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Mercury and methyl mercury release in soil and water - their relationship with different properties in Skogaryd

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Abstract

In Skogaryd research site, water samples were taken and analysed for total mercury (Hg_{tot}) and methylmercury (MeHg) concentration along with the pH, dissolved oxygen (DO) and total organic content (TOC). This to give the condition of the site before the forest is clear-cute and a part of the land rewetted to a wetland. To complement the analysis of the water, soil samples was analyses for Hg_{tot}, organic matter content and potential demethylation constant rate (k_d). The result of the measurements shows that the Hg_{tot} and MeHg concentrations are low in the area, indicating that Skogaryd is an area with low levels of pollution. Relationship of the Hg_{tot} and MeHg concentrations with pH and DO were found. With higher DO and lower pH levels there will be higher concentrations of Hg_{tot} and lower of the MeHg concentrations, indicating that demethylation of the MeHg under these conditions occurs. The soil analysis showed that there is higher organic content at a depth of 20 – 30 cm, however the highest Hg_{tot} concentrations was found in the 0 – 10 cm depth. The samples with the highest Hg_{tot} also had high organic content, indicating that they might have been needed to do more replicates or that there are other metals bound to the organic matter at the 20 – 30 cm depth. The result of the k_d varies over the area, though due to loses of samples available for analyse makes it hard to see if there is a pattern of the variations. The k_d does however range in what other studies has found in similar settings.

Keywords: Mercury, methylmercury, demethylation, pH, dissolved oxygen and total organic content.

Sammanfattning

I Skogaryds forskningsplats vattenprover var tagna och analyserade for totalt kvicksilver (Hg_{tot}) och metylkvicksilver (MeHg) koncentrationer, tillsammans med pH, upplöst syre (DO) and total organisk halt (TOC). Detta för att få de förhållanden som området har före skogen avverkas och delar av området återvätas till en våtmark. För att komplettera vattenanalyserna, jordprover var analyserade för Hg_{tot}, organiskmaterial halt och den potentiella ständiga demetylerings graderingen (k_d). Resultatet av mätningarna visar att Hg_{tot} och MeHg koncentrationerna är låga i området, vilket indikerar på att Skogaryd är ett område med låg halter av förorening. Förhållande av Hg_{tot} och MeHg koncentrationerna med pH och DO hittades. Med högre DO och lägre pH nivåer kommer det bli högre koncentrationerna av Hg_{tot} och lägre MeHg koncentrationer, vilket indikerar på att demetylering av MeHg sker under dessa förhållanden. Jorden analyserad visar att the det är det högre organisk halt i 20 – 30 cm djupet, dock är de högsta Hg_{tot} koncentrationerna hittade i 0 – 10 cm djupet. Proverna med det högsta Hg_{tot} halten hade även hög organisk halt, vilket antyder på att fler replikat kan ha behövts göras eller att det finns andra metaller som binder till det organiska materialet i 20 – 30 cm djupet. Resultatet för k_d varierar över området, dock på grund av förlorade prover för analys gör att det är svårt att se om det finns en trend inom dessa variationer. Värdet för k_d har dock likande omfattning som vad andra studier har haft i likande omgivningar.

Nyckelord: Kvicksilver, metylkvicksilver, demetylering, pH, upplöst syre och organisk halt.

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1. Introduction

All over the world wetlands has been drained for agricultural and silvicultural purposes. It's been estimated that over half of the world's wetlands has been drained (Kronberg et al., 2012), and in Sweden this is no exception. As wetlands are important for biodiversity, carbon storage, cleaning water and other ecosystem services it has become crucial to protect and restore the wetlands. To accomplice this Sweden has the environmental objective *thriving wetlands* (Naturvårdsverket, 2016).

In water, the rate of oxygen diffusion is 10 000 times slower than in air, this leading to limited oxygen levels in wetlands. The decomposition in wetlands therefore mainly occurs by anaerobic decomposition, which is slower than the aerobic. In anaerobic conditions, microbes use the energy from reactions with elements other than oxygen, such as nitrogen, sulphur, iron and mercury (Schlesinger & Bernhardt, 2013). During this process production of methyl mercury (MeHg) occurs. There is a concern of production of MeHg as it accumulates in food webs and can be found in high concentrations in fish (Kronberg et al., 2016).

A recent study done by Koskinen (2017) on rewetted wetlands in Finland showed that dissolved organic carbon (DOC), nitrogen and phosphorus releases increase in lands that are nutrient rich. DOC is an important transporter of MeHg out in the catchment system (Kronberg et al., 2012), and it is therefore important to observe changes in DOC levels.

There are risks with restoring drained wetlands, where the releases of MeHg is one of them. However, the profits gained by restoring the drained wetlands overcome the risks. If the restoration is performed properly and under high supervision, these risks can be minimized. One of the locations were a drained wetland will be restored by rewetting is in Skogaryd, Vänersborg municipality. The aim is to restore it to its previous state, with the first step to clear-cut the forest that today grow on the land. There are however risks with clear cutting. As the trees are cut down the water level increases, there will be higher availability of electron donors and microbial activity is stimulated, leading to a higher production of MeHg (Kronberg et al., 2016).

1.1. Aim and hypotheses

The aim of this thesis is to study the changes of different chemical properties before, during and after deforestation on an area in Skogaryd, that later will be restored to a wetland. Focus will lay on total organic content (TOC), Hg_{tot} and MeHg that are released into a ditch that goes through the area.

- Higher up in the ditch the chemical concentrations will not change as the deforestation will occur further down. Some smaller changes will be found within the area of deforestation, though the highest changes and concentration levels will be found bellow the area of deforestation.
- As the water level rises due to the deforestation, higher amount of DOC and MeHg will be released, though there will not be a significant change in Hg_{tot} released.
- Demethylation will occur in the oxygenated stream water below the deforested area and will thus reduce the amount of MeHg that reach the lake Skottenesjön.

1.2. Study area

In 2006 the Skogaryd research site was created in the southwest of Sweden (58° 23’ N, 12° 09’ E; 60 m.a.s.l.) and from then the type of measurements conducted has increased as well for over the area they are performed on (Klemedtsson, 2012). Since 2013 the research site has been a part of SITES (Swedish Infrastructure for Ecosystem Science, www.fieldsites.se) and today it has 7 different sub-sites. The study conducted in this thesis was performed within sub-site E. “Forest on drained organic soil” (University of Gothenburg, 2019). This area was drained during the 1870s and then used for agriculture until 1951 (Klemedtsson et al., 2010). At this point they planted the area with mainly Norway spruce (Tarvainen et al., 2015), which is still standing and are now planned to be clear-cut.

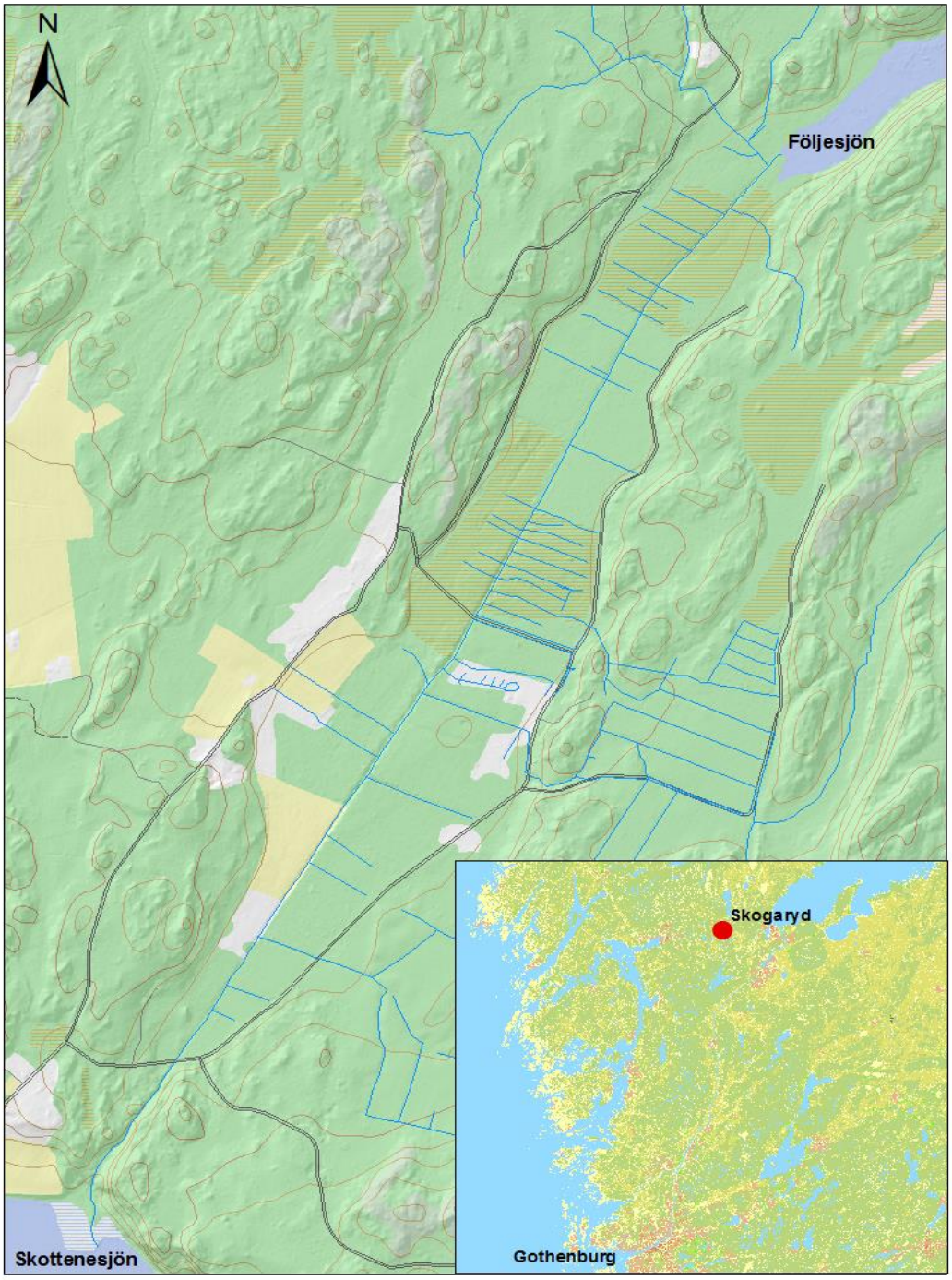

Figure 1. Map over the study area in Skogaryd, with a smaller map showing its location in Sweden.

When the area was drained a mainstream was created, called *Krondiket*, which starts at the outlet of subside D. "Följesjön". Along the stream several other streams are connected to it from drainage streams and other areas such as subside F." Forest on mineral soil" (University of Gothenburg, 2019). Krondiket then flows down to the inlet of Skottenesjön (Fig. 1).

1.3. Methylmercury and mercury

Through thousands of years of human impact, mercury (Hg) do not only exist naturally in aquatic and terrestrial ecosystems. By mining, its use of products; such as paint and electrical devices, and as a trace contaminant in many materials, Hg has been mobilized and spread around the world. The primary transport of Hg is through the atmosphere (Fig.2), where the chemical and physical forms of Hg plays a big role in how far and how much of the element is transported. Elemental Hg (Hg(0)) has a residence time in the atmosphere of several months to a year, before oxidation and deposition in dry gas-phase form or by precipitation. In contrast reactive gaseous Hg (RGM) and particulate bound ionic Hg (Hg(II)), have an atmospheric residence time of hours to a few days. Combined, the Hg is spread and deposited all around the world. The biggest input into the ecosystems comes from the Hg(II), and deposited Hg can be reduced to Hg(0) and reemitted to the atmosphere (Driscoll et al., 2013).

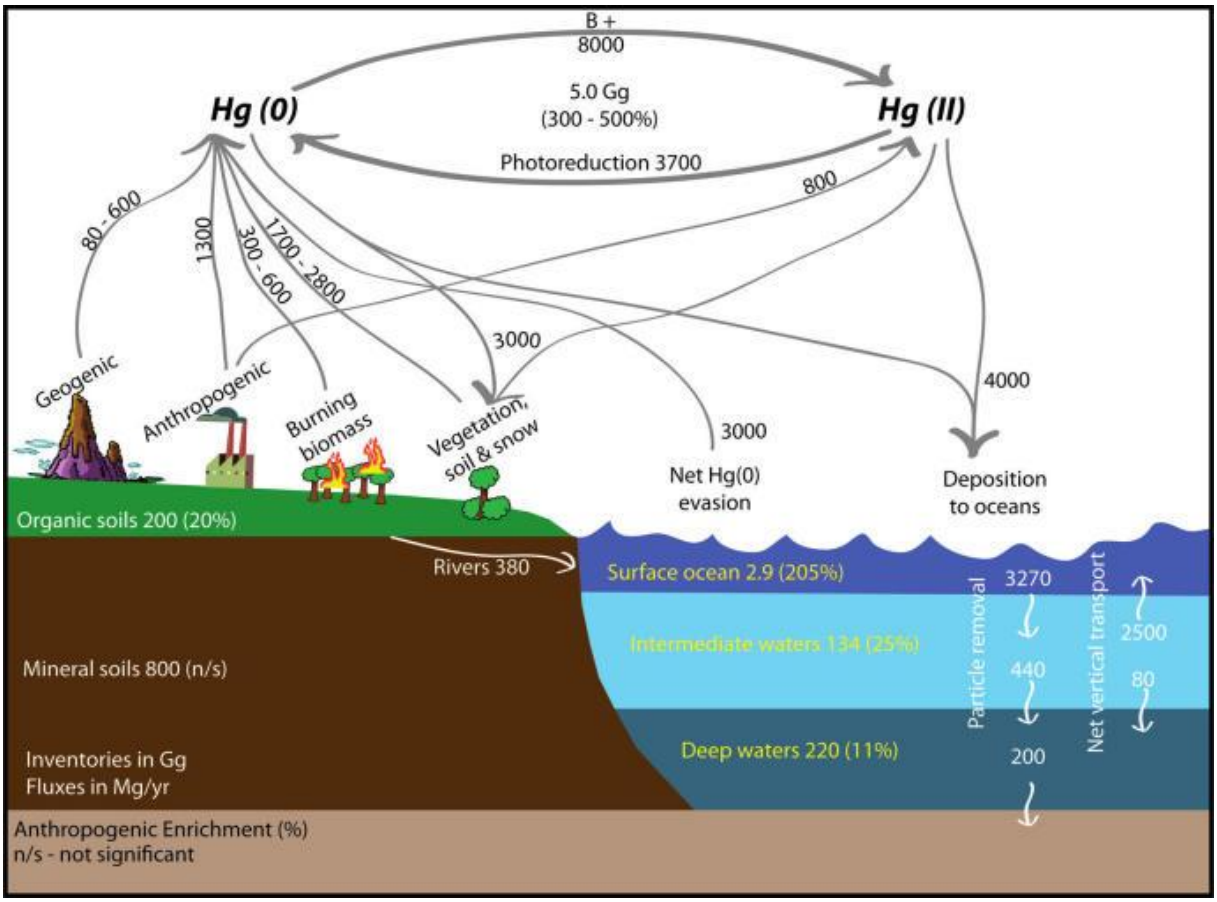

Figure 2. Estimates of the fluxes and pools of Hg at the Earth's surface. Figure taken from Driscoll et al. (2013).

Methyl mercury (MeHg) are mainly produced within the ecosystem, especially those that are dominated by forest and wetlands (Skyllberg et al., 2009). For the production (methylation) of the Hg studies has shown that it is primarily linked to anoxic freshwater environments and the activity of

sulphate-reducing bacteria (SRB). There are also other factors that controls the methylation in the water and soil. Nutrient and pH conditions have been shown to affect the production of MeHg, along with temperature and redox potential. At intermediate levels of pH and nutrients, the methylation is higher than the demethylation (destruction of MeHg), whereas the demethylation is higher in conditions with low or high levels of nutrients and pH (Tjerngren et al., 2012; Hall et al., 2005).

While the methylation of Hg to MeHg occurs in anaerobic conditions primarily, the demethylation of the MeHg back to Hg occurs in both anaerobic and aerobic conditions. There are different ways for the MeHg to form and both abiotic and biotic demethylation exists. Today, there are two known pathways for biotic demethylation. The first are the combined actions of organomercurial lyase (*MerB*) and mercuric reductase (*MerA*), where MeHg are converted to Hg(0) and methane (CH_4). The second pathway are oxidative, MeHg are degraded to Hg(II) and either CO_2 (carbon dioxide) or CH_4. The Hg(II) can potentially be recycled to MeHg (Kronberg et al., 2018).

To study the release of MeHg and Hg are important as it bioaccumulate in the food web. Studies has have shown that the MeHg levels are elevated in fish and shellfish, piscivorous fish such as tuna has had particularly high levels. As fish is one of humans most important sources of protein and are life important in parts of the world, humans are exposed to the MeHg and the dangers of consuming to high amounts of it. An incident in Minamata, Japan, where over 2000 people consumed fish with high concentrations of MeHg led them to suffer severe consequences from neurological disorders, collectively called the Minamata Disease. High levels of pre- or postnatal exposure to MeHg has shown to cause long-term psychiatric symptoms in adults and fetuses exposed have suffered from cerebral palsy-like symptoms (Driscoll et al., 2013).

2. Materials and Methods

2.1. Site description

The site that has been studied have been divided into three different parts (Fig. 3), based on the plans to rewet parts of the area. The first part is the reference forest (Ref.F), this forest is between the outlet of Följesjön and the start of the clear-cut. This section of the area is the most representative forest to use as a reference, though there are some part that differ. Closer to Krondiket there are a bog and then there is a forest that goes from the bog to the roads around the study site. The lower most part of the area is however without the bog and are more similar to the rest of the site.

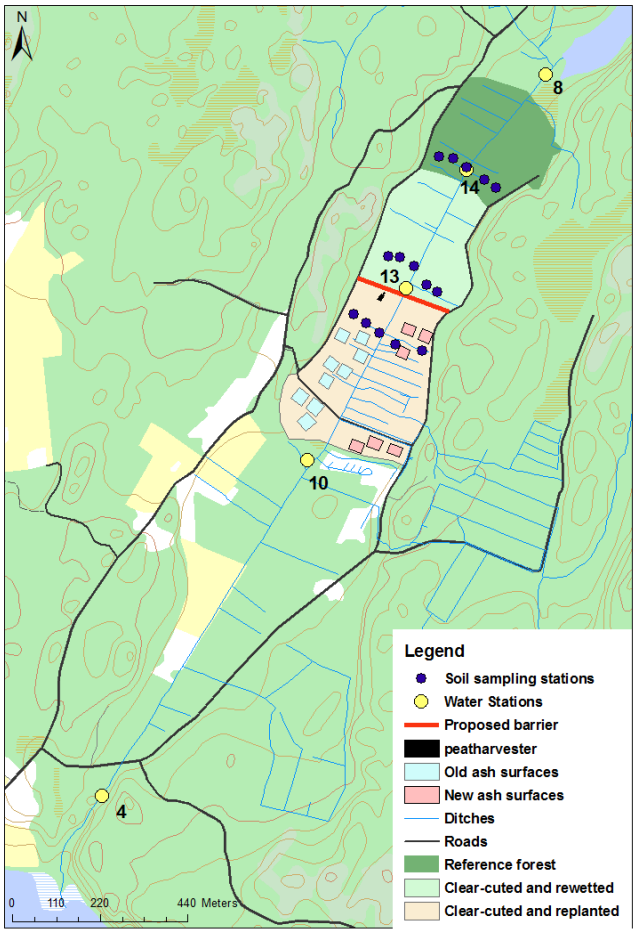

Figure 3. Map over the study site, with its different section and sampling locations.

The second site are the area that will be clear-cute and then rewetted (CReWe). Notable with this section is that in its north-eastern part it has younger forest, due to storm felled trees and a flooding caused by beaver dam that since been removed. The third section is the southernmost one and this part will be clear-cuted and then replanted (CReP). Within this part the peat harvester and the land where peat has been extracted lays in the north-western part. The north-eastern part has pine trees grown in contrast the fir trees that grows in the rest of the study area. Below the part with the pine trees there is an area that they failed to drain properly it are therefore wetter than its surrounding. There are also old bio-ash experimental plots in this are as shown in Figure 3.

2.2. Water sampling

Water sampling were performed with different types of bottles. For the total organic carbon (TOC) analyses, glass bottles were used. These had been washed with deionized water at the end of the washing program and then stored in plastic bags until use. The bottles for the analyses of Hg_{tot} and MeHg was provided by IVL Swedish Environmental Research Institute. These bottles were made of Teflon, cleaned at IVL and then filled with SQ-water and 0.5% HCL. The bottles used for Hg_{tot} were stored in two zipping plastic bags and those for MeHg in one to minimize the risk of contamination.

In the field, the content in the bottles from IVL was poured out downstream from the sampling location (Fig. 3) to not contaminate the water sampling. These bottles and the glass bottles were then field with water had the cap closed, where shaken and then emptied downstream form the sampling site three

times to neutralize the bottles. Thereafter the bottles were filled with the water sample and put back into the zip lock bags. During sampling plastic gloves were used to minimize contamination of the samples. Minimum contact of the bottles outside the bags was also important, in particular for the bottles used for Hg_{tot} as those are easily contaminated.

The properties of pH and dissolved oxygen (DO) were measured in the field by a Hach HQ40D portable multi meter, with the probes Intellical™ LDO101 and Intellical™ PHC101. The probes were held in the stream water in a vertical angel as far out in the middle as possible.

Sampling started in the end of October 2018 and continued until mars 2019, with a total of five sampling times for the MeHg and Hg_{tot}. The sampling locations were firstly based on already existing stations, where further measurements are taken through SITES water. Station 8 were used to give the information of what levels of MeHg and Hg that comes into the study area. Sampling from this station was performed around 20 meters below the original station. This to get the influences from both station 8; Följesjön outlet and station 7; clear-cut (outlet into Krondiket just below Följesjön). Sampling at this station for MeHg and Hg_{tot} started in December 2018. Station 10 represent the end of the study area and station 4 the outlet of Krondiket into Skottenesjön. Station 13 and 14 were created for this and coming studies in the area. Station 14 are located at the end of Ref.F, though still not to near the border of Ref.F and CReWe to be disrupted from the deforestation. Station 13 represent the border between CReWe and CReP.

2.3. The clear-cutting could not be conducted as planed

Before the planed start of the clear-cutting the ground frost was measured to 15 cm and the water level to 40-100 cm below the ground surface. The deforestation was planned to start in north and then continue downward towards the south on the west side of Krondiket and after that proceed to the east side in the same manner. During the clear-cut the brushwood would have been laid out for the machines to drive on to minimize the damage on the ground from them. The machines were also to be driven in a path that was as homogeneous as possible over the area to give an equal driving damage. Around the measuring instruments that has been marked up, the trees need to be cut down manually to prevent damage of the instruments. In the north-eastern part of the area that will be cut the trees are too small to be harvested with the machinery that are used for the rest of the area and need therefore to be cut down manually.

The deforestation was planned and prepared for a start in the middle of February 2019. However, due to changing weather conditions the plan was not carried thru. The snow melted, creating a flooding 300 % stronger than at a rain event and the ground became too soft to drive on without damaging the land area. The deforestation is now planned to start in august 2019, when the groundwater level has decreased to around 100 cm and it will be possible to deforest without driving damage.

2.4. Soil sampling for incubation studies

To complement the water analyses in Krondiket and due to loss of data after the cancelled clear-cut, transects of soil sampling were performed (Fig. 3). A total of nine transects were created, with tree transect in each area. Each transect have five sampling points, except for the ninth that has four, that goes from west to east. The transects are numbered from north to south, with number one in the north and the ninth in the south. The sampling points were numbered 1 – 45, number 1 – 5 for the first transect in a west eastern direction and 41 – 45 for the ninth.

In the Ref.F area, the sampling was done in the southern part as it is drier and more homogeneous with the areas bellow. In the CReWe, the transects are located along smaller draining streams, that goes out to Krondiket. In CReP sampling took place along streams that connects with Krondiket from both sides as well. Here the transects are located to include the pine area and to avoid the part there they failed to drain the land. The south-eastern part was flooded during marking up the points and measurements and point 44 were therefore not manageable to create at this time.

For this study, soil samples were only taken on transects three, six and seven (Fig. 3), which lays in their separate section of the site. At each of these 15 sampling locations two samples was taken on the depths 0-10 cm and 20-30 cm.

Figure 4. The soil sampler, with its two different parts.

The samples were taken with a soil sampler (Fig. 4), that has two parts. The sampler was set on 60 cm depth, pushed down in the ground, then twisted around before pulled up. The top layer of fauna was removed and then the depths 0-10 cm and 20-30 cm were measured up. The blade, the right part in Figure 4, was put into the sampler from the top to push up the sampled core to make extraction of sample easier. To ensure that no sample was lost or spilled on the ground a plastic bag was put underneath, the samples were then put into zip lock bags. The samples were then transported with ice packs until their preparations started in the field lab.

2.5. Laboratory work

2.5.1. Water analyses

The water analyses were transported in a cooler to Gothenburg the same day the samples were collected and then kept at 4 °C over night. The water collected in glass bottles were run in a Shimadzu TOC-L Total Organic Carbon Analyzer. The samples were run unfiltered and with an amount of 20 – 25 ml water was used in the vials belonging to the instrument. The analyses were done between 7 – 10 days after they were collected.

The samples taken for MeHg and Hg was delivered to IVL the day after they were collected. For both analyses the samples was unfiltered and done by a Cold vapour atomic fluorescence spectroscopy (CVAFS). For the Hg_{tot} the IVL A9 method was used, which has a detection limit of 0.04 ng/L, a quantification limit of 0.1 ng/L. The measurement uncertainty for the Hg_{tot} depends on the amount of Hg_{tot} in the sample, under 0.25 ng/L the uncertainty is 14 % and over 0.25 ng/L it is 8 %. For the MeHg the method used was IVL A10, which has a detection limit of 0.02 ng/L, a quantification limit of 0.06 ng/L and a measurement uncertainty of 12 %. The measurement uncertainty is for the analyse method and are stated with an approximately of 95 % quantification interval.

2.5.2. Soil analyses

2.5.2.1. Incubation study

The soils properties change with change of land usage, to know the changes in the soil in Skogaryd after clear-cut and rewetting the potential methylation rate constant (k_m) and demethylation rate constant (k_d) was calculated. This gives the potential capacity the soil has to methylate or demethylate the Hg and MeHg respectively (Kronberg et al.2016). The potential methylation and demethylation rate constant in the soil where determined by an incubation experiment. 60 falcon tubes were sent from the Stockholm University, each falcon tube had tracer of inorganic Hg(II) (Hg^{198}) and $Me^{200}Hg$ added. After arrival they were kept in a freezer until start of the experiment.

After that the soil samples had been collected, they were taken to the field laboratory in Skogaryd. The falcon tubes were taken out from the freezer and the tracer in the bottom spike were warmed up. The falcon tube was placed on a two-decimal scale and had approximately 10 g of soil added to them. As the water content of soil was high enough to stir the samples with the tracer no additional pore water was added. Each sample taken in the field was added into two falcon tubes, one named T_0 and the other T_{24}. When all the samples had been divided into the falcon tubes the T_0 samples was put into dry ice to freeze. The soil left in the zip lock bags was frozen for further analyses later on. The T_{24} samples had para film placed in the top of the tube before a Pasteur pipette, hocked to N_2 gas, was placed in the tube for one minute to remove the oxygen and replace it with the N_2 gas. The cap was put on the tube immediately after removing the Pasteur pipette, leaving the para film on. The N_2 flushing of the samples was done in a randomly order to minimize unwanted trends in the samples if the equipment would stop work properly. The T_{24} samples was then left to incubate for 24 hours in the field laboratory in 17 – 18 ℃. After the 24 hours the T_{24} samples was placed in dry ice. The samples were then transported to Stockholm University where they were kept in a freezer until analysed.

The samples were thawed and then had 0.125 ml of $Me^{201}Hg$ internal standard added before being stored in the cold for one hour. 10 ml of dichloromethane (DCM), 10 ml of potassium bromide (KBr) and 2 ml of cupper sulphide ($CuSO_4$) was then added to samples that then were slightly stirred before another hour to react with the chemicals. The samples were then put on a shaker for one hour to properly shake them. They were then placed in a centrifuge for 5 min, the DCM had then been separated from the rest of the sample and chemicals and were in the bottom of the falcon tubes. Extraction of DCM, which contains the Hg and MeHg, was done with a Pasteur pipette and placed into a new flacon tube. 10 ml MQ-water was added into the tube and then put in a pressure cocker with a temperature of 45 °C, a Pasteur pipette hocked to N_2 gas are put into the vial to evaporate the DCM. The water and the Hg and MeHg concentration are then left. Into vials belonging to Gas Chromatography – Inductively Coupled Plasma (GC-ICP-MS), 10 ml of MQ-water, 0.225 ml of acetate buffer, 0.030 of sodium tetraethyl borate ($NaBEt_4$) and finally 0.100 ml of the sample was added. The samples were then run in the GC-ICP-MS.

2.5.2.2. *Further soil analyses*

The soil left in the zip lock bags was put into a freeze-dryer for one week, to remove the water in the samples. When this were done the samples were analysed for Hg_{tot}, which was done by a Direct Mercury Analyzer (DMA) 80. Before analysing the samples were grounded down to a powder and put into a so-called boat that belongs to the DMA. The boat was weighted before the sample was added, then zeroed before the sample was added. The amount of sample added were 0.0600 – 0.0800 g, and the scale used for this had four decimals. The samples were then put into the instrument for analyse, which took around 5 min for each sample (Fig. 5). When the analyse was done the boat with sample was weighed again. From the weights the organic content in the soil can be calculated.

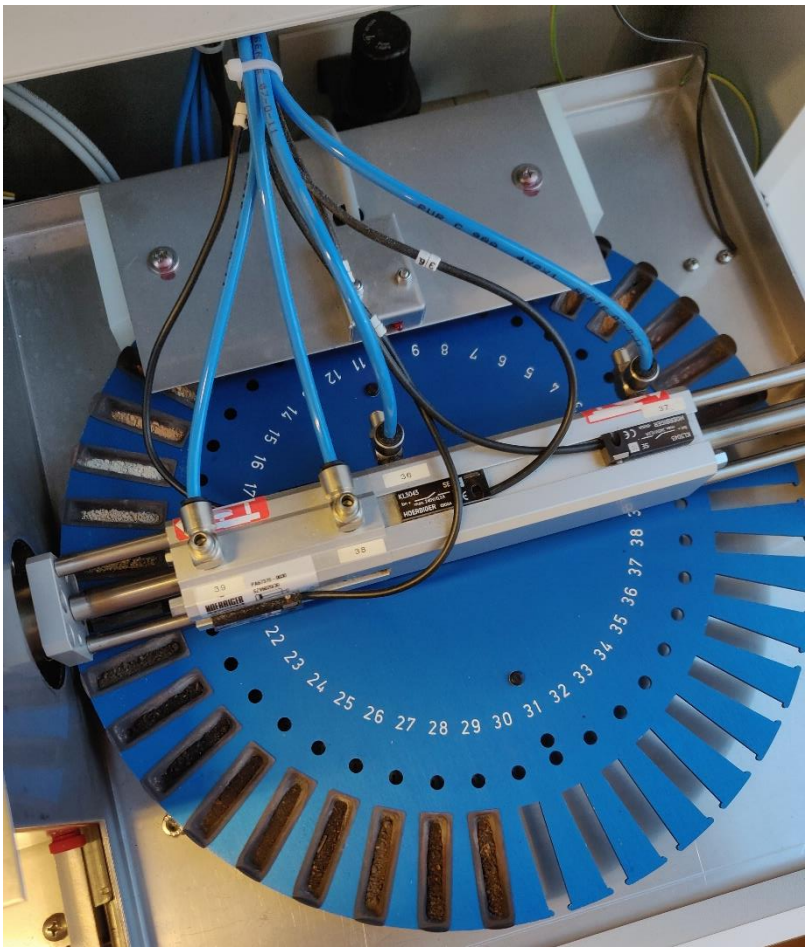

Figure 5. The soil samples in the DMA instrument. Samples positioned at 22 - 29 has not get been analysed and those with a location spot with a lower number has been analysed, giving them a different colour of the material.

2.6. Data management

To calculate the isotopic signal from the GC-ICP-MS into concentrations for the ambient, $Me^{198}Hg$ and $Me^{200}Hg$ the equations from Qvarnstrom & Frech (2002) was used. The concentrations of $Me^{198}Hg$ and $Me^{200}Hg$ was then used to calculate the k_m and k_d. This using eq. 1 and eq. 2, modified from Liem-Nguyen (2016).

$$k_m = \frac{[MeHg^{198}Hg]T_{24} - [Me^{198}Hg]T_0)}{[204HgII]addition \times 1} \quad (d^{-1}) \qquad \text{(eq. 1)}$$

$$k_d = \frac{-1 \times (\ln[MeHg^{200}Hg]T_{24} - \ln[Me^{200}Hg]T_0)}{1} \quad (d^{-1}) \qquad \text{(eq. 2)}$$

Excel was used for data management and the graphs and tables was produced in the program. Statistical analysis in form of mean, standard deviation (SD) was done as well (Devore, J. L., 2012).

3. Result

3.1. Water chemistry

During the five months of water sampling and analysis of Hg_{tot} and MeHg the result received from IVL had at two occasions MeHg concentration levels that were less than 0.06 ng/L, which is under the quantification limit for the method used. During November, there is no measurement for station 8 as

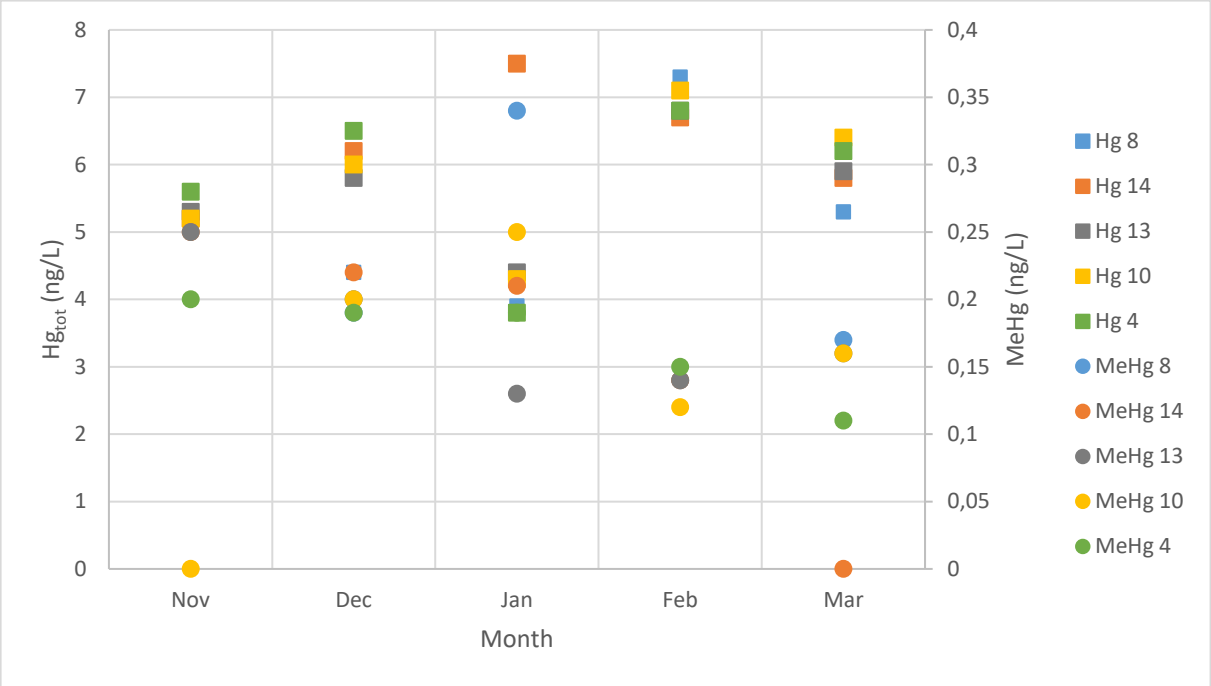

Figure 6. The measured concentrations of Hg and MeHg in the water at the five different sampling stations during the months measured.

the decision of sampling there came later, and for the other stations the Hg_{tot} and the MeHg concentrations are clustered together (Fig. 6). In the later months the clusters start to separate, with an increasing Hg_{tot} concentration and a lower MeHg. In February this can be seen clearly (Fig. 6), were the Hg_{tot} concentrations are around 7 ng/L and the MeHg are around 0.15 ng/L. The DO levels were during the February measurement around 11 mg/L for all the stations and this was during a flooding event from the snow melting. The Mars measurement had similar DO levels as February, however there are a bigger spread within the Hg_{tot} and MeHg concentrations.

During the measurements in January the pattern of separate clusters for Hg_{tot} and MeHg, do not exist as it does for the other months (Fig. 6). There is a bigger spread of the result and the Hg_{tot} and MeHg are mixing. What can be seen is that for station 10 and 8 their Hg concentration levels are low and the MeHg levels for these stations lays higher than the Hg_{tot} in the graph. Station 13 has a Hg_{tot} concentration approximately the same as station 10, however the MeHg concentration is more than 0.10 ng/L lower than the MeHg at station 10.

The standard deviation (SD) within the different station do not differ with more then 0.04 - 0.10 ng/L for the MeHg (Tab. 1). For the Hg_{tot}, the difference is bigger, having the highest SD at station 8 with 1.50 ng/L. Station 8 are also the station with the lowest Hg_{tot} mean concentration and highest MeHg mean concentration.

Table 1. The table shows the mean and standard deviation (SD) for Hg and MeHg at the different stations.

	Mean (ng/L)	SD (ng/L)
Hg 8	5.23	1.50
Hg 14	6.28	0.88
Hg 13	5.64	0.88
Hg 10	5.80	1.08
Hg 4	5.78	1.19
MeHg 8	0.21	0.09
MeHg 14	0.16	0.10
MeHg 13	0.18	0.05
MeHg 10	0.15	0.09
MeHg 4	0.17	0.04

The range of the pH measurement at the five stations was mainly between 5 - 6.5, though station 14 in November had a pH value of 7.07 (Fig. 7.a). Around the pH of 6 the MeHg and Hg_{tot} cluster together, however clear distinction between them are seen as the pH levels decreases. The lower the pH becomes the higher the Hg_{tot} are and the MeHg decreases.

The DO measured at the stations varies between 8 ~ 10.5 mg/L, were it in January is 12.03 mg/L for station 4. During the November measurements the DO was under 8 mg/L for stations 14, 13 and 10. From over 10 mg/L the Hg_{tot} and MeHg cluster separately, and a trend of higher Hg_{tot} and lower MeHg can be spotted with higher DO levels (Fig 7.b).

What can be seen for station 4 in January is that the Hg_{tot} and MeHg levels overlap each other in the graphs in Figure 6 and Figure 7a-c. This measurement time is what has the highest DO level and the lowest total organic content (TOC), where it does not have the highest pH level it does have one of the higher ones with 6.38. The TOC levels are generally between 22 – 25 mg/L (Fig.7.c), though station 10, and 14 has over 25 mg/L. The one for station 14 that has a TOC level of 27.24 mg/L, are from the January sampling. Station 10 have three out of four analysis that are over 24 mg/L, while station 8 has between 21.01 – 23.06 mg/L. Station 4 have like station 8, also lower TOC levels (21.1 – 24.23 mg/L) though it varies more. No trend of Hg_{tot} and MeHg having an increase or decrease can be seen with the amount of TOC in the water.

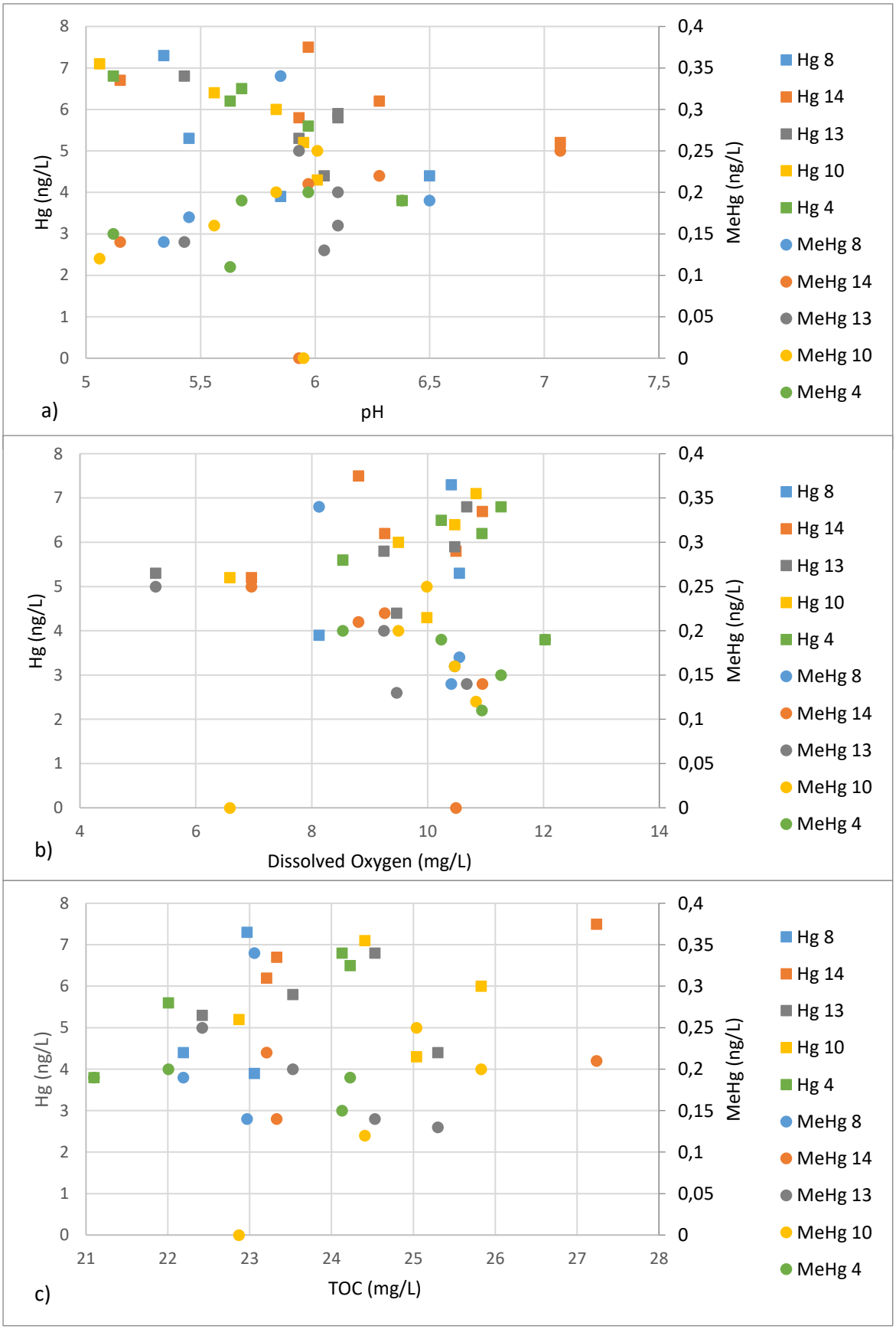

Figure 7. The Hg and MeHg concentrations ploted to a) pH, b), dissolved oxygen and c) the total organic content. Values at zero were occasions when the MeHg concentrations was under detection limit.

3.2. Soil analysis

Due to negative concentration levels of the Hg^{198} the potential methylation constant rate (k_m) were not possible to calculate, leaving only the potential demethylation constant rate (k_d). The reason for the negative values is due to low concentrations of the added tracer. After some complications during laboratory work, 9 of the original 30 samples could be used for the k_d calculations.

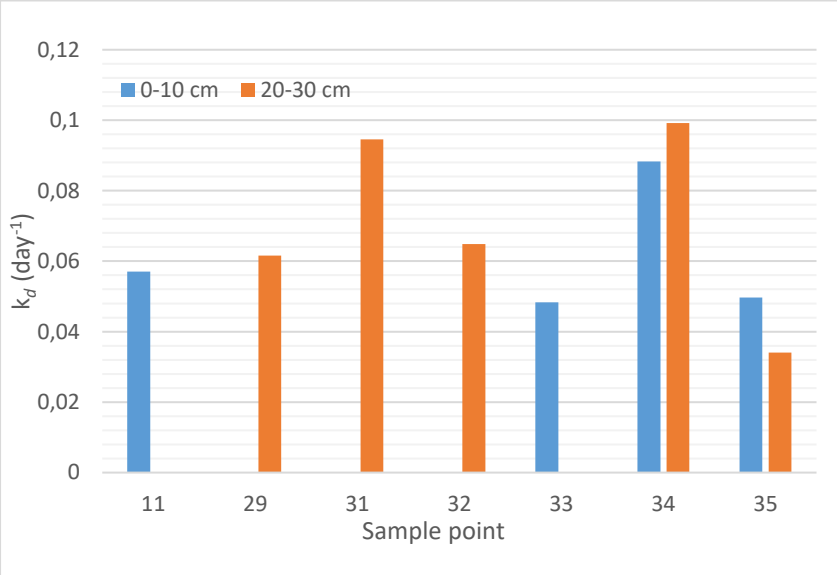

Figure 8. The potential demethylation rate constant for the different depths at the sampling points.

The k_d could be calculated for each sampling point in transect 7, however only from one sampling point in transect 3 and 6. Only for the sampling points 34 and 35 in transect 7 could both 0 – 10 cm and 20 – 30 cm be calculated.

The 0 -10 cm has its lowest k_d at point 33 (Fig. 8), with a k_d of 0.048 day^{-1}. Point 35 have the second lowest with a k_d of 0.049 day^{-1}. At sampling point 35 the lowest value for the 20 – 30 cm are with a k_d of 0.034 day^{-1}. Sampling point 34 has the highest k_d levels are for both 0 – 10 cm and 20 – 30 cm, with 0.088 and 0.099 day^{-1} respectively. From the data available the 0 – 10 cm have, with the exception of sampling point 35, lower k_d values than the 20 – 30 cm.

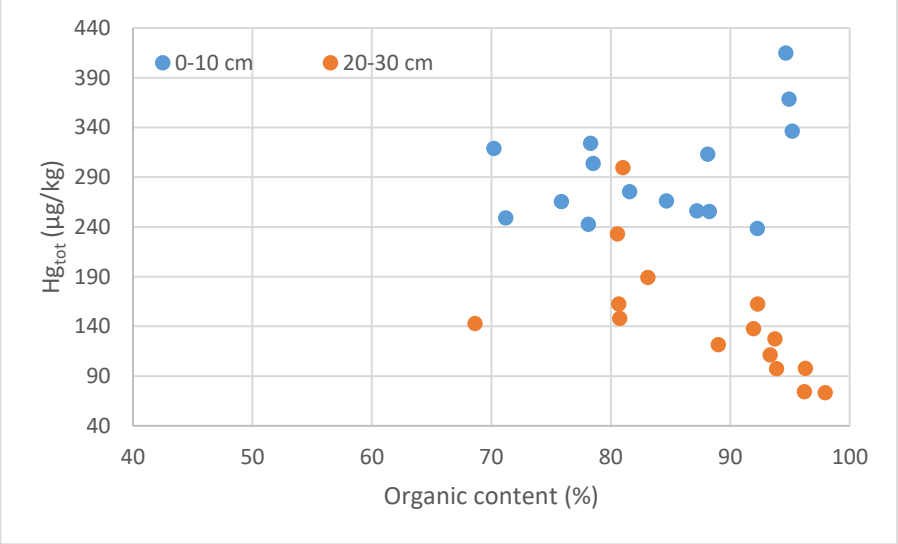

Figure 9. The Hg_{tot} concentrations of the soil against the organic content in the soil samples.

The soil organic matter for the soil samples range between 68.6 – 98 % for the 20 – 30 cm depth and 70.2 – 95.2 % for the 0 – 10 cm. The lower depth cluster together tighter than the shallower one, though the former has a bigger range (Fig. 9). The 20 – 30 cm also has more sampling points that are over 90 %. Sample point 27 and 33 has for the 20 – 30 cm depth higher Hg concentration then the other sampling location at the same depth, with 299.89 and 233.08 µg/kg respectively. These two samples also have lower organic content, compared with the other samples at the 20 – 30 cm depth. For the 0 – 10 cm depth there are a bigger variety of organic content amount, and the Hg concentration are higher than in the lower depth. For this shallower depth the highest concentration also has the highest amount of organic content, while it is the opposite for the deeper depth. The tree sampling

points that has the highest Hg concentration and highest organic content are sampling point 11, 12 and 15, with Hg levels of 414.99, 336.57 and 368.69 µg/kg.

4. Discussion

The mean of the Hg_{tot} and MeHg concentrations at the different stations (Tab. 1) validate what the February measurement in Figure 6 shows. That if there are higher Hg_{tot} concentration there will be lower MeHg concentration. This do not show how big the methylation or demethylation are, and alone it does not show that those processes even occur. However, connecting these concentrations and trends to the pH and DO and seeing their relationship will show whatever methylation or demethylation occurs.

The Hg_{tot} and MeHg result from the February analysis show that there will be demethylation in the water when the oxygen levels of the water are higher. The result from Mars also had high oxygen levels, however the clear spread of the Hg_{tot} and MeHg clusters are not as pronounced as in February. The reason for this is probably that in February there was a flooding event, which was the reason for the stopped clear-cutting. The snow in the area melted in a few days, giving high water levels and high flow rate of the water, helping circulate the water. Comparing with the measurement in November were the Hg_{tot} and MeHg had different relationship to each other (Fig. 6), it had low oxygen level in the water. The water level in Krondiket in November was also lower, decreasing the flow rate of the water and then the circulation of the water will be less then at higher water levels, resulting in lower oxygen levels. During Mars the water level was still high in Krondiket along with the high oxygen levels, the big difference from February is that the water level did not reach over Krondikets boundaries and that leading to lower flow rate in Mars. This giving the smaller separation of clusters in the Mars measurement.

The January results differs from the other months, it has a high spread of the Hg_{tot} and MeHg concentrations and there are stations that have the MeHg values above the Hg_{tot} in Figure 6. This indicates that there is higher methylation during this time, compared to the other months. The DO and pH do not stand out compared to the other months, however the TOC levels are in general the highest for the study site during this month. The organic content has the ability to bond the Hg_{tot} and MeHg to it, which makes the TOC an important transport path for the Hg_{tot} and MeHg (Berndt & Bavin, 2012). Even though other properties that are not measured in this study probably has a high impact on the result, the higher TOC levels in January can explain the higher concentrations of Hg_{tot} and MeHg as there are more transporters at that time.

The result from Table 1 and Figure 7.a show that with lower pH there will be lower concentration of the MeHg at the same time as the Hg_{tot} will increase. This is what Tjerngern et al. (2012) found as well, though their result was also connected to C/N quot. What they also found was that the highest MeHg yield was at pH ~5, which is the same level that this study has had as the lowest pH. However, at this level demethylation was already occurring in the water in Skogaryd. Why this is, may be due to that as the DO levels in the water (Fig. 7.b) can be seen increasing there will be higher Hg_{tot} and lower MeHg concentrations. Tjerngern et al. (2012) did not show how the pH and oxygen alongside each other affect the methylation/demethylation but hade the relationship between pH and nutrients instead. There might be more parameters then the oxygen levels in the water that explains why demethylation can be seen already at pH ~5, which is contradictory to the Tjerngren et al. founding, however what those would be cannot be confirmed with this study.

Skogaryd is an area that do not have any nearby industries and there are not high contaminations in the forest area. This mean the favourable demethylation type that will occurred in the area are the oxidative pathway, which makes the MeHg go back to Hg(II) (Kronberg et al. 2018). Hg(II) can again go back to MeHg later on if it is deposited in an anoxic environment. This means that though the releases of MeHg can be controlled by creating demethylation possibilities, their levels can change after entering Skottenesjön. Hg_{tot} levels should therefore not increase radically at station 4 in the future, to ensure less risk for MeHg exposure.

The Hg_{tot} analysis of the soil showed the highest concentrations in the shallower depth, where the Ref.F had the highest concentrations. This site also had the highest organic content in the study site, this would most likely be due to that this is a wetter area and with the bog present in it has more fauna affecting at the depths measured than in CReWe and CReP.

The highest Hg_{tot} concentrations for the 20 – 30 cm depth are in the CReWe area. That this particular area has the highest concentration might be of concern as this is the area that in the future will be rewetted and restored to a wetland. The plan is that the groundwater level will be at about 10 cm below ground surface. This would mean that the depth of concern would in normal state be saturated with water and therefor anoxic. Tough the concentrations in this layer are higher than in the other areas, the concentration does not vary from the range of Hg_{tot} that Kronberg et al. (2016) measured. They did however the measurements at 0 – 10 cm but had found in earlier studies that in their study area these depths did not very much.

That the 20 – 30 cm has in general higher organic content but lower Hg_{tot} are not what the normal situation in soil are. Soil with high amount of organic matter has pronounced metal binding properties, which make the soil accumulate higher levels of metal (Tack et al., 1997), this would mean that the 20 – 30 cm depth should be located in the same area as the highest values of the 0 – 10 cm (Fig. 9). There is high variability of the concentrations in the soil due to mineral content, climate, land use, age, soil organisms, vegetation and topography (Mikkonen et al., 2017). As the samples are taken in an area with the same climate, topography and to a big extent land use, these should not affect the result. The age of the soil in the shallower layer will differ more around the area and the soil organisms can as well. The vegetation does differ at some locations though not to an extent that the soil at the different soil sampling points would have a big differences in properties. That there might be other metals in the soil that are bound to the organic matter, would explain why there are lower concentrations of Hg_{tot} even with the higher organic content. With further chemistry analysis that hypothesis could be determined.

Even though no direct pattern could be given from the k_d result, the values of it are similar to what other studies has gotten. Kronberg et al. (2016) did incubation studies similar to what performed in this study for forests in northern Sweden with the same age and type of tree stand. In their result for the 0 – 10 cm depth the k_d levels are within the same range as received in this study.

From the k_d result it is hard to see if the different areas Ref.F, CReWe and CReP has different properties. As the soil sampling points 31 – 35 are from transect 7 in CReP and has big variations within the transect the k_d can change within a short distance. The variations are also not influenced by the location to Krondiket as sampling point 31 lays close to the road on the east side and sampling point 34 lays on the west side off Krondiket. Sampling point 34 also has a substantially higher 0 – 10 cm value compared to the other values at the same depth. The location of this point is in an area were other experiments has been conducted and this might have affected the properties of the soil. It is however not the case for the sampling point 31, which is in an area were there has not been any experiment. Unfortunately, the 0 – 10 cm depth are not analysed for this sampling point and if this depth would have a similar

amount as at sampling point 34 cannot be determined. There is not possible though to say if these two points really do stand out in the study site as so many samples from the incubation experiment was lost at the preparation for the analysis.

Besides the 0 – 10 cm k_d at sampling point 34 the remaining sapling points at this depth have similar values to one each other. The 20 – 30 cm depths are higher than the shallower depth with the exception of sampling point 35, were the lowest demethylation rate constant is. Why this value is so much lower than at the other sampling points is unknown. Looking at the result of the Hg_{tot} in the soil and the organic matter it does not differ much between sampling point 34 and 35, though there is bigger difference comparing with the sample from point 31. At this sampling point the organic matter is lower for both depths then they are at the 35th, and the Hg_{tot} are for the shallower depth higher at sampling point 31. The possibility the sampling point 34 has, due to experiments in its surrounding altered properties of the soil are likely as the 35th have high difference in its k_d values and the only other sample with similar value are the 31st, which has both different organic content and Hg_{tot} properties.

That the potential methylation rate constant rate could not be calculated are due to that when the preparation of the tracer was done, an assumption on how much Hg^{198} would be needed was done. As mention, Skogaryd is an area that have not suffered from contaminations do not having any industries located near. This makes the Hg concentrations low, and the concentrations was expected to be higher in the soil then they actually were. The concentration of the tracer would have needed to be higher for the bacteria in the soil to start reacting to it and for a signal in the GC-ICP-MS could have been received. If the soil had been analysed for Hg_{tot} concentrations before the incubation experiment, better knowledge of the amount of Hg^{198} needed would have been possible.

To improve the result of the Hg_{tot} and organic matter content replicates from each sampling point should have been done. As the situation are now were only one is done, the sample analysed could be unrepresentative for the sample collected at the site. There was however no time to make more replicates, which would have made the result more reliable. With the result it still can be clearly shown that the highest concentrations of the Hg_{tot} are in the 0 -10 cm layer.

There are TOC data missing due to problem with the instrument, the last analysis has not been able to be performed and the analyse for November has not been done as the instrument was not running later in that month. Result from an early analyse in November was used instead of the samples taken alongside with Hg_{tot} and MeHg samples, and from this time there was no measurement at station 14.

During the preparation for the analysis of the incubation study, too much of $Me^{201}Hg$ internal standard was added in many of the T_{24} vials, leading to not having enough standard for all the samples. As pairs of T_{24} and T_0 are needed to calculate the potential methylation and demethylation rate constants, the sets that not had standard added to them was used with accurate amount of standard. The other samples could have been used if there would have been more standard and the pair would have had the same amount of standard.

To improve the result for the soil analyses a glove-box should have been used instead of only the head-flushing to exclude the presents of oxygen completely from the samples. This was not possible as the department of Earth Science at the University of Gothenburg due not own one and the incubations needed to be performed with fresh samples that had not been frozen or kept outside of its normal environment for a longer time.

5. Conclusion

Due to the weather the clear-cut could not be performed in the study area, leading to that the wanted changes could not be followed. However, due to a flooding event in February the third hypothesis could be confirmed as the MeHg decreased as the oxygen level of water was higher at the time and the Hg_{tot} increased. The pH and oxygen level of the water showed to have influence on the demethylation of the MeHg. With lower pH and higher oxygen level, the demethylation would increase in the water, giving higher concentrations of Hg_{tot} and lower MeHg.

The demethylation rate constant varies in the area and the different depth, though no specific trends in the three subsites of the area could be seen. The Hg_{tot} of the soil indicate that the highest concentrations are in the 0 – 10 cm depth in the whole area, while the 20 – 30 cm has lower Hg_{tot} but higher content of organic matter.

Further measurements in the area should the continued to improve the reliability of the data and also to see seasonal variations. Studies of other properties should also be included for both the water and soil analyses to give a bigger understanding for how the relationships affect the methylation and demethylation.

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8. Appendix

A.1. Water analysis data

Table 2. The data from the water stations during the months studied.

	Station	8	14	13	10	4
Coordinates WGS84	**x**	58.374181	58.37197	58.369263	58.365286	58.357554
	y	12.15061	12.14738	12.145003	12.141097	12.132919
Hg (ng/L)	**Nov**		5.2	5.3	5.2	5.6
	Dec	4.4	6.2	5.8	6	6.5
	Jan	3.9	7.5	4.4	4.3	3.8
	Feb	7.3	6.7	6.8	7.1	6.8
	Mar	5.3	5.8	5.9	6.4	6.2
MeHg (ng/L)	**Nov**	-	0.25	0.25	< 0,06	0.20
	Dec	0.19	0.22	0.2	0.2	0.19
	Jan	0.34	0.21	0.13	0.25	0.19
	Feb	0.14	0.14	0.14	0.12	0.15
	Mar	0.17	< 0,06	0.16	0.16	0.11
TOC (mg/L)	**Nov**	21.01	-	22.42	22.87	22.01
	Dec	22.19	23.21	23.53	25.83	24.23
	Jan	23.06	27.24	25.3	25.04	21.1
	Feb	22.97	23.33	24.53	24.41	24.13
Dissolved oxygen	**Nov**	-	6.96	5.31	6.59	8.54
(mg/L)	**Dec**	-	9.26	9.25	9.5	10.24
	Jan	8.13	8.81	9.47	9.99	12.03
	Feb	10.41	10.95	10.68	10.84	11.27
	Mar	10.55	10.49	10.47	10.47	10.94
pH	**Nov**	5.83	7.07	5.93	5.95	5.97
	Dec	6.5	6.28	6.1	5.83	5.68
	Jan	5.85	5.97	6.04	6.01	6.38
	Feb	5.34	5.15	5.43	5.06	5.12
	Mar	5.45	5.93	6.1	5.56	5.63

Table 3. Pearson's correlation for, Hg_{tot}, MeHg, pH, dissolved oxygen (DO) and total organic carbon (TOC).

	Hg 8	Hg 14	Hg 13	Hg 10	Hg 4	MeHg 8	MeHg 14	MeHg 13	MeHg 10	MeHg 4	TOC 8	TOC 14	TOC 13	TOC 10	TOC 4	DO 8	DO 14	DO 13	DO 10	DO 4	pH 8	pH 14	pH 13	pH 10	pH 4
Hg 8	1.00																								
Hg 14	0.82	1.00																							
Hg 13	-0.54	-0.26	1.00																						
Hg 10	-0.52	-0.25	0.99	1.00																					
Hg 4	-0.80	-0.49	0.94	0.93	1.00																				
MeHg 8	-0.76	-0.35	0.25	0.29	0.54	1.00																			
MeHg 14	-0.25	0.08	-0.42	-0.55	-0.33	0.27	1.00																		
MeHg 13	-0.76	-0.84	-0.06	-0.13	0.22	0.42	0.46	1.00																	
MeHg 10	0.54	0.79	-0.29	-0.20	-0.36	0.13	-0.17	-0.73	1.00																
MeHg 4	-0.11	0.08	-0.53	-0.65	-0.42	0.24	0.99	0.47	-0.11	1.00															
TOC 8	0.66	0.95	0.09	0.14	-0.17	-0.27	-0.75	-0.99	0.79	-0.63	1.00														
TOC 14	-0.59	-0.05	0.76	0.83	0.74	0.96	-0.30	0.07	0.35	-0.52	0.11	1.00													
TOC 13	0.83	0.99	-0.15	-0.10	-0.42	-0.43	-0.61	-0.98	0.80	-0.47	0.97	-0.11	1.00												
TOC 10	0.12	0.62	0.00	0.08	0.02	0.51	-0.26	-0.53	0.90	-0.14	0.65	0.72	0.56	1.00											

	pH 4	pH 10	pH 13	pH 14	pH 8	DO 4	DO 10	DO 13	DO 14	DO 8	TOC 4
Hg 8	0.49	0.11	-0.03	-0.39	-0.41	0.76	0.42	0.36	0.15	0.42	-0.67
Hg 14	0.20	-0.08	-0.17	-0.63	-0.03	0.89	0.65	0.60	0.37	0.85	-0.18
Hg 13	-1.00	-0.90	-0.65	-0.53	-0.37	-0.06	0.37	0.40	0.66	-0.21	0.89
Hg 10	-0.99	-0.89	-0.55	-0.56	-0.39	0.02	0.45	0.49	0.73	-0.09	0.91
Hg 4	-0.92	-0.69	-0.40	-0.25	-0.10	-0.30	0.16	0.22	0.47	-0.24	0.94
MeHg 8	-0.23	0.13	0.42	0.24	0.83	-0.33	-0.06	0.01	0.02	0.23	0.64
MeHg 14	0.42	0.52	-0.01	0.50	0.62	-0.38	-0.62	-0.65	-0.73	-0.98	-0.50
MeHg 13	0.11	0.43	0.26	0.87	0.43	-0.98	-0.90	-0.87	-0.74	-0.84	0.02
MeHg 10	0.25	0.12	0.34	-0.47	0.26	0.83	0.71	0.70	0.44	0.99	-0.04
MeHg 4	0.54	0.64	0.12	0.57	0.67	-0.37	-0.64	-0.66	-0.78	-1.00	-0.57
TOC 8	-0.15	-0.42	-0.31	-0.89	-0.29	0.98	0.96	0.94	0.81	0.93	0.10
TOC 14	-0.80	-0.94	0.79	-0.27	1.00	0.01	0.40	0.48	0.67	0.48	0.95
TOC 13	0.09	-0.24	-0.19	-0.77	-0.30	0.99	0.85	0.82	0.63	0.82	-0.16
TOC 10	-0.03	-0.01	0.30	-0.44	0.52	0.63	0.71	0.74	0.55	0.95	0.36
TOC 4	-0.88	-0.67	-0.42	-0.42	0.12	-0.06	0.38	0.44	0.64	0.18	1.00
DO 8	0.15	-0.14	0.92	-0.97	0.48	0.88	1.00	1.00	0.97	1.00	
DO 14	-0.69	-0.81	-0.39	-0.92	-0.45	0.68	0.94	0.95	1.00		
DO 13	-0.44	-0.60	-0.20	-0.90	-0.30	0.85	1.00	1.00			
DO 10	-0.41	-0.60	-0.24	-0.92	-0.33	0.89	1.00				
DO 4	0.00	-0.29	-0.12	-0.80	-0.28	1.00					
pH 8	0.38	0.65	0.57	0.51	1.00						
pH 14	0.57	0.80	0.59	1.00							
pH 13	0.67	0.80	1.00								
pH 10	0.92	1.00									
pH 4	1.00										

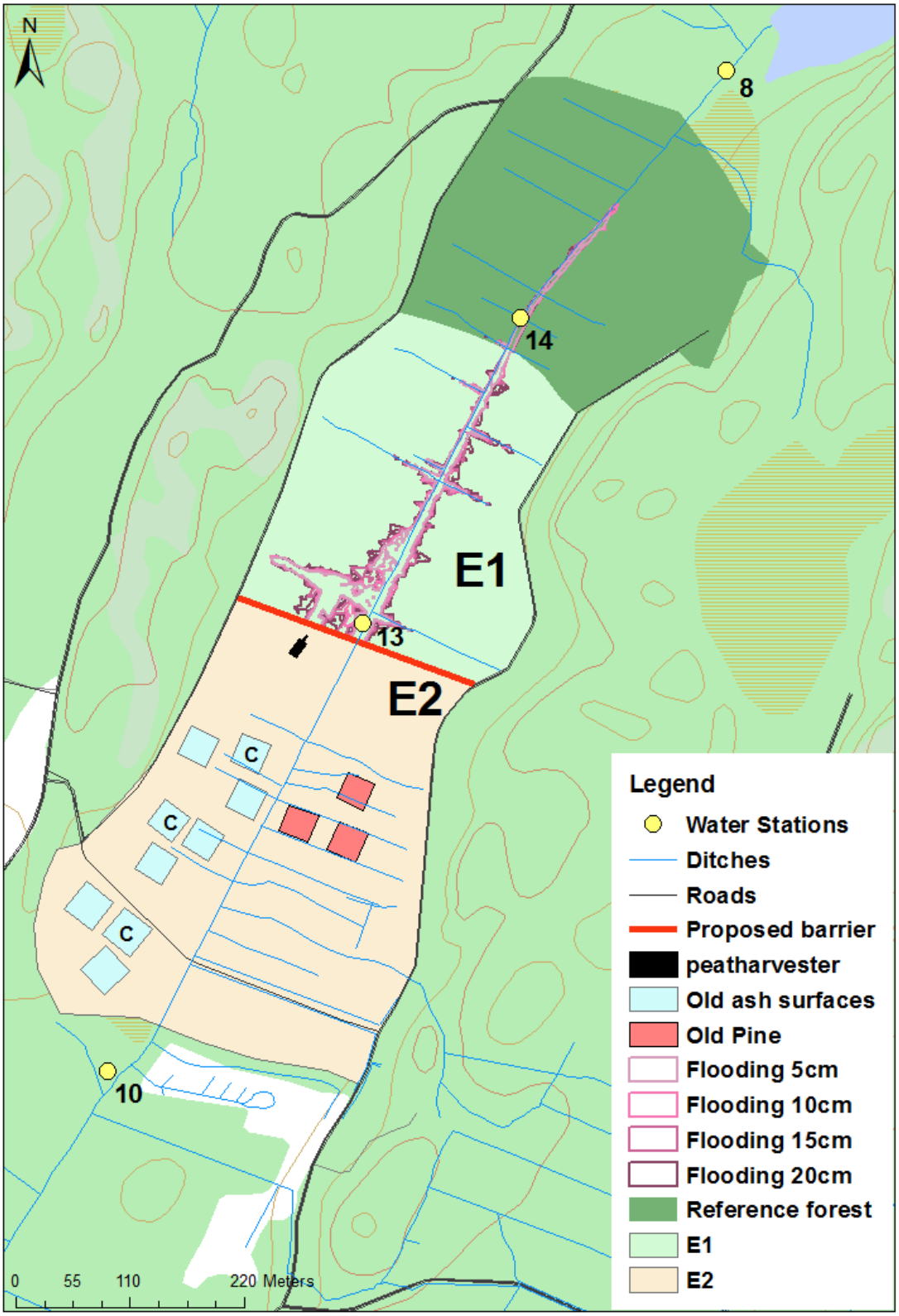

Figure 10. Flooding scenarios for 5, 10, 15 and 20 cm flooding events after the barrier are in place.

A.2. Soil analysis methods

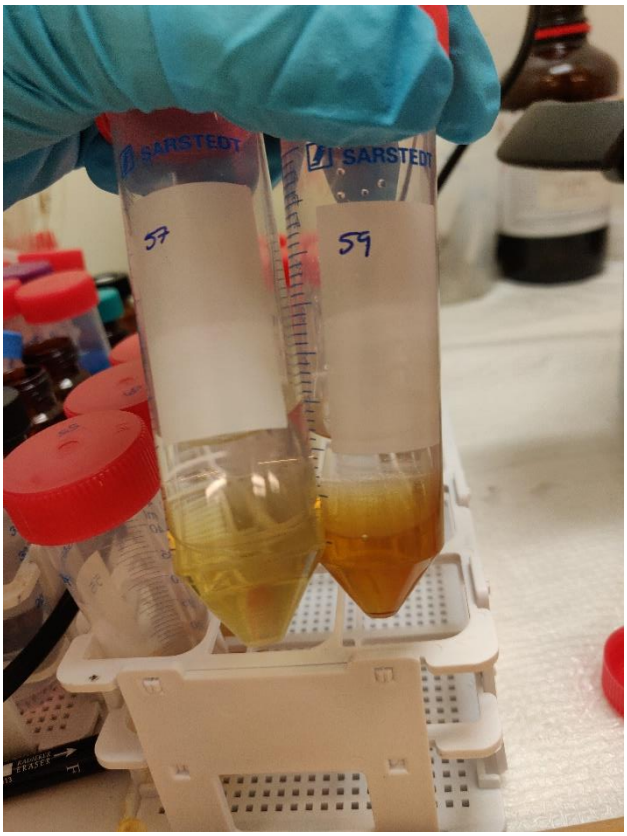

Figure 11. The samples before the DCM are removed. After the DCM are removed the samples becomes clear, without a direct colour.

Figure 12. The DCM being evaporated from the soil, leaving the

A.3. Soil analysis data

Table 4. The data from the soil analysis

	Coordinates (WGS84)		Hg_{tot} (µg/kg)		Organic content (%)		k_d (day-1)	
Sampling point	**x**	**y**	**0-10 cm**	**20-30 cm**	**0-10 cm**	**20-30 cm**	**0-10 cm**	**20-30 cm**
11	58.37224	12.14617	415.0	97.8	94.7	93.9	0.057	-
12	58.37223	12.1468	336.6	127.5	95.2	93.8	-	-
13	58.37204	12.14738	319.0	189.5	70.2	83.1	-	-
14	58.37178	12.14815	249.3	111.3	71.2	93.3	-	-
15	58.3716	12.14866	368.7	97.9	94.9	96.3	-	-
26	58.36995	12.14419	324.3	162.6	78.3	80.7	-	-
27	58.36995	12.1447	303.9	299.9	78.5	81.0	-	-
28	58.36976	12.14533	242.7	148.3	78.1	80.8	-	-
29	58.36935	12.14588	266.1	137.9	84.7	92.0	-	0.062
30	58.36921	12.14635	313.4	162.5	88.1	92.3	-	-
31	58.36862	12.14282	265.7	142.9	75.9	68.6	-	0.095
32	58.36843	12.14336	275.5	233.1	81.6	80.6	-	0.065
33	58.36823	12.14395	256.1	121.7	87.2	89.0	0.048	-
34	58.36799	12.14466	238.5	73.5	92.3	98.0	0.088	0.099
35	58.36788	12.14581	255.5	74.3	88.3	96.2	0.050	0.034

A.4. Elevation illustration

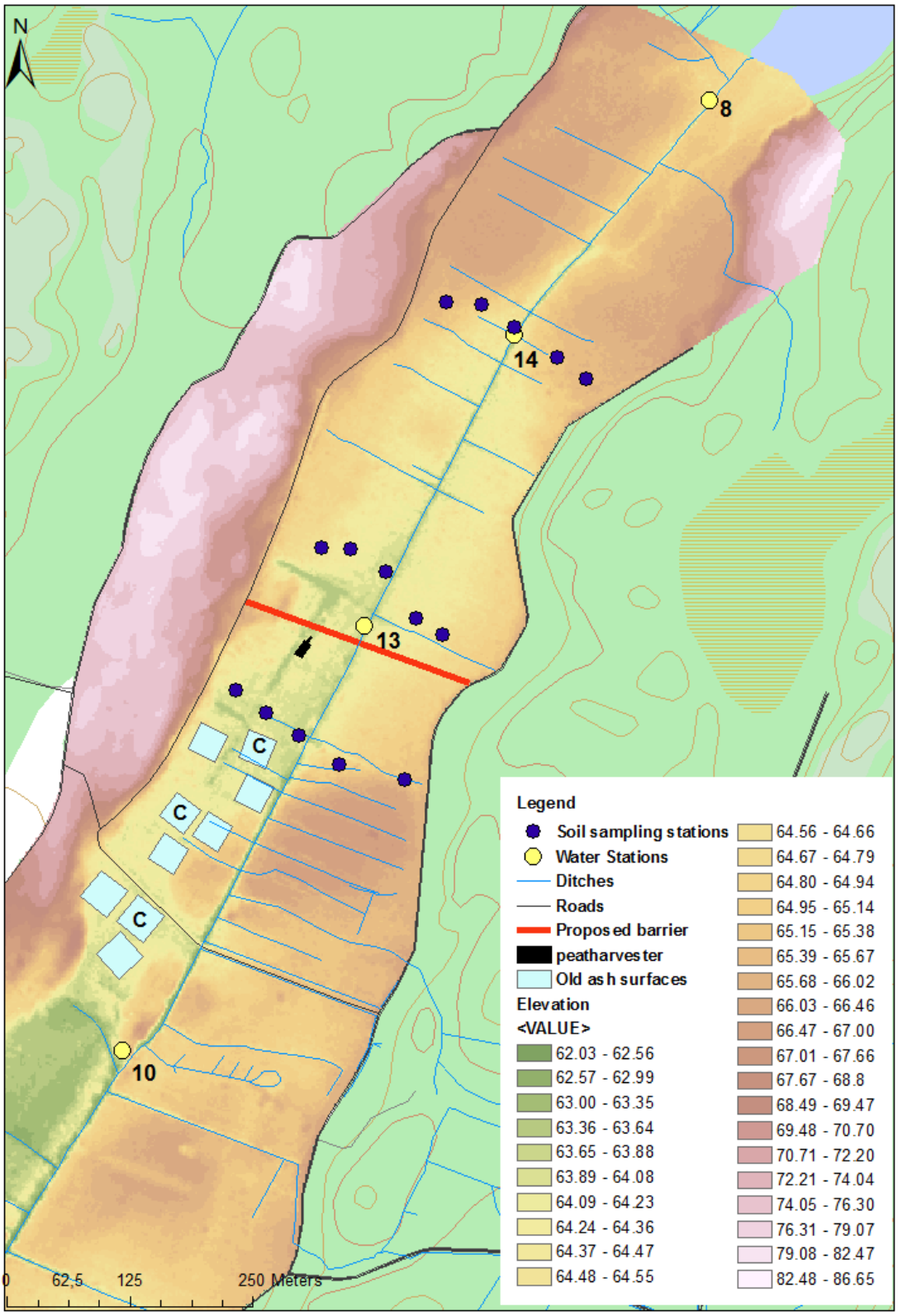

Figure 13. The elevation of the study site.