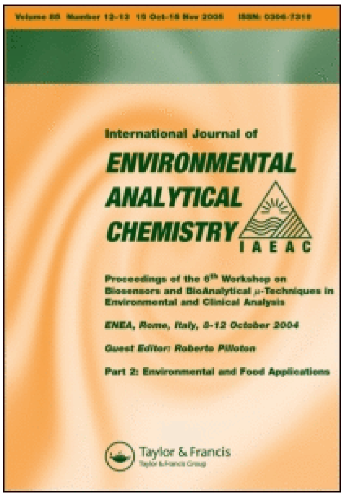

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SAMPLING AND SUBSTRATE APPLICATION METHODS FOR PESTICIDE MINERALIZATION EXPERIMENTS IN UNDISTURBED SOIL SAMPLES

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Different strategies for pesticide mineralization experiments have been proposed in the literature. The structural state of the soil as well as the method for applying pesticide to the soil can be anticipated to affect the mineralization of the pesticide. Also the soil water content during mineralization is important for the degradation of pesticide. The present study focused on different sampling and application methods. A new method was developed which used cores of undisturbed soil, sampled in tubes of 6.1 cm i.d. and 8.55 cm long, avoiding compression of the soil when sampling. ^{14}C-mecoprop was drip applied to soil cores under suction. The new method was compared to our previous method taking samples in 15 cm long tubes with i.d. 5 cm, and applying pesticide by injection to the soil at the water content found when sampled.

The new methodology produced the same mineralization pattern of applied pesticide that was observed for the traditional method. A non-linear model gave a good description of the kinetics of the mineralization. It was concluded, that the degradation kinetics of ^{14}C-mecoprop was not dependent on the sampling and application methods. However, an analysis of variance indicated that the suction application method produced reduced variance between replicates compared to our traditional technique.

Keywords: ^{14}C-mecoprop; mineralization; methods; comparison; undisturbed soil

INTRODUCTION

Detection of pesticides in groundwater has led to great interest in the fate of pesticides following application to soils and crops in agriculture. Sorption, leaching and degradation of pesticides in soil have been studied extensively during the last

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decades. Results from such analysis are claimed by the authorities before permitting the use of new pesticides. A high number of studies concerning the degradation of pesticides have been published. Review papers have been produced as well. Smith[1] summarised the results of 96 phenoxyalcanoic acid degradation studies, and Roeth[2] reviewed enhanced herbicide degradation in soil with repeated application. A review by Fomsgaard[3] and a report by Lindhardt *et al.*[4] showed that until recently degradation of pesticides in soil most often has been studied in disturbed samples (homogenised, sieved and eventually dried soil or soil suspended in water). Soil structural features of disturbed soils have been shown to differ considerably from undisturbed soil structure[5]. This has major implications for microbial turnover of organic matter applied to soil[5,6] and augment the necessity of performing also pesticide degradation studies in undisturbed soil samples. This perhaps is especially important for the subsoil which is never disturbed by tillage.

The soil water regime has major influence on microbial activity in soil[7]. Several studies report the effect of soil water content upon the turnover rate of applied organic matter to soils[8–10] and upon degradation of pesticides[11–14]. Therefore, degradation studies ought to take place at well defined water regimes. Most often degradation studies are reporting the soil water status as quantity of water, e.g. as a percentage of the Water Holding Capacity (WHC)[15], a percentage of the field capacity[16] or the Water Filled Pore Space (WFPS)[17]. However, as textural differences of soils will influence the volumetric water contents at a specific soil water potential, the water quantity of soils used for incubation should be regulated by the water potential in order to get results allowing real comparisons between soils[5,6]. The purpose of the present study was to compare a traditional method of sampling in narrow tubes and application through injection of pesticide – generally used in sandy soils in our laboratory – with a new method avoiding compression of the soil when sampled, and using drip application of pesticide to the soil at a pre-defined water potential.

MATERIALS AND METHODS

Soil samples

All samples were taken at 45 cm depth (37–53 cm for long vertical cores, 40–50 cm for short vertical cores) from a clayey soil in Flakkebjerg situated in East Denmark. The soil texture is shown in Table I. Samples were taken in stainless steel tubes of the dimensions mentioned in Table II. The tubes were forced into the soil in a vertical and a horizontal position, respectively, maintaining aseptic conditions. All tubes were capped and the samples were stored at approximately 5°C until application of pesticide.

TABLE I Texture and pH of soil samples taken in Flakkebjerg, 45 cm depth (37–53 cm for long vertical tubes, 40–50 cm for short vertical tubes)

Humus %	*Clay %*	*Silt %*	*Coarse silt %*	*Fine sand %*	*Coarse sand %*	*pH*
0.3	22.9	11.1	10.9	27.4	27.4	5.6

TABLE II Design of experiment

Tube dimensions	*Sampling*	*Pesticide application*
Short tubes, 8.55 cm length, 6.1 cm diameter	vertical	injection
Short tubes, 8.55 cm length, 6.1 cm diameter	vertical	suction
Short tubes, 8.55 cm length, 6.1 cm diameter	horizontal	injection
Short tubes, 8.55 cm length, 6.1 cm diameter	horizontal	suction
Long tubes, 15 cm length, 5 cm diameter	vertical	injection
Long tubes, 15 cm length, 5 cm diameter	vertical	suction
* Long tubes, 15 cm length, 5 cm diameter	horizontal	injection
Long tubes, 15 cm length, 5 cm diameter	horizontal	suction

* our standard method until now.

Soil drainage system

Experimental setup

The new pesticide application method was based on a traditional ceramic plate system for drainage of soil samples[18], which was slightly modified in order to drip apply the pesticide to the samples following drainage to a specific water potential (Figure 1). The system consisted of container with lid, housing the ceramic plate (Soilmoisture, High-Flow 1 bar ceramics) (number 1 in Figure 1) and the samples (2 in Figure 1). The suction was delivered by an ordinary pneumatic pump and the pressure controlled by a precision vacuum regulator. A graduated plastic cylinder (3 in Figure 1) served as sink, at the same time allowing inspection of outflow from the samples during time.

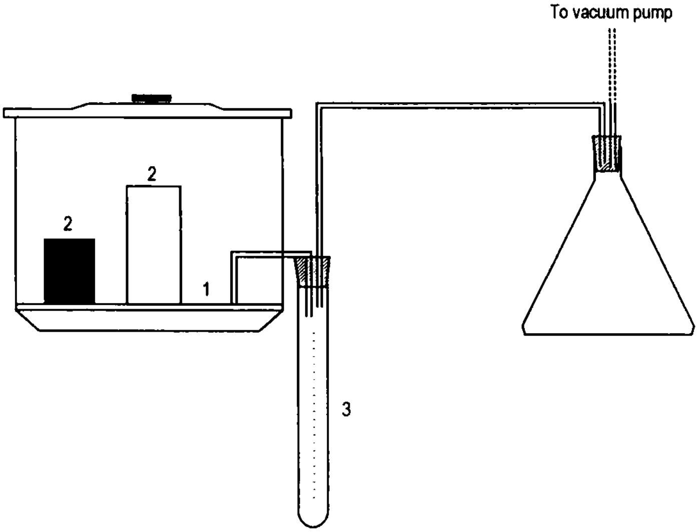

FIGURE 1 Laboratory apparatus used for the application of pesticides to undisturbed soil samples in degradation experiments. 1. Porous ceramic plate with quartz sand, 2. Soil samples in stainless steel tubes, 3. Reservoir

Working procedure

The soil cores were prepared for the experiment by removing about 1 cm of soil from the top of the soil sample. The bottom of the soil core was carefully trimmed in order to have a plain surface without smearing the soil. A piece of a fine meshed fabric of synthetic material was attached to the lower end of the core. Then it was placed on the humidified ceramic plate having a shallow layer of fine quarts sand on top of it in order to secure capillary contact. Water was added to the ceramic plate, and the core (or cores, if more than one sample) was left for at least 24 hours for saturating the soil. Then a suction of 500 hPa (corresponding to a water potential of pF 2.7) was applied to the plate, and the soil was allowed to drain to equilibrium. When equilibrium was reached, suction was turned off, and water again applied to the plate and allowed to re-enter the soil before application of pesticide to the top of the core. The calculated amount of pesticide (to give the desired concentration of 0.04 μg ^{14}C-mecoprop g^{-1} soil) in 200 ml ethanol was added to a piece of filter paper and the ethanol was evaporated in the air. The filter paper was placed at the top of the soil sample, 1.4 ml of water was added to the short cores, 1 ml to the long cores, little by little avoiding saturation at the top of the soil. The cores were then left for 1 hour. Vacuum was turned on, and augmented little by little until the desired vacuum (– 500 hPa) was obtained. The cores were then left with vacuum for 2 hours before removing it for incubation.

Control was made to check if there were traces of pesticide on the filter paper and if break-through had occurred (extraction of filter paper and quarts sand with ethanol and liquid scintillation counting).

Initial trials

To ensure, that the pesticide would be positioned in the soil core without leaking out of the bottom, initial trials were performed. In connection with a field trial[19,20], where leaching of pesticides through clayey soils was determined and compared with simulated amounts, an extension was added to the leaching model CMLS[21]. The extension of the model with simulation of dispersion and diffusion, calculated as described by Leistra[22,23] resulted in a better agreement with the observed concentrations[24]. On basis of the extended model, the penetration of the pesticide in the soil core was simulated. Several trials, with different amounts of water added to the soil core after application of pesticide, followed by suction in different periods, were carried out. Afterwards the cores were cut in levels of 1 cm thickness and the pesticide content through the profile was determined. The most appropriate application of the pesticide turned out to be an amount of 1.4 ml water for the short cores and 1 ml for the long cores. The results further indicated that the draining period following turn on of vacuum should not exceed 2 hours.

Chemicals

Ring ^{14}C-labelled mecoprop (2-(4-chloro-2-methylphenoxy)propanoic acid) with a specific activity of 7.19 MBq mg^{-1} and a radiochemical purity of 99.2 % was obtained from Amersham. ^{14}C-mecoprop was added to the soil to give a concentration of 0.04 μg g^{-1}. 96 % ethanol was obtained from Merck and Ultima Gold scintillation liquid from Packard.

Reference method of application through injection

A quantity of 4 ml of a water solution of ^{14}C-mecoprop was injected into the soil with a long needle at the water content it had at sampling, distributing the solution in the core as even as possible[14]. The concentration of the injected solution was calculated to give a final concentration of 0.04 μg ^{14}C-mecoprop g^{-1} soil (dry weight) and a water content of 50 % WHC (water holding capacity).

Incubation

The sample cores with added ^{14}C-mecoprop were placed individually in upright position in jars and incubated at 10°C. A gentle flux of atmospheric air was passed through the samples 4 hours twice a week, and the mineralization product, $^{14}CO_2$ was absorbed in traps of 1 M KOH[14]. The KOH absorption traps were changed 10 times, the first time 20 days after start of incubation and the last time 90 days after start of incubation. The amount of $^{14}CO_2$ present in KOH was quantified by liquid scintillation counting using Ultima Gold scintillation liquid. Accumulated % ^{14}C from added ^{14}C-mecoprop converted to $^{14}CO_2$ was depicted versus time (Figure 2–9).

Design of experiment

A 2^3 factor design was used, considering: core dimension, sampling, and pesticide application method (Table I). Each factor was investigated at two levels, long (and narrow) or short (and wide) cores, horizontal or vertical sampling, and application by injection or suction. For each combination, 3 replicates were made.

RESULTS

Figure 2–9 show the mineralization curves of the different combinations of experiments, where total accumulated ^{14}C, evolved as the mineralization product $^{14}CO_{2,}$ is shown as a function of number of days of incubation.

The curves generally had the same form, with an increment in $^{14}CO_2$ production at the beginning, and later on a decline in the production, so a non-linear model:

$$P = c_0 - \frac{k_1 c_0}{(k_1 + k_2 c_0)e^{k_1 t} - k_2 c_0} \quad \text{eq.(1)}$$

where

P = Concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$)

c_0 = total concentration of pesticide converted to $^{14}CO_2$ by the modelled process

k_1 = rate constant

k_2 = rate constant

t = time

were fit to the curves to evaluate if the kinetics of mineralization of all samples were the same.[25,26,27] The software used was Jandell Scientific Table curve 2D[28]. The model fit according to eq. (1) is presented as the solid line in Figure 2–9, where the dots show the actual sampled values. The different combi-

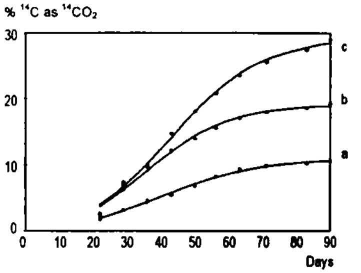

Fig. 2. Short tubes, vertical sampling and pesticide injected

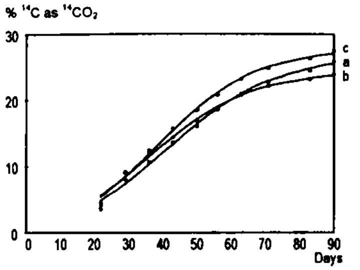

Fig. 3. Short tubes, vertical sampling and pesticide added with suction.

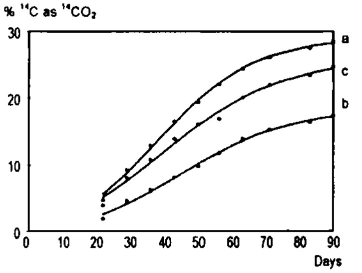

Fig 4. Short tubes, horizontal sampling and pesticide injected.

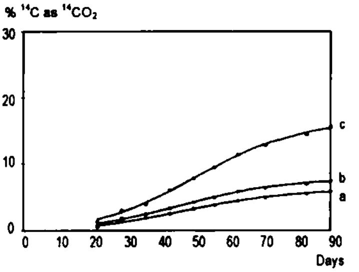

Fig. 5. Short tubes, horizontal sampling and pesticide added with suction

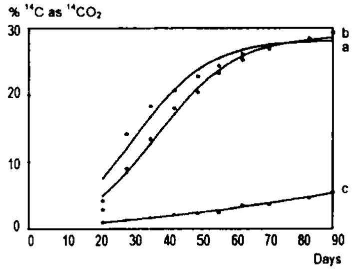

Fig. 6. Long tubes, vertical sampling and pesticide injected.

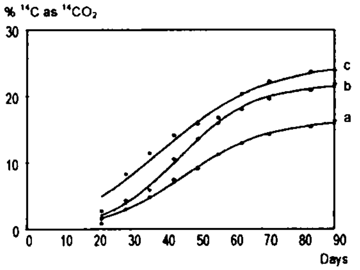

Fig. 7. Long tubes, vertical sampling and pesticide added with suction

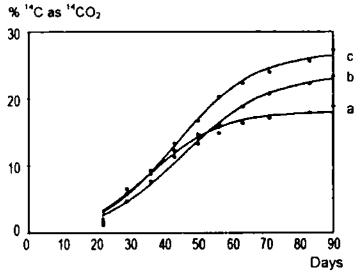

Fig. 8. Long tubes, horizontal sampling and pesticide injected.

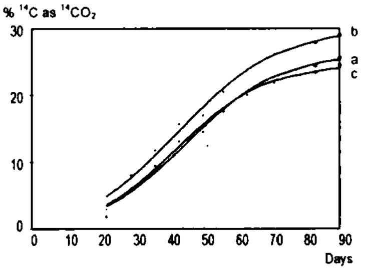

Fig. 9. Long tubes, horizontal sampling and pesticide added with suction.

FIGURES 2–9. Mineralization of 0.04 μg · g^{-1} ^{14}C-mecoprop in clayey soil shown as accumulated % ^{14}C evolved as $^{14}CO_2$. 3 replicates. Dots: sample dates; solid line: fitted model according to Liu and and Zhang [25], Liu et al. [26], and Fomsgaard [27] nations of tube type, sampling and application of pesticide all followed the same model except for replicate c in Figure 6. In that single case the degradation was very slow and it was not possible to estimate the parameters of the model.

The model developed by Liu and Zhang[25] was developed for metabolic degradation, and it has formerly shown to be useful for modelling degradation of pesticides in subsoil[27]. The fit of the model to the present data showed, that independent of the sampling and application method used, the degradation of ^{14}C -mecoprop in clayey soil from 45 cm depth followed metabolic kinetics.

The parameter c_0, determined with the fitted model, was equal to the total amount of ^{14}C evolved as $^{14}CO_2$ except for replicate c in Figure 6. To be able to include replicate c, the statistical analysis was made with the final value for ^{14}C as $^{14}CO_2$ from each curve.

TABLE III Analysis of variance table for three-way factorial pesticide mineralization experiment. Comparison of logarithmic transformed variances

Source of variation	*Df*	*Sum of squares*	*Mean square*	*F value*	*Pr > F*
Tube dimensions	1	0.00809386	0.00809386	0.02	0.9090
Sampling	1	0.60024737	0.60024737	1.54	0.4321
Application	1	6.33980252	6.33980252	16.24	0.1549
Tube dim.*sampling	1	2.63073006	2.63073006	6.74	0.2341
Sampling*application	1	2.49143761	2.49143761	6.38	0.2400
Tube dim.*application	1	0.00522720	0.00522720	0.01	0.9267
Residual	1	0.39044216	0.39044216		
Corrected total	7	12.46598077			

Comparison of variances

The variances of the estimated total concentration of $^{14}CO_2$ were compared assuming a linear model including main-effects and 2-way interaction effects for the logarithm of the variance between replicates for each of the eight combinations. Assuming the estimated total concentration of $^{14}CO_2$ to be normal distributed with constant variance, the logaritmic transformed variances were expected to have a variance of 2/2=1. Comparing variances, no replicate observations exist, s_0^2 cannot be calculated and the possible three-way interactions cannot be tested. The residual showed to be $< 2/f$ (Table III), so it can be concluded that three-way interactions had no importance. Two-way interactions and main effects of tube dimensions and sampling direction were not significant either

(Table III) so an analysis of variance of only the main effect of application was performed, showing that the two different application methods (injection or suction) had differences in variations between replicates (Table IV). The hypothesis of no differences in variances between methods was rejected at the 4.7% level of significance. Suction was the application method with smallest variances as shown in Figures 2–9.

TABLE IV Analysis of variance table for three-way factorial pesticide mineralization experiment. Comparison of logarithmic conversions of variances. Test of the main effect "application method"

Source of variation	*Df*	*Sum of squares*	*Mean square*	*F value*	*Pr > F*
Application	1	6.33980252	6.33980252	6.21	0.0470
Residual	6	6.12617825	1.02102971		
Corrected total	7	12.46598077			

Comparison of means

The estimated total $^{14}CO_2$ concentrations were analysed using a linear model assuming that the variances of the different methods were different:

$$Y_{tsan} = m + a_t + b_s + g_a + (ab)_{ts} + (ag)_{ta} + (bg)_{sa} + E_{tsan}$$

where Y_{tsan} = estimated total $^{14}CO_2$ of the application method "a" using tubes of dimension "t" placed in sampling direction "s" in replicate "n" and $E_{tsan} \sim$ i.i.d. $N(0, s^2_a)$.

The analysis showed that all effects could be assumed to be zero except for the interaction effect of tube dimension times sampling direction, Table V. The short cores sampled horizontally are found to produce significantly lower amounts of $^{14}CO_2$ than the long cores sampled in that direction and the short cores sampled vertically, Table V.

DISCUSSION

The lower variability of pesticide degradation between replicates found for the suggested suction method compared to the injection technique is of major importance for future pesticide mineralization experiments. The application of the pesticide through the filter paper combined with convectional as well as diffusional distribution of the substrate during the 2 hour period of suction to the initially drained soil sample may have caused a better distribution of pesticide within the soil core in investigation. Also the control of the water regime by applying a well defined suction to the samples before application of the pesticide may have attributed to the better performance of the degradation tests.

TABLE V Comparison of means of different combinations of experiments. Figure s denoted with the same letter are not significantly different

Combination	*Least squares mean, % ^{14}C evolved as $^{14}CO_2$*	*Std. Dev.*
Tube dimensions:		
short	19.63^{a}	2.29
long	22.88^{a}	2.29
Sampling:		
vertical	21.85^{b}	2.29
horizontal	20.66^{b}	2.29
Application:		
injection	21.90^{c}	3.06
suction	20.61^{c}	1.05
Tube dim*samling:		
short*vertical	25.07^{d}	2.97
short*horizontal	14.20^{e}	2.97
long*vertical	18.64^{de}	2.97
long*horizontal	27.13^{d}	2.97
Tube dim*application:		
short*injection	21.54^{f}	4.32
short*suction	17.73^{f}	1.49
long*injection	22.27^{f}	4.32
long*suction	23.50^{f}	1.49
Sampling*application:		
vertical*injection	20.48^{g}	4.32
vertical*suction	23.22^{g}	1.49
horizontal*injection	23.32^{g}	4.32
horizontal*suction	18.00^{g}	1.49

Of high importance is also the fact that the new and the old application method produced about identical estimates of pesticide degradation. The water potential suggested in this study is relevant in future use of the new method. Although investigations have indicated the degradation of some pesticides, eg. mecoprop to be rather insensitive to soil water content, from between water holding capacity to above wilting point[14], it it may be regarded as a progress to conduct these experiments under more controlled water regimes than is the case for our previous method. This is of especial importance when comparing results from contrasting soil types having widely different soil water holding characteristics.[5]

Tube dimensions and sampling direction were found to have no effect upon pesticide degradation. Differences in soil characteristics within the 15 cm deep subsoil layer in investigation may have caused the stastically significant interaction between tube dimensions and sampling direction.

Further research is needed to test the performance of the suggested method also for other soil types. It is also desirable to investigate the effect of water potential and perhaps different structural states of the soil (e.g. compacted soil) in order to get a better understanding of the degradation processes in the soil.

CONCLUSION

The mean values for the formation of the mineralization product $^{14}CO_2$ showed no significant difference between our previous method and the new. The new method, however, had smaller variances between replicates, which is a great advantage, when working with soil samples where the heterogeneity of the soil generally increases the variance.

With the new method, compression of soil is avoided, and the application of pesticide resembles natural conditions, because pore size distribution is not changed. The possibility of adjusting the water content of the soil sample according to field capacity (pF values) is another advantage.

The new system for sampling and application of pesticides in pesticide mineralization studies showed to be recommendable.

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