

Water and Environmental Studies
Department of Thematic Studies
Linköping University

Chlorination of Organic Material in Different Soil Types

Malin Gustavsson

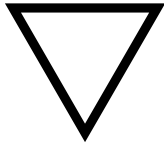
**Master's programme
Science for Sustainable Development**

Master's Thesis, 30 ECTS credits

ISRN: LIU-TEMAV/MPSSD-A--09/006--SE



Linköpings Universitet



Water and Environmental Studies
Department of Thematic Studies
Linköping University

Chlorination of Organic Material in Different Soil Types

Malin Gustavsson

**Master's programme
Science for Sustainable Development**

Master's Thesis, 30 ECTS credits

Supervisor: David Bastviken

2009

Upphovsrätt

Detta dokument hålls tillgängligt på Internet – eller dess framtida ersättare – under 25 år från publiceringsdatum under förutsättning att inga extraordinära omständigheter uppstår.

Tillgång till dokumentet innebär tillstånd för var och en att läsa, ladda ner, skriva ut enstaka kopior för enskilt bruk och att använda det oförändrat för ickekommersiell forskning och för undervisning. Överföring av upphovsrätten vid en senare tidpunkt kan inte upphäva detta tillstånd. All annan användning av dokumentet kräver upphovsmannens medgivande. För att garantera äktheten, säkerheten och tillgängligheten finns lösningar av teknisk och administrativ art.

Upphovsmannens ideella rätt innefattar rätt att bli nämnd som upphovsman i den omfattning som god sed kräver vid användning av dokumentet på ovan beskrivna sätt samt skydd mot att dokumentet ändras eller presenteras i sådan form eller i sådant sammanhang som är kränkande för upphovsmannens litterära eller konstnärliga anseende eller egenart.

För ytterligare information om Linköping University Electronic Press se förlagets hemsida <http://www.ep.liu.se/>.

Copyright

The publishers will keep this document online on the Internet – or its possible replacement – for a period of 25 years starting from the date of publication barring exceptional circumstances.

The online availability of the document implies permanent permission for anyone to read, to download, or to print out single copies for his/her own use and to use it unchanged for non-commercial research and educational purpose. Subsequent transfers of copyright cannot revoke this permission. All other uses of the document are conditional upon the consent of the copyright owner. The publisher has taken technical and administrative measures to assure authenticity, security and accessibility.

According to intellectual property law the author has the right to be mentioned when his/her work is accessed as described above and to be protected against infringement.

For additional information about Linköping University Electronic Press and its procedures for publication and for assurance of document integrity, please refer to its www home page: <http://www.ep.liu.se/>.

Contents

| | |
|---|----|
| Abstract | 1 |
| Introduction | 1 |
| Methods | 2 |
| Soil Sampling | 2 |
| Experimental Setup..... | 3 |
| Soil Extractions..... | 4 |
| Analytical Procedures..... | 5 |
| Organic ³⁶ Cl in the Residual Soil | 5 |
| Results | 6 |
| Soil characteristics | 6 |
| Formation of Cl _{org} | 7 |
| Discussion | 10 |
| Acknowledgements | 12 |
| References | |
| Appendix | |

Abstract

Research has shown that formation of chlorinated organic matter occurs naturally and that organic chlorine is as abundant as the chloride ion in organic soils. A large number of organisms are known to convert inorganic chloride (Cl_{in}) to organic chlorine (Cl_{org}) (e.g. bacteria, lichen, fungi and algae) and some enzymes associated to these organisms are capable of chlorinating soil organic matter. The aim with the study was to compare organic matter chlorination rates in soils from several different locations dominated by either coniferous forest or pasture. Soil from eight samples sites in the southern of Sweden were incubated at 20°C with addition of $^{36}\text{Cl}_{\text{in}}$ in a 138 days long radiotracer experiment. The results show that transformation of $^{36}\text{Cl}_{\text{in}}$ to $^{36}\text{Cl}_{\text{org}}$ occurred and that the amounts of $^{36}\text{Cl}_{\text{org}}$ increased over time. The chlorination rate was higher in the samples from coniferous forest than in samples containing pasture soil, where the specific chlorination rate was 3-4 times smaller. This study contributes new information about chlorination in various soil types and soil from different locations in southern central Sweden. The similarity between the chlorination rates measured in coniferous forest soils so far indicate that up scaling to regional estimates may be less problematic than expected.

Keywords: biogeochemical cycle; chloride, organic chlorine; retention

Introduction

Chloride participates in a complex biogeochemical cycle, and details regarding this cycle have been discussed for several years (Öberg et al, 2005). In the late 80's and early 90's it was revealed that large amounts of naturally formed chlorinated organic compounds were present ubiquitously in the environment (Asplund and Grimvall, 1991; Haselman et al, 2000; Öberg et al, 2005; Svensson et al, 2006). These compounds were earlier believed, to originate from anthropogenic activities only (Öberg, 2002). However, evidence now shows that formation of chlorinated organic matter occurs naturally and that organic chlorine is as abundant as the chloride ion in organic soils (Öberg et al, 2005; Bastviken et al, 2009). A large number of organisms are known to convert inorganic chloride (Cl_{in}) to organic chlorine (Cl_{org}) (e.g. bacteria, lichen, fungi and algae) and some enzymes associated to these organisms are capable of chlorinating soil organic matter (Öberg et al, 2005). Both Cl_{in} and Cl_{org} are found in soil, water and air compartments (Winterton, 2000). Even if there is evidence that Cl_{in} can be transformed to Cl_{org} , the underlying processes are not understood. Cl_{in} imbalances have been seen in several experiments, meaning that the input not is the same as the output of chloride. It seems as Cl_{in} is not only retained but also can be released from the soil (Bastviken et al, 2007). According to Rodstedth et al (2003) this suggests that soil may act both as a sink and a source of chloride but it is unclear under what conditions.

There is a big interest in understanding how and how fast these transformation processes of Cl_{org} take place for a number of reasons. First, because it is still often assumed that chloride is inert and freely mobile in soil and present mainly as chloride ions, and in line with these assumptions chloride is used in hydrological research and biogeochemical modelling. In these cases chloride is used as an inexpensive and easily measured tracer for soil and ground water movements when calculating budget and deposition estimates (Kirchner et al, 2000). Not accounting for the chloride cycling including retention and release can lead to bias when

doing hydrological modelling. Second, the believed inert nature of chloride in soil is translated to radioactive chloride (^{36}Cl) (Kashparov et al, 2005; Levchuk et al, 2008). ^{36}Cl is produced in the irradiated fuel assembly during reactor operations and is a long lived fission product with a half-life of $3,01 \cdot 10^5$ years. Considering the believed functions that it is inert and moves freely as an anion in the environment and its long half-life, makes ^{36}Cl one of the radionuclides of interest in the planning of deep repositories for nuclear waste (Fréchet & Degros, 2004). ^{36}Cl has been identified as an environmental contaminant and have negative effects on human health. A better insight of the movement of the radionuclide through soils is therefore important, both to understand soil uptake and distribution and to be able to trace leakage from storage units (White & Broadley, 2001). Bias can here get serious consequences if ^{36}Cl is retained in the soil and leaks not are observed.

Therefore, a deeper understanding of the underlying processes of formation of Cl_{org} is important to better understand the biogeochemical cycling of chlorine and to support hydrological and risk analysis models. Most detailed studies regarding transformation of chlorine in soils has to my knowledge been performed on coniferous forest soil from a specific site (Johansson et al, 2003a; Johansson et al, 2003b; Rodstedth et al, 2003; Öberg & Sandén, 2005, Öberg et al, 2005; Svensson et al, 2006; Bastviken et al, 2007; Bastviken et al, 2009). It therefore appears important to compare previous results with similar studies in other soils and at other locations. The aim of the present study is to compare organic matter chlorination rates in soils from several different locations dominated by either coniferous forest or pasture. If rates are different this can provide new information about how environmental factors affect the transformation of Cl_{in} to Cl_{org} and patterns between these variables can be found.

A study of Cl_{org} in deciduous and coniferous forest soil indicated that Cl_{org} increased with increasing Cl_{in} concentrations, organic matter content and decreasing pH (Johansson et al, 2003a). Since forest soil in general contains more organic matter than soil in pasture (Eriksson et al, 2005). The hypothesis in this study is that transformation of Cl_{in} to Cl_{org} is more extensive in forest soil where the content of organic material is higher than in pasture soil.

The aim of the present study can be addressed in following research questions:

- How extensive is the transformation of Cl_{in} to Cl_{org} in soils from habitats dominated by coniferous forest and pasture?
- Is there any difference between forest soil and pasture regarding transformation rate of Cl_{in} to Cl_{org} ?

Methods

Soil Sampling

Soil was collected within a radius of 100 kilometres from Linköping, Sweden in September 2008. Eight sites were sampled; at four of the locations podsol soil in coniferous forest was retrieved. The other four sites supplied pasture soil. Pasture is here defined as; land that not

regularly is ploughed but yearly is grazed and the main purpose with land use is not forest production. The sites all had different characteristics in terms of age, texture and area covered by treetops. The age of the trees growing on the forest sites differed from 25 to 50 years old. The area covered by treetops varied from 50 to 80 percent. The age of the pasture was 40 to over 100 years old. The area covered by treetops differed here from 5 to 20 percent (Table 1). Soil was collected with a spade from the topsoil layer 5-15 cm below ground level and transported in polyethylene plastic bags to the laboratory. The soil was then refrigerated at 4°C until it was used in the experiment.

The places for sampling were chosen based on advice by Mats Walheim working as field officer in the Department of Forest Resource Data in the Swedish University of Agricultural science (SLU) in Umeå. The places were selected on the basis that the soils should be representative Swedish forest and pasture soil types according to the criteria of the Swedish National Forest Inventory (<http://www-nfi.slu.se/>). They should also lie in a 100 kilometres radius from Linköping for practical reasons.

Table 1. Soil and vegetation characteristics at the sample sites.

| Sample | Land use class | Soil parent material¹ | Texture | Vegetation field layer⁵ | % of sample area covered by treetops | Stand age (years) |
|---------------|-----------------------|---|---------------------------|---|---|--------------------------|
| F1 | Forest soil | Till | Coarse silty ² | High - low herb types | 80 | 25 |
| F2 | Forest soil | Till | Fine sandy ³ | High – low herb types | 44 | 50 |
| F3 | Forest soil | Till | Fine sandy | Thin leaved grass types | 48 | 50 |
| F4 | Forest soil | Till | Coarse silty | Bilberry | 51 | 50 |
| P1 | Pasture | Till | Sandy ⁴ | Broad leaved grass types | 12 | 74 |
| P2 | Pasture | Till | Coarse silty | High – low herb types | 9 | 55 |
| P3 | Pasture | Till | Sandy | Broad leaved grass types | 19 | 105 |
| P4 | Pasture | Till | Sandy | Broad leaved grass types | 5 | 37 |

¹ = Soil parent material comprises information on genesis and grain-size distribution of the parent material for soil formation.

² = soil with a grain size between 0.06-0.02 mm

³ = soil with a grain size between 0.2-0.06 mm

⁴ = soil with a grain size between 0.6-0.2 mm

⁵ = for explanation see appendix A.

Experimental Setup

The soil was distributed in 50 ml plastic centrifugation tubes (Sarstedt, Germany) and incubated at 20°C with addition ³⁶Cl_{in} according to the methodology of previous studies (Bastviken et al, 2007; Bastviken et al, 2009). Briefly 2,0 g fresh soil was transferred to each tube. ³⁶Cl_{in} diluted to 300 000 disintegrations per minute (DPM; 1 Bq = 60 DPM) diluted with MilliQ-water was added to each centrifuge tube. The volume of added ³⁶Cl_{in} solution varied from 0.4 to 1.3 ml depending on how much water was needed to be able to distribute the

isotope homogeneously (by looking at the solution) in each soil type. After addition of $^{36}\text{Cl}_{\text{in}}$ in solution, the samples were dried under a fan in room temperature to reach original weight. When the right weight was reached the initial samples were taken out and put in the freezer. The rest of the samples were placed in the dark with aeration using aquaria pumps (4 x 15 minutes each day). To prevent drying of the soil, the aquaria pumps pumped air through a separate centrifugation tube filled with water, to moisturize it. Weekly controls of water content (by weighing the tubes) and aeration (by checking air flow function) were carried out. If the water content reached a weight five percent lower/higher than original weight, this was adjusted by either adding or taking away water by drying the soil with an open lid until it reached initial weight. The sampled text tubes were removed from the experimental setup and immediately frozen until further analyses. This resulted in a 138 days long radiotracer experiment. The incubated series were sampled at five occasions day 0 (representing initial samples), 23, 55, 82 and 138 and three replicates were collected each time.

Soil Extractions

To analyze the formed $^{36}\text{Cl}_{\text{org}}$ the soil was first extracted to remove remaining $^{36}\text{Cl}_{\text{in}}$. Before starting the soil extractions a test was made to see if the process from previous experiments (Bastviken et al, 2007; Bastviken et al, 2009) could be improved. In the previous method (method A) eight soil extractions were carried out to be sure that all Cl_{in} leached out from the soil, leaving only particular Cl_{org} behind in the residual soil. Apart from the extractions the method A had three steps that should make sure that microbial cells containing chlorine should break and release the chlorine. Method A contained one step where the soil was frozen for at least 24 hours and one step where the soil was dried for a 24-hour period and then one step where the samples were sonicated with an ultrasound stick. The test performed within this study compared method A with two more compressed methods denoted B and C. Method B contained all three steps (freezing, drying and sonicating) and was the same as method A with the exception that there were only four soil extractions. Method C was the same as method B with the exception that the drying step was taken away. The different soil extraction methods were carried out on two different soils, forest and pasture. The result shows that there was no need to extract more than four times but that the drying step was necessary to leach maximum amounts of Cl_{in} . (for results see appendix B). The following extractions were carried out with method B, which more precisely is explained below and in Figure 1.

Samples taken out on the sample occasions were first frozen for at least 24 hours. The samples were then thawed the same day as the extractions took place. After that 20 ml Milli-Q water was added to each tube and the tubes were placed on an end-over-end shaker for 30 minutes, centrifuged (6000g for 10 min) and the supernatant was transferred by pipette to new centrifuge tubes. After that the samples were dried in 60°C for 24 hours, milled, rewetted with 5 ml water, and sonicated 45 seconds with 50% intensity; (Bandelin Sonorex RK510H). After this, 15 ml water was added and the sample was shaken and centrifuged again. Two more extractions were carried out but this time with 0.01 M KCl (to enhanced exchange with possible adsorbed $^{36}\text{Cl}_{\text{in}}$). The first and second extracts were then poured together in the same tube as well as the third and fourth extract in another tube. The extracts were then frozen and the residual soil was dried in 60°C for 24 hours and stored until further analyses.

Analytical Procedures

Initial content of water, organic matter, Cl_{in} and Cl_{org}

The original soil was sampled before starting the experiment to determine soil water contents (by drying at 105°C for 31h) and initial concentrations of soil organic matter, total organic chlorine, and extractable Cl_{in} . Soil organic matter was measured by loss of ignition at 550°C for 8 hours. To analyze initial concentrations of Cl_{in} and particle bound organic chlorine initial samples without any ^{36}Cl were prepared and extracted the same way as the samples containing ^{36}Cl , with the exception that the last two extractions were done with 0.01 KNO_3 instead of KCl (Figure 1). This was done to avoid Cl_{in} contamination in the initial samples before the subsequent Cl_{in} analysis. The reason to use KCl as extraction solvent in the samples containing ^{36}Cl was to be sure that all ^{36}Cl was leached. The extracts were then frozen and after thawing analyzed for chloride concentrations by ion chromatography with chemical suppression (MIC-2, Metrohm). The extracts were first filtered through a 25 mm easy pressure filter and then analyzed according to Standardization (1995).

The residual soil was analyzed for total organic halogens (TOX) content after being dried and milled according to Asplund et al. (1994). TOX approximately equals total organic chlorine since chlorine is the by far most abundant halogen in soils. In short, a 20 mg sample was added to acidic nitrate solution and shaken for at least one hour on a rotary shaker (180 rpm). The solution was then filtered through a 0.40 μm polycarbonate filter and rinsed with a nitrate solution followed by acidified MilliQ-water. The filter and the sample were then combusted under a stream of oxygen at 1000°C (Euroglas AOX Analyzer). There were two replicates for each sample and MilliQ-water was analyzed as blanks.

Organic ^{36}Cl in the Residual Soil

After the extractions the dried residual soil from the ^{36}Cl amended treatments was milled and approximately 0.2 g soil was combusted to determine the amount of organically bound ^{36}Cl . The soil was combusted in 1000°C under a stream of O_2 gas and formed $H^{36}Cl$ gas was trapped in two scintillation vials in series containing 10 ml 0.1 M $NaOH$ (Laniewski et al, 1999). Previous tests confirmed that the chlorine associated with the residual soil and detected this way was organically bound and associated with humic and fulvic acids (Bastviken et al, 2007).

The $NaOH$ solutions containing trapped ^{36}Cl were analyzed for ^{36}Cl by LSC (Beckman LX 6300). The analysis was corrected for quench (i.e. shielding by the sample matrix resulting in that not all radioactivity is counted) using standard quench curves prepared from solutions with the same matrix composition as the samples (e.g. 0.1 M $NaOH$). Before analyzing the samples a scintillation cocktail (Ultima Gold XR, Chemical Instruments AB) was added to all ^{36}Cl extracts and also to blank controls (milli-Q water and scintillation cocktail). All radioactive measurements were corrected for background radiation by subtracting radioactivity in the blank controls.

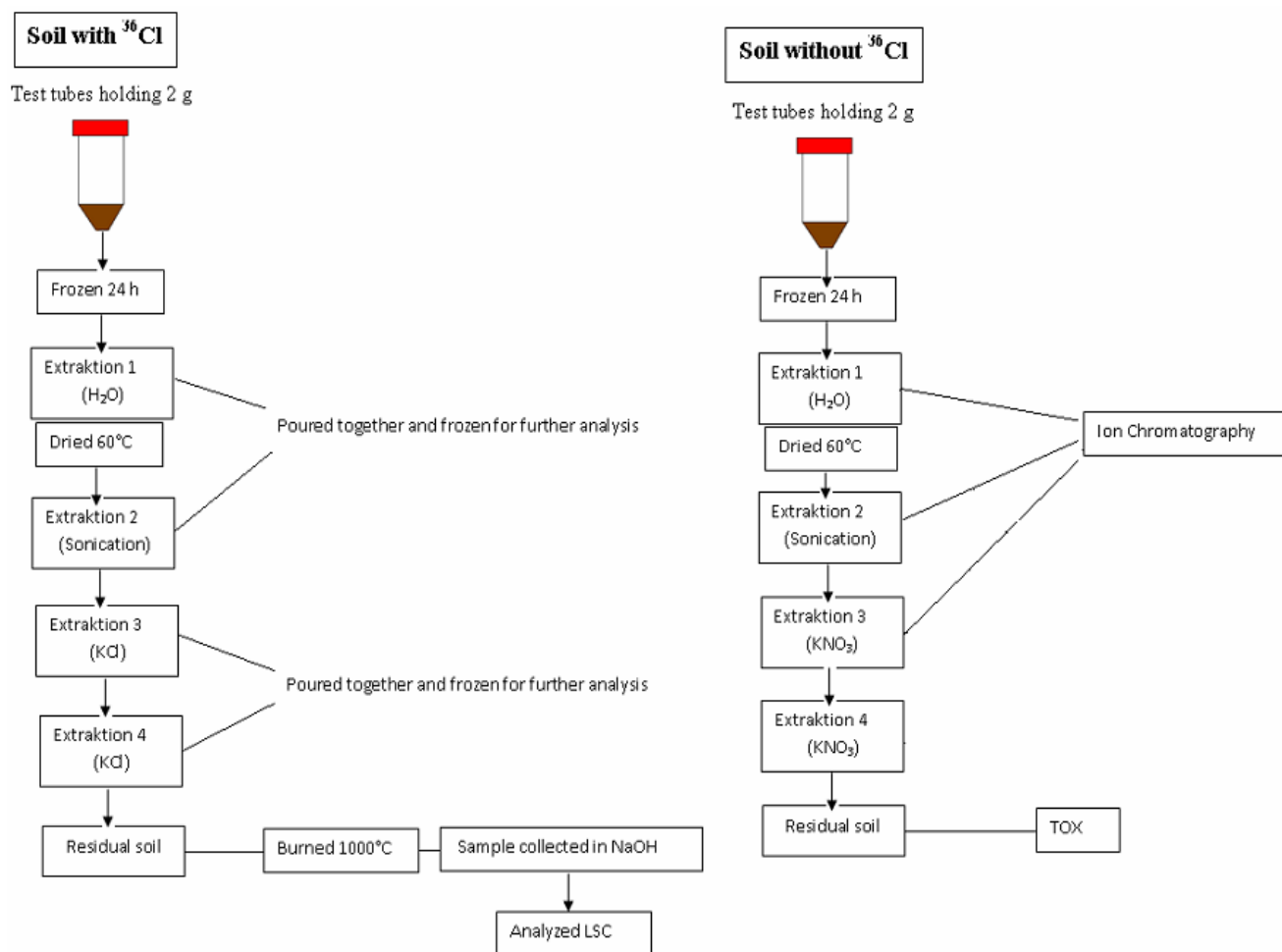


Figure 1. Overview of the extraction procedures and different analysis of soil with and without ³⁶Cl, modified after Bastviken et al (2007).

Results

Soil characteristics

Soil characteristics varied between the eight different samples, but most between the two land use classes' forest and pasture. Soil water content varied between 35-54 % in forest soil and between 24-36 % in pasture. The initial value of total organic chlorine content (TOX) varied from 66 to 191 $\mu\text{g g}^{-1}$ dry weight in forest soil and from 35 to 59 $\mu\text{g g}^{-1}$ dry weight in pasture. The amount of extractable inorganic chlorine varied from 33 to 83 $\mu\text{g g}^{-1}$ dry weight in forest soil and from 6 to 12 $\mu\text{g g}^{-1}$ dry weight in pasture soil (Table 2). The average carbon-to-chlorine ratios in the soil were 3600 carbon atoms per chlorine atom. There was no difference between carbon-to-chlorine ratios when comparing the two land use classes (Table 3).

Table 2. Characteristics of the soil used in the experiment, values reported are averages \pm standard deviation.

| Sample | Land use class | Soil water content (% \pm , n=3) | LOI ¹ (% of d.w. n=3) | Initial TOX (μ g/g) | Extractable Cl _{in} (μ g Cl g ⁻¹ d.w., n=2) | pH (RO-water ²) | pH (KCl) | % added ³⁶ Cl to each test tube ³ |
|--------|----------------|------------------------------------|----------------------------------|--------------------------|--|-----------------------------|----------|---|
| F1 | Forest | 35 \pm 0.2 | 15 \pm 0.1 | 68 \pm 17 | 47 \pm 5 | 5.0 | 4.0 | 8 |
| F2 | Forest | 54 \pm 1 | 41 \pm 2 | 191 \pm 37 | 83 \pm 20 | 4.4 | 2.9 | 6 |
| F3 | Forest | 36 \pm 0.8 | 17 \pm 0.7 | 102 \pm 14 | 37 \pm 4 | 4.7 | 3.7 | 10 |
| F4 | Forest | 47 \pm 1 | 26 \pm 2 | 66 \pm 5 | 33 \pm 11 | 4.8 | 3.8 | 14 |
| P1 | Pasture | 25 \pm 0.03 | 7 \pm 0.3 | 35 \pm 5 | 6 \pm 0.08 | 5.6 | 4.2 | 51 |
| P2 | Pasture | 33 \pm 0.4 | 15 \pm 0.4 | 40 \pm 5 | 12 \pm 1 | 5.7 | 5.3 | 29 |
| P3 | Pasture | 24 \pm 0.2 | 8 \pm 0.1 | 39 \pm 4 | 6 \pm 3 | 5.2 | 4.2 | 49 |
| P4 | Pasture | 36 \pm 0.07 | 11 \pm 0.1 | 59 \pm 11 | 6 \pm 0.7 | 5.4 | 4.3 | 67 |

¹ = Loss on ignition, heating the soil allowing volatile substances to escape.

² = Reverse osmosis, purified water

³ = The amount of ³⁶Cl added expressed as percent of initial amount of extractable Cl_{in}.

Formation of Cl_{org}

The results show that transformation of ³⁶Cl_{in} to ³⁶Cl_{org} has occurred and that the amounts of ³⁶Cl_{org} increased over time. The total amount formed ³⁶Cl_{org} in forest soil varied from 15.6 \pm 4.0 to 28.8 \pm 4.4% of the initially added ³⁶Cl_{in} (Figure 2). In pasture soil this range was 4.3 \pm 0.27 to 7.6 \pm 1.7% (Figure 3). This corresponds to a specific chlorination rate (proportion of transformed ³⁶Cl_{org}) of 0.0016 d⁻¹ and 0.00042 d⁻¹ median value for forest and pasture respectively (Table 4). In absolute terms the chlorination rates were 9.0 \pm 0.85 to 12.9 \pm 3.3 μ g Cl g⁻¹ dry weight d⁻¹ for forest soil and 0.24 \pm 0.0015 to 0.57 \pm 0.051 μ g Cl g⁻¹ dry weight d⁻¹ for pasture soil.

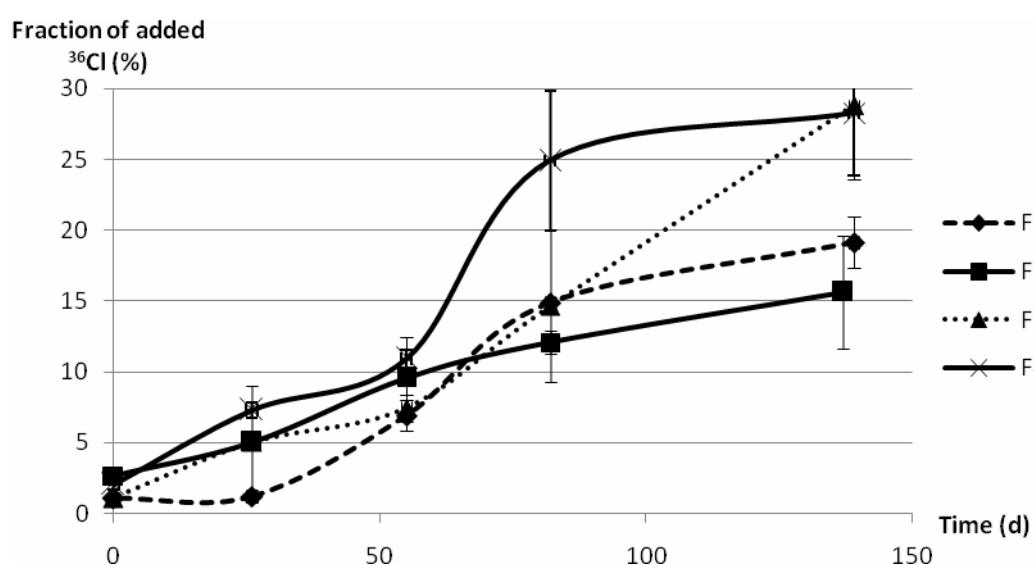


Figure 2. Fractions of added ^{36}Cl recovered as Cl_{org} over time in forest soil samples (F1-F4) (average \pm ISD, $n=3$). Note that the smoothed lines between the sampling points are only to clarify which points are connected and that there is no information between these data points.

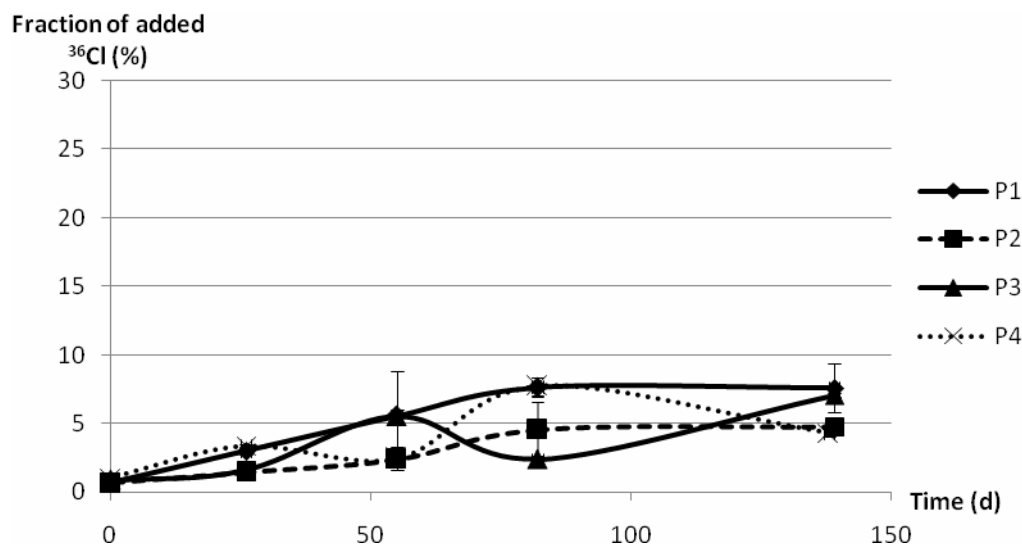


Figure 3. Fractions of added ^{36}Cl recovered as Cl_{org} over time in pasture soil samples (P1-P4) (average \pm ISD, $n=3$). Note that the smoothed lines between the sampling points are only to clarify which points are connected and that there is no information between these data points.

A linear regression analysis revealed that the obtained chlorination rates in all four forest soils F1-F4 and two of the pasture soils P1 and P2 were significant $P < 0.05$ (Table 3).

The highest chlorination rate (i.e. $\mu\text{g Cl g}^{-1}$ dry weight day^{-1}) was found in one of the forest soils called F2 ($0.081 \mu\text{g Cl g}^{-1}$ d.w. d^{-1}). The chlorination rates in the forest soils had a median value of $0.073 \mu\text{g Cl g}^{-1}$ d.w. d^{-1} . The slowest chlorination rate was found in a pasture soil. The significant rates in the pasture soils were very similar and had a median value of $0.0035 \mu\text{g Cl g}^{-1}$ d.w. d^{-1} . The median values of the chlorination rate in forest soil are approximately 20 times more extensive than the rates in pasture soil.

Table 3. Chlorination rates and carbon:chlorine ratio in the different soils, R^2 and p -values regard the regressions used to obtain specific chlorination rates (see Figures 2 and 3).

| Sample | R^2 | p-value | Specific chlorination rate (d^{-1}) | Chlorination rate ($\mu\text{g Cl g}^{-1}$ d.w. d^{-1}) | C-to-Cl ratio ¹ |
|--------|-------|---------|--|--|----------------------------|
| F1 | 0.93 | 0.0082 | 0.00146 | 0.0686 | 3151 |
| F2 | 0.97 | 0.0023 | 0.00098 | 0.0806 | 3190 |
| F3 | 0.98 | 0.0025 | 0.00200 | 0.0736 | 2427 |
| F4 | 0.91 | 0.012 | 0.00202 | 0.0672 | 5851 |
| P1 | 0.82 | 0.034 | 0.00051 | 0.0032 | 3037 |
| P2 | 0.88 | 0.018 | 0.00032 | 0.0039 | 5485 |
| P3 | 0.67 | 0.091 | 0.00041 | 0.0026 | 3172 |
| P4 | 0.34 | 0.300 | 0.00028 | 0.0015 | 2771 |

¹ = Number of carbon atoms per chlorine atom.

Descriptive statistics (Pearson correlation) for two rates of chlorination and different environmental variables are presented in Table 4. Chlorination rate was associated with all of the environmental variables except for stand age of the sample site.

Table 4. Correlation coefficients for Pearson correlations between specific chlorination rate, chlorination rate, extractable Cl_{in} , pH(KCl), pH (RO-water), TOX, LOI and water content ($n = 8$).

| | Specific chl. rate (d^{-1}) | Chl. rate ($\mu g Cl g^{-1} d.w. d^{-1}$) | Extractable Cl_{in} ($\mu g Cl g^{-1} d.w., n=2$) | pH (KCl) | pH (RO-water) | TOX ($\mu g/g$) | LOI (% of dw.) | H ₂ O (part of f.w.) | Stand age |
|------------------|---------------------------------|---|---|--------------|---------------|-------------------|----------------|---------------------------------|-----------|
| Spec.chl. rate | - | | | | | | | | |
| Chl. rate | 0.86 | - | | | | | | | |
| Cl_{in} conc. | 0.51 | 0.88 | - | | | | | | |
| pH (KCl) | -0.51 | -0.75 | -0.78 | - | | | | | |
| pH (RO) | -0.70 | -0.90 | -0.86 | 0.90 | - | | | | |
| TOX | 0.33 | 0.73 | 0.91 | -0.82 | -0.84 | - | | | |
| LOI | 0.39 | 0.73 | 0.88 | -0.70 | -0.78 | 0.89 | - | | |
| H ₂ O | 0.49 | 0.75 | 0.81 | -0.63 | -0.75 | 0.80 | 0.94 | - | |
| Stand age | -0.39 | -0.51 | -0.43 | 0.43 | 0.14 | -0.31 | -0.31 | -0.54 | - |

The chlorination rates for the different soils were statistically tested for possible linear relationships with stand age of the sample site area, Cl_{in} concentration, LOI, TOX, water content and pH (RO-water and KCl). A significant correlation $P < 0.05$ was found between all environmental factors with an exception for stand age on the sample site (Cl_{in} concentration $P = 0.0039$, LOI $P = 0.047$, TOX $P = 0.040$, water content $P = 0.032$, pH (RO-water) $P = 0.0021$ and pH (KCl) $P = 0.032$). The relationship between chlorination rate and Cl_{in} concentration (Figure 4), chlorination rate and LOI (Figure 5) and between chlorination rate and pH (KCl) (Figure 6) can be seen in the figures below.

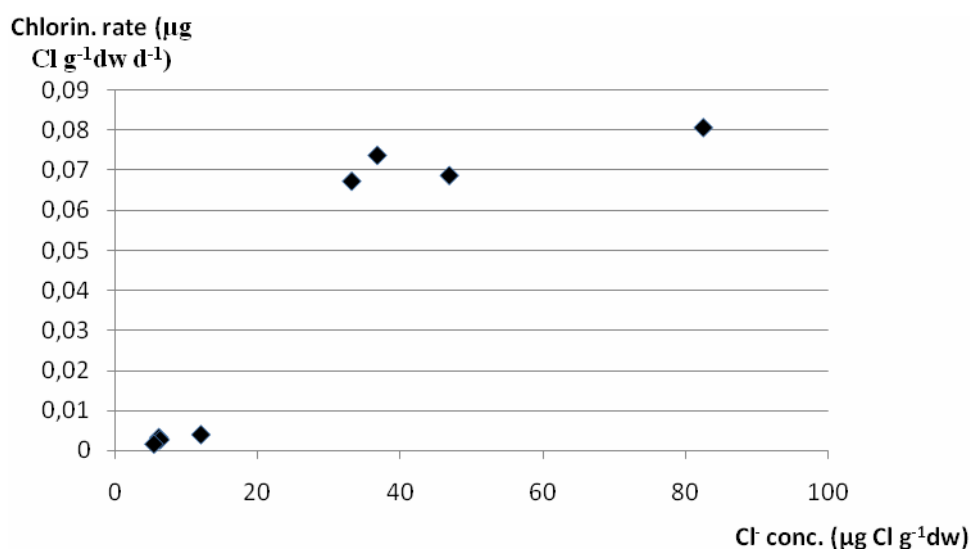


Figure 4. Relationship between chlorination rate and Cl_{in} concentration.

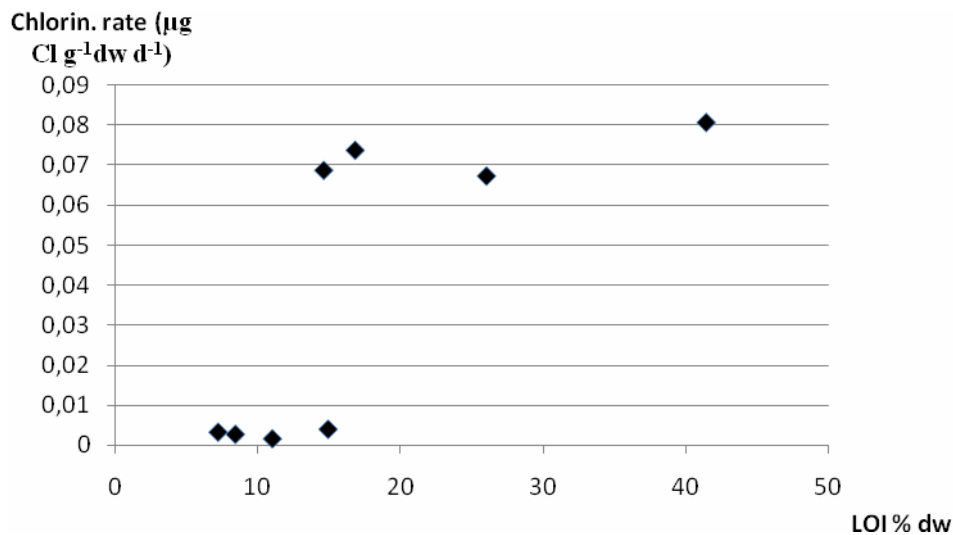


Figure 5. Relationship between chlorination rate and LOI.

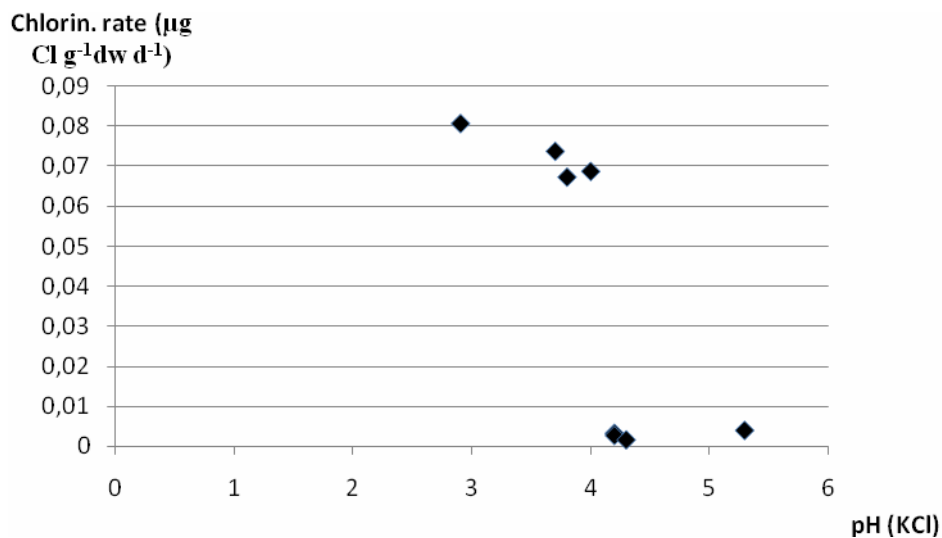


Figure 6. Relationship between chlorination rate and pH (KCl).

Discussion

The transformation of Cl_{in} to Cl_{org} in soils from habitats dominated by coniferous forest was extensive and 29 percent of the added $^{36}Cl_{in}$ was transformed to $^{36}Cl_{org}$ over a 138 day period. The specific chlorination rate was higher in the samples from coniferous forest than in samples containing pasture soil, where the specific chlorination rate was 3-4 times smaller. The highest absolute chlorination rate was $0.081 \mu\text{g Cl g}^{-1}$ dry weight d^{-1} (assumed that all extractable Cl_{in} that originally was present in the soil was transformed at similar rates as the added $^{36}Cl_{in}$) and the absolute chlorination rate in forest soil was 20 times higher than the absolute rate in pasture soil (Table 3).

The chlorination rate in the coniferous forest soil is similar to results from previous studies based on the same methodology as the present study. Bastviken et al (2009) based their results on a coniferous forest soil with approximately the same amount of organic matter content and the total amount formed $^{36}\text{Cl}_{\text{org}}$, there varied from 10-37% and the chlorination rate ranged from 0.012 to 0.078 $\mu\text{g g}^{-1}$ dry weight d^{-1} . In comparison the total amount formed $^{36}\text{Cl}_{\text{org}}$ in the present study was 16-29% in coniferous forest soil and the median chlorination rate for the forest soils were 0.073 $\mu\text{g g}^{-1}$ dry weight d^{-1} . These results, together with the study by Bastviken et al (2009) are in line with a previous study and therefore strengthen their suggestion that 20-60 % of the chloride added to the soil is converted to organic matter chlorine over a period of one year (Rodstedth et al, 2003).

The less extensive transformation rate in pasture soil supports my hypothesis that Cl_{in} transforms to Cl_{org} faster in coniferous forest soil than in pasture soil. The different formation rates of Cl_{org} in the two soil types can depend on several factors according to the analysis, where a significant correlation was seen between chlorination rates and several environmental variables; concentration of Cl_{in} , organic matter content, total organic halogens (TOX), water content and pH. From the results in this study it is difficult to assess the relative importance of the different studied environmental factors. Many of the factors are significantly correlated with each other and it is hard to say if these correlations directly contribute to formation of Cl_{org} or if the patterns just reflect correlated effects from other studied variables. However, results from the present study correspond with results from previous studies, which in different ways also have found that three of these environmental variables; organic matter (Asplund & Grimvall, 1991; Johansson et al, 2003b), pH (Öberg et al, 1996) and Cl_{in} (Rodstedth et al, 2003) are central for the formation of Cl_{org} . (Johansson et al, 2003a). The fact that the variation in concentration of Cl_{org} seem to differ more between the soil types than the carbon-to-chlorine ratio does have been observed in earlier studies (Johansson et al, 2003b). They then proposed that another environmental variable was the cause of the observed pattern. Two other studies suggested that Cl_{in} and organic matter are the main two substrates when Cl_{org} is formed during degradation of organic matter through enzymatic processes that catalyze the formation of reactive species such as hypochlorous acid (Johansson et al, 2000; Johansson et al, 2003a). These enzymes (e.g. chloroperoxidases) can be produced by soil microorganisms and fungi (Öberg et al, 1997, Öberg & Sandén, 2005). The activity optimum of the enzyme lies in the pH range of 3-4 and the highest concentrations of Cl_{org} may be found in acid environments (Reina et al, 2004). This implies that the correlation seen between chlorination rates and pH in the present study might be due to a higher activity of the enzymes, especially because coniferous forest soil in general has a higher organic matter content and a lower pH than pasture soils. This also go in line with a study by Öberg et al (1996) which found a significant increase in the amount of originally bound chloride with decreasing pH. Possibly organisms producing the chlorinating enzymes are more common in coniferous forest soils than in pasture environments. The positive relationship with water content probably indirectly reflects influence of other variables correlated to the water content. A high water content leading to diminished oxygen concentrations in the soil should not in itself lead to increased chlorination rates since previous studies have shown a negative effect of anoxia on chlorination (Bastviken et al, 2007; Bastviken et al, 2009). The relationship with TOX probably reflects that more TOX will accumulate in soils with higher chlorination rates.

Limitations

For several reasons it is clear that the results in the present study have limitations. First, chlorination rates should be regarded as potential rates and not in situ actual rates due to the experimental conditions with long isolation of the soils during the incubation. Second, if Cl_{in} limits chlorination the $^{36}Cl_{in}$ addition itself to the original soil may have stimulated chlorination. If so the rates should be most biased (overestimated) where the addition was highest relative to initial Cl_{in} . The additions of $^{36}Cl_{in}$ in solution correspond to 9.64 μg Cl_{in} per test tube. The added amount radioactive chloride consists of 7 to 67 % of the total amount of chloride in the soils (Table 2). Then the difference between forest and pasture soils may be even larger than shown in this experiment. Third, the results only regard particulate Cl_{org} and leached Cl_{org} in dissolved organic carbon (DOC) is not considered. Previous work has shown that this extracted Cl_{org} is proportional to the particulate Cl_{org} and are in order of 2-10% of the Cl_{org} (Bastviken et al, 2007; Bastviken et al, 2009).

Conclusion

This study has discovered that chlorination occurs in both coniferous forest and in pasture soils. It has therefore contributed to new specific site information about different soil types, compared to previous work which was primarily performed on coniferous forest from one specific site. The present study and previous work show that existing chlorination rates from coniferous soil were quite similar. Hence, since the soil for these measurements were collected within a circle of 200 km diameter in southern central Sweden it seems possible that similar soil conditions result in similar chlorination rates regardless of the location within this area. This means that estimates of regional chlorination rates may be less problematic than expected, once the link between chlorination rates and soil conditions (e.g. moisture, temperature etc) has been established. In addition the study provides the to my knowledge first chlorination estimates from pasture soil. Further research is needed to increase the understanding of the underlying processes that drive/inhibit the cycling of chlorine in soil and to raise the awareness about retention of chlorine in soil. Particularly, when it comes to hydrological ground water models and especially when it comes to predict transfer of radioactive chlorine from source contamination (from fuel or accidents) into the food chain or when tracing leaks from fuel storages.

Acknowledgements

I would like to thank David Bastviken, Lena Lundman, Susanne Karlsson, Monica Petersson, Teresia Svensson and of course Malin Andersson, Cecilia Göransson and Salar Valinia for their help in the laboratory and with this thesis.

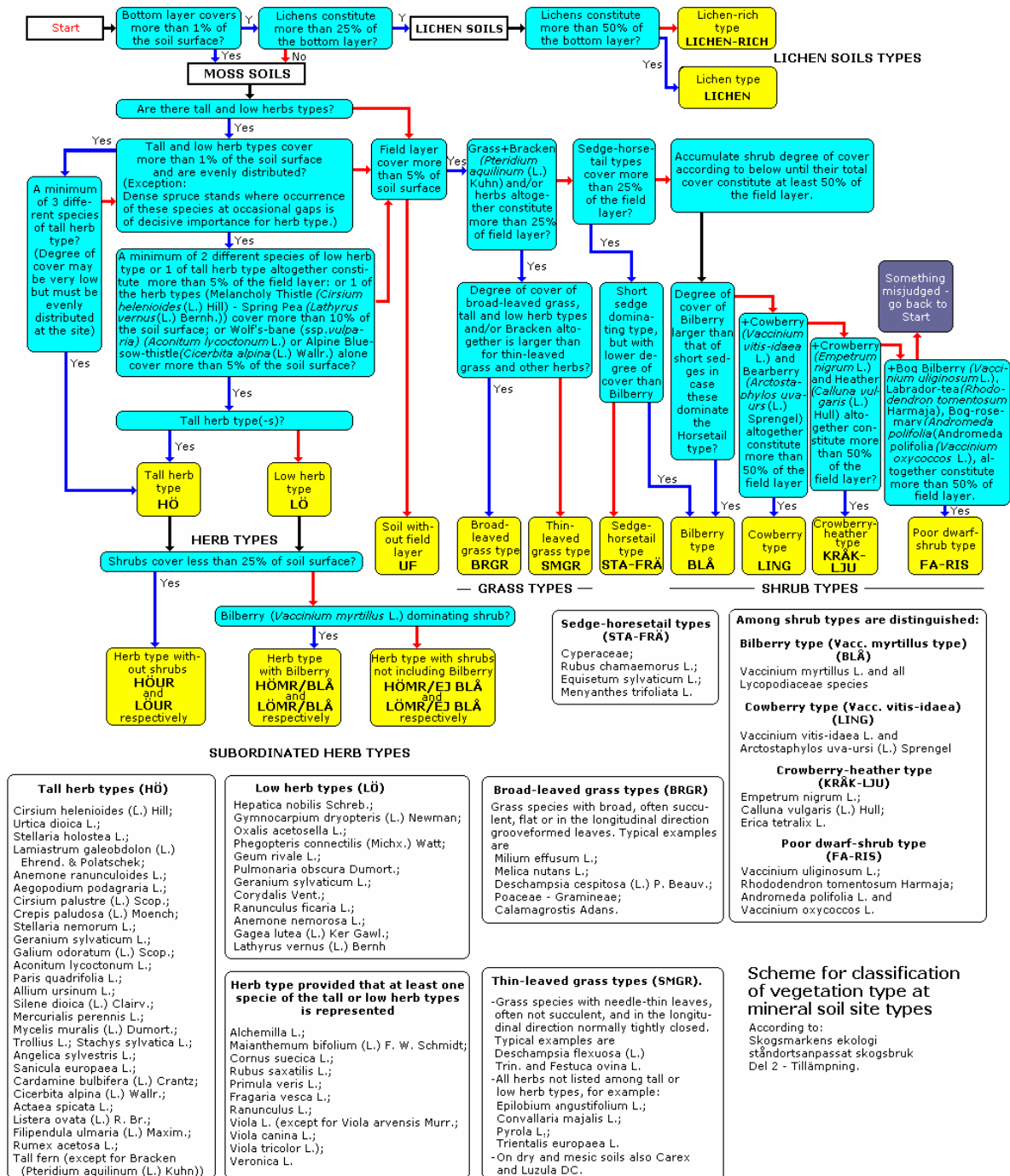
References

- Asplund, G., (1994). Determination of the total and leachable amounts of organohalogens in soil. *Chemosphere* 28, 1467-1475.
- Asplund, G. & Grimvall, A., (1991). Organohalogens in nature – more widespread than previously assumed. *Environ. Sci. Technol.* 25, 1346-1350.
- 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*, in press. Available on line at: <http://pubs.acs.org/journal/esthag>.
- 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.
- Eriksson, J., Nilsson, I. & Simonsson, M., (2005). *Wiklanders Marklära*. Studentlitteratur: Lund, Sweden.
- Fréchou, C. & Degros, J-P., (2004). Measurement of ³⁶Cl in nuclear wastes and effluents: Validation of a radiochemical protocol with an in-house reference sample. *Journal of radioanalytical and nuclear chemistry*, Vol. 263, No. 2, 333-339.
- Haselman, K., Ketola, R., Laturus, F., Lauritsen, F. & Grøn, C., (2000). Occurrence and formation of chloroform at Danish forest sites. *Atmospheric Environment*, 34, 187-193.
- Johansson, E., Sandén, P. & Öberg, G., (2003a). Organic chlorine in deciduous and coniferous forest soils in southern Sweden. *Soil science*, vol. 168, no.5, 347-355.
- Johansson, E., Sandén, P. & Öberg, G., (2003b). Spatial patterns of organic chlorine and chloride in Swedish forest soil. *Chemosphere* 52, 391-397.
- Kashparov, V., Colle, C., Zvarich, S., Yoschenko, V., Levchuk, S. & Lundin, S., (2005). Soil-to-plant halogens transfer studies 2. Root uptake of radiochlorine by plants. *Journal of Environmental Radioactivity*, 79, 233-253.
- Kirchner, J. W., Feng, X. H., Neal, C., (2000). Fractal stream chemistry and its implications for contaminant transport in catchments. *Nature* 403, 524-527.
- Laniewski, K., Dahlén, J., Borén, H., Grimvall, A., (1999). Determination of group parameters for organically bound chlorine, bromine, and iodine in precipitation. *Chemosphere* 3, 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.
- Mark info, SLU, available at: <http://www-markinfo.slu.se/eng/vegeta/vegtyp/images/vegsch5.gif>, [2009-05-27].

- Reina, R.G., Leri, A.C. & Myneni, S. C. B., (2004). Cl K-edge X-ray Spectroscopic investigation of enzymatic formation of organochlorines in weathering plant material. *Environ. Sci. Technol.*, 38 (3), 783-789.
- Rodstedth, M., Ståhlberg, C., Sandén, P. & Öberg, G., (2003). Chloride imbalances in soil lysimeters. *Chemosphere*, 52, 381-389.
- Standardization, E. C. (1995). f. *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.
- Svensson, T., Sandén, P., Bastviken, D. & Öberg, G., (2006). Chlorine transport in a small catchment in southeast Sweden during two years. *Biogeochemistry*.
- Swedish National Forest Inventory, available at <http://www-nfi.slu.se/>, [2009-05-28].
- Öberg, G., Börjesson, I. & Samuelsson, B., (1996). Net change in organically bound halogens in relation to soil pH. *Water Air Soil Pollut.* 89, 351-361.
- Öberg, G., Brunberg, H. & Hjelm, O., (1997). Production of organically-bound chlorine during degradation of birch wood by common white root fungi. *Soil Biol. Biochem.* Vol 29, no 2, 191-197.
- Öberg, G., (2002). The natural chlorine cycle – fitting the scattered pieces. *Appl Microbiol Biotechnol.* 58, 565-581.
- Öberg, G. & Sandén, P., (2005). Retention of chloride in soil and cycling of organic matter-bound chlorine. *Hydrol. Process.* 19, 2123-2136.
- Öberg, G., Holm, M., Sandén, P., Svensson, T. & Parikka, M., (2005). The role of organic-matter-bound chlorine in the chlorine cycle: a case study of the Stubbetorp catchment. Sweden. *Biogeochemistry.* 75, 241-269.
- White, P. & Broadley, M., (2001). Chloride in soils and its uptake and movement within the plant: A review. *Annals of Botany*, 88, 967-988.
- Winterton, N. (2000). Chlorine: the only green element – towards a wider acceptance of its role in natural cycles. *Green Chem* 2, 173-225.

Appendix

A: Scheme for classification of vegetation type at mineral soil site types.



Source: Mark info, SLU

B: Results from the method test

Table 5. Total radiation in DPM (disintegrations per minute) per g fresh weight and tube.

| Method | Replicate 1 | Replicate 2 |
|---------------|--------------------|--------------------|
| A | 325972 | 336210 |
| B | 303795 | 373270 |
| C | 220096 | 261208 |

The table show that method B gave similar results as method A (when comparing how much $^{36}\text{Cl}_{\text{org}}$ that was found in the soil), even though less soil extractions were carried out.