH$	—	15
Palmitic acid	$CH_3—(CH_2)_{14}—COOH$	7.17	25
Pentadecanoic acid	$CH_3—(CH_2)_{13}—COOH$	—	25
Myristic acid	$CH_3—(CH_2)_{12}—COOH$	6.11	30
Tridecanoic acid	$CH_3—(CH_2)_{11}—COOH$	—	30
Lauric acid	$CH_3—(CH_2)_{10}—COOH$	4.6	40
Undecanoic acid	$CH_3—(CH_2)_9—COOH$	—	50
Capric acid	$CH_3—(CH_2)_8—COOH$	4.09	50
Pelargonic acid	$CH_3—(CH_2)_7—COOH$	—	60
Caprylic acid	$CH_3—(CH_2)_6—COOH$	3.05	50
Heptylic acid	$CH_3—(CH_2)_5—COOH$	1.92	60
Caproic acid	$CH_3—(CH_2)_4—COOH$	1.87	60
Valeric acid	$CH_3—(CH_2)_3—COOH$	1.39	60
Butyric acid	$CH_3—(CH_2)_2—COOH$	0.79	60
Propionic acid	$CH_3—CH_2—COOH$	0.33	60

[a] From Reference 16.

Table 1. Characteristics of the degradation products of methyl oleate

3.5 Formation of degradation products

The metabolites resulting from the degradation of methyl oleate were identified and their spatial and temporal distributions determined. All those found were shorter carbon-chain fatty acids (Table 1).

The distributions of these metabolites in space and time changed in a more diffuse manner than that of the parent compound (Figs 4 and 5). Figure 4 shows the progressive appearance of the first metabolite, oleic acid, formed in a reaction catalysed by an esterase. Oleic acid concentration increased until day 21 and then decreased, disappearing totally after day 42. Similar phenomena were observed for all of the metabolites listed in Table 1, but over different time scales.

The spatial migration of metabolites seemed to be directly linked to the size of the carbon chain because the shortest fatty acids, such as the six-carbon caproic acid (Fig 5), tended to migrate further than the longer chain acids, such as the 18-carbon oleic acid (Fig 4). The maximum depths to which the compounds migrated are listed in Table 1, along with log K_{ow} values, an index of their hydrophobicity. These K_{ow} data, available in the literature,[16] correspond to the protonated form of the acids. In soil, these compounds will be in the ionized form and present lower K_{ow} values and consequently higher solubility and leaching potential. Nevertheless, the migration tendency of the metabolites is similar for protonated and for ionized forms: the metabolites with the highest K_{ow} values

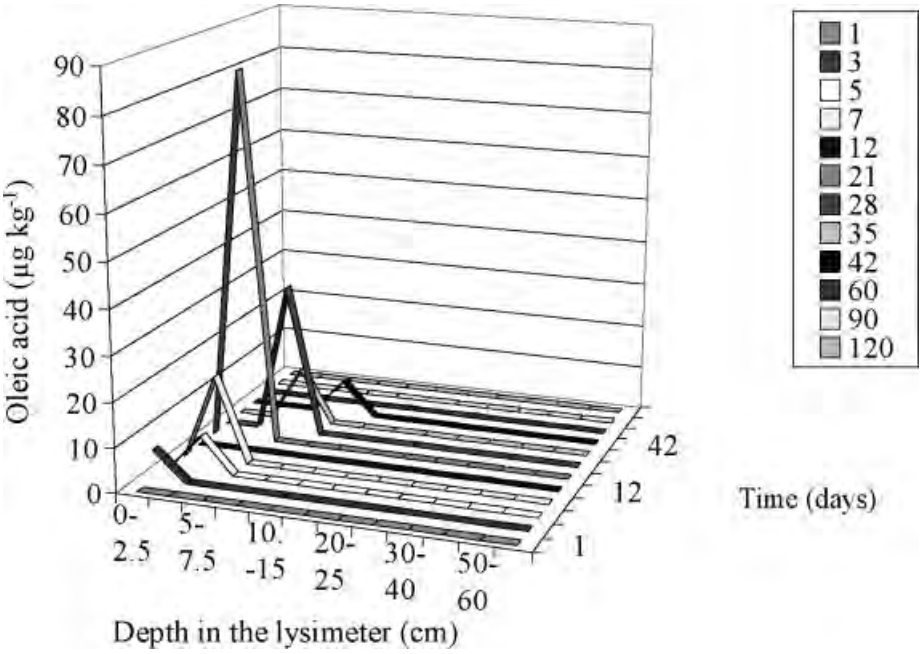

Figure 4. Appearance and disappearance of oleic acid.

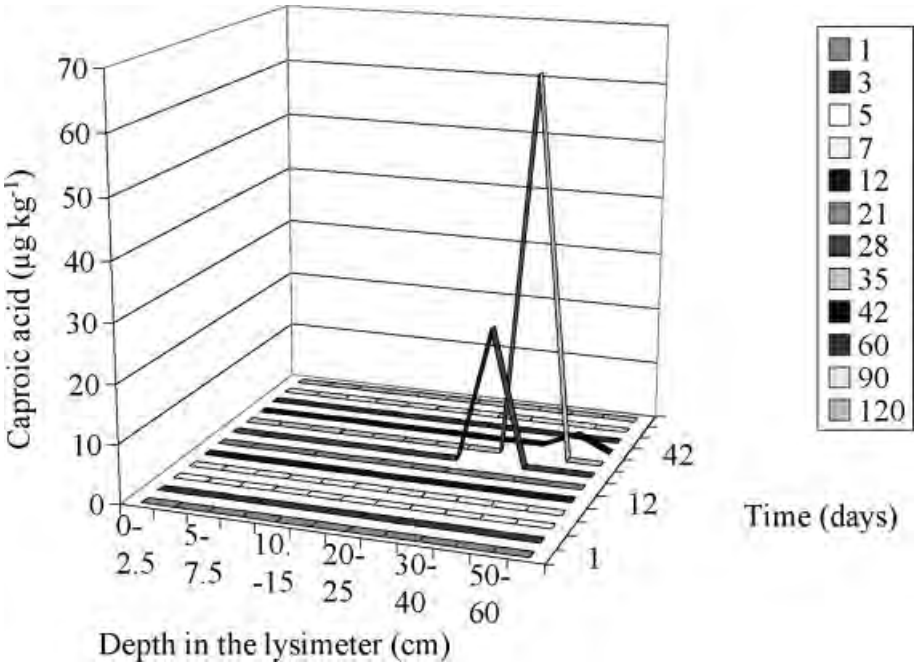

Figure 5. Appearance and disappearance of caproic acid.

tend to stay in the first 20 cm, whereas those with lower K_{ow} values tend to migrate to greater depths.

None of the metabolites were detected after 60 days, suggesting that the methyl oleate was completely degraded at this point.

3.6 Primary degradation of the mineral oil

The mineral oil was completely degraded after 90 days (Fig 6); degradation therefore took 50% longer for the mineral than for the plant oil. This observation was consistent with the observed half-life of this oil, which was 11 days. The mineral oil migrated through the first 20 cm. This migration to slightly greater depths than observed for plant oil may result from the longer time required for degradation, leading to a longer time being available for migration of the intact molecule.

3.7 Water percolating through the soil

No traces of methyl oleate, its degradation products or of mineral oil were detected in the water percolating through the soil during the 120-day experimental period. This confirms that none of these products migrate deeply into the soil and that they are therefore unlikely to contaminate subterranean water.

4 DISCUSSION

This lysimeter-based study showed that methyl oleate disappeared totally within 60 days of its application at the recommended rate (2 litres ha^{-1}) and that this compound migrated through the first 15 cm of soil. This plant oil is therefore readily degraded in the soil.

We used quantitative chromatography of all of the

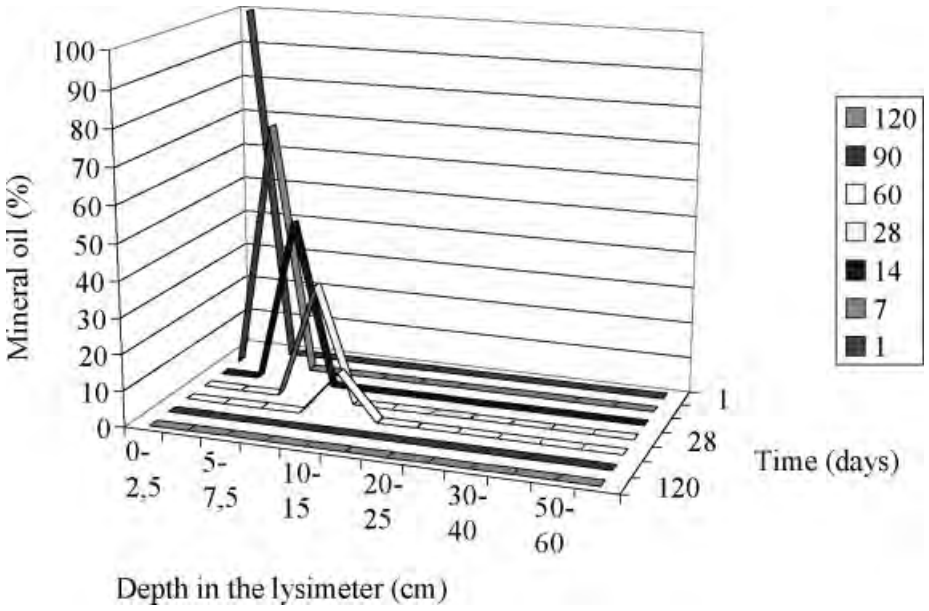

Figure 6. Changes in the amount of mineral oil over space and time.

organic compounds present in the soil during the experiment to identify the metabolites formed and to check that methyl oleate was degraded as described in the literature. It is known that the degradation of methyl oleate by soil micro-organisms may involve two types of reaction: β-oxidation and ω-oxidation.[17–19] These reactions generate different degradation products such as shorter carbon-chain fatty acids. For both types of oxidation, the final product is carbon dioxide. Langbehn and Steinhart[20] showed that the same type of reactions occur during the degradation of hydrocarbons (diesel).

The appearance of methyl oleate metabolites in the soil at different depths during the experiment may be due to storage of the molecules within micro-organisms, which then metabolise these molecules, excreting the resulting products into the soil.[21]

The spatial migration of metabolites depended on the length of the carbon chain: the shorter the chain, the further the molecules migrated in the soil. The C5–C9 acids migrated up to 60 cm. We can explain this behaviour in terms of two factors. The smaller metabolites are formed lower in the profile due to being the later degradation products of the oil. However, the migration of the metabolites may also correlate with the hydrophobicity of the molecule: the longer the chain, the more hydrophobic is the molecule. This trend can be evaluated by calculating the *n*-octanol/water partition coefficient K_{ow}. It is clearly seen that metabolites with high log K_{ow} values (ie the C13–C18 acids) tended to remain in the first 30 cm. Conversely, metabolites with log $K_{ow} \leqslant 3$ migrated further, probably due to the water percolating through the soil columns.

The most important finding was that none of the metabolites were detected after 60 days, proving that methyl oleate is completely degraded at this time. Furthermore, no traces of additive oil or metabolites were found in the water percolating through the soil. This suggests that methyl oleate presents no pollution risk for soils and subterranean water.

The plant oil was degraded more quickly than the mineral oil (60 days *versus* 90 days, Fig 7), with the two oils having half-lives of 7 and 11 days, respectively.

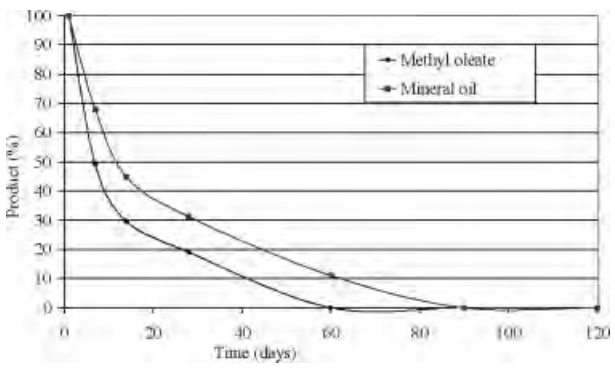

Figure 7. Comparison of the overall degradation of the two oils in lysimeters.

Methyl oleate can therefore be considered to belong to the category of readily biodegradable products.[22] The mineral oil metabolites were not monitored and there are no accurate data in the literature on this topic, so that no conclusions can be drawn about the true environmental impact of this product. However, even if the time of degradation is longer than that for the plant oil, we can consider that the product does not really pollute soil because of its total disappearance.

We also calculated the GUS coefficient for methyl oleate and for the mineral oil to confirm the previous results. The values obtained were -0.72 and -0.55, respectively, confirming that these oils were not very mobile[23] and that they spent enough time in soil to be degraded by micro-organisms.

5 CONCLUSION

We used lysimeters 20 cm diameter $\times$ 70 cm in height to study the fate of two types of pesticide additive in soil: one derived from a plant and the other from petroleum oil.

We carefully checked the hydrodynamic behaviour of the lysimeters before the experiments and simulated the rainfall at the study site to ensure that the conditions were as close as possible to natural conditions. We then used quantitative chromatography to monitor spatial and temporal changes in the amounts of the two oils.

Primary degradation was more rapid for the plant oil (60 days) than for the mineral oil (90 days), the plant oil having a half-life of 7 days and the mineral oil having a half-life of 11 days. Degradation thus took about 50% longer for the mineral oil. This may be because the mineral oil has a longer carbon chain, leading to slower degradation by soil micro-organisms.

The plant ester did not migrate very deeply into the soil because it was rapidly broken down by the micro-organisms in the soil and did not have time to migrate. β- and ω-oxidation led to the appearance of metabolites that migrated to depths of up to 60 cm and were completely degraded within 60 days. The mineral oil migrated slightly further in the lysimeters, and this was correlated with a slower degradation.

The environmental balance sheet for the methyl ester of oleic acid was largely positive in this study because the recommended amount of this additive oil was totally degraded within 60 days, as were its biodegradation products. We also showed that the molecules did not migrate very far and that there was therefore no risk of subterranean water pollution. A more detailed study has confirmed these results on different soils in lysimeters and in the field,[24] validating the environmental advantages of the oil of plant origin.

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