

The Tema Institute
Campus Norrköping

Chlorination of Organic Material in Agricultural Soil

Vali nia, Salar

Bachelor of Science Thesis, Environmental Science Programme, 2009



LINKÖPINGS UNIVERSITET

Linköpings universitet, Campus Norrköping, SE-601 74 Norrköping, Sweden

**Institution, Avdelning**

Department, Division
Tema vatten i natur och samhälle,
Miljövetarprogrammet
Department of Water and Environmental Studies,
Environmental Science Programme

Datum

Date 2009-06-22

Språk

Language

- Svenska/Swedish
 Engelska/English

Rapporttyp

Report category

- Licentiatavhandling
 Examensarbete
 AB-uppsats
 C-uppsats
 D-uppsats
 Övrig rapport

ISBN

ISBN LIU-TEMA/MV-C—09/15--SE

ISSN**Serietitel och serienummer**

Title of series, numbering

Handledare

Tutor David Bastviken

URL för elektronisk version

<http://www.ep.liu.se/index.sv.html>

Titel

Klorering av organiskt material i åkerjord

Title

Chlorination of organic material in agricultural soil

Författare

Author

Salar Vali nia

Sammanfattning**Abstract**

Chlorine is an essential building block in the environment and can be found in most places. Chlorine participates in a complex biogeochemical cycle and has been discussed for many years and it is well documented that natural chlorination of organic compound takes place in many parts of the ecosystem. Chlorine can be inorganic (Cl_{in}) and organically bound (Cl_{org}). Previous studies have shown that the transformation of Cl_{in} to Cl_{org} is connected with the amount of organic matter and the microbial activity in the soil. So far, studies have been focused on forest soil and there is a need for analysing the natural chlorination in other soil types. The aim of this study was to provide chlorination rates in agricultural soil which does not seem to have been done previously. Three common agricultural soils experiencing different agricultural practice and different cropping systems were incubated with $Na^{36}Cl$ at $20^{\circ}C$ in a 56 days radiotracer experiment. The results show that a chlorination of $^{36}Cl_{in}$ to $^{36}Cl_{org}$ in agricultural soil occurred and the Cl_{org} levels increased over time. The chlorination rates ranged from 0,040 to 0,063 $\mu g Cl g$ dry weight soil $^{-1} d^{-1}$. This was 10-fold lower than rates previously measured in coniferous forest soil. However, when expressed as $\mu g Cl g$ dry weight organic carbon $^{-1} d^{-1}$, rates in the agricultural soil was only slightly (at the most 2-fold) lower than in coniferous forest. This study contributes with new knowledge of natural chlorination rates in agricultural soil and gives further evidence that the natural chlorination can be connected to the amount of organic matter in the soil.

Nyckelord**Keywords**

biogeochemical cycle; chlorination; organic chlorine; organic material

Abstract

Chlorine is an essential building block in the environment and can be found in most places. Chlorine participates in a complex biogeochemical cycle and has been discussed for many years and it is well documented that natural chlorination of organic compound takes place in many parts of the ecosystem. Chlorine can be inorganic (Cl_{in}) and organically bound (Cl_{org}). Previous studies have shown that the transformation of Cl_{in} to Cl_{org} is connected with the amount of organic matter and the microbial activity in the soil. So far, studies have been focused on forest soil and there is a need for analysing the natural chlorination in other soil types. The aim of this study was to provide chlorination rates in agricultural soil which does not seem to have been done previously. Three common agricultural soils experiencing different agricultural practice and different cropping systems were incubated with Na^{36}Cl at 20°C in a 56 days radiotracer experiment. The results show that a chlorination of $^{36}\text{Cl}_{\text{in}}$ to $^{36}\text{Cl}_{\text{org}}$ in agricultural soil occurred and the Cl_{org} levels increased over time. The chlorination rates ranged from 0,040 to 0,063 $\mu\text{g Cl g dry weight soil}^{-1} \text{d}^{-1}$. This was 10-fold lower than rates previously measured in coniferous forest soil. However, when expressed as $\mu\text{g Cl g dry weight organic carbon}^{-1} \text{d}^{-1}$, rates in the agricultural soil was only slightly (at the most 2-fold) lower than in coniferous forest. This study contributes with new knowledge of natural chlorination rates in agricultural soil and gives further evidence that the natural chlorination can be connected to the amount of organic matter in the soil.

Table of Content

ABSTRACT	1
INTRODUCTION	3
METHODOLOGY	4
SOIL COLLECTION	4
EXPERIMENTAL SETUP	4
SOIL EXTRACTIONS	6
ANALYSIS FOR SAMPLES WITHOUT ADDED ³⁶ CL.....	8
ANALYSIS FOR SAMPLES WITHADDED ³⁶ CL	9
RESULTS	9
RESULTS SAMPLES WITHOUT ADDED ³⁶ CL.....	10
RESULTS SAMPLES WITH ADDED ³⁶ CL	10
DISCUSSION	13
EXPERIMENTAL EVALUATION	14
SUMMARY	15
THE MAJOR CONCLUSIONS	15
ACKNOWLEDGEMENTS	15
REFERENCES	16

Introduction

Chlorine is a common element in the environment, and occurs in soil, ground and surface water, precipitation, vegetation, sediments and the atmosphere (Asplund & Grimvall 1991). Chlorine can be inorganic (primarily occurring as chloride; Cl^- , Cl_{in}) or organically bound (organic chlorine; Cl_{org}) (Hägg 1979). The complexity of the biogeochemical chlorine cycle where chloride participates has been discussed for many years (Öberg et al 2005). It is now well documented that a natural chlorination of organic compounds takes place in the environment (Öberg et al 2005, Svensson et al 2006).

Studying the transformation of Cl_{in} to Cl_{org} in soil is important since it will cause retention of Cl_{in} . Cl_{in} is often assumed to be non-reactive and is often used as a tracer of soil water movement. Such estimates of soil water transport, and thereby possibly contaminant transport may be biased by chloride retention due to Cl_{org} formation. In addition $^{36}\text{Cl}_{\text{in}}$ is an important part of low level radioactive waste due to its mobility and long half life (3.01×10^5 years). In nuclear reactors ^{36}Cl is formed by neutron capture by stable ^{35}Cl that is present in the reactor material. Therefore, when decommissioning the nuclear plant material $^{36}\text{Cl}_{\text{in}}$ is released to the environment (Hou et al 2008). In fact there are studies claiming that $^{36}\text{Cl}_{\text{in}}$ is the second most important compound in nuclear waste and that it deserves more attention in risk assessment models (Sheppard et al 1996)

Another reason for studying ^{36}Cl is the negative impact it can have on the environment and human health due to its long half life, 3.01×10^5 years (Sheppard et al 1996). Especially if $^{36}\text{Cl}_{\text{in}}$ reaches surface soils and is retained there for long time by e.g. transformation to $^{36}\text{Cl}_{\text{org}}$ being less mobile. Such retention would mean that the $^{36}\text{Cl}_{\text{in}}$ spends much longer time in the soils allowing uptake by plants during longer time frames than if Cl_{in} had been rapidly washed away from the soil. Kashparov et al (2007a) have done experiments of $^{36}\text{Cl}_{\text{in}}$ uptake in agricultural crops near the Chernobyl grounds. These experiments showed that a wide variety of crops like wheat, oat and clover, efficiently took up ^{36}Cl in its biomass from the roots and therefore the retention time of possible released $^{36}\text{Cl}_{\text{in}}$ in soils are of importance for risk assessment models.

There are different factors that affect the natural chlorination. Previous studies have shown clear evidence that there is natural chlorination of Cl_{in} to Cl_{org} by microbial activity in forest soil (Bastviken et al 2007). Temperature and the presence of O_2 seem to be important for the natural chlorination (Bastviken et al 2009). Other factors of importance are Cl_{in} concentrations and the amount of organic matter in the soil. Previous studies have shown that the binding of Cl_{in} to Cl_{org} is correlated to the amount of organic matter in forest soils (Johansson 2003 a; Johansson 2003 b; Öberg et al 2005).

So far, transformations of Cl_{in} to Cl_{org} have primarily been studied in forest soils. Therefore there is a need of analysing the chlorination in many different soil types. This study focuses on rates of organic matter chlorination in agricultural soil. The aim of the thesis is to investigate the magnitude and rate of natural chlorination of $^{36}\text{Cl}_{\text{in}}$ in agricultural soil. The rates will be compared to previous measurements in coniferous forest soil. The hypothesis is that the chlorination in agricultural soil in relation to

chlorination in forest soil will largely depend on the amount of organic matter in the various soils. Since the organic matter content in agricultural soil typically is lower than in upper coniferous forest soils, low chlorination rates are expected in this study.

Methodology

The methodology used in this thesis was used and verified in Bastviken et al (2007). The principle is the same but some smaller adjustments from the original methodology have been made to meet the specific aspects of this thesis. The difference between Bastviken et al (2007) and this study is the number of soil extractions. Bastviken et al (2007) uses eight soil extractions while this study uses four. Method evaluation has shown that four extractions as described below is enough and that no difference between four and eight extractions can be found.

Soil Collection

Soil was collected in the Lanna catchment in Västergötland Sweden in 2008 by SLU (Swedish university of agricultural sciences). For these experiments three different types of agricultural soil were chosen to cover some common Swedish types of agricultural soil. The different soil types had some things in common; all three were loamy, the water content was between 23 % - 25,2 % and they were collected in the same area. The soil types are called M21, M22 and M24.

M21 is used for organic farming of wheat since 1997. The area is not used as pasture for animals. The initial water content of M21 at the experimental setup was 25, 2 %.

M22 is an area where levee has been grown for approximately four years. Two to three weeks before soil collection the area was sprayed with roundup which is a herbicide. The initial water content of M22 at the experimental setup was 24,4 %.

M24 is an area where grain is produced, the area is treated with artificial fertilizer; 17, 5 kg/ha superphosphate was added yearly since 1941. The area has not been limed. The initial water content of M24 at the experimental setup was 23, 3 %.

The author is aware that calling M21, M22 and M24 for different soil types is not entirely accurate. It might be more appropriate calling the M21, M22 and M24 for soils with different cropping systems or agricultural practices. The term “different soils” is used to simplify for the reader by additionally distinguishing M21, M22 and M24 from each other.

Experimental Setup

The retention of $^{36}\text{Cl}_{\text{in}}$ in the soil was tested using an experiment focusing on the natural transformation of Cl_{in} to Cl_{org} over time.

The three different soils were treated the same during all steps of the preparation of the experiment. 70 g agricultural soil was weighted and homogenized with 15 ml Milli-Q water in a glass beaker by stirring. The homogenized soil slurry was put in a plastic syringe and injected into a 50 ml centrifuge tube (Sarstedt, Germany). The amount of soil transferred to each tube corresponded to $2,4 \pm 1$ g fresh weight agricultural soil (see figure 1). The exact weight in each tube was accounted for in all

calculations to correct for the different amount of soil in each tube. The $^{36}\text{Cl}_{\text{in}}$ tracer (Na^{36}Cl specific activity $0,231 \text{ MBq mgCl}^{-1}$) was diluted in Milli-Q water and 1.2 ml of this solution was added to each tube. This corresponded to an addition of 203 291 DPM (disintegrations per minute; $1 \text{ Bq} = 60 \text{ DPM}$) and $21,62 \mu\text{g Cl}_{\text{in}}$ to each centrifuge tube.

After adding ^{36}Cl to the homogenized soil the isotope solution was stirred in the wet soil by a syringe needle to evenly distribute the $^{36}\text{Cl}_{\text{in}}$. Two crossing slots were made in the soil to increase the aeration of the soil in the bottom of the centrifuge tube (see figure 1). The centrifuge tubes were placed under a fan at approximately 20°C to regain their original fresh weight. M22 and M24 regained their fresh weight after roughly 16 h and M21 after 24 h. Directly after the fresh weight of the soils was reached three samples of each soil were frozen to determine the initial ^{36}Cl distribution in the soil. This represented the start sample of the experiment (day 0). For each soil three samples without any added ^{36}Cl were frozen at day 0 to determine soil water content, initial concentration of organic matter (LOI), total organic halogens (TOX), adsorbable organically halogens (AOX), and extractable chloride (Ion chromatography).

A total of 54 tubes were prepared with $^{36}\text{Cl}_{\text{in}}$. For each soil three tubes were frozen at the beginning of the experiment, while the remaining tubes were connected to an aeration system (see figure 1). Each system consisted of 15 centrifuge tubes with soil and ^{36}Cl along with two centrifuge tubes with Milli-Q water. The cap of the centrifuge tubes were modified to have rubber stoppers allowing airflow into and from the tubes through synergic needles. The tubes were connected with tubing so that all air going to the soil tubes first bubbled through two centrifuge tubes with Milli-Q to increase air moisture and minimize the drying of the soil (see figure 1). The air flow was driven with an aquarium pump. The airflow was sustained trough the systems for 20 minutes every fourth hour, this was regulated by a timer. To insure that the water content did not increase or decrease the centrifuge tubes were weight every week. If the water content deviated more than 5 % from the original weight measures were taken to adjust the water content by water additions or drying through removal of the water tubes.

The first test tubes were withdrawn from the setup and preserved by freezing when the added ^{36}Cl reached the fresh weight of the agricultural soil (referred to as day 0). The second sampling occurred after 27 days and samples were withdrawn from the laboratorial setup a third time after 56 days. Two more samplings will occur but could not be included in this study due to time limitations.

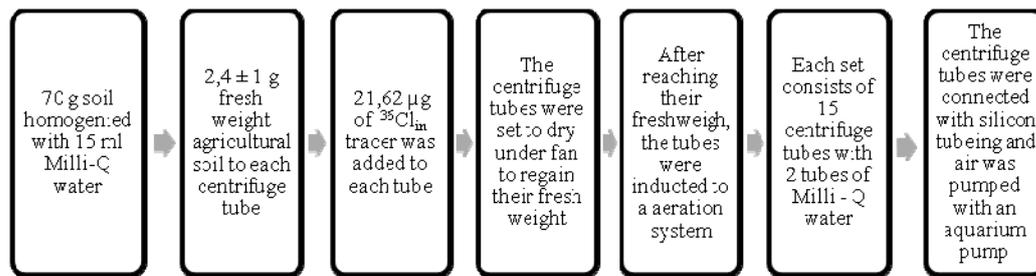


Figure 1. An schematic view of the different steps of the experimental setup.

Soil extractions

In this experiment 27 soil samples with added ^{36}Cl and 16 soil samples without added ^{36}Cl were extracted. The extractions were made to separate $^{36}\text{Cl}_{\text{in}}$ (being leachable) and non-leachable particle bound ^{36}Cl which previous research has shown to be organically bound (Bastviken et al 2007).

Soil extractions of soil samples with added ^{36}Cl

Extraction 1

The frozen samples were taken out of the freezer approximately 2 h before the extractions. When the soil reached room temperature 20 ml Milli-Q water was added to each tube and placed on an end-over-shaker for 30 minutes (see figure 2). The tubes were centrifuged at 6000 (g) for 10 minutes to separate the supernatant from the soil. The supernatant was removed from the centrifuge tube with a pipette and poured in another centrifuge tube. The residual soil was placed in a drying cupboard in 60°C for 24 h (see figure 2).

Extraction 2

After drying the soil in the tubes, the soil was crushed into small particles with a mortar. 5 ml H_2O was added to all tubes and after which they were sonicated with ultrasound for 45 seconds at 50 % (Bandelin Sonorex RK510H) intensity to destroy cells in the soil and thereby release the $^{36}\text{Cl}_{\text{in}}$ in the cells (see figure 2). After exposing the soil to ultrasound additional 15 ml H_2O was added to the soil and the soil was shaken in an end-over-shaker for 30 minutes. The tubes were centrifuged at 6000 (g) for 10 minutes and the supernatant was removed with a pipette and poured to another centrifuge tube (see figure 2).

Extraction 3

20 ml 0,01 M Potassium chloride (KCl) was added to the centrifuge tubes (see figure 2), the soil was shaken with an end-over-shaker for 30 minutes then centrifuged at 6000 (g) for 10 minutes, the supernatant was removed from the soil with a pipette and poured in to another centrifuge tube.

Extraction 4

After the third extraction 20 ml KCl was added to the centrifuge tubes, the soil was shaken with an end-over-shaker for 30 minutes then centrifuged at 6000 (g) for 10 minutes. Then the supernatant was removed from the soil with a pipette and poured in to another centrifuge tube.

When all four extractions were made, extractions 1 and 2 were poured together and extractions 3 and 4 were poured together (extracts from each original soil tube were pooled separately) and the extracts were frozen until further analyses (see figure 2).

Soil extractions of samples without ^{36}Cl

The steps between the extractions are very similar. Extraction 1 and 2 are exactly the same but there is a difference in extraction 3 and 4, the difference is the use of chemical. For the samples without added ^{36}Cl the chemical use for extraction 3 and 4 is KNO_3 . Adding KCl would interfere with the chloride analysis of these extracts and therefore 0.01 M KNO_3 was used instead (see figure 2).

Extraction samples with added ^{36}Cl

Extraction samples without added ^{36}Cl

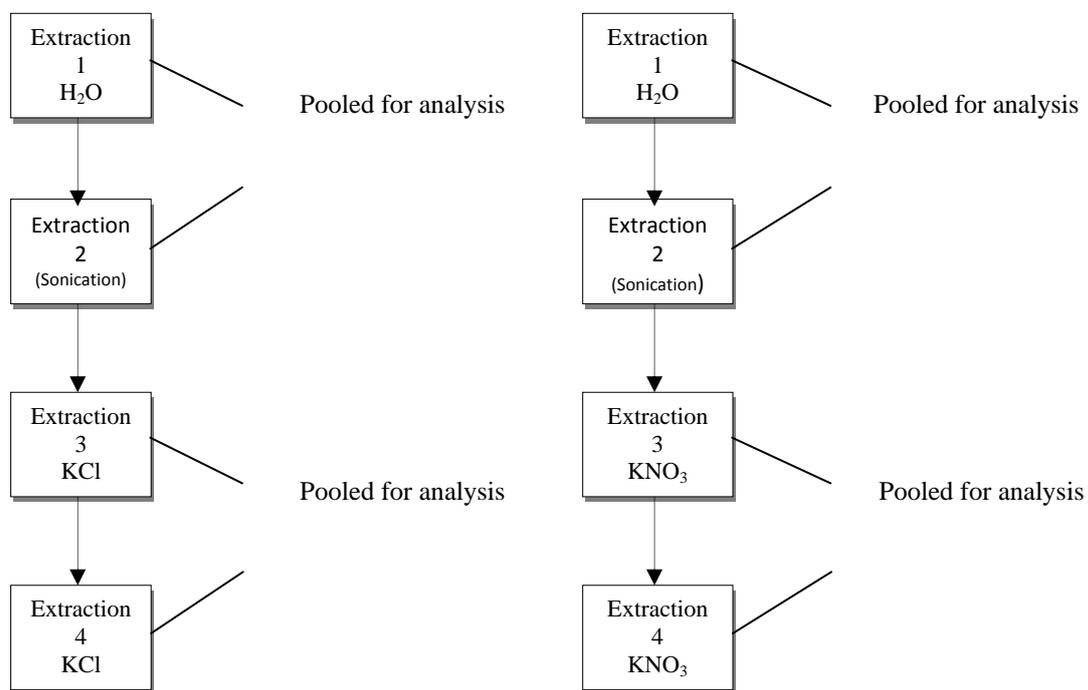


Figure 2. Shows the extraction procedures for separate $^{36}\text{Cl}_{\text{in}}$ (being leachable) and non-leachable particle bound ^{36}Cl before analyzing the samples.

Analysis of samples without ^{36}Cl

Adsorbable organic halogens (AOX)

An AOX instrument (Euroglas) was used to examine the content of extractable Cl_{org} in the extracts. Two replicates of each soil type were analysed. The AOX analysis was carried out according to Svensk Standard (SEEN 1485 1997), which is the standard procedure for the analysis of adsorbable organic halogens. Chlorine is the most abundant halogen in soil environments (Brady & Weil 2002). Therefore the analysis is made with the assumption that chlorine dominates the halogen pools in the soil samples.

The procedure for preparing the extraction for the analysis of AOX is the following; 10 ml of the extract was diluted with Milli-Q water to a final volume of 100 ml in a 300 ml –Erlenmayer flask. 50 mg \pm 3 mg activated carbon was put into the solution. An acidified nitrate solution was added to the flasks, 20ml: 0,22 M KNO_3 , 0,02 M HNO_3 . Between 5-7 drops of HNO_3 was added to the solution, this would yield a pH $<$ 2. The pH level was controlled with litmus paper. When the preparation was finished the flasks were put on a rotary shaker at 180 rpm for 60 \pm 5 minutes. The solution was filtered through a 0,45 μm polycarbonate filter (Euroglas). After the solution was filtered the flasks and the filter were washed (2*10 ml) with two different solutions: 20 ml 0,01 M KNO_3 , 0,001M HNO_3 , and 20 ml acidified Milli-Q water with approximately 5-7 drops of HNO_3 regulating the pH to $<$ 2. In addition a blank consisting of 100 ml Milli-Q water was analysed. After the two washing steps the filter was combusted through a stream of $\text{O}_2(\text{g})$ at 1000° C in an AOX analyser (ECS 3000; Euroglas). To determine the halogens the analysis uses a titration with silver ions.

Total organic halogens (TOX)

The analysis of TOX was done according to Asplund et al (1994). There are similarities between AOX and TOX analysis but the main difference is that in TOX residual soil is used instead of the extracts. 20 mg of residual soil was milled and poured in a 100 ml Erlenmayer flask. The residual soil was mixed with an acidic nitrate solution 20 ml (0,2 M KNO_3 , 0,02 M HNO_3). The mix of soil and nitrate solution was shaken on a rotary shaker 180 rpm for 60 \pm 5 minutes. The soil solution was filtered through a 0,45 μm polycarbonate filter (Euroglas). A blank consisting of 20 nitrate solution was analysed. The washing and analysis steps are the same as the AOX and described above.

Ion chromatography (IC)

To determine the chloride concentration in the different agricultural soils an analysis with ion chromatography was performed according to the standard procedure for determination of chloride concentrations of water with low contamination levels (European Union Standard 1995). A MIC – 2-chromatography system was used with a chemical suppression (Metrohm), a metrosep A Supp 5 IC column (150 * 4.0 mm), and an autosampler for 10 ml test tubes. To transport the ions, an eluent consisting of 0,0024 M Na_2CO_3 and 0,003 M NaHCO_3 was prepared. A calibration curve was prepared with the chemicals: NaF (F^-), KBr (Br^-), KH_2PO_4 (PO_4^{2-}), NaCl (Cl^-), NaNO_3 (NO_3) and Na_2SO_4 (SO_4^{2-}), diluted to concentrations of 10 ppm, 5ppm, 2,5 ppm, 1,25 ppm and 0,625 ppm (ppm in this case corresponds to mg L^{-1}). Two blanks

with Milli- Q water were set as first sample and after this sample the calibration curve samples were analysed. Every 30: th sample a 10 ppm standard sample and two blanks with Milli-Q water were analysed.

Loss of ignition (LOI)

To determine the amount of organic matter in the agricultural soil an LOI analysis was performed. Approximately 2-3 g (f.w.) of soil was weighed up in a crucible and dried in 60° C for 24 h and in 105° C for an additional 24 h. The samples were weighed and put in an oven at 620° C for 2 h to ignite all the organic matter. LOI represents the weight loss of the samples during the ignition. When calculating the amount of organic carbon (mg organic carbon g d.w. soil) the organic matter values were multiplied by 0,5 under the assumption that 50% of the organic matter weight consisted of carbon.

Analysis for samples with added $^{36}\text{Cl}_{\text{in}}$

Liquid scintillation counter (LSC)

To analyse the activity of the soil bound $^{36}\text{Cl}_{\text{org}}$ a liquid scintillation counter was used. The residual soil after the extraction 1-4 was sub sampled (0,15- 0,25 g_{d.w.}) and transferred to small crucibles. The soil was combusted in an AOX analyser 1000° C under a stream of O₂ for 20 minutes. The combustion result in transformation of all ^{36}Cl to H ^{36}Cl (g) following the gas stream. This H ^{36}Cl was trapped by bubbling the gas trough two scintillation vials with 0,1 M NaOH. More than 98 % of the ^{36}Cl in the combusted soil was trapped by this procedure (Bastviken et al 2007). After combustion, 10 ml scintillation cocktail (Ultima Gold XR, Chemical instruments AB) was added to each scintillation vial. The scintillation vials were analysed in a Beckman LX 6300, counting the β - activity in the solution. Four blanks containing the scintillation cocktail were analysed, two blanks in the beginning and two blanks as the last samples, the enable correction for background radiation.

Statistical analysis

The statistical analysis conducted in this study is a regression according to Weater & Cook (2000). The regression analysis was performed to obtain the slope of the regression line for data indicating the proportion of the added ^{36}Cl found as $^{36}\text{Cl}_{\text{org}}$ (y-axis) at different time points (x-axis) for the different soil types. The slope corresponds to the specific chlorination rate (d⁻¹). A significance level of 5 % was accepted. The R² value of the different soil types explain how well the y – axel variation is explained in the regression model.

Results

Results samples without added ^{36}Cl

The water content of the different soil types was quite similar (see table 1) and the values varied from 25,21 – 24,40 %. Since the difference was so small, the water content is not likely to be responsible for possible differences in chlorination rates between the soils. The loss of ignition gives important background information about the organic matter content in the soil. The values differed from 24,32 to 58,57 mg org. matter g dw soil⁻¹. Calculating the organic carbon content the values varied from 12,16 to 29,28 mg org. carbon g d.w. soil.

The results from TOX were similar between the different soils, the concentrations varied from 37,75 $\mu\text{g Cl}_{\text{org}} / \text{g soil d.w.}$ - 40,57 $\mu\text{g Cl}_{\text{org}} / \text{g soil d.w.}$. The results from AOX varied from 33,54 $\mu\text{g Cl}_{\text{org}} / \text{l}$ - 48,11 $\mu\text{g Cl}_{\text{org}} / \text{l}$. Concentrations of extractable Cl_{in} ranged from 7,21 $\text{Cl g d.w. soil}^{-1}$ - 19,81 $\text{Cl g d.w. soil}^{-1}$ (Table 1).

Table 1. Shows the background values of the agricultural soil without added ^{36}Cl with the different analysis. The results are shown as mean value \pm 1 standard deviation. n is number of replicates

Variable	Soil		
	M21	M22	M24
Water content (%; n=3)	25,2 \pm 0,003	24,6 \pm 0,005	24,4 \pm 0,008
LOI (mg org. matter $\text{g}_{\text{d.w.}} \text{soil}^{-1}$; n=2)	58,6 \pm 33,7	34,4 \pm 0,003	24,3 \pm 4,0
LOI (mg org. carbon $\text{g}_{\text{d.w.}} \text{soil}$; n=2)	29,3 \pm 16,9	16,7 \pm 0,006	12,1 \pm 2,0
TOX ($\mu\text{g Cl}_{\text{org}} / \text{g soil d.w}$ n = 4)	37,8 \pm 9,0	40,5 \pm 6,2	40,1 \pm 2
AOX ($\mu\text{g Cl}_{\text{org}} / \text{l}$ n = 2)	48,1 \pm 7,7	34,3 \pm 9,5	33,5 \pm 3,4
IC ($\mu\text{g Cl}^- / \text{g soil d.w}$ n = 4)	10,6 \pm 2,0	19,8 \pm 2,0	7,2 \pm 1,7

Results samples with added $^{36}\text{Cl}_{\text{in}}$

Specific chlorination rate

The specific chlorination rate indicates the fraction of the Cl_{in} being transformed to Cl_{org} per time unit. The rate was estimated from the slope of regression in figures (3, 4, 5, and 6) yielding specific chlorination in the unit d^{-1} . All regressions were statistically significant and R^2 values are shown in Table 2. M24 had the fastest specific chlorination rate with a value of 0,0063 d^{-1} while rates for M21 (0,0040 d^{-1}) and M22 (0,0031 d^{-1}) were somewhat slower (see table 2).

Chlorination rates

The chlorination rate is expressed in $\mu\text{g Cl g}_{\text{d.w.}}^{-1} \text{d}^{-1}$. The chlorination rate thereby indicates the actual transformation of Cl_{in} to Cl_{org} . For calculating the chlorination rate, the following formula was used; **Chlorination rate = specific chlorination rate * Cl_{in} concentration** (for Cl_{in} results, see table 1). The chlorination rates ranged from 0,0040 to 0,0063 $\mu\text{g Cl g}_{\text{d.w.}}^{-1} \text{d}^{-1}$. Hence the rates in all soils were in the same order of magnitude (see table 2).

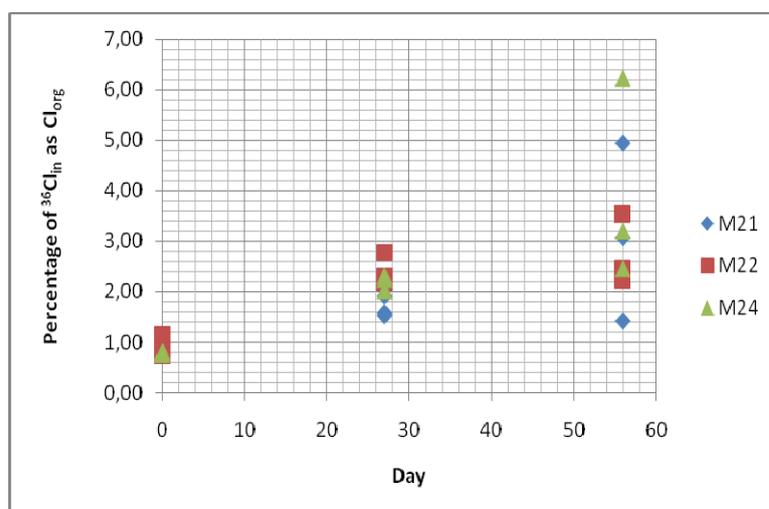


Figure 3. Percentage of added $^{36}\text{Cl}_{\text{in}}$ recovered as $^{36}\text{Cl}_{\text{org}}$ over time in the different soil types. All nine replicates from each soil are represented in the figure.

Chlorination rate normalized to organic carbon content

To enable comparison between different soil types e.g. agricultural soil and forest soil where the content of organic matter differs significantly the chlorination rate was normalized to organic carbon content and expressed in the unit $\mu\text{g C g OC}^{-1}\text{d}^{-1}$. When calculating this type of chlorination rate the following formula was used:

Chlorination rate normalized to organic matter content = Chlorination rate* $M_{d.w.}$

where $M_{d.w.}$ equals the mass d.w. agricultural soil needed for 1 g organic carbon.

M21 showed the lowest rate with 0,14 unit $\mu\text{g C g OC}^{-1}\text{d}^{-1}$ followed by M24 0,34 $\mu\text{g C g OC}^{-1}\text{d}^{-1}$ and M22 0,37 $\mu\text{g C g OC}^{-1}\text{d}^{-1}$.

Table 2. Shows the specific and estimated chlorination rates in M21, M22 and M24

Variable	Soil		
	M21	M22	M24
Specific Chlorination rate (d^{-1})	0,00040	0,00031	0,00056
p-value	0,024	0,0048	0,0083
R ² - value	0,53	0,70	0,65
Chlorination Rate ($\mu\text{g Cl g}_{d.w.}^{-1} \text{d}^{-1}$)	0,0042	0,0063	0,0040
Chlorination rate normalized to OC content ($\mu\text{g C g OC}^{-1}\text{d}^{-1}$)	0,14	0,37	0,33
For comparison with Bastviken et al 2009			
Org. carbon (mg org. carbon $\text{g}_{d.w.}$ soil)	0,135		
Chlorination Rate ($\mu\text{g Cl g}_{d.w.}^{-1} \text{d}^{-1}$)	0,077		
Chlorination rate normalized to OC content ($\mu\text{g C g OC}^{-1}\text{d}^{-1}$)	0,57		

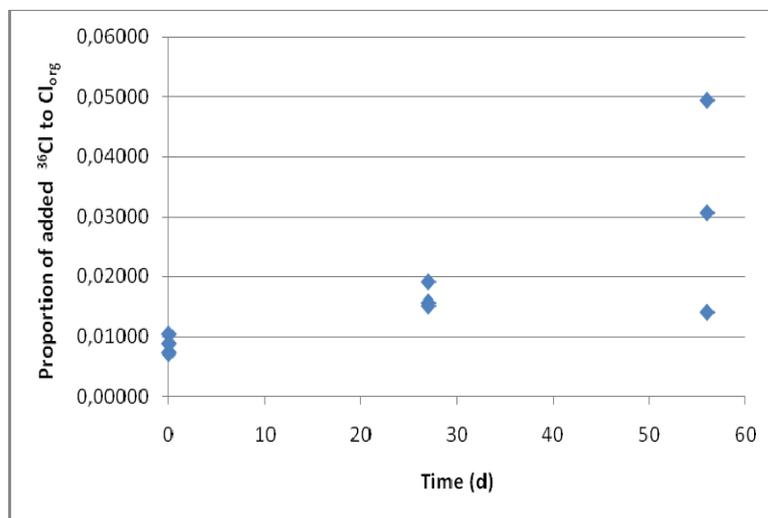


Figure 4. Shows proportion of added ³⁶Cl to Cl_{org} of M21. The specific chlorination rate is the chlorination from 0 – 56 days. The chart is expressed in (d^{-1}).

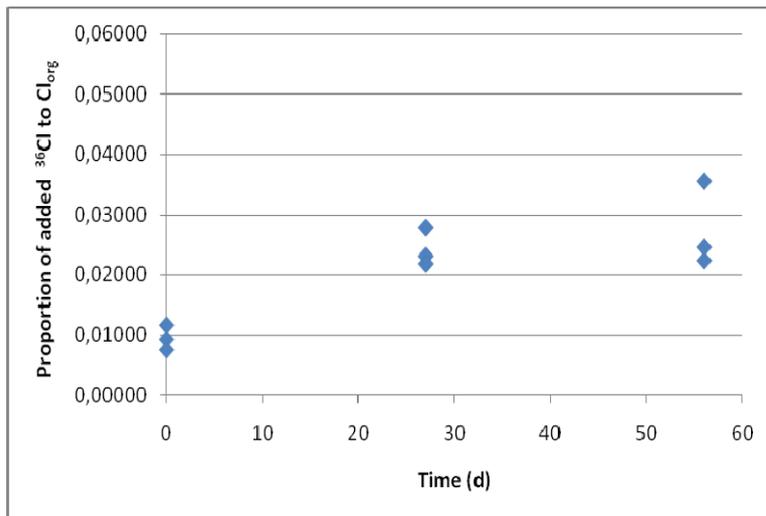


Figure 5. Shows proportion of added ^{36}Cl to Cl_{org} of M22. The specific chlorination rate is the chlorination from 0 – 56 days. The chart is expressed in (d^{-1}).

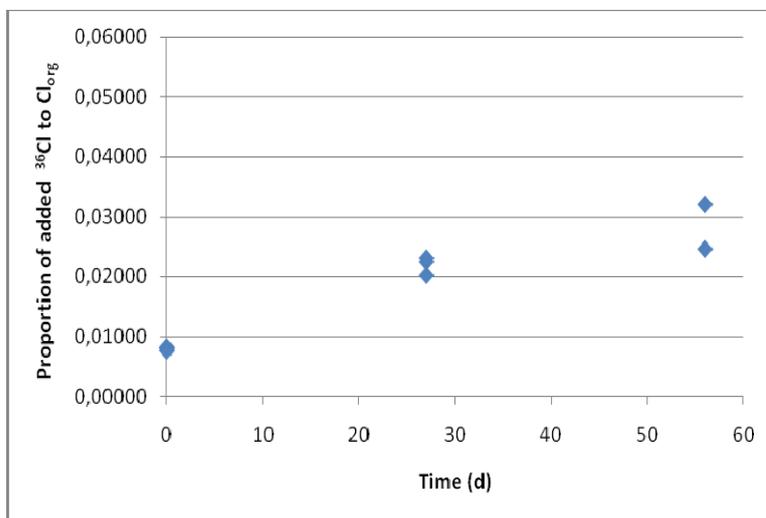


Figure 6. Shows proportion of added ^{36}Cl to Cl_{org} of M24. The specific chlorination rate is the chlorination from 0 – 56 days. The chart is expressed in (d^{-1}).

Discussion

When performing this study the main focus was to the magnitude and rate of natural chlorination of $^{36}\text{Cl}_{\text{in}}$ in agricultural soil. This thesis presents clear evidence that a chlorination of Cl_{in} to Cl_{org} occurs in agricultural soil. The hypothesis was that the chlorination of Cl_{in} to Cl_{org} in agricultural soil would be slower compared to forest soil due to lower organic matter content (Öberg et al 2005; Johansson et al 2003 a; Bastviken et al 2007; Bastviken et al 2009). With the background information about the soil and comparisons made with Bastviken et al (2007) and Bastviken et al (2009) it is clear that the amount of organic matter is lower in agricultural soil compared to forest soil. The chlorination in the agricultural soil was 10-fold slower than in e.g. the study of Bastviken et al. (2009) see table 2. With these findings the hypothesis presented in this thesis could not be falsified.

Percentage of added ^{36}Cl to Cl_{org}

In all samples the percentage of Cl_{in} to Cl_{org} increased over time indicating significant chlorination from Cl_{in} to Cl_{org} in M21, M22 and M24 (see figure 2). The chlorination in M21, M22 and M24 were quite similar and no substantial difference can be shown (see table 2 and figure 2).

Soil characteristics and Chlorination rates

The soil characteristics may influence the chlorination between the different soil types. M22 and M24 are sprayed with pesticides and fertilizers while M21 is an organic culture, where the use of pesticides and fertilizers is restricted. According to EG (834/2007) that specifies the demand on organic cultures, the chosen agricultural practice has to increase or maintain the amount of organic matter in the soil. Results from LOI indicate that M21 has higher organic matter content than M22 and M24 (see table 1). In spite of this no clear difference in chlorination rate between soils can be distinguished. Hence, although chlorination may be related to the organic matter content when comparing M21, M22 and M24 the organic matter content varies with quite large numbers. The differences between the studied soils are apparently too small to indicate such a relationship considering just agricultural soils. Accordingly the differences of farming practices including organic farming, treatment with artificial fertilizers, roundup or the crops grown did not result in large differences regarding the chlorination rates and other factors may have been more important. It should also be noted that the agricultural soils came from nearby locations and differed mainly in terms of previous agricultural practice and not due to their location. Hence the original soil may be similar in many aspects.

Specific chlorination rate

A linear regression was used to calculate the coefficient of line to examine the chlorination of Cl_{in} to Cl_{org} d^{-1} . All regressions were significant ($p < 0,05$) but the R^2 values (Table 2) and Figures 2-5 show that the data did not follow the line very closely. Figure 4 and 5 actually indicates that the chlorination may level off with time and then fitting a linear relationship to the data can be questioned. However, to data originates from three sampling times only and more sampling times would be needed to support the decision of the most appropriate non-linear relationship to use. Therefore the simple linear regression was used in this case anyway awaiting further data from the experiment.

Experiment evaluation

When distributing the soil to the tubes at the start of the experiment some difficulties occurred due to the loamy soil, therefore the amount of soil is not coherent throughout the centrifuge tubes. The exact amount of soil added to each tube was considered in all calculations and there were no indications that the variation of soil_{f.w.} influenced the results or caused the observed variability.

This is the first time agricultural soil has been in this type of experiment under well aerated conditions. There were some difficulties maintaining the water content at the same level throughout the whole experiment. One of the tubes with Milli-Q water was taken away due to an increase of water content in the beginning of the experiment, further on in the experiment both of the tubes were taken away. The experiment allowed a 5 % weight difference. The differences in water content were found early on and watched closely during the experiment, for that reason the small variability over time in water content should not have influenced the results.

Chlorination rates normalized to organic carbon content

When normalizing the chlorination rate to investigate the chlorination rate / organic matter content a comparison between different soil types like agricultural soil and forest soil can be done accounting for the large difference in organic matter content (Table 1 and 2). Bastviken et al (2009) did similar experiments on chlorination of forest soil in different temperatures. It is of interest to compare the different chlorination rates in a similar temperature rate: the experiment in this thesis was conducted in 20 ° C and the same temperature was tested in Bastviken et al (2009). When looking at the chlorination rate which indicates the chlorination / g_{d.w.} soil approximately a factor 10 differs (0,078 µg Cl g_{d.w.}⁻¹ d⁻¹ compared to M21 0,0042, M22 0,0063 and M24 0,0040 µg Cl g_{d.w.}⁻¹ d⁻¹) between the results in this thesis and Bastviken et al (2009). With these results actual chlorination of forest soil is more extensive than in agricultural soil / g_{d.w.} soil. When calculating the chlorination rate normalized to organic carbon content another picture emerge. The value calculated for Bastviken et al (2009) is 0,5777 µg C g OC⁻¹d⁻¹ and for M21 0,1466 µg C g OC⁻¹d⁻¹, M22 0,3782 µg C g OC⁻¹d⁻¹ and M24 0,3367 µg C g OC⁻¹d⁻¹ (see table 2). With these numbers the chlorination connected to the amount of organic carbon is more similar between forest soil and agricultural soil. If the organic matter content was similar between forest soil and agricultural soil the chlorination rate could possibly have been similar. I therefore hypothesize that the natural chlorination in soil is dependent of the amount of organic matter in the soil and that this pattern is most clear when looking at soils having large differences in terms of organic carbon. The similar rates between the agricultural soils indicates that such a relationship between organic carbon content and chlorination rates is less clear when comparing soils having more similar organic matter content.

Summary

The chlorination of agricultural soil is an important factor to recognize and further discuss due to the implications that agricultural soil has on the environment and the effects it can have on the whole ecosystem including humans. Kashparov et al (2007 a) and Kashparov et al (2007 b) have made studies on how ³⁶Cl transfers in plants and agricultural foodstuffs to make risk assessments following a potential radioactive

waste contamination. With an uptake of Cl_{in} in plants the movement of the ^{36}Cl will differ from what is estimated from assumptions that Cl_{in} is non-reactive. An accumulation in tissue of dairy cattle and pigs is tested by Levechuk et al (2008) and an accumulation is presented with a use of equilibrium isotopic ratio. Combining these research fields with the research now available about the chlorination, uptake and accumulation of ^{36}Cl in agricultural soil, a discussion about the possible movement of ^{36}Cl in the different food chains can be enhanced. Considering the chlorination of Cl_{in} to Cl_{org} , uptake of plants and accumulation in dairy cattle can be better understood and in addition lead to improved knowledge about chloride cycling in soil.

This study shows that chlorination of organic material occurs in agricultural soil, somewhat slower than previous research of forest soil but still a substantial chlorination is shown. With this knowledge, contributions to the mapping for specific soil types can be enhanced and a greater understanding of chlorination in different soil types is reached. It has also been shown that the amount of organic matter could play an important role in the chloride cycle in the soil. Further studies in this area are of great importance, first to understand the underlying processes of chlorination in different soil types. Second, with the new knowledge about retention in agricultural soil is when determining ^{36}Cl as source contamination from radioactive fuel or decommissioning of nuclear plants. The ^{36}Cl could with its long half life (more than 300 000 years) and since chlorination move up in the food chain, cause harm to the ecosystem and humans. Therefore the result of this study has implication within several fields including radioactive risk management and general biogeochemistry.

The major conclusions of this study include

- A substantial natural chlorination of Cl_{in} to Cl_{org} occurs in agricultural soil.
- The amount of chlorination can be connected to the amount of organic matter in the soil.
- This thesis cannot identify any clear differences between the agricultural soil types studied.

Acknowledgements

I would like to show great appreciation to my tutor David Bastviken for all his guidance, help and knowledge when conducting this study. I would also like to thank Lena Lundman, Susanne Karlsson, Monica Petterson, Teresia Svensson, Cecilia Göransson, Malin Andersson and Malin Gustavsson for all practical assistance during this study.

References

- Asplund, G. Determination of the total and leachable amounts of organohalogens in soil. *Chemosphere* 28, 1467–1475.
- Bastviken, D., Svensson, T., Karlsson, S., Sandén, P. & Öberg, G., (2009). Temperature sensitivity indicates that chlorination of organic matter in forest soil is primarily biotic. *Environmental Science & Technology*, 43, 3569-3573.
- Bastviken, D., Thomsen, F., Svensson, T., Karlsson, S., Sandén, P., Shaw, G., Matucha, M., Öberg, G., (2007). Chloride retention in forest soil by microbial uptake and by natural chlorination of organic matter. *Geochimica et Cosmochimica Acta*, 71, 3182-3192.
- Brady N, Weil R (2002) The nature and properties of soils. New Jersey, Pearson Education Inc.
- Europeiska rådets förordning. (2007). (EG) nr 834/2007. Om ekologisk produktion och märkning av ekologiska produkter och om upphävande av förordning (EEG) nr 2092/91. *Europeiska unionens officiella tidning*, L 189/1.
- European Union Standard, (1995). Water quality-Determination of dissolved fluoride, chloride, nitrite, orthophosphate, bromide, nitrate and sulphate ions, using liquid chromatography of ions – Part 1: Method for water with low contamination (ISO 10304-1, 1992). Brussel.
- Fréchou, C. & Degros, J-P., (2004). Measurement of ^{36}Cl in nuclear wastes and effluents: Validation of a radiochemical protocol with an in-house reference sample. *Journal of radioanalytical and nuclear chemistry*, 263, 333-339
- Hou, X., Ostergaard, L-F., Nielsen S-P., (2007). Determination of ^{36}Cl in nuclear waste from reactor decommissioning. *Ana. Chemistry*, 79, 3126-3134.
- Hägg, G. (1979) Allmän och oorganisk kemi. Uppsala, Almqvist & Wiksell
- Johansson E, Sanden P, Öberg G (2003a) Organic chlorine in deciduous and coniferous forest soil in southern Sweden. *Soil Science* 168, 347–355.
- Johansson E, Sanden P, Öberg G (2003b) Spatial patterns of organic chlorine and chloride in Swedish forest soil. *Chemosphere* 52, 391–397.
- Kashparov, V., Colle, C., Levchuk, S., Yoschenko, V., Svydynuk, N., (2007a). Transfer of chlorine from the environment to agricultural foodstuffs. *Journal of environmental radioactivity*. 94, 1-15.
- Kashparov, V., Colle, C., Levchuk, S., Yoschenko, V., Zvarich, S., (2007b). Radiochlorine concentration ratios for agricultural plants in various conditions. *Journal of environmental radioactivity*. 94, 10-22.

Laniewski K., Dahlen J., Boren H., and Grimvall A. (1999) Determination of group parameters for organically bound chlorine, bromine, and iodine in precipitation. *Chemosphere*. 38, 771–782.

Levchuk, S., Kashparov. V., Lazarev. N., Colle. C., Howard. B., Yoschenko. V. & Yoschenko, L., (2008). Transfer of Cl from herbage into tissues and milk products of dairy cattle and pigs. *Radiat Environ Biophys*. 47, 111-119.

Sheppard, S.C., Johnson, L.H., Goodwin, B.W., Tait, J.C., Wuschke, D.M., Davison, C.C., (1996). Chlorine-36 in nuclear waste disposal – 1. Assessment results for used fuel with comparison to ¹²⁹I and ⁴C. *Waste Management* 16, 607–614.

Svensk standard, SS-EN 1485 (1997). Vattenundersökningar- Bestämning av organiskt bundna halogener (AOX).

Svensson, T. Sanden, P. Bastviken, D. Öberg, G. (2006). Chlorine transport in a small catchment in southeast Sweden during two years. *Biogeochemistry* 82, 181–199.

Wheater, C. P., Cook, P. A., (2000) Using statistic to understand the environment. London: Routledge 2002

Öberg G, Sanden P (2005) Retention of chloride in soil and cycling of organic matter-bound chlorine. *Hydrol Process*, 19, 2123–2136.