

# Comparison of Mineralization Dynamics of 2,4-Dichlorophenoxyacetic Acid (2,4-D) and 4-Chloro-2-Methylphenoxyacetic Acid (MCPA) in Soils of Different Textures

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## Abstract

The degradation behaviour of 2,4-D and MCPA in four types of soil were determined from measurements of <sup>14</sup>C<sub>2</sub>O evolution over a period of 100 days. The total <sup>14</sup>C-organic volatile compounds evaporated from the soils during the experimental period and the residual <sup>14</sup>C in the soils at the end of the incubation period was also determined. The degree of mineralization was different for tested pesticides, and did not exceed 30% for 2,4-D or 46% for MCPA. The greatest mineralization of 2,4-D occurred in sandy soils containing the least amount of organic carbon, while in the case of MCPA, the highest level of mineralization was observed in loamy sand and silt loam soils. Volatilization was the most important mechanism of 2,4-D loss from soils and accounted for 46.6% of the total applied dose for sandy loam soil. The emission of volatile organic substances from MCPA-treated soils was lower, with the maximum value of 10.5% being emitted from silt soil. A significant amount of the introduced radioactive material was recovered as residues. The level of <sup>14</sup>C-extractable residues for pesticides was low and ranged from 0.9% to 4.9% of total radioactivity. However, the level of <sup>14</sup>C-bound residues was significantly greater and ranged from 14.6% to 43.2% of total radioactivity.

**Keywords:** 2,4-D, MCPA, mineralization, extractable residues, bound residues

## Introduction

Most intensive agricultural practices rely on the use of herbicides to increase crop productivity and improve food quality, and a large proportion of these chemicals reach the soil. However, continuous and extensive pesticide application creates environmental concerns due to the effect on the biological function of the soil. Some of the most common-

ly used pesticides in the world are phenoxy herbicides that have been widely used for weed control since World War II. Important derivatives of the selective systemic phenoxyacetic acid include 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA) (Fig. 1). The first is widely used in cultivated agriculture, pasture and forest management, and to control aquatic vegetation, while the second is used for post-emergence control of broadleaf weeds [1]. Their main mode of action is the inhibition of cell division in new tissues, interfering with

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Table 1. General characteristics of soils used in the experiment.

| Parameter   | Soil I      | Soil II      | Soil III    | Soil IV      |
|---|-------------|--------------|-------------|--------------|
| Sand (2000-50 µm) (%)                                   | 91.0±4.2    | 18.0±2.0     | 69.0±3.2    | 4.0±0.4      |
| Silt (<50-2 µm) (%)                                     | 6.0±1.1     | 76.0±3.8     | 21.0±1.3    | 85.0±2.2     |
| Clay (< 2 µm) (%)                                       | 3.0±0.9     | 6.0±1.3      | 10.0±1.0    | 11.0±0.8     |
| Density (g·cm <sup>-3</sup> )                           | 1.02±0.03   | 1.28±0.09    | 1.11±0.04   | 1.46±0.09    |
| pH <sub>(in water)</sub> (1:5)                          | 6.8±0.4     | 6.0±0.3      | 5.3±0.2     | 7.0±0.2      |
| Cation exchange capacity (CEC) (cmol+kg <sup>-1</sup> ) | 3.300±0.043 | 11.500±0.098 | 8.000±0.079 | 22.000±1.321 |
| Water holding capacity (WHC) (%)                        | 32.0±2.2    | 48.0±1.5     | 36.0±1.9    | 54.0±1.6     |
| Organic carbon (%)                                      | 1.1±0.2     | 2.4±0.6      | 1.2±0.02    | 3.2±0.04     |
| Total nitrogen (%)                                      | 0.07±0.01   | 0.18±0.03    | 0.09±0.02   | 0.22±0.03    |
| Microbial biomass (mg·kg <sup>-1</sup> d.w.)            | 610±23      | 793±38       | 684±32      | 1,020±56     |
| Soil texture classification (US/FAO System)             | Sand        | Silt loam    | Sandy loam  | Silt         |

The values are the means of three replicates with the standard deviation which was within 5% of the mean.

growth and causing apical necrosis, cell disruption, and plant death. These and other phenoxyalkanoic acids have been reported to be mutagenic agents [2]. The herbicides are mobile, easily leached, and weakly persistent, with degradation times ranging from 1 to 3 weeks for 2,4-D and from 1 to 16 weeks for MCPA [3-6]. The average half-life of 2,4-D is approximately 10 days, but Gaultier et al. [7] reported that the field half-life dissipation in 123 subsurface soils was much lower, ranging from 1.7 to 3.5 days. In turn, the half-life of MCPA was reported to be approximately 14 days to 1 month [8].

Many processes are involved in the dissipation of 2,4-D and MCPA in soil. In general, the fate of pesticides in soils is determined by processes such as volatilization, uptake by plants, leaching and runoff, sorption and binding by soil components, and chemical and microbial degradation [9-13]. MCPA is also susceptible to photodegradation in sunlight, but this process is not considered important in 2,4-D loss [14]. The rate of pesticide loss is related to soil properties including organic and mineral content, pH, temperature, soil texture, and the amount of oxygen and water present. By controlling the extent and strength of sorption processes these factors govern the mobility and availability of pesticides for both abiotic and biotic transformations [7, 15-17].

The most important process responsible for 2,4-D and MCPA dissipation is microbial degradation. Many species of bacteria and fungi are capable of degrading phenoxy acid herbicides for use as carbon and energy sources.

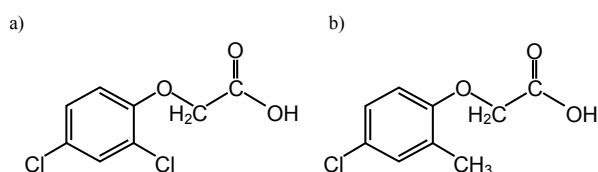


Fig. 1. Chemical structure of 2,4-D (a) and MCPA (b).

Degradation by various strains has been extensively studied and is well documented and reviewed. Bacterial and fungal genera reported to degrade these herbicides include *Acitenobacter*, *Arthrobacter*, *Burkholderia*, *Flavobacterium*, *Pseudomonas*, *Variovorax*, *Trichoderma*, and *Aspergillus* [18-21]. Many reports are also available for discussing the degradative pathways of phenoxy pesticides characteristic of each microbial strain [22-24].

We evaluated the mineralization dynamics of 2,4-D and MCPA over a 100-day period in four soils with different physical and chemical properties. The experiments were performed under controlled laboratory conditions. The degree of 2,4-D and MCPA mineralization was determined at various times from measurements of evolved <sup>14</sup>C<sub>2</sub>O<sub>2</sub>. The total amount of <sup>14</sup>C-organic volatile compounds evaporated from the soils during the experimental period and the levels of <sup>14</sup>C-extractable and -bound residues remaining at the end of the experiment were also determined.

## Materials and Methods

### Soils

Four different composite samples of soils, prepared from ten different sub-samples taken from the areas of 25 m<sup>2</sup>, were collected from the top layer (0-20 cm) at grass-covered fields located in Upper Silesia, southern Poland. The sampling places have not been used for agricultural purposes during the past five years, no application of 2,4-D or MCPA, as well as organic and inorganic fertilizers have been used. Detailed physical and chemical properties of the soils are presented in Table 1. Based on the following analysis the soils used were classified according to the US/FAO System [25]. Particle size of soils was determined by areometric method [26], while the pH value of the aqueous soil extracts (1:5, w/v) were measured in triplicate with

a glass electrode by a Jenway pH-meter at 20°C [27]. The barium chloride method was used for determining cation exchange capacity [28] and concentrations of analyzed ions were estimated by using atomic absorption spectrometry (AAS). Water holding capacity (WHC), organic carbon content ( $C_{org}$ ), and total nitrogen content ( $N_{tot}$ ) were determined by gravimetric method [29], dichromate oxidation in the presence of concentrated sulfuric acid [30], and the Kjeldahl method [31], respectively. Microbial biomass in the soils was measured by glucose-induced respiration method [32]. In the laboratory, the soils were gently air-dried to the point of soil moisture suitable for sieving. After sieving to a maximum particle size of <2 mm, the soils were pre-incubated for 2 weeks in darkness at 20±2°C, before they were used for the experiment.

### Chemicals

Certified standards of 2,4-dichloropenoxyacetic acid (2,4-D, 99.6% chemical purity) and 2-methyl-4-chlorophenoxy-acetic acid (MCPA, 99.6% chemical purity) were purchased from IPO Warsaw, Poland, while [U-ring- $^{14}C$ ]-2,4-D (specific activity 55 mCi mmol<sup>-1</sup>, radiochemical purity 99.0%) and [U-ring- $^{14}C$ ]-MCPA (specific activity 100 µCi mg<sup>-1</sup>, radiochemical purity >99.0%) were obtained from American Radiolabeled Chemicals Inc., USA, and the Institute of Isotopes Co. Ltd., Hungary, respectively. All other chemicals were of analytical grade and purchased from Merck, Germany.

### Test System and Experimental Conditions

The test system consisted of 250 ml biometer flasks containing 50 g dry weight of soil. The flasks were hermetically sealed and connected to a vial containing 10 ml of ethylene glycol to trap  $^{14}C$ -organic volatile compounds and to a second vial containing 10 ml of 0.1 M NaOH to trap  $^{14}CO_2$  (Fig. 2). Stock solutions of 2,4-D or MCPA (200 µl,  $^{12}C+^{14}C$ ) were sprayed on the soil surface by means of a micro-syringe that dispensed very small droplets and ensured thorough mixing. The applied volume contained 500 µg 2,4-D or MCPA, corresponding to a soil concentra-

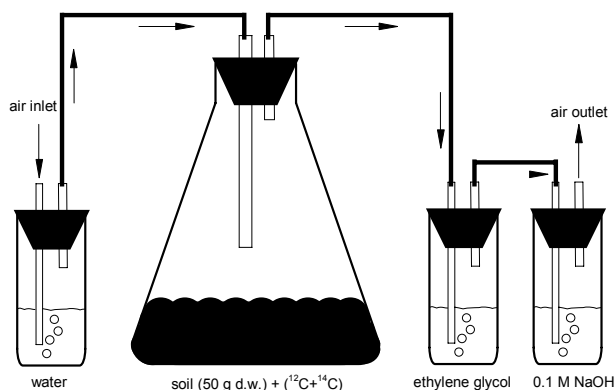


Fig. 2. Schematic of biometer flask used in experiment of 2,4-D and MCPA mineralization dynamics.

tion of 10 mg·kg<sup>-1</sup>. Three replicates of each soil treatment were prepared. The level of introduced radioactivity was checked using a Beckman LS 5000TD Liquid Scintillation Analyzer (Beckman Instruments, Inc, USA). The radioactivity introduced into each sample was approximately 2.48 µCi for 2,4-D and 2.77 µCi for MCPA. The water content of the soil was adjusted to 50% of the maximum water holding capacity. Control samples of each soil consisting of the same 50 g dry weight of soil and the same volume of water were also prepared. Throughout the incubation period, the deionized water was added to the soil to compensate for any water losses exceeding 5% of the initial amount added. The soil samples were incubated for 100 days in a darkened thermostatic chamber (New Brunswick Scientific Co., Inc., USA) maintained at 20±2°C.

### Mineralization Study

After 1, 2, 4, 8, 16, 32, 64, and 100 days of incubation, the NaOH solutions were removed for analysis of evolved  $^{14}CO_2$ . The traps were then refilled with 10 ml of 0.1 M NaOH fresh solution. The radioactivity of 2 ml aliquots of the trap solutions was measured using a Beckman LS 5000TD Liquid Scintillation Analyzer (Beckman Instruments, Inc, USA) after the addition of 10 ml of scintillation cocktail (Ready Gel™ Liquid Scintillation Cocktail, Beckman Coulter, USA) and a 24-hour stabilization period. The mean radioactivity recoveries for compounds introduced into NaOH were 90.6±1.2% for  $^{14}C$ -2,4-D and 90.2±0.2% for  $^{14}C$ -MCPA.

### Evaporation Study

After 100 days of incubation, the total amount of  $^{14}C$ -organic volatile compounds absorbed in the ethylene glycol trap was measured. The radioactivity of 1 ml portions of the trapped solutions was measured using liquid scintillation counting after the addition of 10 ml of scintillation cocktail and a 1-hour stabilization period. The mean recovery percentages were 89.6±0.4% for  $^{14}C$ -2,4-D and 88.4±1.0% for  $^{14}C$ -MCPA.

### Extractable and Bound Residues Analyses

The level of extractable residues in the soil samples was determined after 100 days of incubation. The residues were analyzed by extraction of a 5 g sample of dry soil in a 50 ml glass centrifuge tube with a Teflon cap. The soil samples were extracted with six 10 ml portions of methanol and agitated using a vortexer (Vibrofix VF1, Janke & Kunkel-Ika Labortechnik, Germany). The samples were centrifuged at 5,000 g for 5 min (Janetzki T52, Germany) and the supernatants were filtered through filter paper into a 250 ml flask. The extracts were reduced to 5 ml using a rotary evaporator (Rotavapor® R-210, Büchi Labortechnik AG, Switzerland) at 40°C and then diluted with methanol to a final volume of 25 ml. The radioactivity of a 2 ml aliquot of each sample was measured using a liquid scintillation counter after the addition of 10 ml of scintillation cocktail

and stabilization for 2 hours. The mean recovery of radioactivity introduced into the soil samples was  $84.9 \pm 7.0\%$  for  $^{14}\text{C}$ -2,4-D and  $85.8 \pm 2.8\%$  for  $^{14}\text{C}$ -MCPA.

The level of  $^{14}\text{C}$ -bound residues in the soil samples was determined after 100 days of incubation. After extraction of the residues with methanol, the soil samples were air-dried. The portions of soil samples (2 g) were placed in a 500 ml flask and oxidized using 25 ml of a 3:1 mixture of concentrated  $\text{H}_2\text{SO}_4$  and  $\text{H}_3\text{PO}_4$  and 3 g of  $\text{K}_2\text{Cr}_2\text{O}_7$  for 50 min at  $80^\circ\text{C}$ . The  $^{14}\text{CO}_2$  released by the samples was absorbed in 0.1 M NaOH. The radioactivity of the NaOH solution (2 ml) was measured using liquid scintillation counting after the addition of 10 ml of scintillation solution and stabilization for 24 hours. The mean recovery of radioactivity for bound residues was  $94.3 \pm 5.4\%$  and  $91.9 \pm 1.2\%$  for  $^{14}\text{C}$ -2,4-D and  $^{14}\text{C}$ -MCPA, respectively.

### Calculations

The results from three replicates of each treatment were evaluated using analysis of variance and statistical analysis. The statistical significance ( $P < 0.05$ ) of the differences between pesticides and the effects of soil type on the measured parameters were treated statistically using two-way ANOVA and assessed by post hoc comparison of means using the lowest significant differences (LSD) test. Statistical analysis was carried out using the Statistica 6.0 PL software package. After the experiment, a mass balance to evaluate the total recovery of  $^{14}\text{C}$  introduced into the soils was also performed.

## Results

### Degree of Mineralization

Although differences in mineralization were observed, the degree of 2,4-D mineralization after 100 days did not exceed 30% of the introduced radioactivity in any of the soils (Fig. 3). The mineralization curves were characterized by a lag phase during which the mineralization occurred at a low and linear rate followed by exponential  $^{14}\text{CO}_2$  evolution. In sandy soil, where the rate of 2,4-D dissipation was the highest, the initial phase lasted only one day and pesticide disappearance was observed as early as the second day after application. The  $^{14}\text{CO}_2$  evolution increased linearly until reaching a plateau after 8 days, when the cumulative radioactivity reached 26.2% of the total introduced activity, nearly 90% of the cumulative radioactivity measured on day 100. Upon reaching the plateau, the mineralization process proceeded very slowly, and at the end of the experiment, 29.3% of the applied dose was mineralized (Fig. 3).

A similar 2,4-D mineralization was observed in silt loam and silt soils (Fig. 3). In contrast to sandy soil, the lag phase in these soils lasted nearly 4 days and the mineralization rate during the first days was significantly lower. After two days, only 1.5% of the applied radioactivity had evolved from the silt loam samples, and only 1.0% had evolved from the silt soils. After this point, the mineralization rates for silt loam and silt soils diverged and by day 8 the total radioactivity evolved was 13.9% for silt loam and 18.8% for silt soils. At the end of the experimental period,

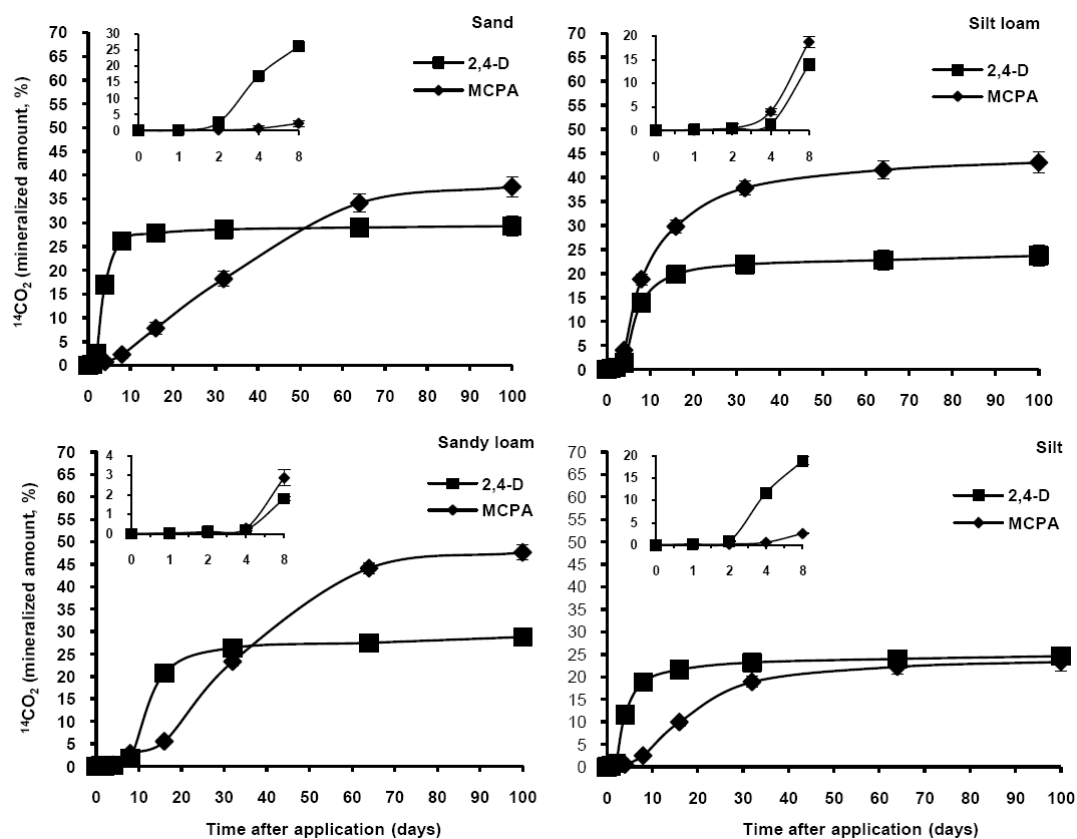


Fig. 3. Mineralization dynamics of 2,4-D and MCPA in different soils. Symbols are the means of three replicates. Error bars represent the standard deviation, which was within 5% of the mean.

Table 2. The total amounts of <sup>14</sup>C-organic volatile compounds (% total radioactivity) evaporated from the soils during the experimental period.

| Soil       | Pesticide             |                       |
|------------|-----------------------|-----------------------|
|            | 2,4-D                 | MCPA                  |
| Sand       | 37.4±3.0 <sup>a</sup> | 0.8±0.2 <sup>b</sup>  |
| Silt loam  | 30.4±2.2 <sup>c</sup> | 5.4±0.3 <sup>d</sup>  |
| Sandy loam | 46.3±6.1 <sup>e</sup> | 4.8±0.1 <sup>f</sup>  |
| Silt       | 35.3±2.6 <sup>a</sup> | 10.5±0.1 <sup>g</sup> |

The values are the means of three replicates with the standard deviation which was within 5% of the mean. The significant differences between pesticides and the effect of soil were indicated by different letters (two-way ANOVA; P<0.05; LSD test).

23.7% of the radioactivity introduced into the silt loam samples and 29.6% of the radioactivity in the silt soil samples had evolved as <sup>14</sup>CO<sub>2</sub> (Fig. 3). The lag phase for 2,4-D mineralization in sandy loam soil lasted nearly 8 days, during which the mineralization rate was very low and did not exceed 1.6% of the applied dosage (Fig. 3). From this point, <sup>14</sup>CO<sub>2</sub> evolution sharply increased, and during the next 24 days 24.6% of the added 2,4-D was mineralized. After 10 days, total radioactivity evolved as <sup>14</sup>CO<sub>2</sub> amounted to 28.8% of the introduced radioactivity.

The rate of MCPA mineralization in terms of <sup>14</sup>CO<sub>2</sub> evolution is presented in Fig. 3. For this pesticide, a linear increase in <sup>14</sup>CO<sub>2</sub> evolution over the experimental period was observed. However, the course of this process was different in various soils. In sand, sandy loam, and silt soils the lag phase preceding MPCA dissipation lasted 4 days, whereas in silt loam soil this phase was shorter (2 days), and by day 4 approximately 5% of the added radioactivity had evolved as <sup>14</sup>CO<sub>2</sub> (Fig. 3). After 8 days, 18% of the introduced radioactivity had been recovered from silt loam samples, while the recovery in the remaining soils did not exceed 3% of the applied radioactivity. Beyond this point, MCPA mineralization accelerated in all soils and differ-

ences in the kinetics of <sup>14</sup>CO<sub>2</sub> evolution from MCPA mineralization became better defined. After 16 days, the recovered radioactivity levels were 7.8%, 29.8%, 5.5%, and 10% of the initial radioactivity for sand, silt loam, sandy loam, and silt soils, respectively (Fig. 3). The highest increase in MCPA mineralization occurred in sandy loam soil, where after 64 days the amount of evolved <sup>14</sup>CO<sub>2</sub> reached 41% of the total radioactivity added to the soil. Over the course of the experiment, the highest MCPA mineralization occurred in sandy loam and silt loam soils, in which 47.6% and 43.1% of the total applied radioactivity were recovered as <sup>14</sup>CO<sub>2</sub>. In the remaining soils, the mineralization of MCPA was significantly lower, with 37.6% of the applied radioactivity being recovered as <sup>14</sup>CO<sub>2</sub> from sand and 23.4% of the applied dose being recovered from silt soil. The mineralization rate in tested soils was dependent on pesticide (P<0.0001), soil type (P<0.0001), time (P<0.0001) and interactions between these factors (P<0.0001).

### Amounts of the Total Volatile <sup>14</sup>C-Organic Compounds

The total amounts of <sup>14</sup>C-organic volatile compounds recovered at the end of the trials are presented in Table 2. The results of evaporation studies reveal that this process was significant in the case of 2,4-D dissipation from soils. A large fraction of the introduced radioactivity was isolated in the form of volatile <sup>14</sup>C-organic compounds, ranging from 30.4% for silt loam soil to 46.3% for sandy loam soil. For MCPA, the amount of radioactivity corresponding to volatile organic compounds was lower, with a maximum value of 10.5% of the introduced radioactivity obtained for silt soil (Table 2). The total amount of <sup>14</sup>C-organic volatile substances recovered was dependent on pesticide (P<0.0001), soil type (P<0.0001), and interactions between these factors (P<0.0001).

### Amounts of Extractable and Bound Residual <sup>14</sup>C

The levels of extractable and bound residual <sup>14</sup>C present in the soils are listed in Fig. 4. A significant fraction of the

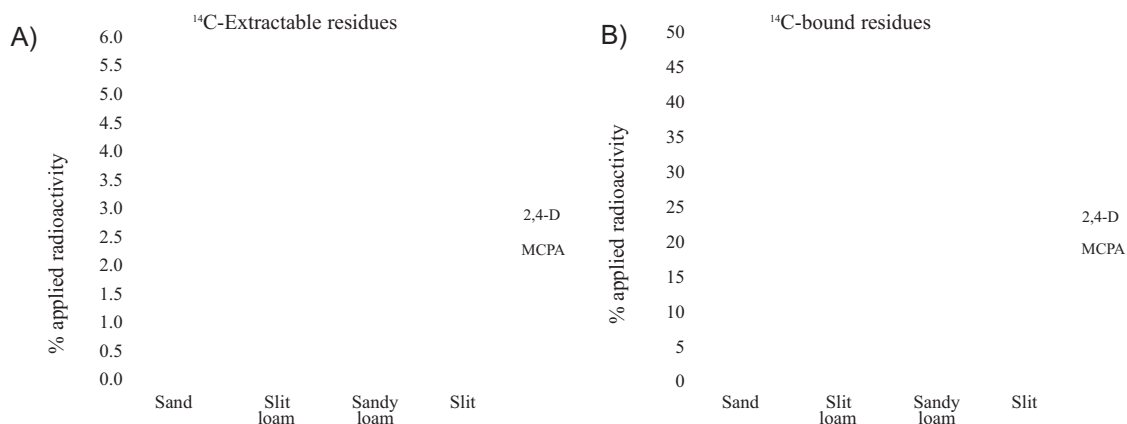


Fig. 4. <sup>14</sup>C-Extractable (A) and <sup>14</sup>C-bound residues (B) in soils after 100 days of 2,4-D and MCPA mineralization. The values are the means of three replicates. Error bars represent the standard deviation, which was within 5% of the mean. The significant differences between pesticides and the effect of soil for a given kind of residue formation were indicated by different letters (two-way ANOVA; P<0.05; LSD test).

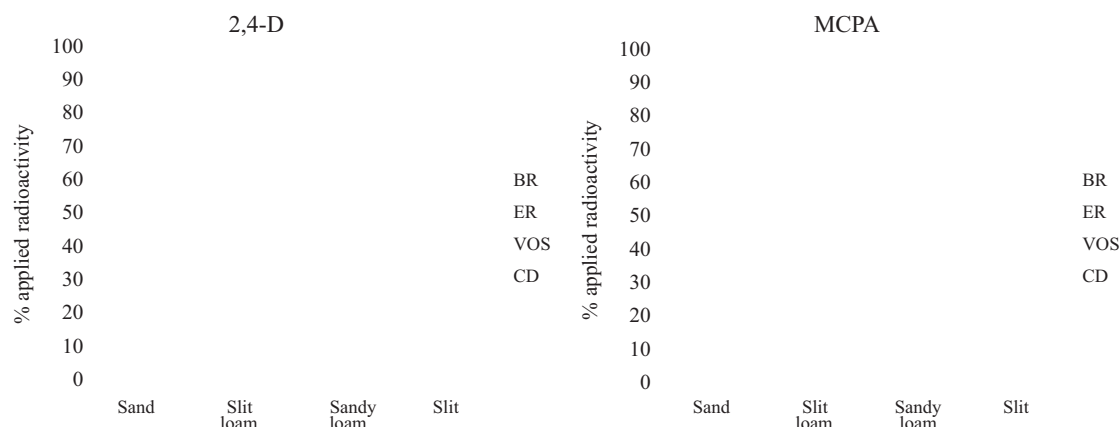


Fig. 5. Mass balance for tested pesticides after 100 days of experiment. Error bars represent the standard deviation, which was within 5% of the total recovery of three replicates. Abbreviations: CD –  $^{14}\text{CO}_2$ ; VOS –  $^{14}\text{C}$ -organic volatile substances; ER –  $^{14}\text{C}$ -extractable residues; BR –  $^{14}\text{C}$ -bound residues.

introduced pesticides were converted to extractable and bound residues. Moreover, significant differences were observed between soils in the formation of pesticide residues. In general, the level of  $^{14}\text{C}$ -extractable residues for all pesticides was low and ranged from 0.7% (silt soil) to 1.2% (sandy loam soil) for 2,4-D, and from 2.0% (silt loam soil) to 4.9% (sandy soil) for MCPA. Levels of bound residual  $^{14}\text{C}$  were greater in all soil types. For 2,4-D the non-extractable residues ranged from 14.6% in sandy soil to 31.2% in silt loam soil after 100 days, while in the case of MCPA the amount of bound residual pesticides ranged from 23.0% (sandy loam soil) to 43.2% (silt soil). The amounts of residual radioactivity were dependent on pesticide ( $P < 0.0001$ ), soil type ( $P < 0.0001$ ), and interactions between these factors ( $P < 0.0001$ ).

### Mass Balance

The total recovery of  $^{14}\text{C}$  in 2,4-D samples ranged from 85.4% (sandy soil) to 96.1% (sandy loam soil), while in the case of MCPA samples the total recovery ranged from 77.5% (sandy loam soil) to 85.4% (sandy soil) (Fig. 5).

### Discussion

Our studies demonstrate that 2,4-D and MCPA are readily degraded in various soils. However, the extent and rate of pesticide mineralization and the amount of residue varied between soils. Differences in pesticide dissipation were especially apparent during the first days after application. The lag phases for 2,4-D and MCPA degradation varied in length, but in all soils adaptation phases were followed by rapid mineralization. Mineralization of 2,4-D was low in all soil types, and after 100 days of incubation less than 30% of the total radioactivity introduced into the soils was evolved as  $^{14}\text{CO}_2$ . Other studies have claimed much higher degrees of mineralization for 2,4-D. For example, Gonod et al. [33] observed mineralization of 35-50% of the total pesticide applied to soil aggregates of various sizes. Even more

effective 2,4-D mineralization (from 54% to 66% for clay, loamy, and sandy soils) was reported by Boivin et al. [34]. On the contrary, a lower extent of 2,4-D mineralization was observed in Egyptian clay and clay loam, where only 10-14% of the applied dose was degraded over a period of 90 days [35].

We observed that the kinetics of 2,4-D mineralization were highly variable during the first 16 days of incubation. Later differences between degradation curves for the different soils were much smaller, suggesting that a balance between the available 2,4-D and the microbial potential for pesticide degradation had been established. The occurrence of a short lag phase (1 day) followed by a strong increase in mineralization in sandy soil indicated that the population of 2,4-D degrading microorganisms was sufficient. It has been reported that microorganisms capable of utilizing 2,4-D *via* growth-linked degradation or cometabolism occur both in pristine soils and soils previously exposed to this pesticide [19, 21]. The short lag phase in sandy soil might also be attributed to the high availability of the pesticide for transformations, since this soil is characterized by a small amount of clay and organic matter. Clay and organic matter serve as sites for pesticide adsorption and can exert a large influence on bioavailability [16, 36-38]. Although it has been shown that phenoxyacid pesticides are weakly sorbed by various solid soil components [34] and dissolved organic matter [5], adsorption is probably the most important mode of interaction between soil and pesticides and controls their concentration in the soil liquid phase. Many studies of pesticide degradation kinetics have underscored the complex interactions between sorption and degradation. Guo et al. [39] have reported that increasing the sorption potential of soil by adding activated carbon significantly decreased the degradation rate of 2,4-D. Gaultier et al. [6] studied the degradation of 2,4-D in 114 agricultural soils and found that 2,4-D degradation was strongly correlated with the amount of soil organic carbon present. The authors divided the soils into two groups based on their soil organic carbon (SOC) content. The first group includ-

ed soils with less than 1% SOC and the second those with greater than 1% organic carbon. Soils in the first group generally had greater 2,4-D degradation rates. A similar phenomenon was observed in our study, where the greatest total degradation of 2,4-D occurred in sandy soils containing approximately 1% organic carbon, whereas the lowest mineralization was observed in silt loam and silt soils containing 2.4% and 3.2% of organic carbon. However, the variability in 2,4-D and MCPA mineralization may be caused by a complex set of interacting parameters such as bioavailability of the compound, pH, moisture, microbial diversity, degree of microorganism specificity, and soil properties [1, 40, 41].

The mineralization of MCPA proceeded differently from 2,4-D. Differences in the extent and rate of MCPA mineralization with respect to soil type were greater than those observed for 2,4-D mineralization. MCPA degradation began later in the experiment, suggesting that the structure of the pesticide strongly influences the degradation. The dissipation of MCPA ranged from 23.4% in silt soil to 47.6% in loamy sand. Similar or higher degrees of MCPA mineralization were reported by Sørensen et al. [42], ranging from 44% for sandy soil to 72% for clay soil. Similarly, Fredslund et al. [43] found that 49% to 62% of MCPA was converted to  $^{14}\text{CO}_2$  during a 67-day incubation period in sandy soil. In our study the mineralization of MCPA in sandy soil was lower than that observed for silt loam or loamy sand soil, suggesting that pesticide availability is not a key parameter regulating MCPA degradation. Sørensen et al. [42] observed that even when sorption was high mineralization proceeded rapidly and that the highest rate of MCPA dissipation correlated with the largest sorption and the lowest desorption. An opposing relationship between sorption strength and mineralization was described by Jensen et al. [44]. The authors suggested that mineralization only occurred in the water-extractable fraction of MCPA. In turn, Juhler et al. [45] studied 18 parameters in 62 soil samples from different sites and observed that MCPA degradation was strongly related to total organic carbon and microbial activity. The influence of organic carbon content on MCPA mineralization was also described by Thorstensen and Lode [9]. The authors explained the faster degradation of MCPA in sandy loam soil compared to loam soil as a consequence of the higher pH and higher organic content in the second soil, which increases pesticide sorption. However, in highly decomposed material with the greatest sorption capacity they observed increased MCPA degradation rates. In our study the MCPA mineralization varied between soils, but we did not find any useful correlation to any of the determined parameters. It seems that other factors, such as available nutrients or the number and activity of the microorganisms, influence the MCPA degradation to a greater extent.

One of the known mechanisms of pesticide loss is volatilization, particularly when pesticides are applied to soil or plant surfaces. Taylor and Spencer [46] reported that volatilization might exceed pesticide dissipation through chemical degradation, runoff, or leaching. We observed that pesticides differed distinctly in their volatility. This process

played the most important role in the case of 2,4-D dissipation, where almost 50% of the applied radioactivity in sandy loam soil later appeared as volatile  $^{14}\text{C}$ -organic compounds. For MCPA, the amount of radioactivity corresponding to volatile organic substances was much lower and the highest recovery of  $^{14}\text{C}$ -organic carbon (10.5%) evaporated from soils occurred in silt soil. The physical and chemical properties of the pesticide and the environmental conditions exert significant influences on this process. The higher evaporation of 2,4-D is probably due to weaker binding of 2,4-D to soil components and the formation of low molecular mass degradation products that evaporate more easily than the parent pesticide or 2,4-dichlorophenol, the primary degradation metabolite [34].

A large fraction of the introduced radioactivity was recovered in the form of extractable and non-extractable residues. However, the level of extractable  $^{14}\text{C}$ -residues was low for all pesticides, with maximum values of 1.2% for 2,4-D and 4.9% for MCPA. Similar results have been reported by Boivin et al. [34], who studied the sorption and degradation dynamics of 2,4-D in sandy, clay, and loamy soils. They found that more than 93% of the applied 2,4-D could be desorbed from all soil types using 0.01 M calcium chloride, confirming that 2,4-D sorption is reversible. During the incubation period the level of extracted residues markedly decreased and after 60 days in each soil they constituted less than 2% of the applied dosage. Kristensen et al. [47] studied the mineralization of 2,4-D and other pesticides, reporting that the recovery of water-extractable 2,4-D fractions after 141 days of incubation was less than 8%.

The level of bound  $^{14}\text{C}$ -residues was significantly greater. Depending on the pesticide and soil type, this quantity ranged from 14.6% to 43.2% of the total applied radioactivity. For 2,4-D, the level of bound residues in sandy soil was significantly lower ( $P < 0.05$ ) than in the other soils, indicating the important role of organic matter in the binding of this pesticide. A lower level of bound residues in sandy soils also was observed by Boivin et al. [34]. A significant proportion (typically ranging from 20 to 70%) of a pesticide or its degradation products may remain in the soil as a persistent residue bound to the soil colloids [48, 49, 51]. The ability of soil to retain these compounds is due to strong adsorption and chemical reactions occurring on the surfaces of mineral particles and humus [38, 50]. Van der Werf [2] stated that many pesticides formerly believed to be readily degraded and 'lost' from the soil were later shown to be present in soil as bound residues. From an ecotoxicological point of view, binding of pesticides to organic soil fractions results in a decrease of active substance available to interact with biota; a reduction in the pesticide toxicity; and a reduction in their leaching [48, 49, 51]. These interactions are strongly correlated with aging. Increased contact time between chemicals and soil leads to the formation of a larger fraction of pesticides permanently retained in the soil. There is evidence that with longer residence times in the soil, bound pesticide residues tend to lose their biological activity and become more resistant to degradation and extraction [52, 53].

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