

Mineralization Dynamics of Chlormequat Chloride (CCC) in Soils of Different Textures

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Abstract

The aim of this study was to evaluate the mineralization rates of chlormequat chloride (CCC) in soils of different textures from measurements of ¹⁴CO₂ evolution over a period of 100 days. The level of extractable and bound residual ¹⁴C in the soils at the end of the experiment was also determined. The degradation pattern of CCC was similar in all soil types and degree of its mineralization ranged from 53.4 to 55.7% over a period of 100 days. This process in all soils was described by first-order kinetics and CCC was mineralized with a similar constant rate (*k*) on the level of 0.029-0.034 day⁻¹, and DT₅₀ ranged from 67.7 days for sandy soil to 71.0 days for silt loam soil. Evaporation was not significant in the removal of CCC from soils, and the total amount of ¹⁴C-volatile organic compounds recovered at the end of the trials was similar to the background level. The level of ¹⁴C-extractable residues was low and reached the maximum value of 7.2% of the introduced radioactivity for sand and sandy loam soils. Levels of ¹⁴C-bound residues were higher in all soil types. The highest value (39%) was obtained for silt soil, while in the remaining soils it was lower and ranged from 30 to 33.5% of the introduced radioactivity. The relatively large amount of bound residues of CCC is probably connected with a strong affinity for soil components despite of its high water solubility and potential to be leached.

Keywords: chlormequat chloride, mineralization, bound residues, extractable residues, soil

Introduction

Modern agriculture, apart from pesticides inhibiting the growth of pathogenic microorganisms and pests, uses many compounds that act as plant growth regulators. These chemicals are applied to modify plant growth, flowering and fruit yield. One of the most often used representatives of such substances is chlormequat, which in six out of 18 European countries is among the five chemicals applied most frequently in culture wheat [1]. Chlormequat was included in Annex I of Directive 91/411/EEC with the

restriction to be used only on cereals and ornamental plants. However, it has been approved for use on pears and other fruits in many parts of the world.

Chlormequat (2-chloroethyltrimethylammonium) belongs to the quaternary ammonium pesticide family and is generally applied as a highly water-soluble chloride (chlormequat chloride, also known as chlorocholine chloride and CCC) (Fig. 1). It is used as a plant growth regulator to promote sturdier growth in cereals and reduce the risk of lodging. Chlormequat chloride acts by inhibiting gibberellin synthesis and the restricted elongation of the stem results in shorter and more compact plants [2]. It has been found that CCC application may also increase the number of

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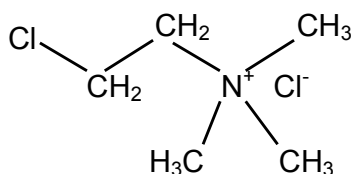


Fig. 1. Chemical structure of chlormequat chloride.

grains per ear and higher ear numbers, and their size appeared to be responsible for the increased yield of barley and triticale [3, 4]. Additional responses of plants to chlormequat application involve increased root size, higher number of leaves, and their delayed senescence [5, 6], as well as changes in biochemical parameters in plants and fruits, such as content of chlorophylls, carotenoids, and concentrations of some microelements [7].

It has been shown that chlormequat may be accumulated in plant tissues and various analytical methods were used to estimate the concentration of CCC in many fruits and agricultural products [8-12]. The results of these studies have revealed that the chlormequat concentrations in fruits treated with recommended CCC dosages were generally under the maximum limit permitted by European regulations. However, the widespread and continuous use of CCC is of great concern since some publications indicated the possible impact of CCC on mammalian health and fertility [13-15]. Results of the *in vitro* experiments conducted to estimate the effect of chlormequat-treated wheat on the reproduction of mice showed that CCC reduced the functional competence of their spermatozoa [16].

In the soil, CCC exhibits moderate persistency, with half-life ranging from 13 to 34 days [17, 18]. Juhler et al. [18], studying the fate of chlormequat in the subsurface layers of sandy and clay soils, calculated that depending on the soil type half-life of CCC ranged from 21 to 61 days. Chlormequat is easily adsorbed to soil particles and the value of distribution coefficient (K_d) reflecting the concentration-dependent sorption ranged from 2 to 566 $\text{cm}^3\cdot\text{g}^{-1}$, reaching the highest values for clayey till soils [18]. In an artificially constructed water/montmorillonite system the values of K_d were significantly lower and ranged from 1.3 to 2.2 $\text{cm}^3\cdot\text{g}^{-1}$ [19]. 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 [20-25]. Adsorption in soil avoids the presence of pesticides in surface and ground waters, but hinders their volatilization and biodegradation; on the other hand, desorption into the soil solution facilitates runoff and leaching into water bodies, but makes the pesticide available to soil microorganisms and facilitates biodegradation as a result [26-29]. Formulation of pesticide is a great factor for its fate in the soil environment as well [30].

Concerning the potential risk for environment and human health, it is important to assess the mechanisms involved in dissipation of CCC in soils. Therefore, the aim of the study was to determine, under laboratory conditions, mineralization dynamics of chlormequat chloride over a

100-day period in four soils with different physical and chemical properties. The degree of CCC mineralization was calculated at various points of the experiment from measurements of evolved $^{14}\text{CO}_2$. Additionally, the total amount of ^{14}C -volatile organic compounds evaporated from soils and the amount of ^{14}C -extractable and ^{14}C -bound residues remaining at the end of the experiment were determined.

Materials and Methods

Characteristics of Soils

Four different composite samples of soils, prepared from ten different sub-samples taken from areas of 25 m^2 , 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, nor for the application of chlormequat chloride as well as organic and inorganic fertilizers. 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 [31]. Particle size of soils was determined by areometric method [32], 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 [33]. Barium chloride method was used for determining cation exchange capacity [34] and concentrations of analyzed ions were estimated 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 [35], dichromate oxidation in the presence of concentrated sulfuric acid [36], and Kjeldahl method [37], respectively. Microbial biomass in the soils was measured by glucose-induced respiration method [38]. 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

The certified standard of chlormequat chloride (2-chloro-ethyltrimethylammonium chloride, CCC, 99.0% chemical purity) was purchased from IPO Warsaw, Poland, while [methyl- ^{14}C]-CCC (specific activity 55 $\text{mCi}\cdot\text{mmol}^{-1}$, radiochemical purity 99.0%) was obtained from American Radiolabeled Chemicals Inc., USA. 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

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 \pm 4.2	18.0 \pm 2.0	69.0 \pm 3.2	4.0 \pm 0.4
Silt (<50-2 μm) (%)	6.0 \pm 1.1	76.0 \pm 3.8	21.0 \pm 1.3	85.0 \pm 2.2
Clay (< 2 μm) (%)	3.0 \pm 0.9	6.0 \pm 1.3	10.0 \pm 1.0	11.0 \pm 0.8
Density ($\text{g}\cdot\text{cm}^{-3}$)	1.02 \pm 0.03	1.28 \pm 0.09	1.11 \pm 0.04	1.46 \pm 0.09
pH _(in water) (1:5)	6.8 \pm 0.4	6.0 \pm 0.3	5.3 \pm 0.2	7.0 \pm 0.2
Cation exchange capacity (CEC) ($\text{cmol}\cdot\text{kg}^{-1}$)	3.300 \pm 0.043	11.500 \pm 0.098	8.000 \pm 0.079	22.000 \pm 1.321
Water holding capacity (WHC) (%)	32.0 \pm 2.2	48.0 \pm 1.5	36.0 \pm 1.9	54.0 \pm 1.6
Organic carbon (%)	1.1 \pm 0.2	2.4 \pm 0.6	1.2 \pm 0.02	3.2 \pm 0.04
Total nitrogen (%)	0.07 \pm 0.01	0.18 \pm 0.03	0.09 \pm 0.02	0.22 \pm 0.03
Microbial biomass ($\text{mg}\cdot\text{kg}^{-1}$ d.w.)	610 \pm 23	793 \pm 38	684 \pm 32	1020 \pm 56
Soil texture classification (US/FAO System)	Sand	Silt loam	Sandy loam	Silt

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

to a second vial containing 10 ml of 0.1 M NaOH to trap $^{14}\text{CO}_2$ (Fig. 2). Stock solutions of CCC (200 μl , $^{12}\text{C}+^{14}\text{C}$) 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 CCC, corresponding to a soil concentration of 10 $\text{mg}\cdot\text{kg}^{-1}$. 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 2.11 μCi . 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 \pm 2 $^\circ\text{C}$.

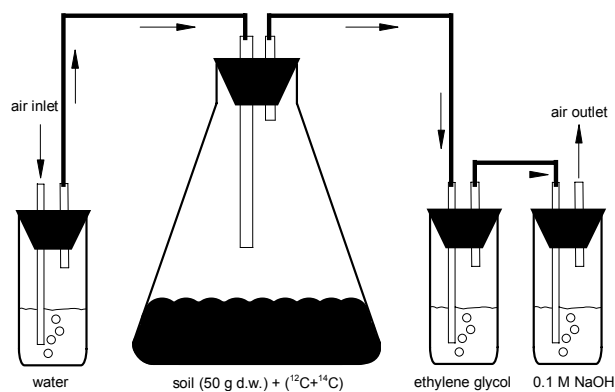


Fig. 2. Schematic of test system used in the experiment of CCC mineralization.

Mineralization Study

After 1, 2, 4, 8, 16, 32, 64, and 100 days of incubation, the NaOH solutions were removed for analysis of evolved $^{14}\text{CO}_2$. 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 after the addition of 10 ml of scintillation cocktail (Ready GelTM Liquid Scintillation Cocktail, Beckman Coulter, USA) and a 24-h stabilization period. During validation studies, the mean radioactivity recovery for ^{14}C -CCC introduced into NaOH was 90.3 \pm 0.4%.

Evaporation Study

After 100 days of incubation the total amount of volatile ^{14}C -organic 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. During validation studies, the mean recovery percentage for ^{14}C -CCC introduced into ethylene glycol was 89.9 \pm 0.1%.

Extractable and Bound Residue Analysis

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 h. During validation studies, the mean radioactivity recovery for ^{14}C -CCC introduced into the soil samples was $89.6 \pm 5.1\%$.

The level of non-extractable ^{14}C -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 H_2SO_4 and H_3PO_4 and 3 g of $\text{K}_2\text{Cr}_2\text{O}_7$ for 50 min at 80°C. The $^{14}\text{CO}_2$ released by the samples was absorbed in 0.1M 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 h. During validation studies, the mean radioactivity recovery for ^{14}C -CCC introduced into the soil samples was $89.9 \pm 3.3\%$.

Kinetics and Statistical Analyses

Mineralization of chlormequat chloride in soils was fitted to a first-order kinetic model. The rate constant (k) (day^{-1}) was determined using the algorithm $C_t/C_0 = e^{-kt}$,

where C_0 is the amount of CCC in soil at time zero, C_t is the amount of CCC in soil at time t (day). Times in which the CCC concentration in soils were mineralized by 50% (DT_{50}) were calculated from the linear equation obtained from the regression between $\ln(C_t/C_0)$ of the chemical data and time (t).

The results from three replicates of each treatment were evaluated using analysis of variance and statistical analysis. The significance ($P < 0.05$) of differences were assessed by post hoc comparison of means using the least significant differences (LSD) test using the Statistica 6.0 PL software package. The data obtained in mineralization studies were treated statistically by two-way ANOVA, considering the effect of soil type, time and interactions between these factors, while the results concerning the residue formation in soils were analyzed by one-way ANOVA, considering the effects of soil type. Following the experiment, a mass balance to evaluate the total recovery of ^{14}C introduced into the soils was also performed.

Results and Discussion

The kinetics of $^{14}\text{CO}_2$ evolution from CCC displayed a similar pattern in all soil samples, but small differences were apparent (Fig. 3). For all soils, the mineralization curves were characterized by a lack of lag phase, and the total

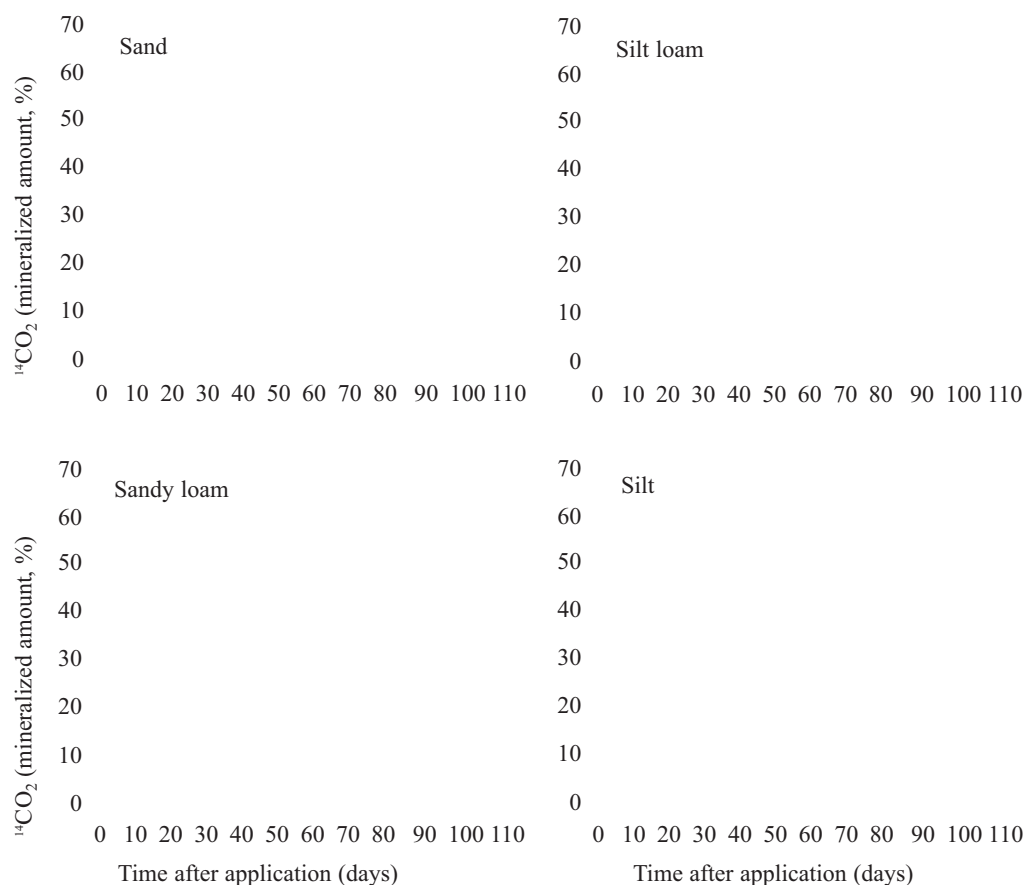


Fig. 3. Mineralization dynamics of CCC in different soils. Symbols are the means of three replicates. Error bars represent the standard deviation, which was within 5% of the mean.

amount of $^{14}\text{CO}_2$ evolved increased linearly over time. After the first day, the lowest CCC mineralization rate occurred in sandy loam soil, where the evolution of $^{14}\text{CO}_2$ did not exceed 3% of the applied dose. For the remaining soils the $^{14}\text{CO}_2$ evolution ranged from 8% to 10%. This observation might result from the lower number of microorganisms and their activities in sandy soils as compared to silt and loam soils. The differences in the dynamics of CCC mineralization were apparent until day 16, after which the amount of evolved $^{14}\text{CO}_2$ was similar for all soil types. After 100 days of incubation 55.7% of the CCC was mineralized in sand, 53.4% in silt loam, 54.8% in sandy loam, and 54.7% in silt soil. Mineralization appeared to be the main CCC dissipation process in soils and it did not depend on the soil type. As indicated by data obtained from kinetic studies, the mineralization process in all soils was described by first-order kinetics with a similar constant rate (k) on the level of 0.029-0.034 day^{-1} (Table 2). Statistical analysis also showed that there were no differences between period of time for a 50% decline of the initial CCC concentration added. The values of DT_{50} calculated from the linear equation obtained from the regression between $\ln(C_t/C_0)$ of the chemical data and time (t) ranged from 67.7 days (sandy soil) to 71.0 days (silt loam soil) (see Table 2). Similar or slightly lower values of DT_{50} for CCC dissipation in clayey till soils were estimated by Juhler et al. [18]. However, in sandy soil the DT_{50} was significantly lower and reached a value of 21 days. Rapid degradation of chlormequat was also observed by Keller [39] in a field experiment with a sandy loam soil and in a greenhouse trial with a clay soil. He found that the applied radioactivity decreased to 88% of the total amount in loam and 33% in clay after three weeks, and to 22% and 33% after six weeks, for loam and clay soils, respectively. In other studies it has been observed that 4 months after CCC usage at the permitted dosages its soil concentration corresponded to 12-23% of the total doses applied [41]. In turn, Guo et al. [17] reported that about 90% of added CCC dissipated during 21 days. The similarity of the degradation curves from day 16 indicates that in presented studies the type of soil had little effect on the mineralization rate of CCC.

One of the known mechanisms of pesticide loss is volatilization, particularly when pesticides are applied to soil or plant surfaces. Taylor and Spencer [41] reported that volatilization might exceed pesticide dissipation through chemical degradation, runoff, or leaching. However, in our studies the evaporation was not significant in the removal of CCC from soils. The total amount of ^{14}C -volatile organic compounds recovered at the end of the trials was similar to the background level. This observation may be explained by chloramequat properties, that the similarity to other quaternary ammonium pesticides is characterized by low volatility (vapour pressure $< 1 \times 10^{-6}$ Pa at 20°C) [2]. It has been shown that pesticides distinctly differed in their volatility, and the physical and chemical properties of the pesticide and the environmental conditions exert significant influences on this process. The higher evaporation of many pesticides is probably due to weaker binding to soil com-

Table 2. Kinetic data of CCC mineralization in different soils.

Soils	Time intervals (days) / k (day^{-1})								Average k (day^{-1})	Linear equation (1^{st} -order)	R^2	DT_{50} (days)*
	0-1	1-2	2-4	4-8	8-16	16-32	32-64	64-100				
Sand	0.103	0.084	0.039	0.016	0.014	0.008	0.005	0.002	0.034 ± 0.008^a	$\ln(C_t/C_0) = -0.0073t - 0.1987$	0.8247	67.7 ± 4.9^b
Silt loam	0.091	0.094	0.041	0.017	0.014	0.008	0.004	0.002	0.034 ± 0.006^a	$\ln(C_t/C_0) = -0.0069t - 0.2035$	0.7825	71.0 ± 3.6^c
Sandy loam	0.029	0.112	0.041	0.015	0.015	0.010	0.005	0.002	0.029 ± 0.009^a	$\ln(C_t/C_0) = -0.0076t - 0.1612$	0.8291	70.0 ± 2.7^b
Silt	0.095	0.087	0.041	0.015	0.015	0.010	0.004	0.002	0.034 ± 0.008^a	$\ln(C_t/C_0) = -0.0071t - 0.2014$	0.7964	69.3 ± 3.1^a

*The values were calculated from the linear equation obtained from the regression between $\ln(C_t/C_0)$ of the chemical data and time (t).

^aThe values marked with the same letter (a) do not differ significantly (one-way ANOVA; $P < 0.05$; LSD test).

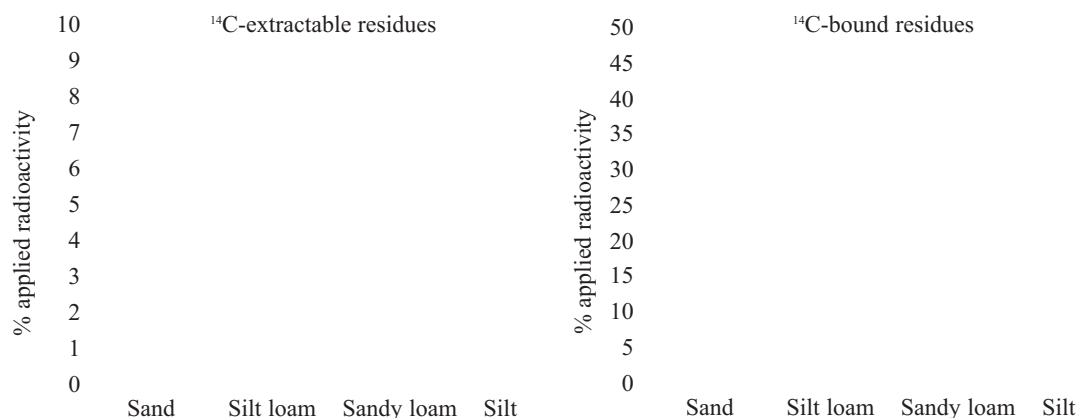


Fig. 4. ¹⁴C-extractable and ¹⁴C-bound residues in soils after 100 days of CCC mineralization. The values are the means of three replicates. Error bars represent the standard deviation, which was within 5% of the mean. The significant differences between soils for a given kind of residue formation were indicated by different letters (one-way ANOVA; $P < 0.05$; LSD test).

ponents and the formation of low molecular mass degradation products that evaporate more easily than the parent pesticide [22, 41, 42].

Results of our studies showed that a large fraction of the introduced ¹⁴C-CCC was converted to extractable and bound residues, and significant differences between soils in the formation of ¹⁴C-CCC residues were observed (Fig. 4). In general, the level of ¹⁴C-extractable residues was low and reached the value of 3.5% of the introduced radioactivity for silt loam and silt soils, while for sand and sandy loam soils this value was two times higher, and reached 7.2% of the introduced radioactivity. Levels of ¹⁴C-bound residues were greater in all soil types. The highest value (39% of total radioactivity) was obtained for silt soil, while the amount of bound residual CCC in the remaining soils was lower and ranged from 30% to 33.5% of the applied radioactivity. The large amount of bound residues is probably connected with a strong affinity of ammonium quaternary herbicides for soil components [43]. Strong retention of the quaternary herbicides to soil greatly limits any leaching or surface run-off, but also reduces their availability for microbial breakdown in the soil water, so they are very persistent in soil.

The mobilization and transport of quaternary herbicides-enriched soil erodes particles may generate downslope and downstream environmental problems that mainly affect detritivore ecology. Therefore, quaternary herbicides have the potential to become re-available in the soil environment and/or to move from the soil compartment to another environmental compartment [44-46]. 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 [23, 24, 47-51]. Strong binding of CCC to soil particles confirmed results by Juhler et al. [18], who found that less than 10% of the sorbed pesticide could be desorbed from the soil phase after water addition to the system studied. Their results obtained from sorption/desorption experiments clearly showed that the

amount of chlormequat retained on soil colloids correlated with soil type. Sorption was highest in the clayey till soil than that in sandy soils, which the authors explained by the larger content of clay and iron in the first soil. Important participation of clay in CCC binding was earlier documented by Maqueda and Morillo [19] in studies with montmorillonite as standard clay mineral. In studies by Henriksen et al. [40], who used pressurized liquid extraction and LC-MS/MS analysis, the recovery of chlormequat reached 46-77% for sandy soils and 13-40% for clayey soils. Higher recovery of CCC ranging from 75% to 82% was reported by Guo et al. [17]. The big differences in published results are attributable to various solvents used for CCC extraction from soils, methods of its detection, and type of soil used in the experiments. Moreover, quaternary ammonium herbicides are a difficult chemical group to analyze [52]. Also in our studies, the highest amount of

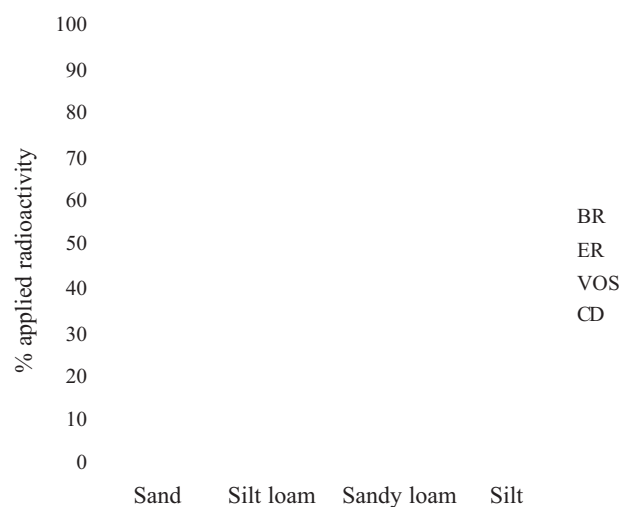


Fig. 5. Mass balance for chlormequat chloride after 100 days of experiment. Error bars represent the standard deviation, which was within 5% of the total recovery of three replicates. Abbreviations: CD – ¹⁴CO₂, VOS – ¹⁴C-volatile organic substances, ER – ¹⁴C-extractable residues, BR – ¹⁴C-bound residues.

CCC-bound residues occurred in silt soil containing the highest content of clay. 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 [42, 50-53]. 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 [54, 55].

A mass balance performed following the experiment revealed that the total recovery of ^{14}C in CCC samples ranged from 88.5% (silt loam soil) to 97.2% (silt soil) (Fig. 5).

In summary, the present study showed relatively fast dissipation of CCC in soils. Mineralization appeared to be the main CCC dissipation process in soils independently of their texture. Due to the low volatility of CCC, no organic compounds evaporated from soils were determined. The content of clay and organic carbon distinctly influenced the amount of ^{14}C -extractable and ^{14}C -bound residues. The relatively large amount of bound residues of CCC is probably connected with a strong affinity for soil components despite its high water solubility and potential to be leached.

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