Thesis for the Master's degree in chemistry

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Determination of MEA-nitramine in Soil Water and Assessing the Sorption Potential of MEA-nitramine to Soil

60 study points

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Abstract

Small amounts of amines are emitted with the flue gas from amine based carbon capture plants. The amines atmospheric degradation have been studied in detail. The environmental impact of the degradation products, e.g. the possibly carcinogenic and mutagenic nitramines, is however insufficiently studied. This thesis investigates the capacity of nitramines to sorb to soil, depending on the soils physiochemical characteristics. To accomplish this, the analytical challenges with regards to determination of nitramines in soil water had to be solved.

Soil samples were collected in the vicinity of Technology Centre Mongstad (TCM) ($\sim$ 15 km) as this is a possible future deposition site for nitramines. The sampling sites were chosen with the aim of collecting soils with different physiochemical properties, with special emphasis on the content of soil organic matter and soil texture. All of the collected soil samples (n=16) were analysed for explanatory parameters expected to influence soil sorption, such as organic matter (OM) content, dissolved natural organic matter (DNOM) leached from the soil, texture, mineralogy, pH and conductivity. A selection of the samples (n=5) were used in soil sorption experiments to assess the sorption potential of N-Nitroethanolamine (MEA-nitramine). A batch experimental set-up was used, and sorption was measured as loss of MEA-nitramine from the aqueous phase after being added at known concentrations (24 h equilibrium). For these samples, elemental composition was also determined. Possible correlations between the sorption coefficients and the soils physiochemical characteristics were assessed. Determination of MEA-nitramine in soil water with LC-MS/MS proved to be a challenge due to matrix effects. Different calibration methods with and without matrix matched calibration solutions were tested. Another nitramine, N-nitromethylamine (MMA-nitramine), was tested as internal standard. Additionally, a couple of sample pretreatment techniques were tested to try and separate the analyte from the matrix (solid phase extraction (SPE), filtration).

Sorption partitioning coefficients between soil and aqueous phase were determined (K_d) and the results imply that a significant amount of MEA-nitramine will remain in the aqueous phase. Correlation between the partition coefficients for the five studied soils and the soils OM content was observed ($r > 0.788$). Loss of internal standard (MMA-nitramine) was also observed, this loss correlated strongly with the DNOM concentration in the sample supernatant ($r = 0.9924$), implying that sorption to DNOM could be important. The relation between sorption and OM content implies that sorption will generally be higher in the top soil (organic) horizons than in the lower (mineral) soil horizons. As deposition of atmospherically formed nitramines will be to the soil surface, this can serve to hinder mobility.

The analytical determination method for nitramines in soil water was improved, although not considered satisfactory at the end of the study. Major improvement resulted from the used of matrix matched calibration curves, which compensate for both ion suppression/enhancement effects and possible sorption to DNOM. Even though this is time- and labour consuming, the method was proven more successful than attempts made to separate the analyte from the matrix prior to analysis. The used of MMA-nitramine as an internal standard for determination of MEA-nitramine was not successful, possibly due to the difference in retention time and therefore possible different effect from the matrix components. An isotope-labelled internal standard should therefore be acquired. Investigation into other sample pretreatments could also be of interest. Note that previously reported sorption partition coefficients for sorption of nitramines to soil could be inaccurate if matrix matched calibration curves were not employed.

List of Abbreviations and Definitions

AcA	Acetic acid
CCS	Carbon dioxide capture and storage
CID	Collision-induced dissociation
CEC	Cation exchange capacity
CID	Collision-induced dissociation
cLOQ	Concentration limit of quantification
DDL	Diffuse Double Layer
DC	Direct current
DDL	Diffuse double layer
DOC	Dissolved organic carbon
DMA-nitramine	N-Nitrodimethylamine
DNOM	Dissolved natural organic matter
EDXRF	Energy dispersive X-ray fluorescence
ESI	Electrospray ionization
GC	Gas chromatography
h	Hour(s)
H-ESI	Heated electrospray ionization
LC	Liquid chromatography
LC-MS	Liquid chromatography - mass spectrometry
LC-MS/MS	Liquid chromatography - tandem mass spectrometry
IS	Internal standard
LD_{50}	Lethal dose for 50 % of the population
LLE	Liquid-liquid extraction
MEA	2-aminoethanol
MEA-nitramine	N-Nitroethanolamine
MMA-nitramine	N-Nitromethylamine
MeOH	Methanol
MP	Mobile phase
MS	Mass spectrometry
m/z	Mass-to-charge ratio
NDMA	N-nitrosodimethylamine
OM	Organic matter
PCC	Post combustion CO_2 capture
PES	Polyethersulfone
PSD	Particle size distribution
PZC	Point of zero charge

RC	Regenerated cellulose
RF	Radio frequency
RMS	Root-mean-squared
RP	Reversed phase (chromatography)
rpm	Rotations per minute
RSD	Relative standard deviation
S/N	Signal-to-noise
SP	Stationary phase
SPE	Solid phase extraction
SRM	Selected reaction monitoring
STD	Standard deviation
TCM	Technology Centre Mongstad
UV	Ultraviolet
Q1q2Q3	Triple quadrupole
WDXRF	Wavelength dispersive X-ray fluorescence
XRD	X-ray diffraction
XRF	X-ray fluorescence

List of Definitions Employed in the Sorption Studies

Blank	Blank sample containing soil water, without added MEA-nitramine.
Control	Solution made in the same manner as the samples, without soil, but with added MEA-nitramine.
Soil water based calibration solution	Matrix matched calibration solution.
Spiked soil blank	MEA-nitramine (same concentration as added to the samples) spiked blank sample.
Water based calibration solution	Calibration solution with only water, $CaCl_2$, NaN_3 and MEA-nitramine.

Contents

1 Introduction

1.1 Global Warming and Future Energy Demands

Climate change due to anthropogenic emissions of greenhouse gases is one of the largest challenges faced by the global community. Carbon dioxide (CO_2) is the most important of the anthropogenic greenhouse gases [1]. About 90 % of the anthropogenic greenhouse gas emissions are related to energy production, of which fossil fuel combustion accounts for 80 % [2].

Improving living standards and industrial expansion in developing countries will continue to increases the energy demand. Initially this demand will mainly be met by combustion of fossil fuels releasing increased amounts of CO_2 into the atmosphere [3, 2]. The CO_2 equivalent concentration[1] in the atmosphere was estimated to be 430 ppm in 2011 [5]. Without abatement strategies to reduce greenhouse gas emissions CO_2-equivalent concentrations are expected to reach between 750 and 1300 ppm by 2100 [5]. This change in climate is estimated to result in a higher average global temperature constituting an increase of 3.7 - 4.8 ℃ compared to preindustrial levels [5]. In general terms climate change is expected to cause changes in the quality and quantity of water resources, due to changes in precipitation levels and patterns, and increased snow and ice melt [6]. Shifts in the geographic range, seasonal activities, migration patterns, and/or abundance of terrestrial, freshwater and marine species is also expected, as is changes in crop yield and increased occurrences of extreme weather [6].

Abatement strategies in order to reduce greenhouse gas emissions are thus urgently needed in order to stabilize and curb the concentrations of atmospheric greenhouse gases. Abatement strategies that reduce CO_2 emissions from fossil fuel power plants, refineries and other large-scale industrial sources are required since a continued increase of CO_2 emissions from fossil fuels appears inevitable in order to supply the worlds increasing demand for energy. Carbon dioxide capture and storage (CCS) is one of these strategies. In the mitigation scenarios assessed by the intergovernmental panel on climate change (IPCC) CCS is considered essential in reducing the greenhouse gas emissions needed for achieving the low atmospheric concentration level scenarios; 450 ppm CO_2-eq. concentration in 2100 [5]. Large scale implementation of CCS is required for it to become an significant contribution to mitigating climate change in the future [5].

[1] "CO_2-equivalent concentration: The concentration of CO_2 that would cause the same radiative forcing as a given mixture of CO_2 and other forcing components" [4].

1.2 CO_2 Capture and Formation of Nitramines

CCS *"is a process consisting of the separation of CO_2 from industrial and energy-related sources, transport to a storage location and long-term isolation from the atmosphere"* [3]. Issues pertaining to the transport and storage of CO_2 are beyond the scope of this thesis and will not be discussed further.

Different CO_2 capture technologies are available. Among them Post-Combustion Capture (PCC) has the advantage that it can be retrofitted on existing CO_2 combustion sources and is a mature, commercially available technology [3]. Several separation techniques are available for PCC [7, 8]. Chemical absorption through the use of a solvent is well suited for industrial flue gasses and was the first technology to be used commercially [8, 9].

A full scale commercial PCC plants is already in operation by SaskPower at a coal-fired power station at Boundary Dam, Canada. Several other commercial PCC plants are under construction or planned all over the world [9]. In Norway a large testing facility, Technology Centre Mongstad (TCM), has been in operation since May 2012, and a post-combustion CCS plant is planned on Svalbard [10, 11].

At TCM two different liquid chemical solvents, amine and ammonia, are tested [10]. The environmental consequences of the use of amine based chemical absorption PCC is the basis for this thesis. 2-aminoethanol (MEA) is the most studied amine solvent. MEA forms a carbamate complex with CO_2 in the absorber column, the complex proceeds to a desorber column where the reaction is reversed by high temperature (Figure 1).

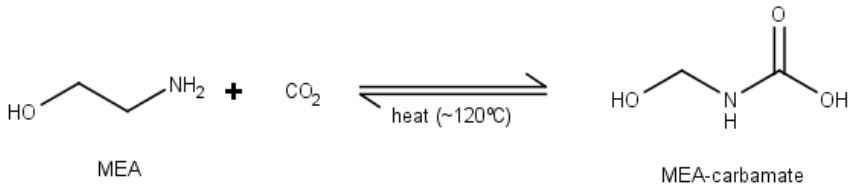

Figure 1 – MEA absorption/desorption of CO_2. Made based on [12].

The process of liquid solvent based PCC technology is illustrated in Figure 2. As this figure illustrates the liquid amine goes through a regeneration step. However, some solvent and alkane-amines will inevitably be emitted with the cleaned flue gas [13]. Escaped amines rapidly degrade through atmospheric photo-oxidation. Characteristic removal time through reaction with OH or NO_3 radicals is 6 hours

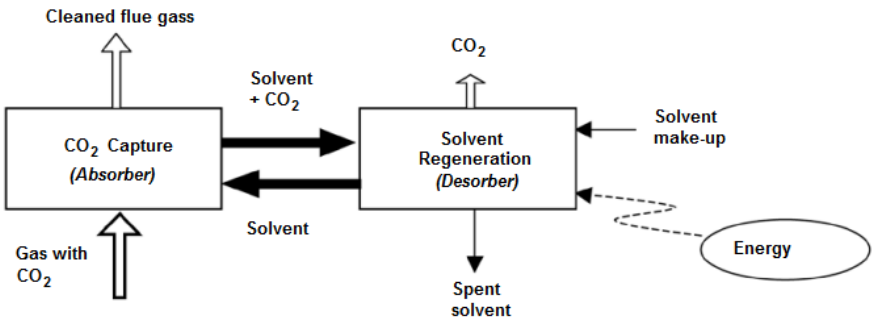

Figure 2 – Illustration of the liquid solvent based PCC process. Modified from IPCC [7].

[14]. Degradation products include the potentially carcinogenic nitramines. One of the degradation products of MEA is the nitramine N-Nitroethanolamine (MEA-nitramine) [15].

1.2.1 Emissions from Technology Centre Mongstad

The dispersion of the atmospheric emissions from the amine plant at TCM has been modelled [16]. A clear pathway of dispersion in air to the north and south-east is seen, with a general reduction in concentrations with increased distance from TCM (Figure 3, left). For wet-deposition the largest concentrations of amines and their degradation products are expected east of TCM (Figure 3, right). The average rain water concentration of nitramines are expected in the ng/l range in the worst case scenario calculations [16].

A baseline study conducted for TCM reports that nitramines, if present in the environment close to Mongstad, are below detection limits (ng/L, ng/g ranges) [17].

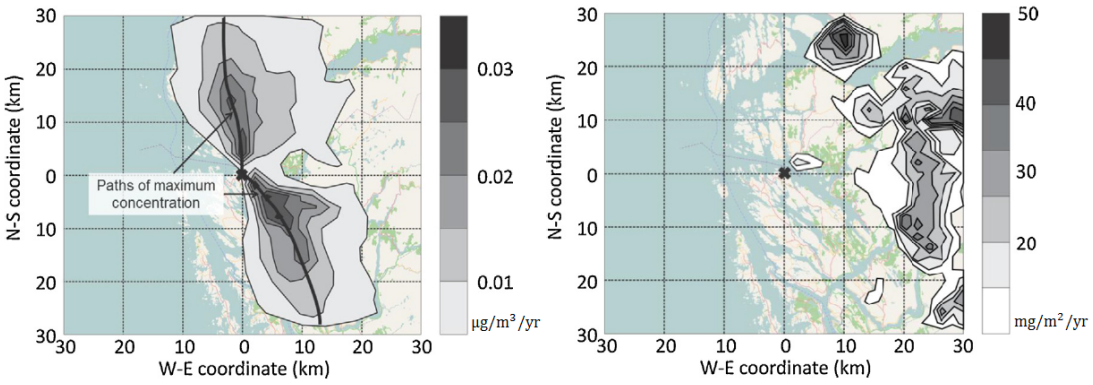

Figure 3 – Dispersion in air (left) and wet deposition (right) of emissions from TCM. The cross indicates the position of TCM. Modified from [16].

1.3 Environmental Fate of Nitramines

Large scale implementation is needed for CCS to be an important contribution to mitigate climate change. This entail that the total emissions of amines will increase world wide. Deposition of the atmospherically formed nitramines will largely be to landmass or sea water, rather than directly to fresh water lakes. Since deposition to sea water does not give a direct pathway to human exposure it has not been the focus of any studies related to TCM [18], and the consequences of deposition of nitramines to sea water is beyond the scope of this study. If nitramines sorb to soil they can accumulate in the soil column, causing possible toxic, mutagenic and carcinogenic impacts on soil organisms, plants and animals, including humans. On the other hand, if they do not sorb to the soil they can be transported to groundwater and eventually to freshwater lakes in labile and bioavailable forms and thus have possible toxic and mutagenic effects on aquatic plants and organisms, as well as become an issue for drinking water quality.

The magnitude of sorption of nitramines to soil depends on the physical and chemical properties of both the nitramines and the soil.

1.4 Objective

The overall goal of this thesis has been to asses the soil physiochemical characteristics that govern the ability of soil to sorb nitramines. The soil samples were collected close to TCM, as this will be a future deposition site for nitramines. Early on it became apparent that accurate determination of N-Nitroethanolamine (MEA-nitramine) in soil water was a huge challenge. Hence the focus of this thesis shifted towards acquiring an accurate liquid chromatography tandem mass spectrometry (LC-MS/MS) method for determination of MEA-nitramine concentrations in soil water.

2 Theory

2.1 Previous Research on Sorption of Nitramines to Soil

A pilot assessment study on sorption of nitramines to soil was performed by Mohr and Vogt at the University of Oslo in 2012 [19]. This study showed that two nitramines, MEA-nitramine and N-Nitrodimethylamine (DMA-nitramine), were more sorbed to organic rich soils (organic matter (OM) content > 75 %) than to mineral soils [19]. The sorption of the nitramines was examined after 24, 48, 96 and 192 hours [19]. For MEA-nitramine no temporal trends in sorption was observed. This implies that equilibrium between the nitramine in aqueous phase and soil was achieved within the first 24 hours [19]. For DMA-nitramine the sorption rate seemed considerably slower, indicated by the fact that stability was not achieved within the studied time frame [19]. It was therefore not possible to establish a constant distribution coefficient for DMA-nitramine [19].

At the 2^{nd} Post Combustion Capture Conference in Bergen in 2013, Sørensen et al. from SINTEF held a presentation on the environmental fate of nitramines and nitrosamines [20]. This presentation included a section on soil adsorption, no adsorption for MEA- and DMA-nitramine was reported to four different soils. An important note here is that their studied soils were inorganic with organic carbon contents less than 7 % [20]. These results thus comply with the pilot sorption study by Mohr and Vogt [19] addressed above. To the authors knowledge no other published material is available on the topic of sorption of nitramines to soil in the open literature.

2.2 Analytical Theory

2.2.1 LC

Liquid chromatography (LC) is a chromatographic technique for separating compounds in solution based on their partition between a stationary phase (SP) and a mobile phase (MP) [21]. The separation used in this thesis is based on the hydrophobicity of the analytes. The most common separation principle is reversed phase (RP) chromatography due to its superiority regarding chromatographic efficiency and achievement of good chromatographic separation for a variety of mixtures [21]. In RP LC the SP is more hydrophobic than the MP. The SP is commonly aliphatic carbon chains, for example C_{18} or C_8, covalently bound to the surface of the silica particles packed in the column. The MP is a polar solvent, often consisting of a mixture of two different liquids so the elution strength can be adjusted according to the analyte of interest. One of the liquids is most often water, and

the pH is adjusted with buffers or acids.

2.2.2 MS

A triple quadrupole mass spectrometer (MS) with heated electrospray ionization (H-ESI) was used in this thesis. MS detectors provide both quantitative and qualitative information on the analytes. Specific molecules or molecule fragments are distinguished based on their mass-to-charge ratios (m/z). MS detectors are the most powerful detectors for chromatography [21].

In standard ESI the MP carrying the analyte from the LC column enters a nebulizer steel capillary along with a coaxial nebulizing gas flow (N_2) [21]. The coaxial N_2 gas flow and a strong electric field at the nebulizer outlet creates a charged particle aerosol [21]. With the instrumental set-up used in this thesis the nebulizer is held at a potential of $\sim$ 3500 V, while the spray chamber has a potential of around 0 V in positive mode, for detection of positively charged ions [21]. For detection of negative ions a negative voltages is used at the nebulizer, generally with a lower potential ($\sim$ -2500 V) [21].

The charged particle aerosol forms a Taylor cone and then breaks into a spray of fine droplets [21]. The fine droplets breaks apart and finally evaporate leaving the ions in gas phase. Different theories exist on how this occurs. Two of the theories are illustrated in Figure 4. Ion evaporation theory applies for ions with relatively low m/z, while charge residue theory is believed dominating for ions with very high m/z [22]. The difference in these two theories occur in the last step. In ion evaporation theory the ions are desorbed from the droplet surface, while in charge residue theory the solvent is completely evaporated [22].

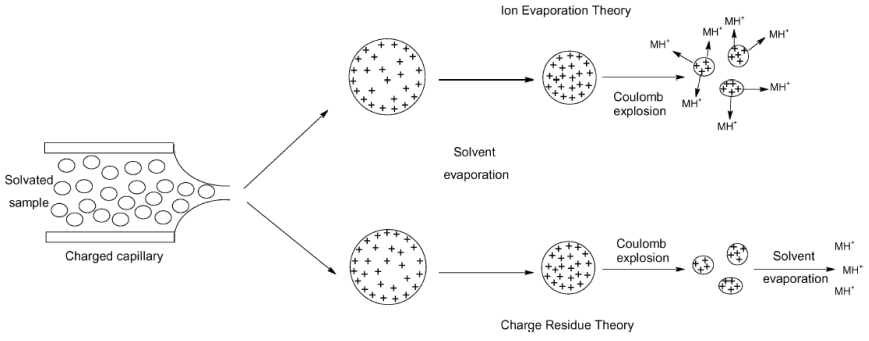

Figure 4 – Illustration of two theories describing ion formation with ESI [22].

A (representative) fraction of the ions in gas phase enters the first quadrupole of the MS. A single quadrupole consists of four parallel electrical rods producing an electric field between them [22, 23]. A radio frequency (RF) voltage is used to create the electric field and confine the ions to stable trajectories, either trapping them within the quadruple or allowing them to pass [23]. While a direct current (DC) potential is superimposed on the field, at a given RF voltage, to define the range of m/z values that have stable trajectories [23]. The RF and DC potentials of same phase or polarity are applied to opposing rods, making adjacent rods 180 degrees out of phase and of opposite polarity [23].

Using three quadrupoles (triple quadrupole - Q1q2Q3, Q = quardupole mass analyser, q = quadrupole radio frequency collision cell) coupled together makes tandem MS (MS/MS) possible. A schematic presentation of a triple quadrupole is shown in Figure 5.

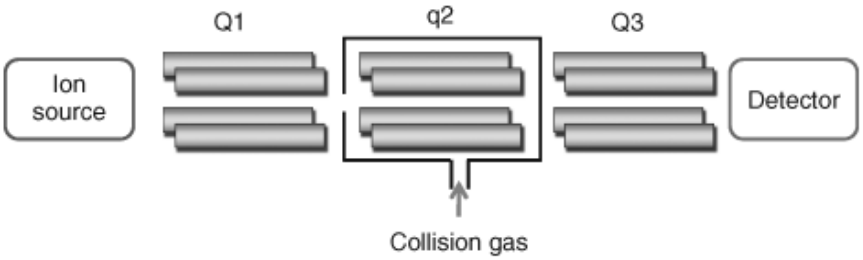

Figure 5 – Schematic of a triple quadrupole mass spectrometer [23].

The MS/MS technique utilized in this thesis was selected reaction monitoring (SRM) which is highly selective for the analyte(s) of interest [21]. The first quadrupole (Q1) is used as a mass filter allowing one or several chosen *precursor* ions (m/z ratios) to pass, these *precursor* ions can be subjected to a reaction in the second quadrupole (q2), often referred to as a collision cell [23, 21]. q2 is used in RF-only mode and therefore acts as a high pass filter allowing all ions with m/z ratio greater than the low mass cut-off[2] to pass [23]. The third quadrupole (Q3) filters the fragments and allows selected *product* ions to reach the detector [23, 22]. Several types of reactions can be achieved in the collision cell (q2). Most

[2]The low mass cut-off is given by the Mathieu parameters (derived from the Mathieu second order differential equations describing the motion of ions). When RF-only mode is used the amplitude of the DC voltage is 0, and the Mathieu parameter $q_u = \frac{4eV}{m\Omega^2 r_0^2} = 0.908$, where e is the charge of ion, Ω is the frequency of the RF voltage, V is the amplitude of the RF voltage, r_0 is the radius of the rods inscribed circle and m is the mass of the ion. In other words ions with m/z ratios that have q_u values lower than 0.908 are allowed to pass [23].

commonly used is the collision-induced dissociation (CID) (sometimes called collision activated dissociation, CAD). In CID reactions the precursor ion collides with a non-reactive collision gas inducing a dissociation (fragmentation) of the precursor ion. The main advantage of using SRM is that it provides the best possible sensitivity for a target analyte in mixture [23].

2.2.3 Determination of Selected Nitramines with LC-MS/MS

The few available publications regarding analytical methods for qualitative and quantitative determination of aliphatic nitramines were recently reviewed by Lindahl et al. [24]. The separation methods used for MEA-nitramine are LC based with different sample pretreatments. In most of the methods the studied sample matrix was water, lab-scale PCC samples, wash water lab-scale PCC samples or standards [24]. Pretreatments include solid phase extraction (SPE) with and without pH adjustment, filtration, quenching with ascorbic acid, liquid-liquid extraction (LLE), as well as no sample pretreatment [24]. Detection is mainly by MS, but ultraviolet (UV) detection at 254 nm is also used [24]. Nitramines are known to absorb UV light around 230 nm [25]. Both atmospheric chemical ionization (APCI) and electrospray ionization (ESI) ion sources are used. Concentration limit of quantification (cLOQ) is mainly reported to be in the µg/L range [24].

In the pilot sorption study by Mohr and Vogt [19], the decanted soil water samples containing either MEA-nitramine or N-Nitrodimethylamine (DMA-nitramine) were filtered through a 0.2 µm pore size membrane filter before analysis, in addition DMA-nitramine was extracted from solution with dichloromethane [19]. MEA-nitramine was analysed on LC-MS, while DMA-nitramine was analysed on gas chromatography (GC)-MS [19]. Matrix matched soil water based calibration curves were not employed (C. W. Mohr, pers. comm.). The analyses were performed at the National Environment Research Institute (NERI), Denmark, but no further method information is available [19, 24].

Different sample pretreatments have been discussed in the literature. Extraction of the nitramine analyte from the sample matrix by SPE (activated carbon sorbent) was found to be the preferable pretreatment step for determination of water soluble nitramines in water wash samples in a study by Dye et al. [26] at NILU. However a later study, with some of the contributing authors in the study bye Dye et al., report that the extraction of nitramines from solution by SPE was tested and rejected due to breakthrough in the SPE column and subsequent loss of sample [27]. Preconcentration of the samples by evaporation was found to be possible for MEA-nitramine (b.p. 266 ℃) in samples where deuturated internal standard was added [27].

2.2.4 Matrix Effect in LC-MS Measurements

The matrix of a sample can influence the ionization of analytes in ESI MS. The term *matrix effects* is in analytical chemistry defined as the combined effect on measured quantity from all other compounds in the sample than analyte [28]. If a specific compound is identified as causing an effect it is called an interference [28]. When matrix components co-elute with the analyte(s) and suppress or enhance the signal of the analyte, what is termed ion suppression or ion enhancement occur. The interfering compounds can also be from compounds added to the samples during sample preparation and/or reagents added to the MP. The matrix effects are compound specific. Ion suppression can lead to higher limit of detection, lower signal-to-noise (S/N) ratio, underestimation of analyte concentration (not detected due to ion suppression), overestimation of analyte concentration (if ion suppression affect the internal standard more than the analyte), bad precision and/or bad linear response between concentration and signal [29].

Theories on how ion suppression occur include competition between co-eluting matrix components and analyte for available charge, competition for access to droplet surface for solvent evaporation (through increased droplet viscosity and/or surface tension), as well as co-precipitation of analytes (particularly for small polar analytes) [29]. Ionic compounds (e.g salts), polar compounds and organic molecules are all potential ion suppressors [30, 29]. If a matrix component has high concentration, high mass, is basic and co-elutes with the analyte the potential for induced ion suppression is higher [29]. A recent review concludes that ion suppression/enhancement is influenced by the properties of the analyte, the matrix, the matrix/analyte concentration ratio, chromatographic conditions, ionization conditions and MS instrumentation [30]. In other words; the causes of ion suppression/enhancement are complex.

Suggestions for reducing ion suppression/enhancement include switching ion source from ESI to APCI, as ion suppression is generally a smaller problem in APCI as the analyte(s) are already in gas phase before ionization occurs [30, 29]. Using negative ionization mode, as negative mode is more selective and therefore associated with lower matrix effects [30, 29]. As well as improving LC separation and testing different sample pretreatments to reduce the presence of interfering compounds e.g. SPE and/or LLE [30, 29]. Internal standard(s), sample dilution, matrix matched calibration curves, and/or standard addition can be used to compensate for the matrix effects if elimination is not possible [30, 29].

2.3 Nitramine Properties

Nitramines are organic compounds with the generic structural formula RR'NNO_2. Nitramines have two distinct functional groups, an amine group (R_3N:) and a nitro group (-NO_2) bound to the amine group, as well one or two variable groups. Amines are derivatives of ammonia (NH_3), wherein one or more hydrogen atom have been replaced by a substituent such as an alkyl or aryl groups. The main nitramines discussed throughout the thesis are the primary nitramines MMA- and MEA-nitramine. Their structures are shown in Figure 6. As MEA is the most common amine solvent, MEA-nitramine was chosen to be the focus of this thesis.

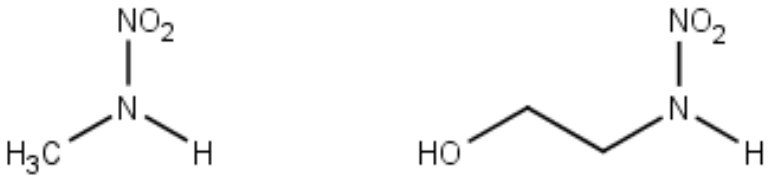

Figure 6 – Structures of MMA-nitramine (left), and MEA-nitramine (rigth).

Atmospheric formation of MEA-nitramine occurs when a hydrogen is abstracted from the amino group of MEA, followed by reaction with NO_2 (Equation 1 and 2) [15]. When MEA is photo-oxidized it is predicted that MEA-nitramine is formed in 0.3-1 % of the reactions in urban regions and 0.005-0.3 % of reactions in rural areas, as its formation is dependent on local NO_2 concentrations [14].

$$NH_2CH_2CH_2OH + {}^{\bullet}OH \rightarrow {}^{\bullet}NHCH_2CH_2OH + H_2O \quad (1)$$

$$ {}^{\bullet}NHCH_2CH_2OH + {}^{\bullet}NO_2 \rightarrow O_2NNHCH_2CH_2OH \quad (2)$$

The mutagenicity and carcinogenicity of nitramines is an insufficiently studied subject. Nitramines are structurally close to nitrosamines (RR'NNO) which are known potent carcinogens [31], though they are not considered persistent in the environment due to rapid photolysis [14]. Investigations into the potential mutagenicity and carcinogenicity of nitramines have therefore been of interest. Several of the nitramines are proven mutagenic and carcinogenic in rodents or bacterial assays [32, 31, 26], and they are therefore treated as suspected human mutagens and carcinogens.

The toxicity of selected nitramines[3] was tested in a large study by Dye et al. [26]. Depending on the type of toxicity (acute oral, cytotoxicity, skin irritation, skin corrosion or eye corrosion) the outcome varied between the different nitramines tested. Most were classified as harmful if swallowed (LD_{50} for rats: 200 - 20000 mg/kg body weight), mildly cytotoxic[4] at concentration around 550 µg/mL, not irritating or corrosive to skin, and mild to severe eye irritants [26].

The Norwegian Institute of Public Health (NIPH) based their nitramines risk estimate on the much more studied nitrosamine; N-nitrosodimethylamine (NDMA). They state that the amount of nitramines and nitrosamines should not exceed 0.3 ng/m^3 in air or 4 ng/L in drinking water [31]. As NDMA is expected to be more potent than any nitramine this is considered a conservative estimate [31].

Reported, theoretical, pK_a values for MEA- and MMA-nitramine are 6.24 $\pm$ 0.1 and 6.51 $\pm$ 0.1, respectively [24], which is slightly higher than the expected soil pH in the collected soil, thus indicating that MEA- and MMA-nitramine are weakly acidic compound and mainly uncharged in soil solution. To the authors knowledge the only measured[5] pK_a values available in the literature for these nitramines is a reported pK_a of 6.2 for MMA-nitramine [33]. Nitramines are polar because of their non-linear structure and the difference in electronegativity between the central N and C or H atoms. Along with their small size this renders them rather soluble in water. Nitramines are thus conceptually considered hydrophilic molecules due to their small size and dipolar property.

In the environment nitramines are found to be persistent: no significant hydrolysis occur for primary and secondary nitramines [34], and a low photo-degradation potential is measured [35]. Moreover, five studied nitramines[3] were classified as not "ready biodegradable" according to OECD Guidelines 301, though large differences were reported between nitramines with or without a hydroxyl group [26]. MEA-nitramine was found to be more biodegradable than most other nitramines with a reported biodegradation of 34 % after 28 days [26]. A newer study on the same nitramines, as well as N-nitrodiethylamine, reported similar trends with negligible biodegradation for nitramines without hydroxyl groups, while nitramines with hydroxyl groups, like MEA-nitramine, had half-lives of about 1 month in water at 20 ℃, and 2 months at 5 ℃, regardless of tested concentration [27]. As the hydroxyl group seems to be subject to faster biodegradation than the amine/nitramine

[3]N-nitromehylamine, N-nitroethanolamine, N-nitrodimethylamine, N-nitropipeazine and 2-methyl-2-nitroamino-1-propanol [26].

[4]Toxic to cells.

[5]The article does not explicitly state that the values are measured, but due to the fact the article is published in 1980, it is assumed that they are [33].

group, biodegradation to a more persistent nitramine is a possibility. Even though some of the nitramines will biodegrade in water with time, in general nitramines can be considered persistent in the environment.

Bioaccumulation is defined as the net accumulation of a chemical in an organisms compared to the concentration in the environment surrounding it [36]. It can be approximated by the octanol-water partition coefficient, K_{OW}, with octanol being used as a proxy for adipose tissue (lipophilicity/hydrophobcity). Large coefficients correspond to high bioaccumulation potential. A compound is defined as bioaccumulative if log K_{OW} is larger than 5 [37]. Dye et al. [26] calculated log K_{OW}, based on structure-activity relationship (SAR), to be in the range -0.52 to -1.51 for the tested nitramines[3]. While More and Vogt [19] reported values for the closely related log partition coefficient between organic carbon and water, log K_{OC}, calculated based on K_d values, to be in the range 1.5-1.8 for MEA-nitramine. In other words, nitramines are not expected to bioaccumulate.

2.4 Sorption Theory

2.4.1 Soil Properties

Soil is operationally defined as particles smaller than 2 mm [38]. The proportions of sand (0.06 - 2.0 mm), silt (0.002 - 0.06 mm) and clay (< 0.002 mm) constituting the soil, are used to define the soil texture (Figure 7).

Soil texture is mainly governed by the manner in which the unconsolidated soil material was deposited. Most of the soil material in Norway is poorly sorted moraine till deposited by the glacial erosion [40, 41]. Below the marine limit (i.e. where the unconsolidated deposits in the catchment were under sea level during the last ice age) this material was redistributed as the shoreline moved through the landscape with the land heaving. Hill tops were washed clean and left as rock outcrops, slopes were left with thin sandy soils, while the valley bottoms mainly have thick clayey marine deposits [41]. Depending on the climate and topography an organic layer has evolved. On the dry hilltops a thin humic cover directly on the bedrock is common, while in well drained soils, typically found on the slopes, only a shallow forest floor O-horizon is usually found. In moist or water saturated parts of the watershed, such as the valley bottom, a thick layer of organic matter (OM) would have typically accumulated constituting a peat soil.

Soil is distinguished into generic horizontal layers. The assemblage of horizontal layers, called soil horizons, constitutes a soil profile and defines the soil type. Soil

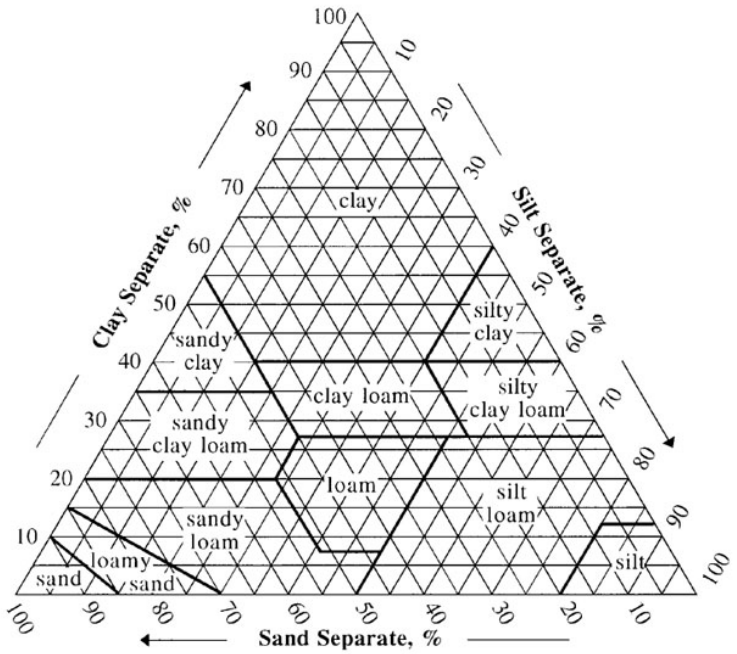

Figure 7 – Triangle for determination of soil texture. Retrieved from [39].

properties have large spatial variation governed by differences in primary material, manner in which the unconsolidated material was deposited, climate, vegetation as well as history and so on.

Podzols (or spodosols) represent a typical forest soil profile in regions devoid of carbonate minerals where dissolved natural organic matter (DNOM) redistributes the iron and aluminium in the soil profile, they are generally associated with regions covered by ice in the Pleistocene Epoch (Ice Age), which includes most of Norway [42, 41]. An illustration of a podzol soil profile is provided in Figure 8.

The top layer of a podzol soil profile is an organic (O) horizon containing plant material in various stages of decomposition [1]. The decomposition processes in the O-horizon releases DNOM and CO_2 into the soil solution [1]. The DNOM contains many weak organic acid functional groups and the CO_2 hydrolyse to carbonic acid (H_2CO_3). Complexation of polyvalent ions by the DNOM combined with the protolysis of the weak organic acids serves to acidify the soil, and the soil water that drains through the O-horizon [1]. The second layer is a black A layer, which is a mix of well decomposed organic material and mineral soil. Below this layer an eluvial (E) horizon is often seen. This layer is bleached from the leaching of iron

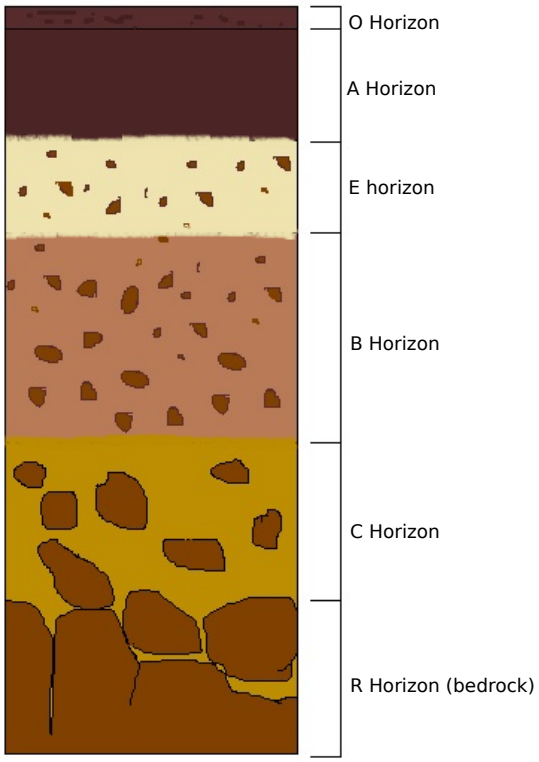

Figure 8 – Illustration of a podzol soil profile. Made based on descriptions from [1, 41].

and aluminium that occurs due to complexation with DNOM in the acidic water [1]. Most of the leached DNOM and minerals will accumulate in the lower illuival horizon, called a B horizon, due to e.g. higher pH and adsorption capacity [1]. Below the B horizon(s) is a horizon consisting of relatively unaltered subsoil referred to as the C horizon, reaching down to the bedrock (R horizon) [1]. Organic soils comprised of a deep H horizon, is found in bogs and peats forming what is termed a histosol soil profile consisting of high amounts of organic material in a thick dark upper layer [41]. The soils content of aluminium and iron oxy-hydroxides is mainly governed by the mineralogy of the primary material. Within a podzol soil profile the highest content of these sesquioxides[6] are found in the B-horizon and the lowest in the E- and O-horizon [1]. Large pools of iron sulphides (e.g. pyrite: FeS_2) are typically found in peats, especially in bogs developed from lakes [1].

Soil is a three-phase mixture of solid particles with pore space filled with air and/or

[6]The prefix *sesqui* means one and a half, i.e. there is 1.5 oxygen for each metal in iron(III) and aluminium(III) oxide [1].

water [1]. The redox potential in soil is governed by water saturation. Oxygen is the primary oxidizing agent for decomposition of organic matter, and will therefore become depleted in the soil if not replenished [1]. In dry soil the oxygen is replenished by diffusion of oxygen through the air filled pore space. In soil saturated with water, diffusion of oxygen is a much slower process and oxygen depletion leads to a decline in redox potential (pE). Soils with a high water table, like peat and bog, therefore generally have reducing conditions.

2.4.2 Sorption to Soil

Sorption to soil is defined as the retention of a compound by the soil. Sorption of compounds to soil occurs through a variety of mechanisms depending on the physiochemistry of the compound, water matrix and the soil. The amount of organic matter (humus) in the soil can be a key parameter influencing sorption to soil, and will vary in the different soil horizons. Humic material can sorb compounds due to its acidic character, negative charge and ability to function as a complexing agent [1]. DNOM is also known to form metal complexes with e.g. iron and aluminium and thus sorb negatively charged compounds [1]. The amount of clay minerals in the soil can also play a role as their small size (diameter: 10^{-5} - 10^{-8} m) give a very large specific surface areas that generally has high sorption capacity due to a net negative surface charge caused by isomorphic substitutions within the crystal lattice [43]. The amount and nature of the clay minerals will depend on the elemental composition and mineralogy of the primary soil materials. Positively charged polyvalent ions, like iron and aluminium ions, can also be important due to their ability to form metal ion bridges between the negatively charged clay or humate sites and negatively charged compounds [1]. The surface of the clay particles can also be coated with humic material through covalent interactions with clay surface metals (Al^{3+}, Fe^{3+}) or metals in the water (Ca^{2+}, Al^{3+}), thus increasing the sorption capacity of the humic material.

In addition there are pH dependent charges due to the protonated or deprotonated weak acid functional groups (e.g. oxide or hydroxy groups) on the humic material and on the clay mineral surface [43]. Such pH dependent charge is especially associated with colloidal iron and aluminium oxy-hydroxy minerals [1], which can also be found in the clay fraction. The net clay charge will thus depend on the soil properties and on the solution pH. The pH at which the solids have no net charge (pH_0) is referred to as the point of zero charge (PZC) [1]. The expected soil properties and acidic soil pH of the soils studied in this thesis would render the clay with a net negative charge, as is the case for most soils in the world. This net negative charge is balanced by a diffuse layer of cations i.e. diffuse double layer

(DDL) [43]. This generates a cation exchange capacity (CEC). The contribution of the clay minerals to the CEC of the soil depend on the mineral structure, the structural substitutions and on the water accessible surface area [43].

The ability of a compound to sorb to a given soil is normally reported as its distribution coefficient between soil and water, K_d, or with a sorption isotherm. K_d is the slope of the isotherm for a given concentration, or for a concentration area if a linear relationship exist. A sorption isotherm displays a compounds relationship between the concentration in soil and solution at a fixed temperature. The Freundlich or Langmuir isotherms are normally used to describe this relationship. The Langmuir relationship describes sorption as a solute occupying a fixed number of equivalent sorption sites on colloid surfaces (monolayer sorption) [1], while the Freundlich relationship describes multilayer sorption with infinitely increasing sorption as the concentration of solute increase [1]. Normally this relationship is therefore only applicable for small molecules at low concentrations. Sorption can also be reported as the percentage sorbed to soil of an added known concentration of analyte [44].

2.4.3 Sorption of MEA-Nitramine to Soil

The key parameters governing the sorption of nitramines to soil are conceptually considered to be soil texture, organic matter content, iron and aluminium oxide content, as well as the soils pH and redox potential. These parameters differ between soil horizons and soil types. The soils capacity to sorb nitramines is therefore also expected to exhibit substantial spatial variation.

Physical sorption of nitramines to soil through cation-exchange due to a net negative charge of the soil is likely not an important mechanism considering that nitramines are mainly uncharged or negatively charged molecules at the expected acidic pH of the studied soils. Still clay minerals and iron and aluminium sesquioxides in the soil provide surface for adsorption and are thus likely to be important explanatory factors for the soils capacity to sorb nitramines. Moreover, the organic nature of the nitramines render them susceptible to be sorbed to the organic matter in soil. Hydrogen bonding between e.g. the hydroxy (-OH) and carbonyl (-C=O) functional groups on humic material and the amine (-NH) and nitro ($-NO_2$) groups on the nitramines constitute a possible sorption mechanism [1]. Coating of OM on the clay mineral colloids causes the large surface area of the clay fraction to enhance sorption to organic matter [1].

The effect of the pH of the soil and its associated water may affect sorption of nitramines. However, this is not straight forward to asses conceptually. The ex-

pected pH in the studied soil is acidic. If the point of zero charge (PZC) in the main mineral constituents of the soil is below the soils pH, the soil will retain a net negative charge generating a net CEC. At the expected low pH range (< 6) the nitramines will mainly be uncharged, but their polarity means they can physically adsorb to the soil through e.g. van der Waals interactions. Based on the pK_a value for MEA-nitramine, approximately 35 % will be negatively charged at pH 6, while the rest will be uncharged. Compounds with a negative charge have been found to be adsorbed to soils through metal ion bridges (ligand exchange/salt bridges) constituted by polyvalent cations like Al^{3+} and Fe^{3+} bound to negative charged sorption sites on the soils [1]. Sorption to alkaline soil is thus expected to be greater than sorption to acidic soil, though as stated approximately 35 % of MEA-nitramines will be negatively charged at pH 6. In addition to pH the explanatory factors in the soils associated water are ionic strength and the concentration of DNOM. Increased ionic strength causes a decreased repulsion between nitramines and reactive surfaces and compounds in solution. This serves to increase sorption.

If nitramines sorb to humic material, the results of the sorption experiments should show a higher degree of sorption to the organic rich soil in the H-, O- and A-horizons. If, on the other hand, the nitramines form complexes with iron and aluminium, higher sorption should be expected in the B-horizons. As DNOM is expected to sorb nitramines it may act as a confounding factor, as organic rich soil horizons also generally generate high concentrations of DNOM in the associated soil water. If DNOM sorbs nitramines this enhances their solubility, and thus total concentration in solution.

2.4.4 Calculation of Sorption

The sorption capacity of MEA-nitramine at a given concentration for a specific soil is represented by the partition coefficient between soil and aqueous phase, K_d (L/kg), at equilibrium (Equation 3).

$$K_d = \frac{C_s\ (mg/kg)}{C_{aq}\ (mg/L)} \tag{3}$$

Where C_s is the amount (mg) of MEA-nitramine sorbed per kilo (kg) soil, and C_{aq} the concentration in aqueous phase (mg/L). C_s is calculated indirectly as the difference between the concentration of added MEA-nitramine and the concentration remaining in aqueous phase.

Sorption can also be calculated using the formula for adsorption from OECD guidelines 106 for testing of chemicals [44]. The word adsorption have been exchanged with sorption, as the specific sorption mechanisms are unknown. The modified equation is presented in Equation 4 [44].

Sorption, S, in % (w/w) is given by:

$$S = \frac{m_s^{ads}}{m_0} \times 100 \tag{4}$$

Where m_0 is the mass of test substance (mg) at the beginning of the test, defined as:

$$m_0 = C_0 \times V_0 \tag{5}$$

Where C_0 is the initial mass concentration of the substance in contact with the soil (mg/dm^3) and V_0 the initial volume of test solution in contact with the soil (dm^3).

m_s^{ads} is the mass (mg) of test substance at the time the analysis is performed, defined as:

$$m_s^{ads} = m_0 - C_{aq}^{ads} \times V_0 \tag{6}$$

Where C_{aq}^{ads} is the mass concentration of the substance in aqueous phase at the end of the test (mg/dm^3).

Note that this formula does not take the liquid to soil ratio into account. Direct sample comparison of S values can therefore only be applied to samples with the same liquid to soil ratio.

The relationship between K_d and S is shown in Equation 7.

$$K_d = \frac{S}{100 - S} \times \frac{V_0}{m_{soil}} \tag{7}$$

Where m_{soil} is the mass of the dry soil (kg).

3 Materials and Methods

3.1 General Reagents and Equipment

Deionized water was used for all experiments. Type II water (10-15 MΩ cm at 25 ℃, Purelabs Option-R, Elga-veolia water, Paris, France) was used to rinse all equipment after washing and for dilution of standards and reagents. The exception is for dissolved organic carbon (DOC) analysis, in which Type I water (18.2 MΩ cm at 25 ℃, Milli-Q Integral s Water Purification System, Merck Millipore, Billerica, MA, US) was used.

Automatic pipettes were used for most experiments. The pipettes were calibrated according to their respective manuals and regularly controlled.

The experiments were carried out by the author in the laboratories at the Department of Chemistry, University of Oslo, unless otherwise specified.

3.2 Nitramines

Nitramine standards of $\sim$ 99 % purity were synthesized by Prof. Y. Stenstrøm, Norwegian University of Life Sciences (NMBU, Norway). Standard solutions were prepared in Type II water. The MEA-nitramine standard came as its precursor, 3-nitro-oxazolidin-2-one, which was hydrolyzed for 24 hours prior to use (Figure 9).

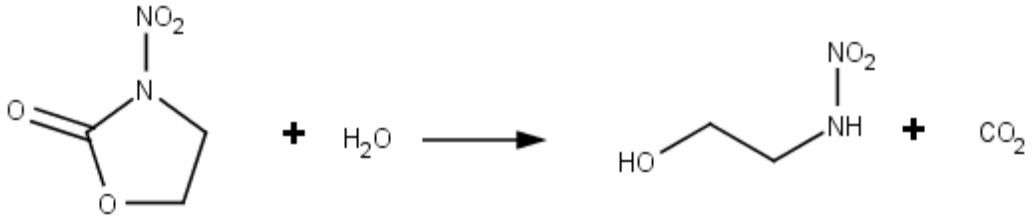

Figure 9 – Hydrolyzation of MEA-nitramine's precursor.

The CAS numbers for MEA- and MMA-nitramine are provided in Appendix A.

3.3 Sampling Site Description

The soil samples studied in this thesis were collected in *Vyrkesdalen* and *Vyrkesdalsdalen*, Gulen, Sogn og Fjordane, Norway. The sample site was chosen within 30 km of TCM, as this is were (future) deposition of nitramines is expected, see

Chapter 1.2.1. The sample site is approximately 15 km in linear distance from TCM. The specific catchments and the sampling plots therein were selected on the basis of a quaternary geological map of the area, aiming to capture the span in especially soil organic matter content and texture (Figure 10). 16 soil samples were collected from 5 locations in this catchment. The locations constitute three different types of unconsolidated material: thin moraine (tM), thick moraine (TM) and peat and bog (P). The samples are labelled with type of material and horizon, e.g. tM_1-O is the sample collected from the O-horizon at thin moraine sample Site 1. Site designation and their locations are shown in Figure 10. GPS coordinates and sample site details are provided in Appendix B.

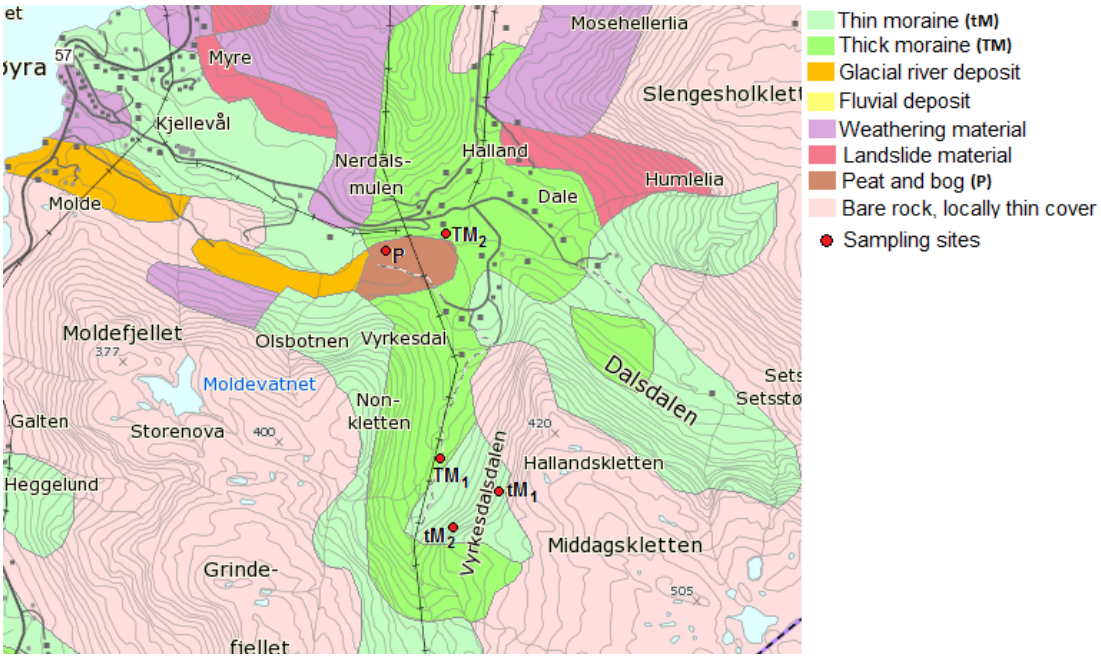

Figure 10 – Quaternary geological map of the sample site area [45]. Sample plots are indicated by red dots and sample site designation.

3.4 Physiochemical Analysis

3.4.1 Sample Pretreatment

The soil samples were air dried and sieved through a 2 mm sieve, according to ISO 11464:2006 [38]. The air dried soil samples were stored in paper boxes at room temperature, in the dark.

3.4.2 Soil Dry Matter Content

The moisture content in the soil must be determined in order to obtain correct values for the dry mass of the soil. This information is needed for determination of e.g. OM content % (w/w). Dry matter content was determined gravimetrically for each soil sample according to ISO 11465:1993 [46]. This was done by drying the soil at 105 ℃ for 6 hours in a TS-80 drying oven (Termaks, Bergen, Norway). The percentage weight difference before and after analysis was calculated.

3.4.3 Soil Organic Matter Content

A proxy for soil organic matter content was determined gravimetrically by loss on ignition (LOI) according to Krogstad [47]. The samples were ignited in a N11 muffle furnace (Nabertherm, Lilienthal, Germany) for 3 hours at 550 ℃. The percentage weight difference before and after ignition was calculated.

3.4.4 Dissolved Natural Organic Matter Concentration

Dissolved organic carbon (DOC) and UV absorbance are used as a proxies for DNOM. The samples were prepared according to NS-EN 1484:1197 [48] and analysed on a TOC-V_{CPH} total organic carbon (TOC) analyzer with an ASI-V auto sampler (Shimadzu, Kyoto, Japan). DOC is defined as organic compounds smaller than 0.45 µm and the samples were therefore filtered through a 0.45 µm membrane filter, see Appendix C.3 for details. Samples contain both organic and inorganic carbon. The instrument therefore removes inorganic carbon by acidifying the samples to pH 2-3 by addition of 1.5 % (v/v) 2M HCl, and then purging the acidified sample with carbon free synthetic air. The remaining non-purgeable organic carbon is measured as CO_2 using an nondispersive infrared sensor (NDIR) detector after all organic matter in the injected sample is oxidized by high temperature combustion. UV absorbance at 254 and 400 nm was measured with an UV-1800 UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan) in the samples prior to DOC analysis so an approximate concentration range could be determined. Details are provided in Appendix C.3.

3.4.5 pH

The pH in soil and supernatant solution was determined with an Orion Research Expandable Ion Analyser EA 920 with a ROSS pH-electrode (Thermo Scientific, Waltham, MA, USA). The pH-electrode was calibrated with pH 4 and pH 7 buffer solutions (AVS TITRINORM, VWR, Radnor, Pa, USA).

The soil pH was determined in a 1+5 (v/v) soil:water suspension after 1 hour shaking at a compact shaker KS-15 table (Edmund Bühler GmbH, Hechingen, Germany) according to ISO 10390:2005 [49].

3.4.6 Conductivity and Temperature

Conductivity and temperature were measured in the supernatant with a Mettler-Toledo FG3 (Greifensee, Switzerland) portable conductivity meter according to ISO 7888:1985 [50]. The conductivity meter was calibrated with a 84 µS/cm conductivity standard (Mettler-Toledo, Greifensee, Switzerland). The conductivity meter automatically corrects the measurement to the reference temperature of 25 ℃.

3.4.7 Particle Size Distribution

Particle size distribution (PSD) is a method for determining the different particle size fractions in a soil sample. Analyses were performed by Eurofins Agro Testing Norway on the mineral soil samples by a combination of dry sieving, wet sieving and sedimentation according to ISO 11277:2009 [51]. Before analyses organic matter is destructed and salts and gypsum ($CaSO_4 \cdot 2H_2O$) are removed if present in significant amounts (electrical conductivity > 0.4 dS/m), as is iron oxides and carbonates if deemed necessary [51]. The report summary from Eurofins is presented in Appendix C.4. Sample tM_2-Bh was not analysed since insufficient sample mass was available.

3.4.8 Mineralogy

X-ray diffraction (XRD) was performed on the samples to determine their crystalline mineralogy. Determination occurs through analysing the diffraction pattern of incident X-rays on a sample. X-rays are diffracted by the planes of atoms as the X-ray wavelengths are similar to atom spacing in crystals and bond-lengths in molecules [52]. The distance between crystalline planes, d-spacing, is distinctive for a a crystalline material. Bragg's law is used to determine the d-spacing for a chosen wavelength and diffraction angel. Instrumentation and determination principles behind XRD are explained in detail in Appendix C.5, as this analysis methods is quite new to the Environmental analysis group.

The soil samples were analysed on a D8 Discover powder diffractometer (Bruker AXS, Karlsruhe, Germany). The soil sample (< 2 mm) was placed in a sample holder and smoothed gently to make the soil surface level with the holder. The soil was then covered with clear plastic to hold it in place. The samples were scanned over an angular range of 2-70 degrees during measurement with Cu Kα radiation

(1.54060 Å). The sample holder was spinning during measurement to reduce the effect of non-randomly oriented particles. EVA version 3.1 and TOPAS version 4.2.0.2 were used to identify and quantify the minerals present in the samples based on the diffractograms.

3.4.9 Elemental Composition

X-ray fluorescence (XRF) was performed on the samples to determine their elemental composition. The sample absorbs high energy X-rays producing excited ions, transition to ground state via fluorescence is measured. A wavelength dispersive XRF system (WDXRF) was employed to look for specific emission lines for the elements of interest. The wavelengths of the peaks are element specific while their net intensities correspond to the concentration of the respective element. Standards with similar matrix were used for calibration. Instrumentation and determination principles behind XRF are explained in detail in Appendix C.6, as this analysis methods is quite new to the Environmental analysis group.

The samples were prepared in steps, resulting in a glass sample called a bead [53]. The samples were ground to a fine powder with a mortar and then heated on a stepwise temperature program and baked for one hour at 1050 ℃ to remove all organic matter. This last step causes oxidation of the sample and loss of volatile elements. The cooled samples were mixed with a flux in a 1+10 ratio, and melted to glass beads in a XRF furnace fusion system. The large flux-to-sample ratio ensures a similar matrix for samples and standards. Melting to sample beads also has the advantage of making the samples homogeneous. The flux is a low atomic number compound with low absorption in the employed X-ray wavelength range. The sample preparation details are given in Appendix C.6. The beads were analysed on an AXIOS^{mAX}-MINERALS (PANalytical, Almelo, Netherlands) WDXRF system with a scintillation counter and an Ar-CH_4 gas flow detector (proportional counter), at the Department of Geosciences, University of Oslo. The scintillation counters measure elements with lower wavelengths (Cu to U), while the proportional detector is used for the elements with emission lines with longer wavelengths (Be to Cu) [53].

Due to baking in the oven loss of organic matter and volatile elements like S, Hg and Cd occur [53]. Determination of this loss is done gravimetrically by weighing the sample before and after burning, as in LOI. The samples were analysed for 21 different elements common in soil. The results were background corrected (to give a net intensity), corrected for overlapping emission lines, matrix corrected (depending on the amount of other elements in the sample) and corrected for drift in the instrument. These corrections were done automatically by the computer soft-

ware; SuperQ version 5 (PANalytical, Almelo, Netherlands), that both controls the instrument and processes the measurements. Correcting the elemental composition, % (w/w), with regards to $LOI_{1050\ °C}$ was done manually. The uncertainty of the reported results (presence of oxidized elements in % (w/w)), before correction with regards to $LOI_{1050\ °C}$, depends on many factors, especially the root-mean-squared (RMS) error of the individual calibration lines for the elements analysed. For most elements, this RMS error is in the order of 0.01-0.03 % (w/w) (heavier elements) , whereas for lighter elements the RMS error can be up to 0.2-0.3 % (w/w), depending on the standards used (Pers. comm., M. Aerts, Department of Geosciences, University of Oslo).

3.5 Sorption Experiment

3.5.1 Method

The experimental design for the sorption experiments was adapted from ISO 21268-1:2007 [54] and ISO 21268-2:2007 [55]; Soil quality - Leaching procedures for subsequent chemical and ecotoxicological testing of soil and soil materials - Part 1(2): Batch test using a liquid to soil ratio of 2 L/kg (10 L/kg) dry matter. Part 1 is for materials with dry matter content ratio higher than 33 % while Part 2 is for materials with lower dry matter content. The procedures in Part 1 and 2 differ only in their liquid to soil ratios used during extraction: 2 L/kg is used in Part 1 and 10 L/kg in Part 2.

In this study Part 1 was used for mineral soils (LOI $<$ 30 % (w/w)) and Part 2 for organic soils (LOI $>$ 30 % (w/w)). This was decided both to achieve good sensitivity as sorption to organic soils is expected to be higher than to mineral soil, and for practical reasons in order to manage to fit the organic soil and solution into the 100 mL bottles (Borosilicate glass, VWR, Radnor, PA, USA) used in the experiments. These small bottles were used due to equipment restrictions (bottle size that fit the end-over-end shaker) and limited amount of available soil sample. The caps of the bottles were lined with polytetrafluoroethylene (PTFE) cap-liners (VWR, Radnor, PA, USA) to prevent adsorption to the plastic (polypropylen) caps. The experimental design is outlined in Figure 11, and deviates from ISO 21268-1/2:2007 [54, 55] in that MEA-nitramine was added in known concentration to the aqueous phase, and amount lost from solution was determined, rather than amount leached from the soil.

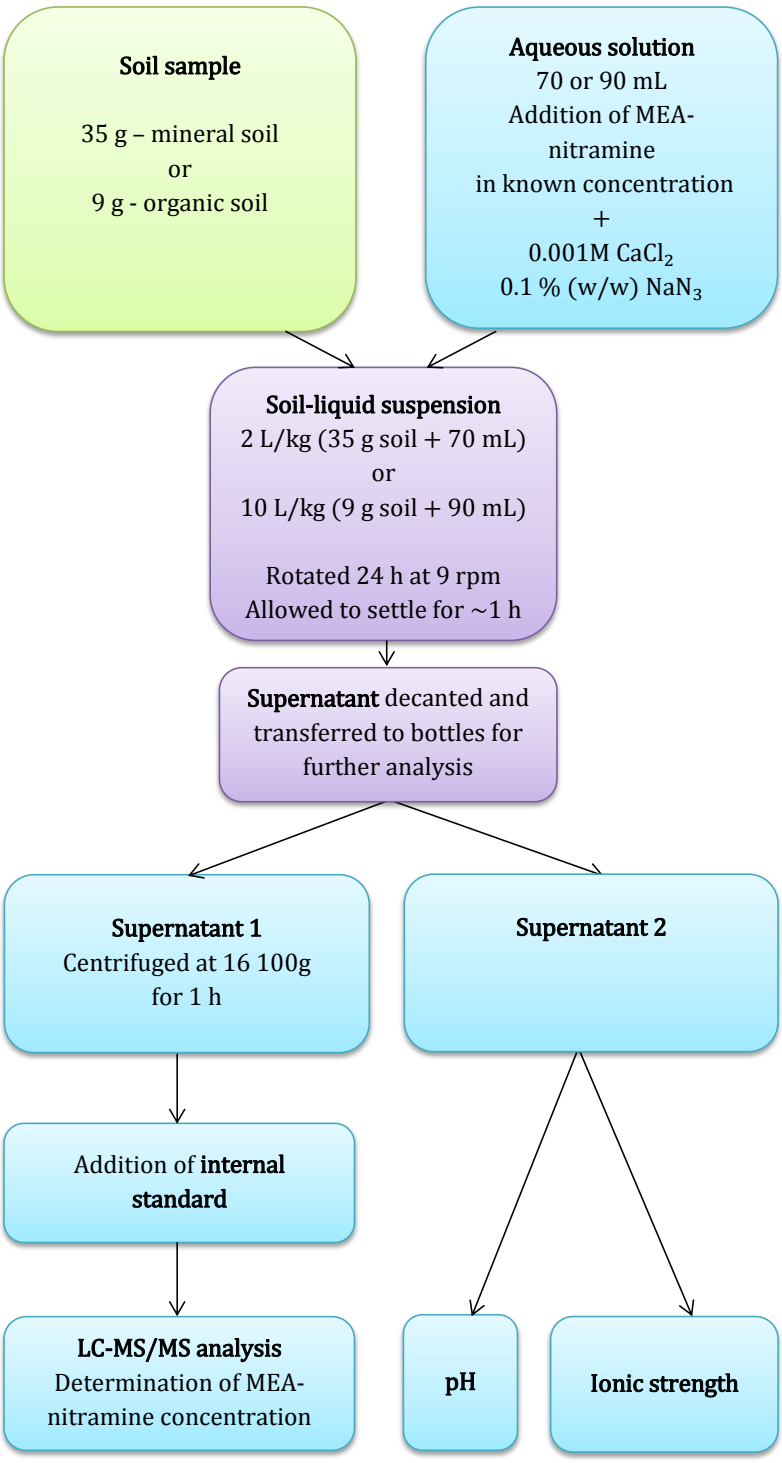

Figure 11 – Experimental design, sorption experiment.

The end-over-end shaker (made in laboratory) was operated at 9 rotations per minute (rpm). The samples were rotated for 24 hours, as it is known that equilibrium for MEA-nitramine between the soil and aqueous phase is reached within that time frame [19]. The samples were allowed to settle for 15-60 min, which deviates from the prescribed 15 min in ISO 21268-1/2 [54, 55], due to the fact that samples with high organic content needed longer time to settle. Sample aliquots for LC-MS/MS analysis were centrifuged (Eppendorf Centrifuge 5415 R, Hamburg, Germany) at 16100 g for 60 minutes. A lower g-force and longer centrifugation time was employed than the prescribed 30 minutes at 20 000 g, as this was the centrifuge available. 0.1 % (w/w) sodium azide (NaN_3)(p.a., Sigma-Aldrich, St. Louis, MO, US) was added as a bactericide. 0.001 M calcium chloride ($CaCl_2$) was added to reduce desorption of DNOM from the soil.

The concentration of MEA-nitramine added to the soil samples was varied, details and justifications are given in results and discussion.

UV absorbance at 254 nm, as a proxy for DNOM, was not measured in the supernatants as nitramines are known to absorb UV light [25] and thus interfere.

LC-MS/MS was employed for determination of MEA-nitramine concentration in the soil water. The analytical method employed in this thesis was based on work by Sofia Lindahl (pers. comm., University of Oslo, 2013/2014). The

3.5.2 Blank samples, Control solutions, Water based - and Matrix Matched Soil Water based Calibration Solutions

Soil water blanks, a water based control solution and water based calibration solutions were prepared and treated in the same manner as the samples. *Blank* samples contained soil, but no added MEA-nitramine, while the *control solution* contained MEA-nitramine (in the same concentration as the samples), but no soil. The *water based calibration solutions* were prepared in volumetric flasks and contained MEA-nitramine in a range of concentrations, they did not contain any soil water extract.

Matrix matched *soil water based calibration* solutions were made from the blank soil samples, which were prepared as described above, and added MEA-nitramine in a range of concentrations.

Spiked soil blanks were also prepared from the blank soil samples, the spiked MEA-nitramine concentration would be the same as added to the soil samples in the sorption experiment. Details on preparation are provided in Appendix D.4.

Calcium chloride and sodium azide concentrations were the same in all the aqueous solutions. The same stock solutions of MEA-nitramine, calcium chloride and sodium azide were used for all solutions within one experiment.

3.5.3 Solid Phase Extraction

To try and separate analyte from matrix SPE was tested. A sample was applied on a 3 mL Bond Elut C_{18} column (Agilent Technologies, CA, US) with 100 mg sorbent material. The flow-through from all applications were collected and analysed separately. The SPE method was adapted from a method provided by PhD candidate T. Vehus (pers. comm., Bioanalytical Chemistry, University of Oslo), details are provided in Appendix D.11.

3.5.4 Analyses and Quantification

The analyses were performed on an LC-MS/MS system. The mobile phase (MP) at a flow of 0.05 mL/min was delivered by the LC system (Thermo Scientific Dionex UltiMate 3000 BioRS system, Waltham, MA, USA), which was equipped with an autosampler, column oven and pump. The MP was composed of a mixture of 70 % HPLC grade water (Optima^{TM} LC/MS, Thermo Fisher Scientific, Waltham, MA, USA) and 30 % methanol (HiPerSolv CHROMANORM gradient grade for HPLC, VWR, Radnor, PA, USA), both with 2 mM acetic acid (eluent additive for LC-MS, Sigma-Aldrich, St. Louis, MO, USA). 1 µL of the sample was injected onto a RP C_{18} silica based Waters Atlantis T_3 column (100 Å, 3 µm, 1 mm x 150 mm) that was used for separation. The detector was a triple quadruple mass spectrometer (Thermo Scientific TSQ Vantage, Waltham, MA, USA) equipped with a heated (H)-ESI ion source operating in negative mode at -3500 V. Thermo Xcalibur version 2.2 software was used during analyses.

With a flow rate of 0.05 mL/min the retention time of MEA- and MMA-nitramine is 2.7 and 2.9 minutes, respectively. The elution time of the mobile phase and possible unretained compounds, t_m, is calculated to be 1.4 minutes. Further details regarding the analytical set-up are provided in Appendix D.1 and D.2.

Quantification of MEA-nitramine in the samples occurred in three different ways during the thesis. In all cases a calibration curve was constructed in Microsoft Excel using linear regression. The details of quantification are provided in the relevant sections of the results and discussion chapter, and in Appendix D.5. MMA-nitramine was employed as internal standard (IS) in some of the analysis. Integration of peak areas was conducted using the Thermo Xcalibur software and the chromatograms were smoothed before integration.

MEA- and MMA-nitramine's precursor and product ion m/z ratios and retention times are shown in Table 1. Product ion for MEA-nitramine with m/z 46.056 and product ion for MMA-nitramine with m/z 59.948 gave the best signal-to-noise of the formed fragments, and were therefore chosen to use for quantification purposes.

Table 1 – Retention time and m/z for MEA- and MMA-nitramines precursor and product ions. Unpublished data from Lindahl (pers. comm.).

Precursor ion (m/z)	Product ion (m/z)	Retention time (min)
MEA-nitramine		2.7
105.054	43.169	
105.054	**46.056**	
105.054	59.939	
105.054	61.192	
MMA-nitramine		2.9
75.069	31.047	
75.069	44.954	
75.069	**59.948**	

3.6 Correlation

For simple linear regression the square of the Pearson correlation coefficient (r) between two variables is equal to the measure of fit for a linear regression line (R^2) [56]. The Pearson correlation coefficient can therefore be found by taking the square root of R^2, the sign of the correlation coefficient will depend on whether the R^2 value is for a positive or negative linear regression line. A correlation coefficient higher than 0.7 (absolute value) generally indicates a strong association between the two variables [56].

3.7 Notation

The term *measurement replicates* is consistently used when several measurements are taken from one sample, while the term *sample replicates* is used when more than one physical replicate is made.

4 Results and Discussion

To access the physiochemical characteristics that govern the soils ability to sorb nitramines, both characterization of the soil and determination of nitramines in soil water have been necessary. The characterization of soil properties will be presented first. The challenges with determination of nitramines in soil water will then be described and assessed in light of the soil and soil water properties, as well as the amounts sorbed to soil.

4.1 Soil Properties

4.1.1 Dry Matter and Organic Matter Content

The moisture content in the soil must be corrected for in determination of organic matter content and in the sorption experiments (Chapter 3.5.1), measured dry matter content is provided in Appendix C.2.

Loss on ignition (LOI) was used as a proxy for the soils organic matter content. LOI differed from 0 to 80.7 % (w/w) in the 16 studied soil samples (Figure 12). Details are provided in Appendix C.2.

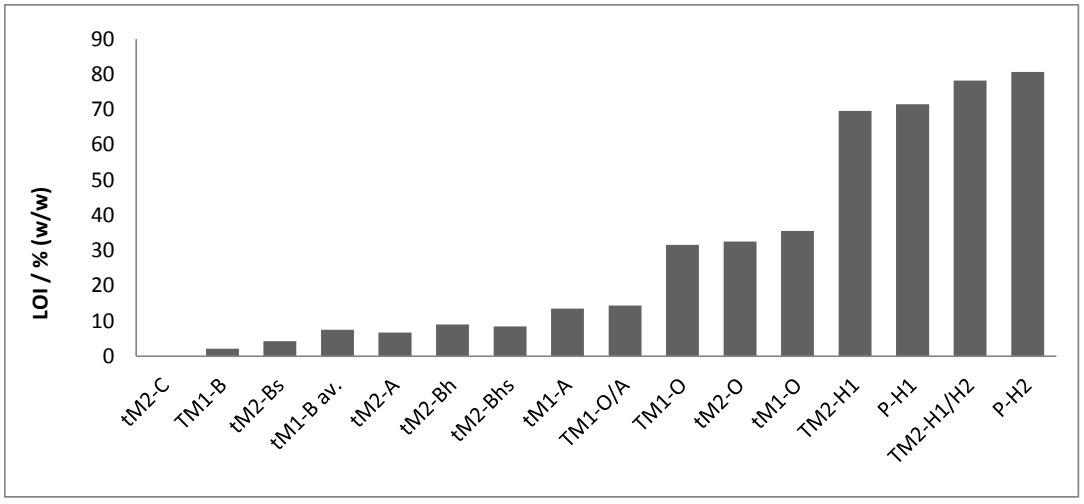

Figure 12 – LOI as a proxy for organic matter content in the soil samples, sorted from low to high. For tM_1-B n=3, while n=1 for the other samples. Soil sample collection details are provided in Chapter 3.3 and Appendix B

Three sample replicates were analysed for sample tM_1-B. The average value (presented in Figure 12) has a relative standard deviation (RSD) of 1 % (w/w) implying

that the analytical precision is good.

4.1.2 Dissolved Natural Organic Matter Content

The concentration of dissolved organic carbon (DOC) was measured as a proxy for DNOM, DNOM is roughly 50 % organic carbon. Ideally DOC should have been measured in the decanted supernatant solution from the sorption experiments. This was not possible due to health concerns, since sodium azide reacts with acids to form toxic gas, and the combustion flue gases (which also may contain MEA-nitramine) are not securely removed from the working environment. Instead blank samples were prepared without MEA-nitramine and sodium azide, using the same liquid to soil ratios as for the sorption experiments, see Chapter 3.5.1. The aqueous extract thus contained 0.001 M $CaCl_2$ in Type I water. Analysis details can be seen in Appendix C.3.

A large variation in values was observed (Figure 13), details are provided in Appendix C.3.

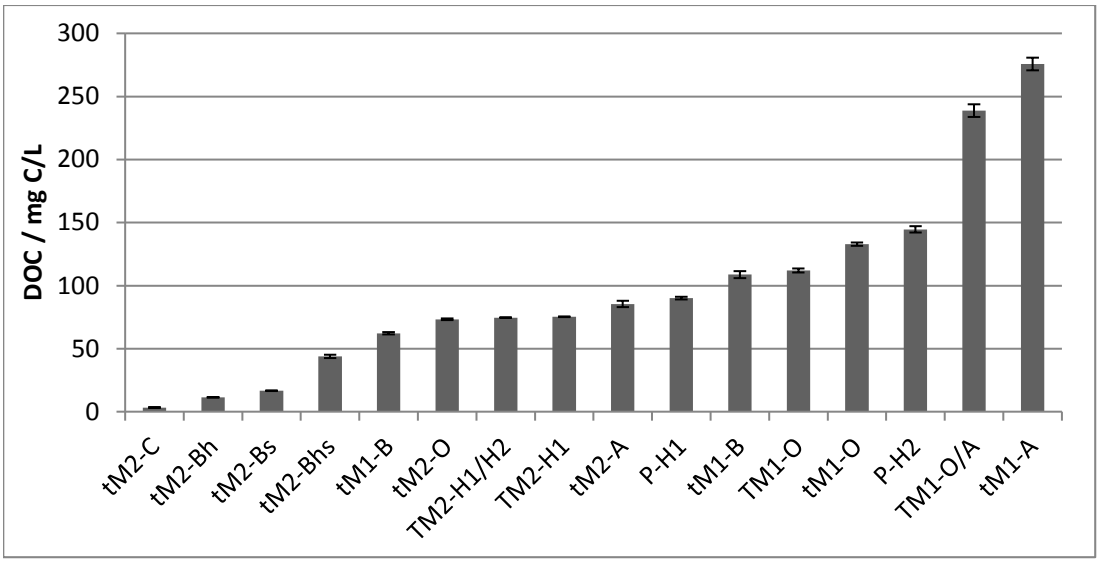

Figure 13 – DOC concentrations in the soil water samples, sorted from low to high. The error bars represent ± 1 standard deviation (STD) calculated from the measurement replicates (n=3-5). Soil sample collection details are provided in Chapter 3.3 and Appendix B.

4.1.3 Particle Size Distribution

Of the collected soil samples, nine are mineral soils. PSD were determined for all but sample tM_2-Bh, as enough insufficient sample mass was available for this soil. The texture of the mineral samples is classified as sandy loam or loam with a clay content less than 11 % (w/w) and silt in the range of 21 - 37 % (w/w). The fractions of sand, silt and clay and the corresponding texture for the analysed mineral soil samples are provided in Table 2, details are provided in Appendix C.4.

Table 2 – PSD of the mineral soil samples given as fractions of sand, silt and clay, and with corresponding texture.

Sample	Sand / % (w/w)	Silt / % (w/w)	Clay / % (w/w)	Total / % (w/w)	Texture [39]
tM_1-A	61	36	3	100	Sandy loam
tM_1-B	63	28	8	99	Sandy loam
tM_2-A	70	21	10	101	Sandy loam
tM_2-Bhs	57	34	9	100	Sandy loam
tM_2-Bs	51	37	11	99	Loam
tM_2-C	69	29	2	100	Sandy loam
TM_1-O/A	61	34	4	99	Sandy loam
TM_1-B	72	22	5	99	Sandy loam

4.1.4 pH

All the soil samples were as expected acidic with pH ranging from 4.2 to 5.4 (Table 3). In order to judge the precision of the method three sample replicates were used for three of the samples, tM_2-C, TM_1-O/A and P-H_2, as these represent samples with low, medium and high OM content, respectively, (Figure 12). Their average pH and RSD, calculated via H^+ "concentration", are reported in Table 3. Details are provided in Appendix C.1. The RSD around 5 % in these samples indicate that the precision of the method is good. Note that the pH value for Type II water (5.4) is a bit lower than the expected 5.6, indicating that the other values might also be a bit lower than accurate. The values measured here were only used to indicate the pH of the soil. After each sorption experiment the pH was measured in the decanted supernatant, and this value was used for the soils in the experiments.

Table 3 – pH in the soil samples.

Sample	Temp. (℃)	pH	RSD (%)
tM_1-O	22.0	4.5	
tM_1-A	22.3	4.5	
tM_1-B	22.3	4.7	
tM_2-O	22.4	4.8	
tM_2-A	22.3	5.0	
tM_2-Bhs	22.4	5.2	
tM_2-Bs	22.4	5.2	
tM_2-Bh	22.4	5.2	
tM_2-C	22.6	5.4	5.4
TM_1-O/A	22.4	4.5	4.5
TM_1-B	22.5	4.8	
TM_1-O	22.1	4.5	
TM_2-H_1	22.1	4.7	
TM_2-H_1/H_2	22.2	4.7	
P-H_1	22.2	4.5	
P-H_2	22.3	4.2	4.2
Blank (Type II water)	21.2	5.4	

4.1.5 Mineralogy and Elemental Composition

Only samples for which sorption results were available were selected for elemental analysis. The elemental composition of these analysed soils were found to be rather homogeneous with a strong dominance of Silicon, constituting about 30 % (w/w) of the mass (Table 4). Aluminium was the second largest constituent, accounting on average for about 7.5 % (w/w) of the total mass. The organic sample, tM_1-O, has a similar composition as the mineral soils, while P-H_2 has very low amounts of reported minerals due to very high organic matter content (80.7 % (w/w)). Details are provided in Appendix C.6

Table 4 – Elemental composition of analysed samples, given as percentage of total sample mass. Only elements that make up 0.1 % or more of the sample mass are reported.

Element	tM_1-O	tM_1-A	tM_2-Bs	tM_2-C	P-H_2
Si % (w/w)	21.8	27.9	29.2	31.2	2.1
Al % (w/w)	5.3	6.8	7.7	8.1	1.1
Fe % (w/w)	1.4	1.9	4.1	2.8	0.6
Na % (w/w)	2.0	2.5	2.5	2.7	0.2
Ca % (w/w)	1.5	1.7	2.0	2.7	0.3
K % (w/w)	1.6	2.1	1.4	1.8	0.2
Mg % (w/w)	0.2	0.2	0.6	1.4	0.1
Ti % (w/w)	0.4	0.4	0.5	0.3	0.1

All the soil samples were analysed for mineralogy. They all contained primary minerals such as quartz and at least one plagioclase feldspar[7] (Table 5). In addition the samples contained smaller percentages (<13 % (w/w)) of other minerals. The details are provided in Appendix C.5. It should be noted that for most samples several unidentified phases were present in the diffractogram, the percentages reported here are therefore not completely accurate. Analysing each individual particle size fraction separately would give more effective characterization [58]. However, this was not prioritised, and the samples were analysed as a single fraction.

It should be noted that accurate quantification of soil mineral composition with XRD is only achieved under ideal conditions. These conditions includes control of degree of preferred orientation, and discrete and well crystalline mineral phases [58]. Neither of these conditions are possible to meet when analysing a soil sample. When the sample is analysed as a total rather than as particle size fractions the clay sized minerals can be difficult to detect and identify due to dilution and interference from coarser minerals in the sample [58].

The high amounts of quartz and plagioclase feldspars combined with the low amounts of Ca and Mg imply that the soils are the weathered materials of igneous rocks like granite and gneiss [41]. The soils are therefore naturally acidic, which is confirmed by the pH measurements (Table 3). That the parent material consists of high amounts of quarts indicate that the yield of clay minerals will be

[7]Oligoclase, labradorite and bytownite are varieties of albite ($NaAlSi_3O_8$) and anorthite ($CaAl_2Si_3O_8$), which are plagioclase feldspars. Oligoclase has a molar ratio of albite:anorthite from 90:10 to 70:30, labradorite has a molar ratio of albite:anorthite from 30:70 to 50:50, and bytownite has a molar ration of albite:anorthite from 10:90 to 30:70 [57].

Table 5 – Main mineral composition of samples. Detailed mineralogy is provided in Appendix C.5.

Sample	Quartz (low) (% (w/w))	Plagioclase feldspars[7] % (w/w)	Other % (w/w)
tM_1-O	28	69	3
tM_1-A	22	65	13
tM_1-B	31	60	9
tM_2-O	34	66	0
tM_2-A	22	69	10
tM_2-Bhs	39	61	0
tM_2-Bs	48	52	0
tM_2-Bh	36	60	4
tM_2-C	48	48	4
TM_1-O/A	44	56	0
TM_1-B	37	61	2
TM_1-O	31	62	7
TM_2-H_1	25	75	0
TM_2-H_1/H_2	31	69	0
P-H_1	23	77	0
P-H_2	30	70	0

small [41]. This is of interest as clay minerals can play an important role in sorption of nitramines, if present. The main mineral constituents of the soil, quarts ($pH_0 = 2$ [1]), albite ($pH_0 < 2$ [59]) and oligoclase ($pH_0 < 1$ [60]) have point of zero charge (PZC) well below the pH of the studied soils. This implies that these soils retain a net negative charge thus generating a net CEC.

4.2 Analytical Challenges and Sorption Experiments

The nitramines ability to sorb to soil will determine if they accumulate in the soil column or are transported to groundwater and freshwater sources. Sorption experiments were conducted to determine the amount of MEA-nitramine sorbed to different types of soil, the experimental design is shown in Figure 11. MEA-nitramine was added in known concentration to the aqueous phase and amount lost from solution was determined. A bactericide (NaN_3) was added to eliminate bacterial degradation as an explanatory factor for reduced MEA-nitramine concentration in the aqueous solution. The soil samples differed in the explanatory variables expected to influence sorption of nitramines to soil, such as pH, OM content and texture, within the range found in local soils close to Mongstad, western Norway. It should be noted that the sorption experiments were not conducted in order to determine accurate sorption coefficients for nitramines in soils. The intention was rather to assess the relative differences between the soil samples and

to relate these to the differences in the soils physiochemical characteristics as well as the differences of the matrix of the supernatant.

An analysis method for determination of selected nitramines, including MEA-nitramine and MMA-nitramine, in Type II water had been partially developed by Dr. Sofia Lindahl (pers. comm., University of Oslo, 2013/2014). This method was developed on the LC-MS/MS instrumentation used in this thesis. The instrument and analysis details are provided in Chapter 3.5.4. The order of a general analysis sequence on the LC-MS/MS is provided in Appendix D.1. Instrumental setting for the LC and the MS are provided in Appendix D.2.

The following results are presented in chronological order as analytical challenges were addressed when they appeared. Several test experiments were conducted with a soil sample from the pilot study by Mohr and Vogt [19], before the method was deemed sufficiently stable for the sorption study using the actual soil samples. Explanations of the expressions *blank*, *control*, *water based calibration solution*, *soil water based calibration solution* and *spiked soil blank* are provided on page vi.

4.2.1 Blank Solutions

To ensure that no MEA-nitramine was present in the soil samples, soil water blanks were prepared and analysed for each soil sample in the sorption experiments. As expected, no signal for MEA-nitramine was detected in any of the soil water blanks in any of the experiments.

MEA-nitramine was below the detection limit in all of the studied soil samples.

4.2.2 Control Solution

Control solution(s) with MEA-nitramine in water were made for each sorption experiment in order to have control of the stability of MEA-nitramine in the aqueous solution, and as a control of possible reduction in concentration due to adsorption to the glass bottles. Primary nitramines, such as MEA- and MMA-nitramine, are not known to hydrolyse [34] so stability should not be an issue, but preparing a water based control should still be done.

A simple sensitivity test was performed to check if rotation and centrifugation of the water based control solution had an effect on the concentration of MEA-nitramine. This was tested as a limited number of bottles (10) could be rotated at the same time in the end-over-end shaker, i.e. shaking the control solution reduced the number of samples that could be simultaneously prepared. Two equal water

based controls were prepared. One was rotated for 24 hours, while the other stood next to the end-over-end shaker for the same time period, i.e. temperature conditions were the same. After 24 hours one aliquot was taken out and centrifuged and one aliquot was transferred directly to an analysis vial, from each of the differently pretreated control solutions. The four differently pretreated control solution aliquots were analysed at the same time, each with three measurements replicates. Details are provided in Appendix D.6.

Figure 14 shows small differences in the measured concentration, these differences are within the uncertainty of the analysis method (approx. 5-10 % for this analysis, based on the RSD for the measurement injections of the controls and the water based calibration solutions). No specific trends can be observed. Regarding the effect of centrifugation the results go in both directions. The concentration in the 24 h rotated control solution show a slight decrease in MEA-nitramine concentration when centrifuged, while the not rotated sample shows an increase in concentration when centrifuged.

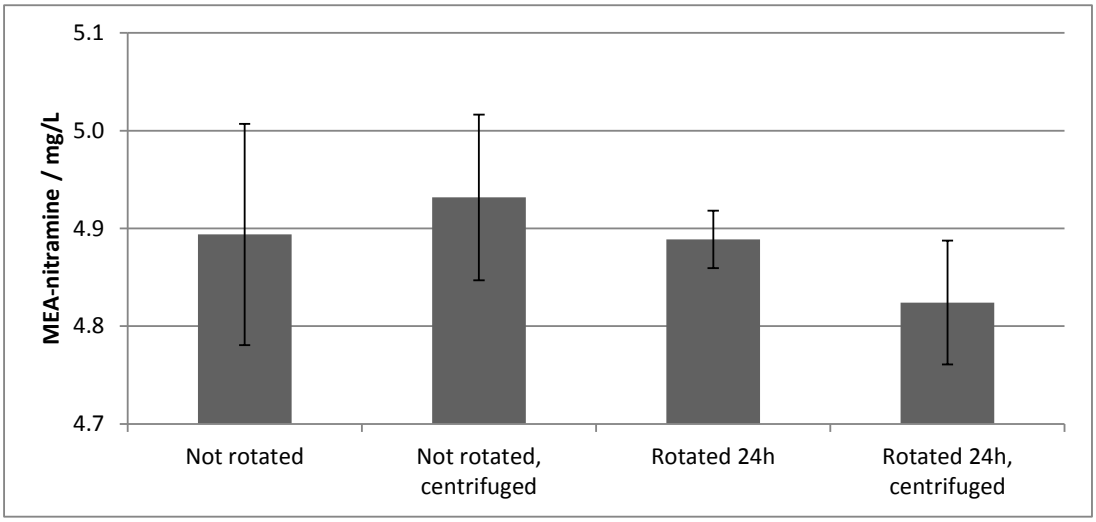

Figure 14 – Concentrations of control samples with different pretreatment. The error bars represent ± 1 STD calculated from the measurement replicates (n=3).

Ideally this test should have been repeated with several independent sample replicates and tested for proper statistical significance. Without sample replicates (only measurement replicates were used) statistical significance can not be determined. However, logic dictates that without any soil, shaking the control solution or centrifuging it should not make any difference to the concentration of MEA-nitramine unless sorption to the glass walls are significant. It was thus chosen

to accept that centrifugation or rotation of the water based controls had minimal effect on MEA-nitramine concentration. The control solutions in the following sorption experiments were therefore neither rotated nor centrifuged. However to check for problems with MEA-nitramine stability and/or adsorption to the bottle walls, the controls were still kept in the bottles for the same time period and at the same temperature as the soil samples.

The concentration of MEA-nitramine in the water based control solution does not seem to have been affected by the rotation or centrifugation pretreatments. The water based control solution was therefore neither rotated nor centrifuged in the following sorption experiments.

4.2.3 Test Experiment: Determination of MEA-nitramine (5.01 mg/L) in Soil Water, 25 L/kg Liquid to Soil Ratio

The method developed by Lindahl (pers. comm.) was for determination of nitramines in Type II water. The first step was therefore to conduct a simple test to determine if a signal could be detected for MEA-nitramine in soil water extract, and to conduct a pilot test of the sorption procedure. As OECD guidelines 106 [44] are made specifically for sorption testing, and differ mainly from ISO 21268-1/2:2007 [54, 55] with regard to liquid to soil ratio. The ratio that theoretically should give 50 % (w/w) sorption according to the OECD guidelines 106 [44] was chosen. 50 % (w/w) was chosen as this should provide a measurable concentration change in the aqueous solution. This ratio was determined based on the approximate distribution coefficient, K_d, of MEA-nitramine for soil sample 005-1 from the pilot study by Mohr and Vogt [19]. This soil was chosen as the test soil in this experiment, as soil 005-1 is an organic soil (69 % LOI, O/A-horizon) with reported K_d of 14 L/kg [19].

A sorption experiment was performed as described in Chapter 3.5.1, with the exception of the liquid to soil ratio. The target sorption of 50 % (w/w) with a K_d of 14 L/kg corresponds to a 25 L/kg ratio [44]. A high MEA-nitramine concentration (5.01 mg/L) was used. Three soil sample replicates were prepared. Sodium azide was not added as a bactericide as soil sample 005-1 had previously been oven dried (105 ℃ [19]) and should thus not contain any large amounts of bacteria. Details are provided in Appendix D.3.

The analysis showed a signal within the range of the water based calibration solutions, corresponding to a concentration of 6 ± 1 mg/L MEA-nitramine. With an added concentration of 5.02 mg/L MEA-nitramine this corresponds to a sorption between 0 and - 39 % (w/w), i.e. an increase in concentration. As no signal for

MEA-nitramine was registered in the soil blank, this reflects the large uncertainty in the method. A large variation between the soil sample replicates were also seen, as represented by the STD of 1 mg/L, with a corresponding RSD of 24 %. The water based calibration curves, which can be seen in Figure 15, also show a large uncertainty with RSD varying between 8-25 % depending on the concentration. It should also be noted that the control solution, which should have had a concentration of 5.02 mg/L MEA-nitramine, gave a signal corresponding to a concentration of 3.9 $\pm$ 0.3 mg/L. This corresponds to a relative error of 21 %. Whether this discrepancy from the expected value and the large STDs for the sample replicates were due to instrument variation or errors during sample preparation is uncertain. However, the fact that large variation occur for the three measurements of the water based calibration solutions does indicate large instrumental variations. Measurement replicates were not used during the analysis, which, in retrospect, was a bad choice. In subsequent analyses all vials (samples, replicates, calibration solutions, etc.) were measured three times consecutively.

Moreover it should be note that the (water based) calibration solutions were not soil water matrix matched, i.e. they were made in water with calcium chloride. This is true for both the experiment conducted here, and the analyses performed in the pilot study by Mohr and Vogt [19]. Possible matrix effects on the analyte itself, the analytes ionization potential is thus not accounted for.

In the pilot study by Mohr and Vogt [19] measurable sorption for sample 005-1 was observed with the liquid to soil ratio (10 L/kg) from ISO 21268-1/2:2007 [54, 55]. As no sorption was observed with the ratio (25 L/kg) calculated according to OECD guideline 106 [44], it was instead decided to try the liquid to soil ratios (10 L/kg for organic soil, 2 L/kg for mineral soil) outlined in Chapter 3.5.1.

MEA-nitramine can be detected in a soil water matrix. A large uncertainty was observed both in the soil sample replicates (RSD 24 %) and the measurement replicates of the water based calibration solutions (RSD up to 25 %). The sorption was calculated to be between 0 and -39 % (w/w) reflecting the large sample replicate variation and method uncertainty, as negative sorption is not theoretically sound. As no significant sorption was observed with a 25 L/kg liquid to soil ratio. The liquid to soil ratios (10 L/kg for organic soil, 2 L/kg for mineral soil) from ISO 21268-1/2:2007 [54, 55] should therefore be tested. The possible effect of the soil water matrix on the analyte itself and on the ionization of the analyte is unknown.

4.2.4 Test Experiment: Determination of MEA-nitramine (4.97 mg/L) in Soil Water, 10 L/kg Liquid to Soil Ratio

To see if the sorption results from the pilot study by Mohr and Vogt [19] could be reproduced for sample 005-1, the test described in Chapter 4.2.3 was repeated, though using a 10 L/kg liquid to soil ratio according to ISO 21268-2:2007 [55]. This is the same liquid to soil ratio that was employed for sample 005-1 in the pilot study by Mohr and Vogt [19]. The concentrations of added MEA-nitramine were different, in the pilot study the concentration of added MEA-nitramine was 0.500 mg/L [19], while 4.97 mg/L MEA-nitramine was employed in this test.

Three sample replicates were prepared. After the sorption experiment, the measured concentration of MEA-nitramine in the aqueous phase was 2.8 ± 0.1 mg/L (RSD: 4 %), corresponding to a sorption of 44 % (w/w). The relative error of the control solution was calculated to be 2 % which indicates the stability of MEA-nitramine in the aqueous phase is good and no absorption to the glass walls occur. In addition the RSD of the water based calibration solutions were lower than 11 %, indicating relatively stable analysis conditions. K_d was calculated to be 9 L/kg, employing the calculus outlined in Chapter 2.4.4. This calculation assumes that the free MEA-nitramine not recovered in the aqueous phase is sorb to the soil. This might however not be accurate as sorption to DNOM may play a role.

The K_d reported for the same soil in the pilot study was 14 L/kg [19]. The difference in K_d can be due different amounts sorbed to DNOM, or because the concentrations of added MEA-nitramine was different. If the two concentrations were not within a linear range on the sorption isotherm for MEA-nitramine in the given soil, the distribution coefficients would necessarily be different. However, the amount of DNOM leached could be significantly higher in the pilot study by Mohr and Vogt, due to employment of a more vigorous shaking (75 rpm) during the sorption experiment [19]. This is significantly higher than the 9 rpm employed in the present study. The measured DOC also reflects this, in the pilot study by Mohr and Vogt sample 005-1 had a reported DOC value between 400-500 mg C/L [19], which is close to twice as high as the highest value measured in the soils in this thesis (see Table 13). It is therefore possible that the different K_d values are due to the difference in DNOM concentration in the aqueous phase.

Significant sorption was observed for soil sample 005-1 with a liquid to soil ratio of 10 L/kg. The stability of the instrument was better than in the test described in Chapter 4.2.3, with RSDs of maximum 11 % for the measurement replicates of the water based calibration solutions. Though as no changes had been made to the method, this was likely arbitrary. The difference in calculated K_d between this

test and the pilot study by Mohr and Vogt [19] is possibly due to differences in DNOM concentration in the aqueous phase, though the fact that different added concentrations of MEA-nitramine were used, could also play a role.

4.2.5 Instrument Variations with Time

Investigation into the instrumental variation was clearly needed as large variation in stability were seen both within and between the experiments described in Chapter 4.2.3 and 4.2.4. In each sorption experiment the water based calibration solutions were measured three times; before, in the middle of, and after the other solutions analysed, or before and twice after. The standard deviation for each water based calibration solution could therefore be calculated. These standard deviations would then represent the instrumental variation over the analysis time for the different concentrations.

The signal from the water based calibration solutions sometimes increased during a sequence and at other times it decreased or was stable. These changes could be abrupt with a sudden loss or increase in signal, or gradual decreases/increases over many hours. The measurements of the water based calibration solutions shown in Figure 15 illustrates a sudden loss in signal sensitivity.

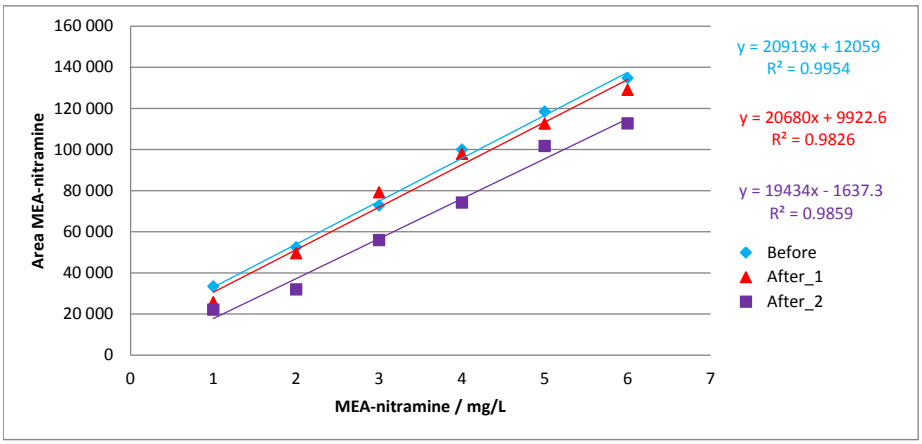

Figure 15 – Area of MEA-nitramine in water based calibration solutions measured before and after samples, plotted against MEA-nitramine concentration (1.00-6.02 mg/L). The water based calibration solutions were measured from lowest to highest concentration. *Before* refers to the set of measurements of the water based calibration solutions before the samples, while *After_1* and *After_2* refer to the sets of measurements of the same calibration solutions, after the samples were analysed.

The water based calibration solutions were measured three times (Figure 15). The first set of measurements form the calibration curve termed *Before*. The second set of measurements form the calibration curve termed *After_1*, and the last set of measurements form the calibration curve termed *After_2*. Between the measurements for *Before* and *After_1* the sorption experiment samples (Chapter 4.2.3) were measured, i.e. there was a time gap of approximately 60 minutes between the two sets of measurements. Between the measurements for *After_1* and *After_2* only a blank sample (5 min.) was measured. It therefore seems to be a sudden decrease in sensitivity between these two last sets of measurements. A test was designed to see if there was a pattern to the variation, as well as to test if this could be due to evaporation from the punctured vials since the vials used in the autosampler had a rubber cap that was penetrated by the needle.

To ascertain that the puncturing of the vial caps did not cause evaporation from the vials, a water based calibration solution of 4.97 mg/L MEA-nitramine was distributed to five identical vials. Injections from each vial was performed 6 times over 12 hours before analysis of the next vial was initiated, making the total analysis time 60 hours. The different vials are indicated by different colours and markers in Figure 16. There seems to be a wave-like pattern, but not a repeatable one within the time frame studied. There is neither a consistent drop nor increase when switching to a new vial. This was as expected as neither water (the matrix) nor MEA-nitramine should evaporate at the temperature in the autosampler (4 °C).

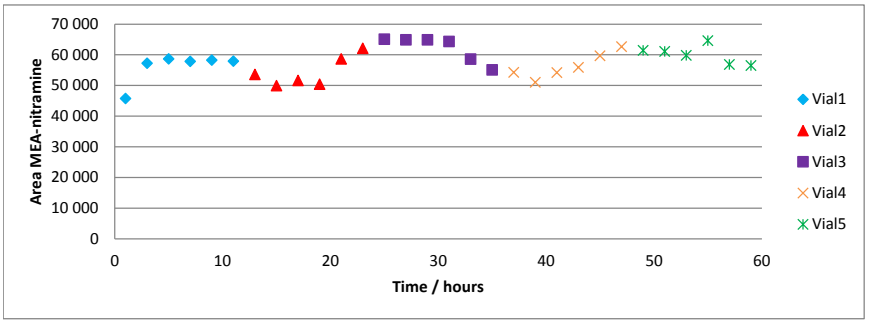

Figure 16 – Instrument variation displayed as the area of the integrated signal peaks for measurements of 4.97 mg/L MEA-nitramine in 6 different vials (Vial1-6) containing the same solution, over time.

The variation seen in Figure 15 correspond to a relative standard deviation (RSD) of 8-25 % depending on concentration, while the variation in Figure 16 correspond to a RSD of 9 %. The magnitude of the variations may therefore be concentration

dependent.

In order to correct for these instrumental variations it was determined to pursue the option of an internal standard (IS), and test if one of the other nitramines available could be used as one. Ideally an IS should be added before the sample preparation to correct for any loss during pretreatment, as well as instrumental variation and matrix. Since the IS should have similar physiochemical characteristics as the analyte it will likely also sorb to the soil and thereby compete with the analyte for sorption sites. This is thus not an option. An IS that does not sorb to soil will on the other hand not be influenced in the same manner as the analyte in other aspects either, and therefore not serve its function. Instead an IS added after the sample preparation should correct for instrumental variation, e.g. differences in injection volume or amount ionized. The ideal IS for this purpose is an isotope-labelled (normally D/^{2}H or ^{13}C) version of the analyte, as this IS will behave in almost the exact same manner as the analyte and is possible to separated by mass for analysis. Unfortunately an isotope-labelled MEA-nitramine was not available at the time. Using a similar nitramine is not ideal as the matrix effects on ionization can be compound dependent, as explained in Chapter 2.2.4.

MMA-nitramine was tested as IS since the physiochemical properties, e.g. pK_a, are rather similar to MEA-nitramine and they are structurally similar. The structures of MEA- and MMA-nitramine are provided in Figure 6. To check if the signal of MMA-nitramine varies in the same manner as MEA-nitramine, one drop of an available MMA-solution (with unknown concentration) was added to a vial containing a water based calibration solution of 4.97 mg/L MEA-nitramine. After checking that the MMA-nitramine signal was above detection limit, the vial was analysed every second hour for 60 hours (Figure 17).

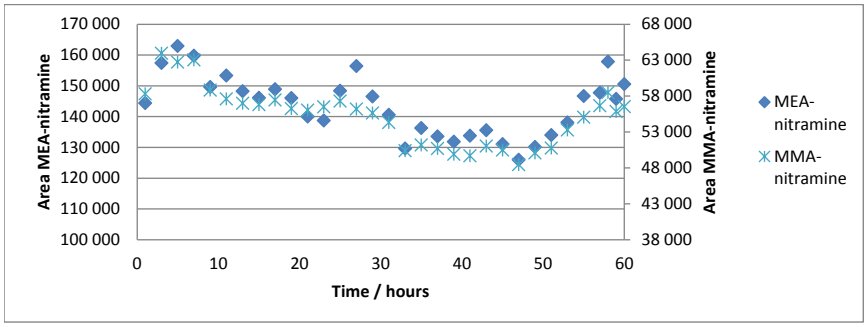

Figure 17 – Instrument variation displayed as the area of the integrated signal peaks for measurements of MEA- and MMA-nitramine over time.

As Figure 17 indicates the analytical signal of the two nitramines follow each other closely, the RSD for the MEA-nitramine measurements were 7 %, while the ratio between MEA-nitramine and MMA-nitramine had a RSD of only 3 %, confirming this. Note that the wavelike pattern seen in Figure 16 is not recognisable during this test. This is further indication that the instrumental variation does not follow a repeatable pattern.

To correct for instrumental variation an IS should be added after sample preparation. MMA-nitramine seem to work well as an IS for MEA-nitramine in water based solutions and should be added to all solutions prior to LC-MS/MS analysis.

4.2.6 Concentration of MEA-nitramine

Realistic concentrations of nitramines in the environment are likely to be very low (ng/L range [16]). The sorption experiment should therefore be conducted with MEA-nitramine concentration in the ng/L range, or the the lowest detectable concentration, if that is higher.

MEA-nitramine in water with concentrations of 5.00 mg/L, 0.50 mg/L, 0.05 mg/L, 5 µg/L, 0.5 µg/L , 0.05 µg/L and 5 ng/L were analysed. The two lowest concentrations are not included in Figure 18, as no signal was detected for concentrations lower than 5 µg/L. Note that the chromatograms have not been smoothed, so that the actually registered signal can be seen. The peak shape gradually deteriorates as the concentration was decreased, until the signal was indistinguishable from the noise at 0.5 µg/L. Even though a signal was seen for 5 µg/L the peak shape was poor, being both unsymmetrical and split. It must also be taken into consideration that sorption to soil and other matrix components in the sample will decrease the concentration in the aqueous phase. Based on this it was chosen to add 0.05 mg/L MEA-nitramine to the samples, as this should result in a sufficient signal for quantification as long as the sorption is less than 80 %.

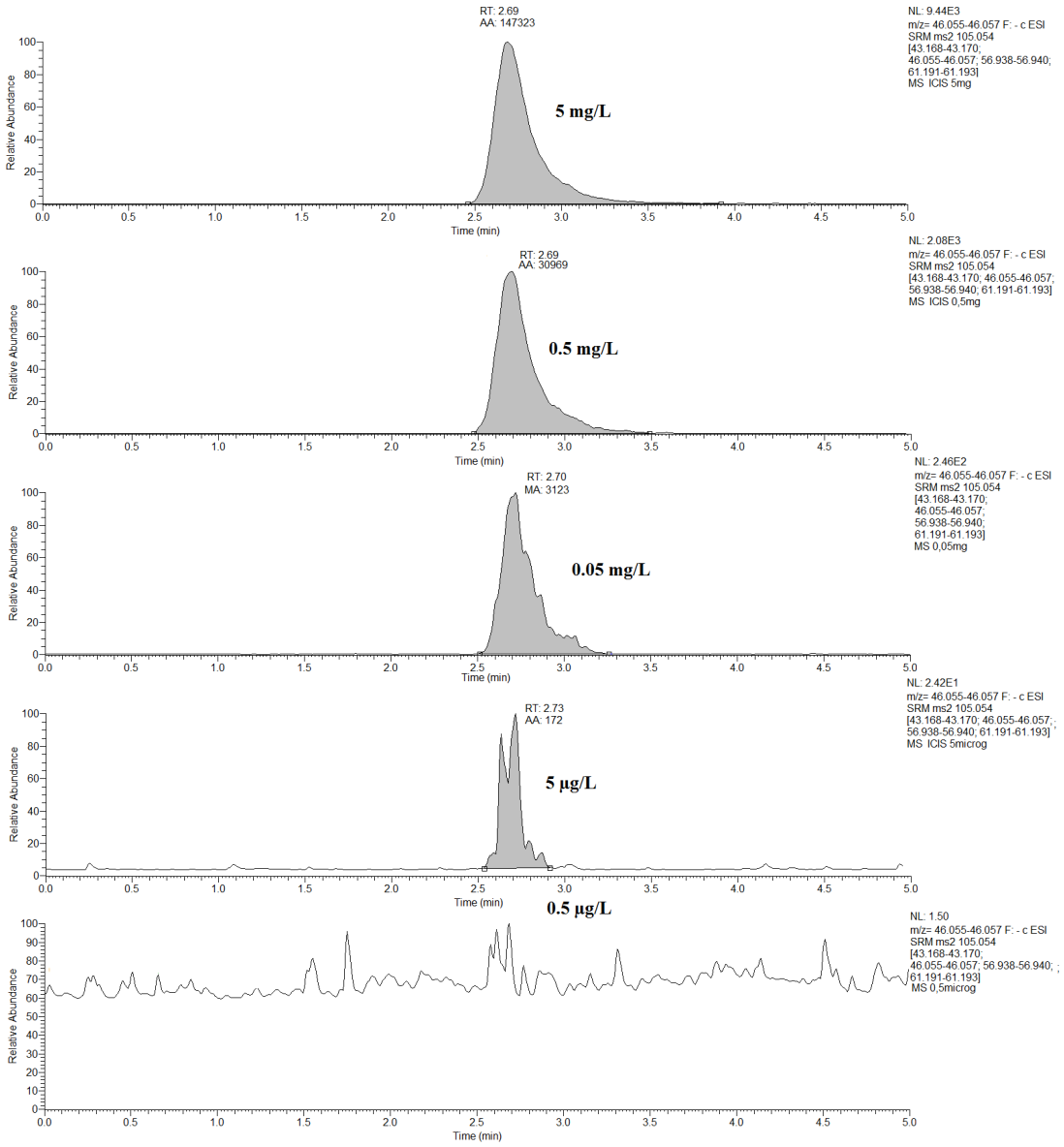

Figure 18 – SRM chromatograms of 5 mg/L, 0.5 mg/L, 0.05 mg/L, 5 µg/L, 0.5 µg/L MEA-nitramine in water (top to bottom) (n=1). 1 µL sample was injected on the C_{18} Water Atlantis T_3 column that was used for separation in a LC-H-ESI-MS/MS system. The flow rate was 0.05 mL/min and the tandem MS was used in SRM mode. The mobile phase composition was 70 % water and 30 % methanol (MeOH), with 2mM acetic acid (AcA). The extracted MEA-nitramine precursor ion peak had m/z 105.054, with product ion of interest at m/z 46.056. The peak area of the product ion was integrated using the Xcalibur software, smoothing was not used.

The concentration of MEA-nitramine added to the soil samples in the sorption experiments should be 0.05 mg/L to ensure sufficient signal for quantification, while still being as low as possible to ensure environmental relevance.

4.2.7 Test Experiment: Determination of MEA-nitramine (0.05 mg/L) in Soil Water with Matrix Matched Soil Water based Calibration Curve

The effect of matrix components on the ionization potential should be assessed, in addition it needed to be checked if a signal could be detected for low concentrations (< 0.05 mg/L) of MEA-nitramine in soil water. The use of MMA-nitramine as IS in soil water also needed investigation. A sorption experiment with 0.05 mg/L added MEA-nitramine was performed according to the procedure outlined in Chapter 3.5.1. Both water based calibration solutions and matrix matched soil water based calibration solutions were prepared. Soil sample 005-1 from the pilot study by Mohr and Vogt [19] was used. MMA-nitramine was added as an internal standard to all the solutions for a resulting concentration of 0.03 mg/L before analysis.

Unfortunately it was assumed that MMA-nitramine would have the same detection limit as MEA-nitramine, this was not the case, and the added MMA-nitramine (0.03 mg/L) could not be detected in any of the solutions.

The signal from the matrix matched calibration solutions are visually lower than the signal for the water based calibration solutions and the slope of the soil water based calibration curve is not as steep (Figure 19). This indicates ion suppression or loss of MEA-nitramine to matrix components.

The concentration of the sample replicates were calculated using both calibration curves. Using the water based calibration curve gave an average concentration of 0.034 ± 0.003 mg/L MEA-nitramine in the supernatant, which corresponds to a sorption of 35 % (w/w). The relative error of the control solution was calculated to be 4 %, indicating that MEA-nitramine is stable and not significantly sorbed to the glass walls. The uncertainty due to instrumental variation was estimated to be maximum 14 %, based on the maximum RSD for the water based calibration solutions during the analysis. From the matrix matched soil water based calibration curve the average concentration in the supernatant from the sample replicates were calculated to be 0.054 ± 0.006 mg/L MEA-nitramine, which corresponds to a sorption of -5 % (w/w). An increase in the concentration is not theoretically sound, but if no sorption occurs, this is within the uncertainty of the analysis. Clearly these calibration curve give significantly different results, and matrix matched soil

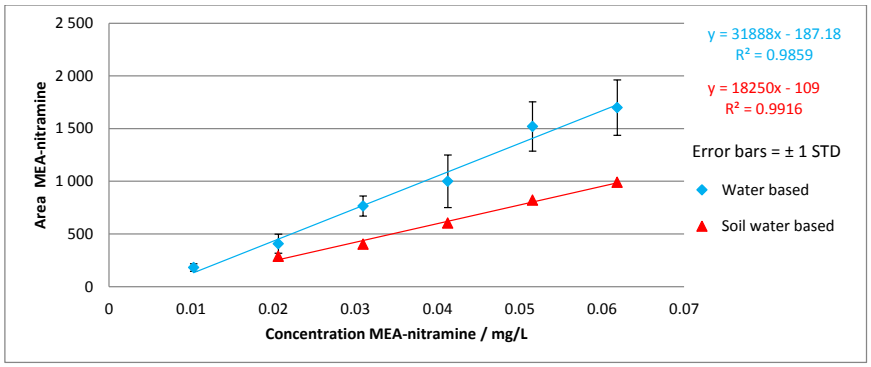

Figure 19 – Calibration curves for water based (blue) and soil water based (red) calibration solutions. The error bars represent ± 1 STD calculated from the measurement replicates of the water based calibration solutions (n=9).

water based calibration curves must therefore be employed.

This also indicates that the reported distribution coefficients for the nitramines to soil in the pilot study by Mohr and Vogt [19] could be incorrect, as matrix matched soil water based calibration curves were not employed. The reported sorption in the pilot study [19] is therefore likely to be due to matrix effects. The same can be said for the reported sorption in the experiment without matrix matched soil water based calibration curve described in Chapter 4.2.4. The analyses carried out in this test, however, do have the drawback that the internal standard was below detection limit and could therefore not be used to correct for instrumental variations. In addition only one soil sample was analysed.

Matrix matching the calibration solutions is a very time consuming process both in preparation and analysis time, as each soil sample will need its own set of calibration solutions. As the original experimental design included testing of 16 soil samples, a compromise to include matrix matching was made. It was decided to make a spiked soil blank for each soil tested. The soil blank for a sample was centrifuged and then spiked to the concentration added in the corresponding sorption experiment soil sample. Its concentration, determined by the water based calibration curve, was used as starting concentration for the sorption calculations. For this to be an option, it must be assumed that the matrix matched soil water based and water based calibration curves are parallel. This principle is illustrated in Figure 20.

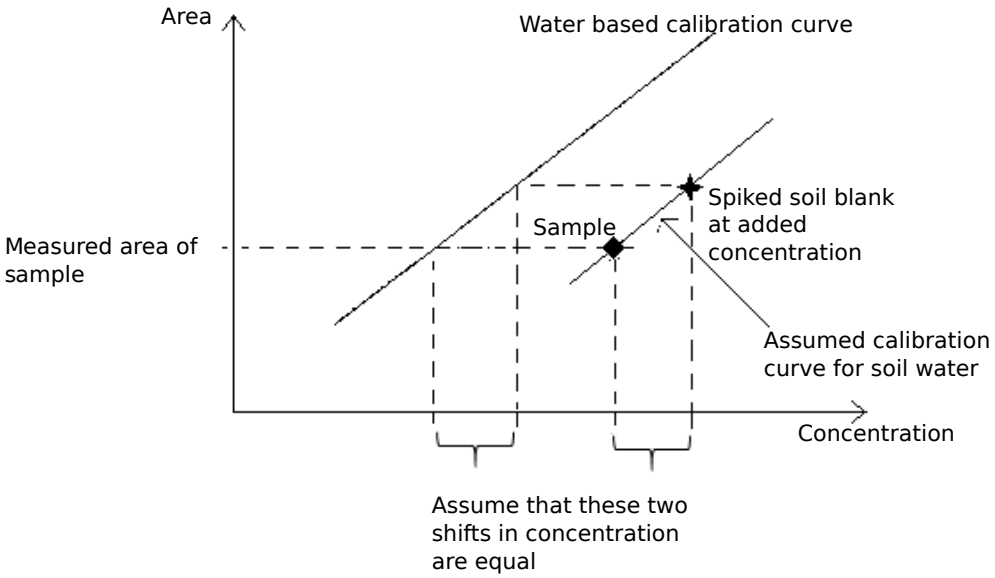

Figure 20 – Principle of sorption calculation using a water based calibration curve and a spiked soil blank. It is assumed that the calibration curve that the spiked soil blank and sample would lie on, is parallel with the water based calibration curve. The difference in concentration between the two measurements should then be equal regardless of whether the "true" (not determined) matrix matched calibration curve or the water based calibration curve is used to determine their concentrations.

Using this method only one spiked sample blank for each soil is needed to correct for the effect of the matrix on MEA-nitramine signal. However, parallel water based and soil water based calibration curves are not likely as matrix effects are concentration dependent and thus will change the slope of the curve, as seen in Figure 19. Calculation in this manner will therefore result in a larger uncertainty in the sorption and K_d values. However, as the opportunity to test all the soil samples (instead of a subset) would make it possible to deduce explanatory parameters, and thus gain a conceptual understanding of the governing processes behind sorption of nitramines to soil, this was acceptable.

The soil water matrix significantly changes the calibration curve compared to the water based calibration curve. The calculated sorption changes from 35 % to -5 % when the water based and matrix matched soil water based calibration curves are employed, respectively. The reported sorption to soil in the pilot study by Mohr

and Vogt [19] could therefore be due to matrix effects. MMA-nitramine was below the detection limit at a concentration of 0.03 mg/L.

4.2.8 Concentration of MMA-nitramine

As MMA-nitramine with concentration 0.03 mg/L was below the detection limit when analysed in the sorption experiment described in Chapter 4.2.7, solutions with 0.33 and 3.00 mg/L MMA-nitramine in water were prepared and analysed. Both concentration were above the detection limit (Figure 21).

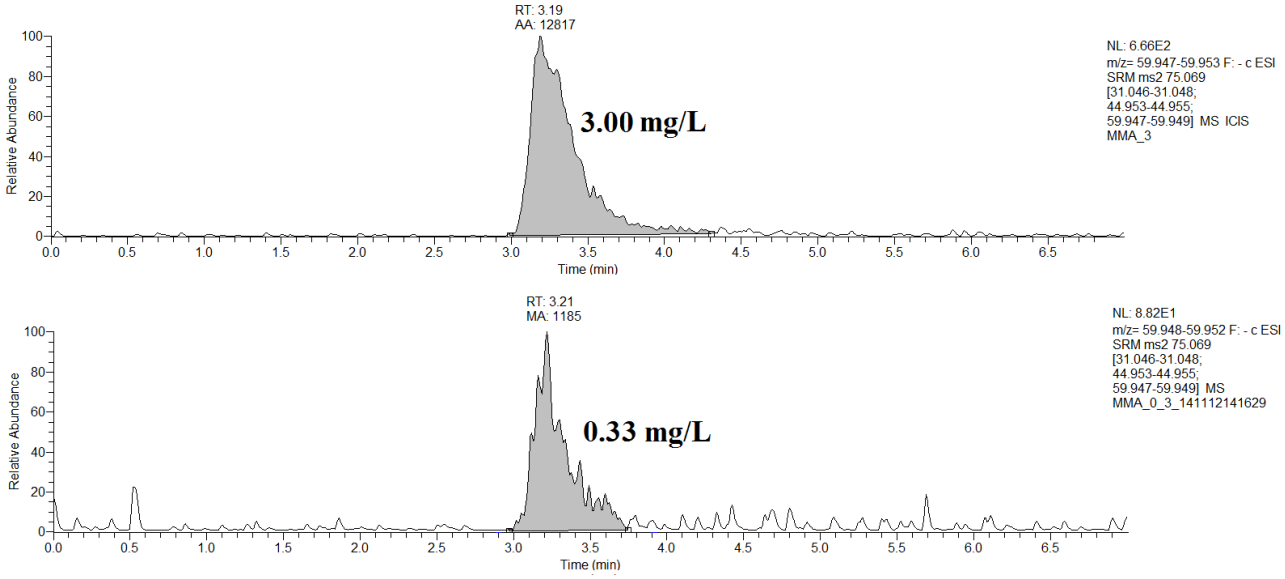

Figure 21 – Extracted SRM chromatograms of 3.00 mg/L (top) and 0.33 mg/L (bottom) MMA-nitramine in water (n=1). The instrumental conditions were as in Figure 18, with exception of the MP composition. The mobile phase composition was 80 % water and 20 % MeOH, with 2mM AcA. The extracted MMA-nitramine precursor ion peak had m/z 75.069, with product ion of interest at m/z 59.948. Note that the shift in retention time (3.2 instead of 2.9 min) shown in these chromatograms were due to the lower amount of methanol employed in the MP, than in the sorption experiments.

The peak shape for 0.33 mg/L MMA-nitramine was not particularly good, and the intensity was quite low (8.82E1). The peak shape for 3.00 mg/L MMA-nitramine was considered satisfactory. As MMA-nitramine was used as internal standard in the following sorption experiments it should be in the same concentration range as MEA-nitramine to ensure similar effect from instrumental variation. When MMA-nitramine is added at a different concentration than the MEA-nitramine present

in the sample the ion suppression/enhancement effect might not be exactly the same, as the effects can be concentration specific [30]. It was thus decided to use a resulting concentration of 1.00 mg/L MMA-nitramine as that should give a satisfactory signal, while being as close to the MEA-nitramine concentration as possible.

The resulting concentration of added MMA-nitramine should be 1.00 mg/L, as this will ensure sufficient signal for quantification, while still being as close to the MEA-nitramine concentration as possible.

4.2.9 Sorption to Soil with 0.05 mg/L MEA-nitramine

As the analytical method at this point was deemed sufficiently stable it was decided to proceed with the sorption study. The large uncertainties observed in the previous test experiments described in Chapter 4.2.3, 4.2.4 and 4.2.7, should be reduced by the use of MMA-nitramine as an internal standard. Seven soil samples were chosen for the first experiments. The samples were chosen to represent a variety of OM content and different textures (sand, clay, etc.), as well as different mineral content based on color, since PSD, mineralogy and elemental composition were not available for the samples at the time.

The experiment was conducted as outlined in Chapter 3.5.1. Three replicates were made for each soil, with 0.05 mg/L MEA-nitramine. Only two soils could be prepared at the same time because of sample number restrictions in the end-over-end shaker. Note that temperature conditions were not ideal as the ventilation system in the building was out of order. The experiment was thus carried out at a 26 ℃. MMA-nitramine (1.03 mg/L) was added as an internal standard to the centrifuged supernatant aliquots before analysis. Spiked soil blanks (0.05 mg/L MEA-nitramine) were made for each soil. Due to the problems encountered with the IS, described below, only four of the seven soil samples were analysed.

The uncertainty caused by stability variation in the instrument during measurement is reflected by the RSD of the measurements for the water based calibration solutions, corrected by the IS measurements (n=9). Their RSDs were lower than 10 %. The RSD of the measurement replicates for the spiked soil blanks varied between 3 and 11 % (n=3). The total uncertainty of the method should therefore be less than 15 %. The difference in signal for IS (MMA-nitramine) in the soil water samples and the water based calibration solutions were larger than what could be attributed to instrumental variation alone. The differences were also soil specific. The measured concentrations of MEA-nitramine in the spiked soil blanks when using IS correction, deviate from the added concentration with 7, 18, 23 and

73 % (Figure 22), calculated values are provided in Appendix D.9. For all the tested soil samples, except tM_2-C in which the supernatant contained very little DNOM (DOC = 3 mg C/L), the deviations from added concentration were larger than what can be contributed to method uncertainty. Keeping in mind that the IS should correct for instrumental variation, these concentrations should be close to the calibration line at MEA-nitramine concentration 0.05 mg/L in water. As this clearly is not the case (Figure 22), loss of MMA-nitramine to matrix components seem to be significant. If MMA-nitramine sorb to matrix components some of the deviation can also be due to instrumental variation not corrected for, as the concentration of MMA- nitramine is not the same in the spiked soil blanks and water based calibration solution.

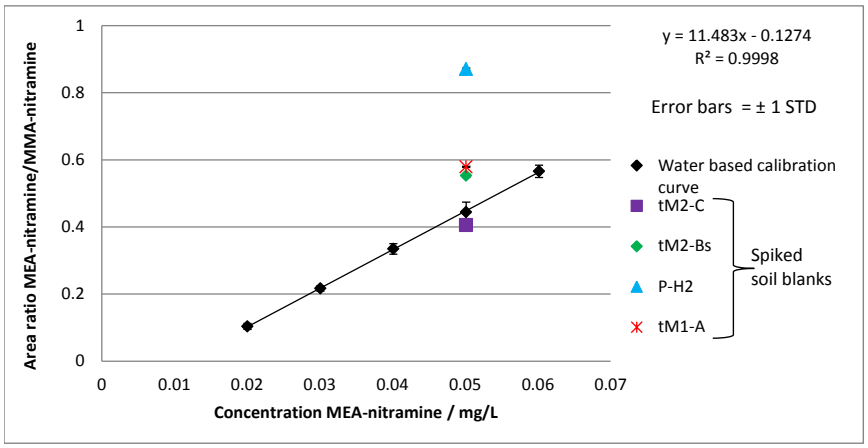

Figure 22 – Water based calibration curve and measurements of the spiked soil blanks shown at the added concentration, 0.05 mg/L MEA-nitramine, to illustrate that the IS is not functioning. The error bars represent ± 1 STD for the measurement replicates (n=9 for the water based calibration solutions, and n=3 for the spiked soil blanks). Not that some of the error bars are smaller than the markers.

In hindsight, loss of IS (MMA-nitramine) to the DNOM in the sample aqueous phase should have been expected. Figure 23 indicates that the IS signal (represented by peak area) decrease $\log_e$ (ln) linearly with increasing DNOM (DOC) concentration in the soil water. If the same trend is true for other nitramines, sorption to DNOM will enhance their mobility in the environment.

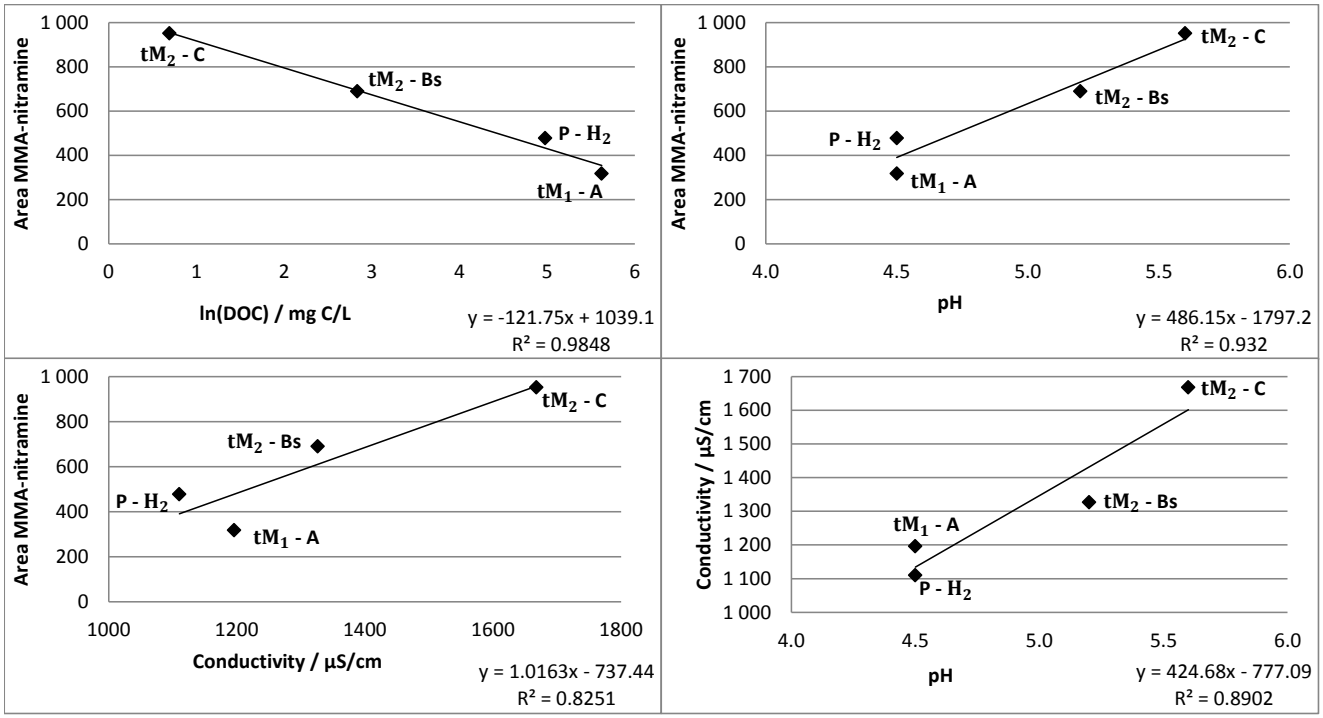

Figure 23 – Relationship between the average decrease in IS signal in the soil samples replicates and the DNOM (DOC) content (top, left), the pH in the solution (top, right) and the ionic strength (conductivity) in the solution (bottom, left). The fourth graph shows the relationship between ionic strength (conductivity) and pH (bottom, right).

A negative linear relationship with increasing pH and ionic strength (conductivity[8]) is also seen. The correlation[9] coefficients (r) are 0.9924, -0.9654 and -0.9084 for the correlations between MMA-nitramine area and ln(DOC), pH, and conductivity, respectively. All the correlation coefficients indicate strong correlations between the variables. The correlation between IS signal and pH and ionic strength may be related to these parameters effect on ionization efficiency. Increasing ionic strength and pH can increase the surface tension of aqueous solutions [52, 61] and therefore effect evaporation of analyte from droplets formed during ionization. Note that there also is a clear correlation between pH and conductivity (r = -0.9435, Figure 23, bottom, right), due (at least partially) to the high specific conductivity of H^+ ions compared to other ions in solution. The correlation with

[8]Considering the high conductivity relative to pH, the conductivity can be used as a measure of the ionic strength as it is not only dependent on the ion conductivity of H^+

[9]Note that all correlation coefficients in this study are based on few samples (n=3-5), the correlation coefficients should therefore be considered together with the scatter plots.

loss of IS to matrix could therefore be to either pH or conductivity with the other working as a confounding factor, or to both. The correlation between matrix effects and DNOM and conductivity concentration in the aqueous solution is in accordance with a study on matrix effects on LC-ESI-MS/MS from fresh and estuarine water. This study shows large matrix effects in samples with high conductivity and high concentration of DNOM [62].

Due to sorption to DNOM and possibly other compounds in the soil water matrix, the concentration of free MMA-nitramine in the water based calibration solutions and the soil water based samples were not equal. MMA-nitramine can therefore not be used to correct for instrumental variation in soil water samples when a water based calibration curve is used. The data were therefore interpreted without using MMA-nitramine as an IS.

The concentrations calculated in the spiked soil blanks using the water based calibration curve, were used as the starting concentration for sorption calculations. The average water based calibration curve and spiked soil blank measurements are shown in Figure 24. The spiked soil blanks should correct for both sorption to DNOM and matrix effects on ionization. The MEA-nitramine signal for the spiked soil blank made for the peat soil sample (P-H_2) is higher than the 0.05 mg/L MEA-nitramine water based calibration solution signal. This indicates that the matrix in sample P-H_2 causes ion enhancement. The magnitude of this effect is difficult to judge as ion enhancement would increase the signal, while sorption to DNOM would decrease the signal as there is less MEA-nitramine in solution.

K_d was calculated using the concentration of the spiked soil blanks determined with the water based calibration curve as starting concentration. The principle of this calculation is described in Figure 20. K_d seem to correlate very well with OM content (LOI)(r=0.9987) and the aluminium content (r=-0.9983) in the soils. Negative correlations with ionic strength (conductivity) (r=-0.8128) in the supernatant and soil iron content (r=-0.6932) were also seen (Figure 25). All the correlation coefficients indicate strong associations between the variables. While the correlation coefficient between K_d and total iron content indicate strong association between the variables, the scatter plots shows that the correlations is mainly due to the one peat sample which is likely to have substantially different physiochemical OM characteristics compared to the other soil samples, due to different decomposition conditions. If this sample is removed the correlation coefficient becomes -0.2929. This is not true for the other correlations, where removal of sample P-H_2 does little to change the strong associations, r becomes 0.9435, -0.9432 and -0.9923 for K_d and LOI, total aluminium content and conductivity, respectively. The corre-

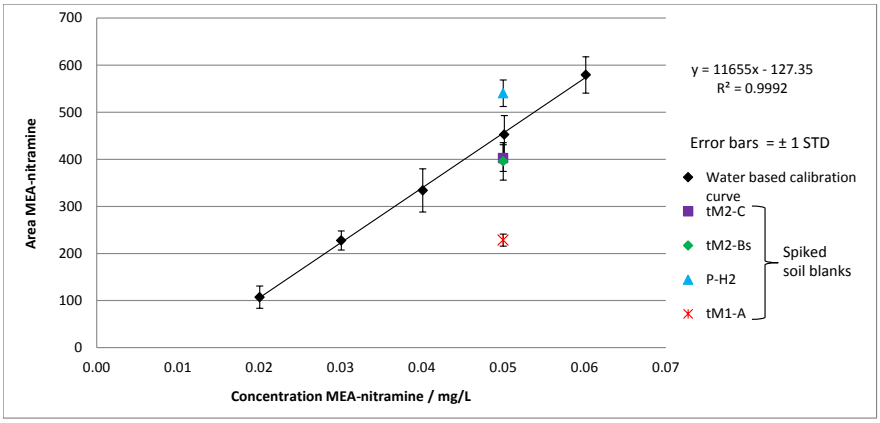

Figure 24 – Water based calibration curve with the measurements for the spiked soil blanks shown at the added concentration, 0.05 mg/L MEA-nitramine, to illustrate the varying effects of the matrices. The error bars represent ± 1 STD for the measurements replicates (n=3 for spiked soil blanks, n=9 for water based calibration solutions).

sponding scatter plots with linear regression lines are provided in Appendix D.9. The slopes of the linear regression curves does however change, approximately 10 % for K_d and LOI, 20 % for K_d and total aluminium content, and 75 % for K_d and conductivity. Note that the K_d values themselves are quite small. For three of the soils the $K_d < 1$ L/kg i.e. more of MEA-nitramine is left in the aqueous solution than is sorbed to the soil. Hence MEA-nitramine can be considered quite mobile in these soils. Also note that the highest K_d occur in the top soil horizon tested (H), and as deposition of atmospherically formed nitramines will be to the soil surface sorption to the the top soil layers can hinder mobility. The K_d values and measured values for pH and conductivity is provided in Appendix D.9.

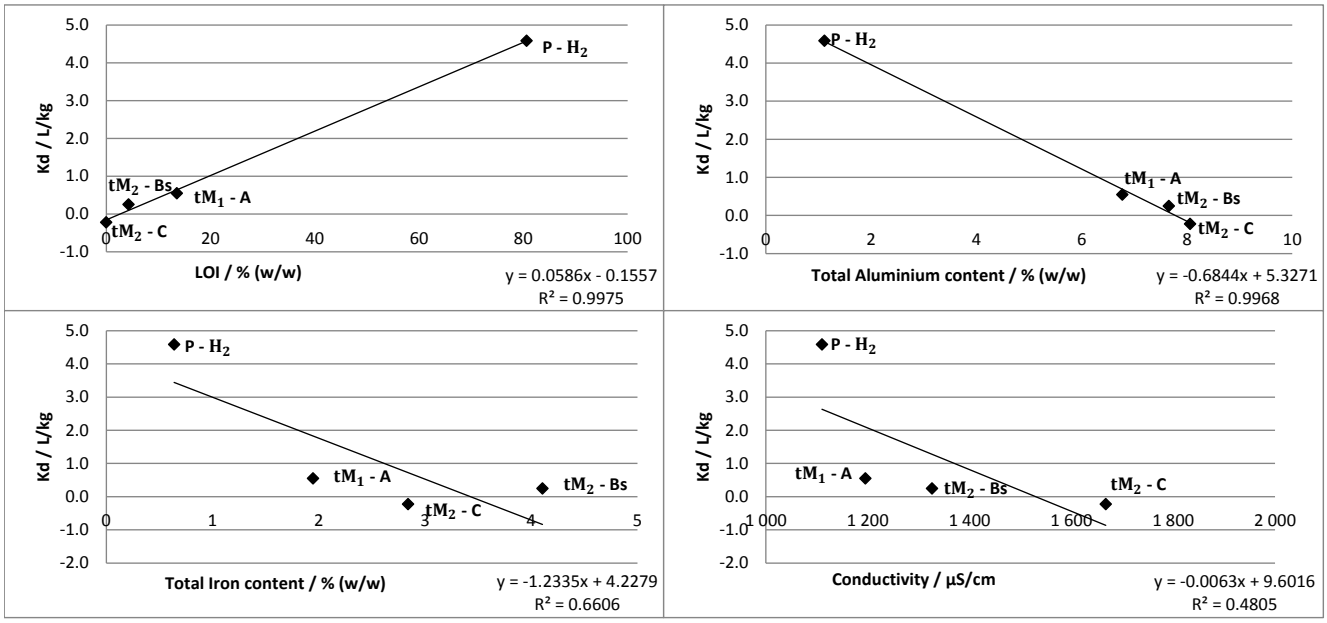

Figure 25 – Relationship between K_d and OM content (LOI) in the samples (top, left), total aluminium content in the samples (top, right), total iron content in the samples (bottom, left) and ionic strength (conductivity) in the samples (bottom, right).

Correlation with organic matter content indicate that the organic matter in the soil could be dominating sorption of nitramines to soil. If sorption to organic matter is dominating, the correlations between K_d and aluminium and iron content could be due to their relation with the organic matter in these samples (Figure 26). The correlation coefficients between LOI and total iron content and total aluminium content were -0.8400 and -0.9998, respectively. The opposite could also be true, that the relationship between K_d and iron or aluminium are confounding factors for the relationship between K_d and organic matter content, though that is unlikely as iron and aluminium ions bound to the organic material should theoretically increase the sorption of negatively charged nitramines through metal ion bridges. It should also be kept in mind that the aluminium and iron contents are given as total amount in samples and that their speciation therefore is unknown.

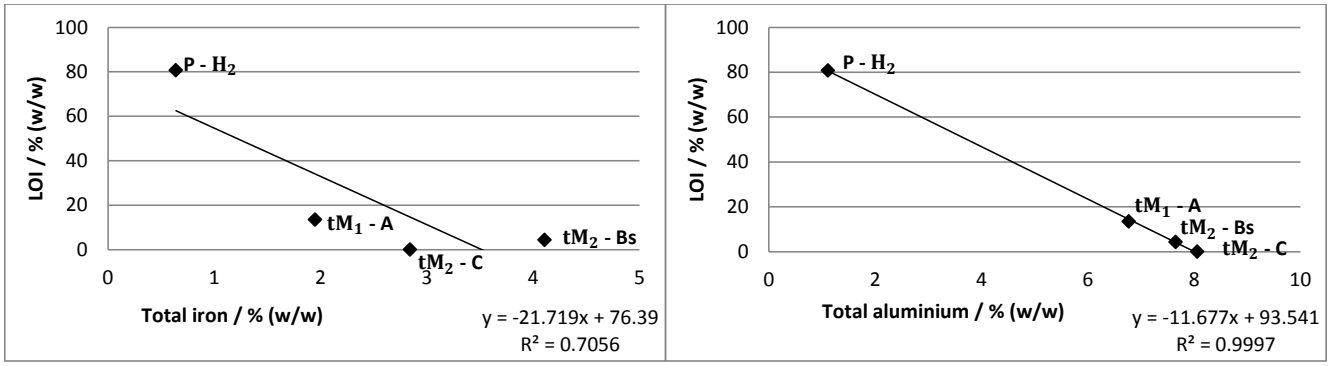

Figure 26 – Relationship between organic matter content (LOI) and total iron content (left) and aluminium content (right).

The uncertainty of the measurements must also be taken into consideration. There are several different aspects that contribute to the method uncertainty that should be considered. The uncertainty caused by the sample preparation and differences in leached DNOM from the soil, is reflected in the RSD for the sample replicates (n=3). In this test that uncertainty was between 7 and 10 %. The uncertainty caused by stability variation in the instrument during measurement is reflected by the RSD for the water based calibration solutions (n=9), their average RSD is 13 %. The relative error of the control solution was calculated to be 7 %, indicating that MEA-nitramine is relatively stable and not significantly sorbed to the glass walls. In addition the uncertainty caused by ion suppression/enhancement due to the sample matrix must be considered. As the spiked soil blanks were used in the sorption calculations, the matrix effects should be accounted for, however their use means another pretreatment and measurements and therefore a general increase in the uncertainty of the method. Considering these uncertainties together the method uncertainty for this experiment was estimated to be no larger than ± 15 %.

The loss of MMA-nitramine signal in three of the four studied soil samples were larger than what could be attributed to instrumental variation. This decrease in MMA-nitramine signal correlates well with the concentration of DNOM in the samples ($r = 0.9924$). If the same trend is true for other nitramines, sorption to DNOM will increase the nitramines mobility in the environment. The fact that MMA-nitramine is lost to DNOM in soil water solutions does however mean that MMA-nitramine can not be used as an IS when a combination of spiked soil blanks and a water based calibration curve is used for quantification. The sorption to soil (K_d), calculated without use of IS, correlate well with the samples OM content. Negative correlations between K_d and iron and aluminium is also observed, though

this is believed to be due to the negative correlations between OM and iron and aluminium. The K_d values are quite small, $K_d < 1$ L/kg for three of four soils, hence MEA-nitramine can be considered quite mobile in these soils. The highest K_d occur in the top soil horizon. Deposition of atmospherically formed nitramines will be to the soil surface, the sorption to the top layers in the soil can therefore hinder mobility.

4.2.10 Investigation into Reduction in Loss of MMA-nitramine to Soil Water Matrix

In order to investigate if loss of IS (MMA-nitramine) to matrix could be reduced by different sample pretreatment a simple filter and centrifugation test was performed. Centrifugation of the sample aliquots for LC-MS/MS analysis was conducted at 16 100 g for 60 minutes. It had not been tested whether this process removed particles as efficiently as filtration with 0.45 µm pore size filters. It would therefore be interesting to check if centrifugation was as effective as filtrating the samples, and if large particles (> 0.45 µm) were present in the centrifuged solution that could increase the sorption of MMA-nitramine. Three soil samples were chosen with different OM content based on soil LOI values, since DOC in the supernatant was not yet measured. One sample with low, one with medium and one with high OM content was chosen, as well as one water based control sample (Samples tM_2-O, tM_2-C and TM_2-H_1, see Table 6). All samples were split into equal aliquots that was either centrifuged or filtered. Equal amounts of IS was added to 1 mL pretreated sample for a resulting concentration of 1 mg/L MMA-nitramine. The water based control sample (no soil) was also analysed without pretreatment (blank). Three available membrane filters with 0.45 µm pore size were chosen; two with regenerated cellulose (RC) membranes from different manufacturers and one with a polyethersulfone (PES) membrane. Filter and centrifuge specifications are provided in Appendix D.10.

The effect of the different pretreatments were judged by the effect the matrix had on MMA-nitramine signal (Figure 27). MMA-nitramine was added after the pretreatment. The average RSD of the measurement replicates (n=3) was 6 %. The different pretreatments appear to have no effect on the matrix composition and thus no effect on the MMA-nitramine signal, as the RSD for the different samples with all pretreatments included are low (5-8 %) and within the same range as the RSD of the measurement replicates (Table 38 in Appendix D.10).

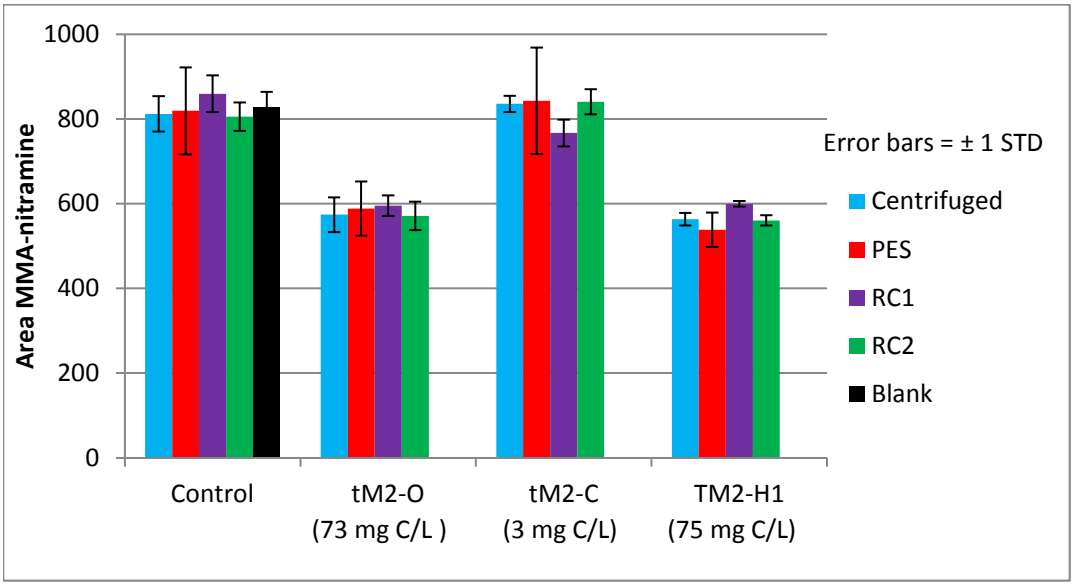

Figure 27 – MMA-nitramine area for each pretreatment for each sample. DOC values (mg C/L) are reported for the soil water samples. The error bars represent ± 1 STD based on the measurement replicates (n=3). Centrifuged = centrifuged at 16 100 g for 60 minutes, PES = polyethersulfone membrane filter (0.45 µm pore size), RC1 and RC2 = regenerated cellulose membrane filters from different manufacturers (0.45 µm pore size), and Blank = no pretreatment. Filter, centrifuge specifications and detailed area measurement values are provided in Appendix D.10.

There are clear differences in recovered concentration (represented by peak area) of the free MMA-nitramine between samples (Figure 27). These differences seems related to the DNOM concentration in the samples. The DNOM (DOC) concentrations for the samples supernatants are provided in Table 6, together with the soil OM content (LOI) as these were the basis for the choice of samples. Unfortunately difference in OM content between sample tM_2-O and TM_2-H_1 were not reflected in different amounts of DNOM. The fact that the loss of MMA-nitramine in these two samples is so similar does however give further evidence to the theory that loss of MMA-nitramine signal is mainly due to sorption to DNOM. Filtering the samples was not expected to change the negative correlation between free MMA-nitramine and DOC, as DOC is operationally defined as particles smaller than 0.45 µm and was as such also measured in 0.45 µm filtered soil water supernatant (Chapter 3.4.4).

Table 6 – The OM content (LOI) and DNOM concentration (DOC) for the three soil samples.

	tM_2-O	tM_2-C	TM_2-H_1
OM content (LOI, % (w/w))	32.6	0.0	69.6
DNOM concentration (DOC, mg C/L)	73	3	75

Pretreatment by centrifugation or by filtration with different types of 0.45 µm filters, did not alter the concentration of MMA-nitramine in the three soil sample supernatants or the water based control solution. Possible sorption to larger particles (< 0.45 µm) do not appear to have any influence on the loss of MMA-signal. The negative relationship between MMA-nitramine signal and DOC should thus be unaltered by the pretreatments. There is therefore no reason to filtrate rather than centrifuge the samples before LC-MS/MS analysis.

4.2.11 Solid Phase Extraction

If the matrix components that influence loss of MMA-nitramine signal (e.g. DNOM) could be separated from the analyte this would simplify the analysis method significantly, since MMA-nitramine concentration in "soil water" samples would be the same as in the water based calibration solutions. If the MMA-nitramine concentration was the same in all solutions it could be used to correct for instrumental variation. Water based calibration curves and spiked soil blanks with MMA-nitramine as IS could then be employed for quantification of MEA-nitramine in the samples, as was tried in the sorption experiment described in Chapter 4.2.9.

Extraction of the MEA-nitramine from the sample matrix by solid phase extraction (SPE) was tested as outlined in Chapter 3.5.3 and detailed in Appendix D.11. A C_{18} column was chosen since MEA-nitramine show some retention on the C_{18} column employed in LC, and as it was available. Soil sample P-H_2 was chosen as test sample as it has a high concentration of DNOM (DOC = 143 mg C/L) and showed a significant loss of MMA-nitramine signal in the sorption experiment described in Chapter 4.2.9 (Figure 23). The SPE method principle is shown in Figure 28, the "names" of the collected flow-through are shown in black boxes.

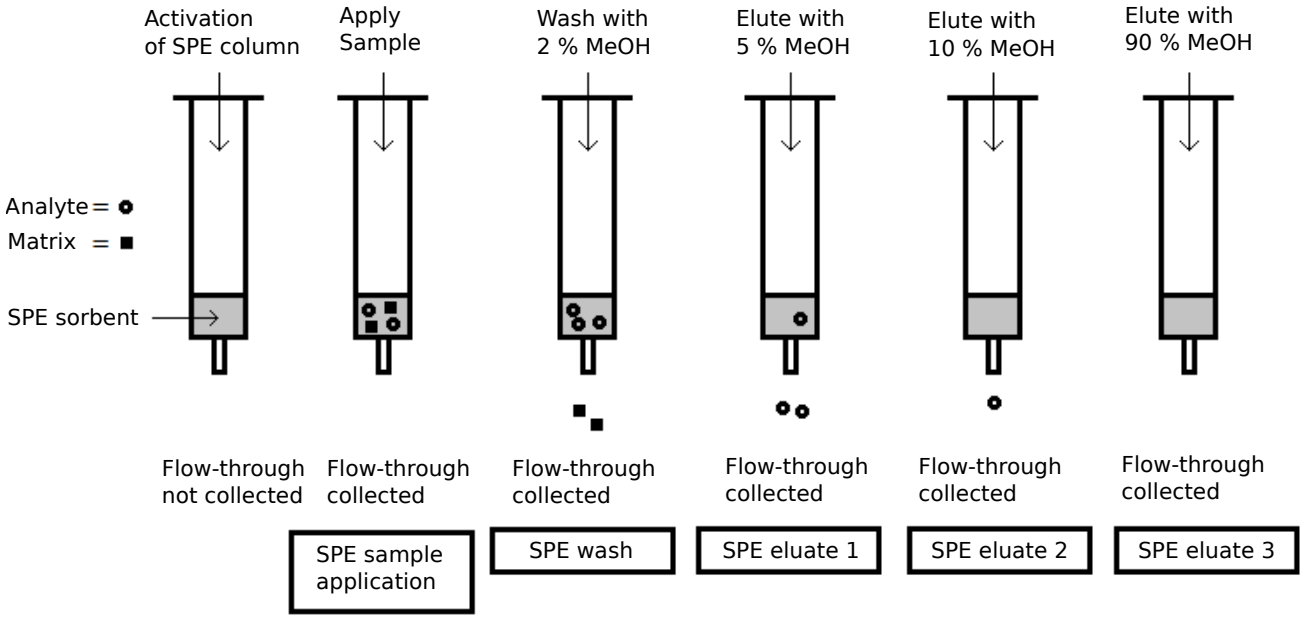

Figure 28 – Principle of SPE. The figure is "read" from left to right. The analyte should be retained on the SPE sorbent, while the matrix is washed out. The analyte is then eluted with a solution with stronger elution strength. The "names" of the collected flow-through are shown in the black boxes.

Breakthrough in the column was observed, i.e. MEA-nitramine was present in the sample application and the wash application flow-through. In the three eluates MEA-nitramine concentration was below the detection limit. Details are provided in Appendix D.11. MEA-nitramine was therefore not retained strongly enough on the SPE sorbent. This same problem was reported by Brakstad et al. [27][10]. If the interfering matrix components were retained on the SPE sorbent, SPE could still be used to separate analyte form matrix, as analyte and matrix would be in different collected flow-through's. The IS (MMA-nitramine) was added to the collected flow-through's from the SPE before analysis, to check if the loss of signal to matrix was eliminated or occurred in a collected flow-through that did not contain MEA-nitramine.

Loss of IS signal to matrix still occurred, as the MMA-nitramine peak area was much lower in the sample application flow-through than in the collected flow-through's from the wash and elution steps (Figure 29). This implies that the matrix components that are mainly responsible for sorption of MMA-nitramine

[10]Column sorbent was not reported, but assumed to be activated carbon as that was used in a prior procedure development study where Brakstad and Zahlsen were contributing authors [26]

are hydrophilic as they are not retained on the C_{18} column and found in the application flow-through. As MEA-nitramine and the matrix components are in the same flow-through, C_{18} based SPE can not be used to separate them.

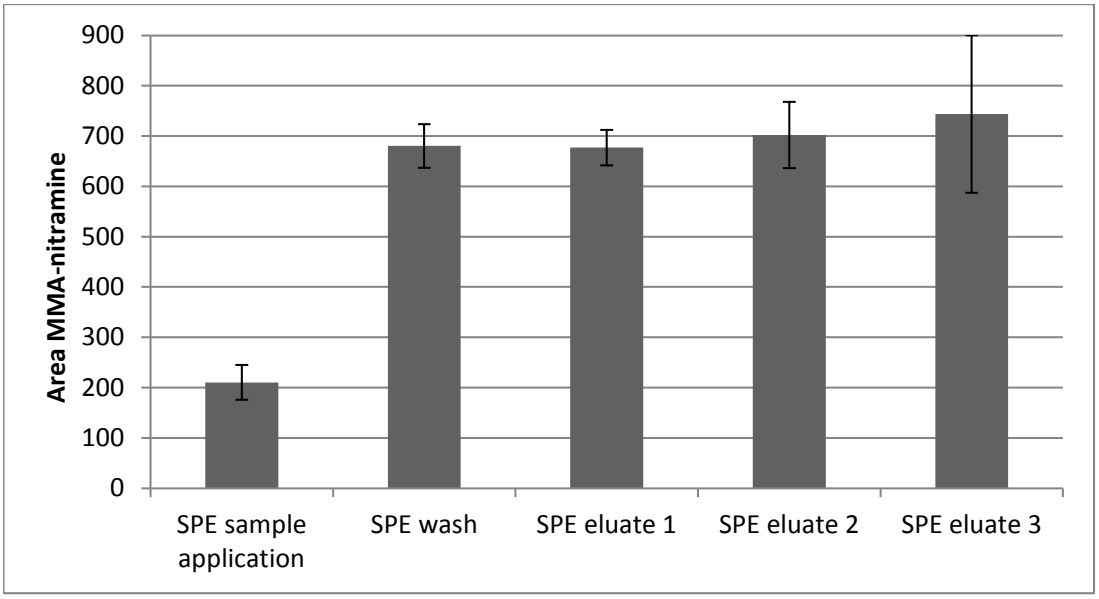

Figure 29 – MMA-nitramine area for the different SPE flow-through's. The error bars represent ± 1 STD calculated from the measurement replicates (n=3). *SPE sample application* is the collected flow-through from the sample application. *SPE wash* is the collected flow-through from the wash step and *SPE eluate 1-3* are the collected flow-through's from the elution steps.

The signal in the SPE sample application flow-through is ∼ 70 % lower than the signal in SPE wash. This is twice as high as the ∼ 34 % loss of signal observed for MMA-nitramine in the same soil sample in the experiment described in Chapter 4.2.9. The same soil, instrumental conditions and concentrations of MEA- and MMA-nitramine are employed. The difference in observed loss does therefore seem to be related to the sample preparation (SPE), how this specifically effects sorption of MMA-nitramine is difficult to assess.

If the SPE with C_{18} sorbent had been able to separate analyte and matrix the challenges with possible loss of analyte to the SPE sorbent would have to be addressed. Generally an IS is added before SPE, so loss to sorbent material can be corrected for. Obviously this would not have been possible with the present analyte, matrix and IS.

SPE with a C_{18} column can not be used to separate MEA-nitramine from the components in matrix that cause loss of signal in the added IS (MMA-nitramine). Matrix matched soil water based calibration curves for each soil sample must therefore be employed. The SPE results does however indicate that the main parts of the DNOM that binds MMA-nitramine are hydrophilic, as the largest loss of MMA-nitramine signal is observed in the collected SPE sample application flow-through.

4.2.12 Sorption to Soil with 5.01 mg/L MEA-nitramine and with Matrix Matched Soil Water based Calibration Curves

Another sorption experiment attempt was made using the same samples as described in the sorption experiment in Chapter 4.2.9, but with higher MEA-nitramine concentration (5.01 mg/L) and matrix matched soil water based calibration solutions. The goal was to access if the K_d values and correlations deduced from the previous sorption experiment (Chapter 4.2.9) could be confirmed with a lower uncertainty due to higher concentration (higher signal-to-noise ratio, S/N) and use of IS with matrix matched calibration solutions in the LC-MS/MS method. The ventilation system in the building was still out of order so that the experiment was carried out at a 26 ℃.

To check the instrument stability for determination of MEA-nitramine in one water based sample and two different soil water based samples (organic sample = P-H_2 and mineral sample = tM_2-C) a test was performed over 10 hours. The measurements showed that the instrument was very stable, with STD for the integrated areas between 2-3 % (Figure 30).

Moreover the variation of the measurements, represented by the STD (2 % water, 3 % organic soil water, and 2 % mineral soil water), does not seem to be influenced by the matrix of the samples. However the magnitude of the analyte signal (peak area) is strongly influenced. Both soil water based samples have a clearly lower signal for MEA-nitramine than the water based sample. This is contradictory to the findings in previous experiments which showed an enhanced signal for MEA-nitramine in P-H_2 soil water, while MEA-nitramine in tM_2-C soil water had a slightly lower signal, both relative to MEA-nitramine in a water based sample/calibration solution (see spiked soil blanks in Figure 24). The only difference between these experiments is the concentration of the added MEA-nitramine. With a much higher MEA-nitramine concentration the effects of the matrix on sorption to DNOM and ionization appears to be different. Making assessments regarding the cause(s) for the differences observed between MEA-nitramine signal in soil water based samples relative to signal in water based samples between different experiments is very difficult due to the combination of various uncertain-

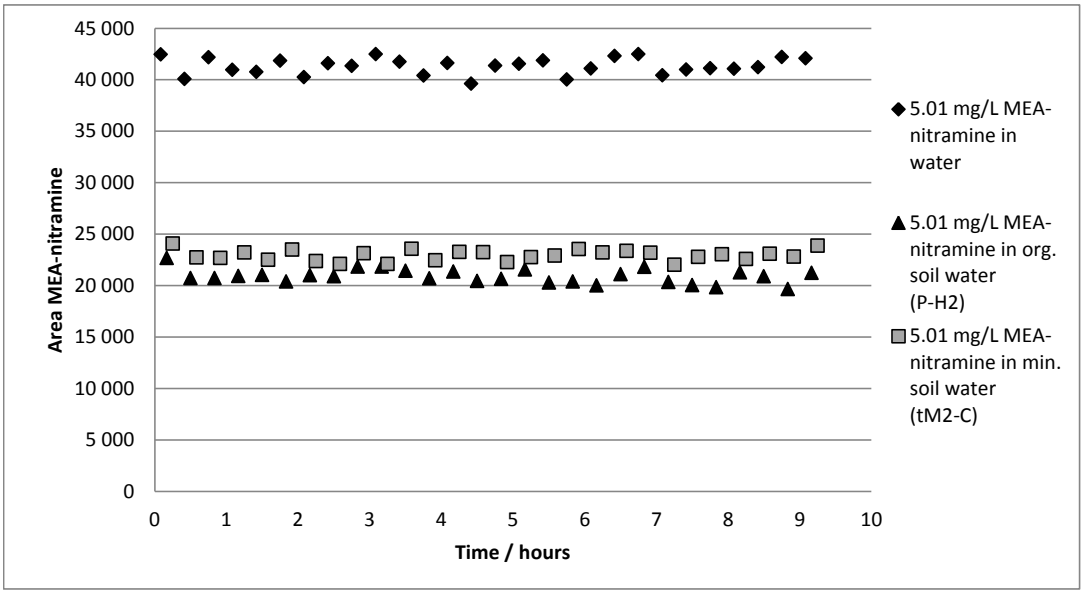

Figure 30 – Proxy for the stability of the instrument displayed as the area of repeated measurements of MEA-nitramine in a water based sample, an organic soil water based sample and a mineral soil water based sample, over time.

ties, especially the effects that influence the sorption of MEA-nitramine to DNOM and ionization of MEA-nitramine, and the lack of control and knowledge of these variables. In addition these observations are from only two different experiments, and could thus just be arbitrary.

A fourth solution with 0.05 mg/L MEA-nitramine in a water based sample was analysed along with the other samples in order to compare the concentration effect on instrumental variation. The measurements for this sample were not reported as the background level gradually increased, during the analysis. S/N started at approximately 50 and decreased to approximately 6. The increase in background was likely due to a build-up of MEA-nitramine somewhere after the column in the LC-MS/MS (probably on the spray needle or cone of the MS, cf. Figure 34). The effect of this could be seen in all the measurements, but did not make a significant impact on the S/N in the samples with higher concentrations of MEA-nitramine (5.01 mg/L).

For the sorption experiment it was prioritised to analyse as many different samples as possible so that an assessment of the results from the previous experiment could be conducted, rather than have sample replicates (only 10 bottles can fit the end-over-end shaker). Five soils (tM_1-O and the four from the previous sorp-

tion experiment described in Chapter 4.2.9) were chosen so that one replicate and one blank for each soil sample could be prepared at the same time. Both matrix matched soil water based and water based calibration solutions were prepared.

Water based calibration solutions were included in order to monitor the stability of the instrument and for determination of control solution concentration. IS was not added to the water based solutions. The graphs in Figure 31 shows the resulting calibration curves for the water based calibration solutions measured before, in the middle of, and after the set of samples for two different analyses on the LC-MS/MS. The first analysis resulted in water based calibration solutions with RSDs greater than 65 % for all concentrations (top graph). The samples were therefore re-analysed (bottom graph). The instrument was more stable during the re-analysis, but still with unacceptably high RSDs for the water based calibration solutions (20-30 %). A large change in stability between the analyses was observed, but the reasons for this is unknown (Figure 30 and 31).

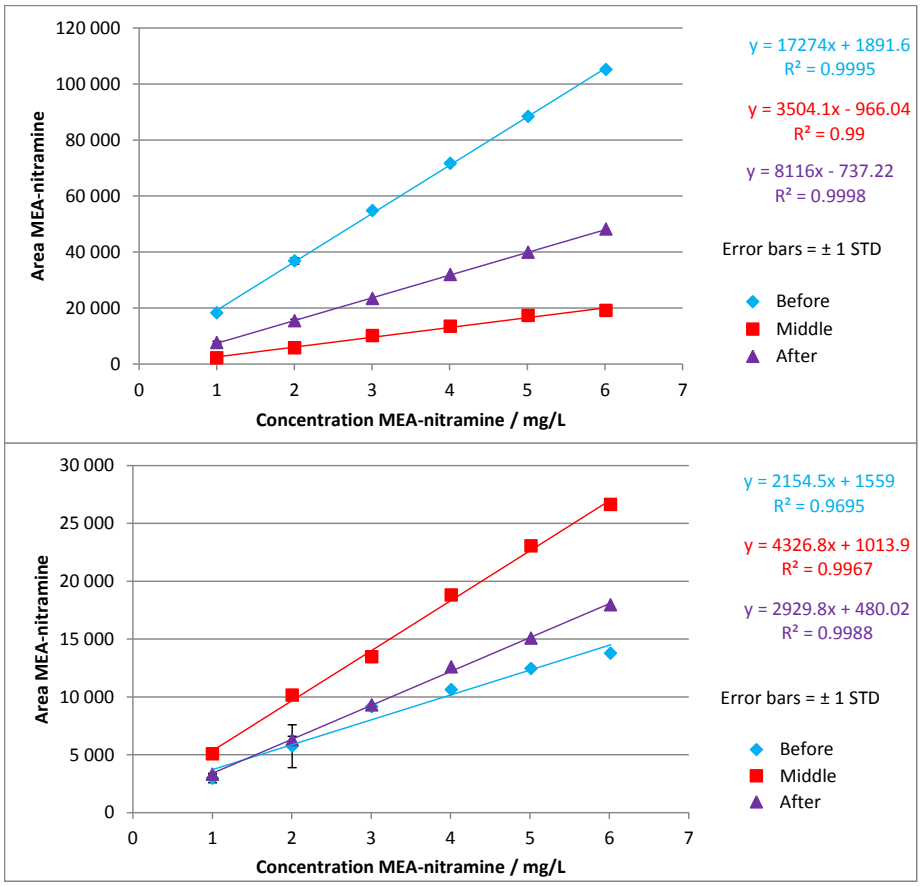

Figure 31 – Water based calibration solutions measured before, in the middle of, and after the set of samples, each time with three measurement replicates for each solution. The error bars represent ± 1 STD for these measurement replicates (n=3), note that for most of the solutions the error bars are smaller than the markers. The calibration curves from the first analysis (top graph) and re-analysis (bottom graph).

During both analyses the 3 mg/L MEA-nitramine water based calibration solution was analysed approximately every 10th injection, so that the stability over time could be monitored (Figure 32). As the first washing step[11] in the first analysis seemed to have had a negative impact on the signal strength, the washing steps were removed from the re-analysis sequence.

[11]Washing step: gradual increase to 100 % (v/v) MeOH (with 2 mM AcA), kept at 100 % (v/v) MeOH for 20 minutes, then a gradual decrease to normal MP ratio (30/70 MeOH/water) and conditioning of column for 20 min.

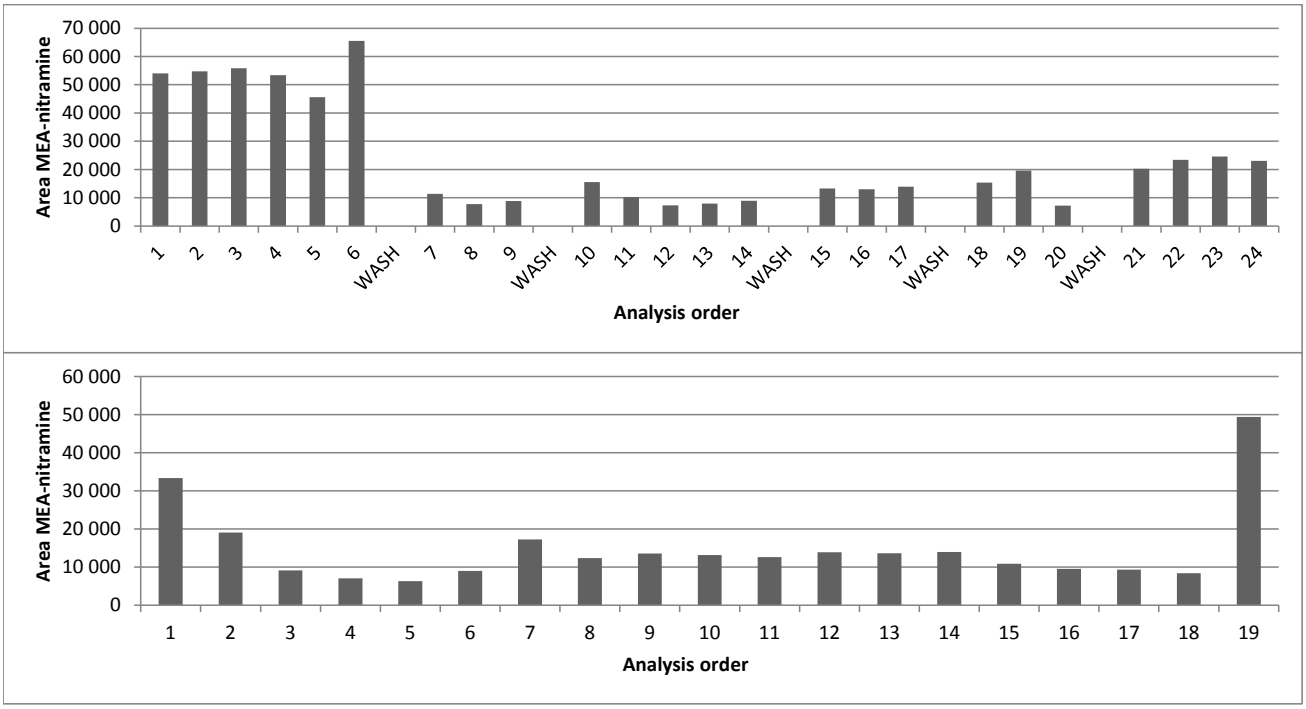

Figure 32 – Signal area over time (analysis order) for the water based 3 mg/L MEA-nitramine calibration solution. First analysis (top bar plot) and re-analysis (bottom bar plot).

Removing the wash steps might have have led to a slightly improved stability, as the RSD for the re-analysis of the water based 3 mg/L MEA-nitramine calibration solution decreased from 79 to 69 %, but this could just as well be caused by random variation.

MMA-nitramine was added to the soil water based samples as IS and the samples were re-analysed again. Figure 33 shows the area for the MMA-nitramine and the 1 mg/L MEA-nitramine equivalent concentration[12] for the spiked soil blank and the matrix matched soil water based calibration solutions for each sample. The signal for MMA-nitramine does not vary in the same manner as the MEA-nitramine signal within each sample and therefore does not correct for the instrumental variation. This is very clear for sample P-H_2 (Figure 33, bottom). Note that between-sample variation should be expected for MMA-nitramine, as the different matrices (different concentration of DNOM) will sorb different concentrations of

[12]1 mg/L MEA-nitramine equivalent concentration is calculated by assuming a linear response between integrated area and concentration, i.e. the integrated areas for 6.02 mg/L calibration solutions were divided by 6.02 etc.

MMA-nitramine.

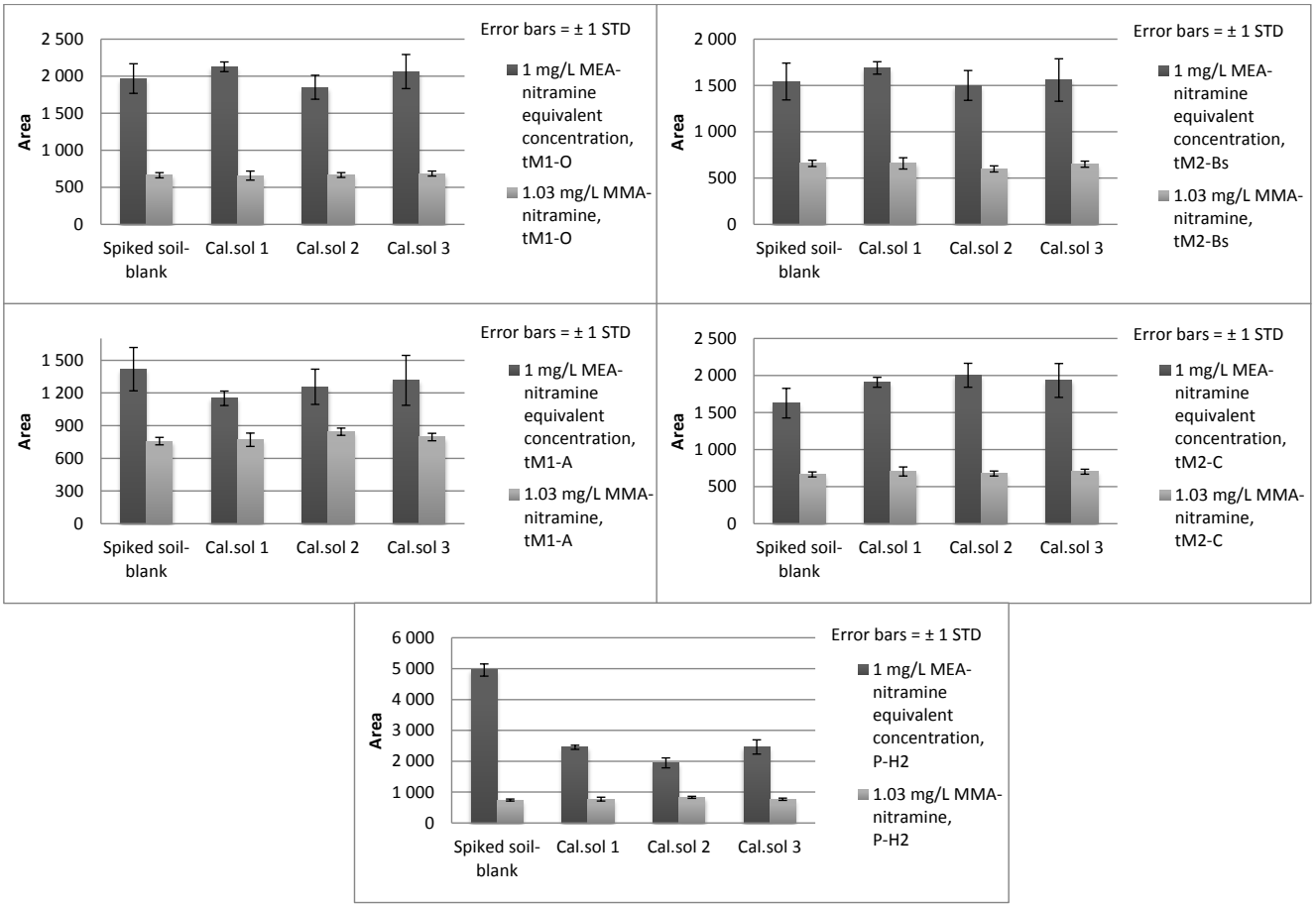

Figure 33 – Area of MEA- and MMA-nitramine for four soil water solutions for each soil sample. The MEA-nitramine areas are all adjusted to an equivalent concentration of 1 mg/L[12], the added MEA-nitramine concentration in the calibration solutions were 1.00 mg/L for Cal.sol 1, 3.01 mg/L for Cal.sol 2 and 6.02 mg/L for Cal.sol 3. MMA-nitramine was added for the same resulting concentration, 1.03 mg/L, in all soil water solutions. The error bars represent ± 1 STD for the measurement replicates (n=3).

The instrumental variation could therefore not be corrected by using MMA-nitramine as an IS. The sorption results using just MEA-nitramine are not presented, as the uncertainties were too large. Instrumental variation alone accounts for an uncertainty of at least 39 %[13] in this analysis.

[13]RSD of repeated measurement of 3.01 mg/L MEA-nitramine in water throughout the second

The difference in retention time between MEA- and MMA-nitramine (20 seconds) could explain why MMA-nitramine is not functioning as an internal standard. The matrix could have different effect on ion suppression/enhancement during the different time intervals of the analysis, i.e. the matrix components are separated by the LC column and thus not eluted simultaneously. This can be checked by a post column infusion experiment, explained in detail in Appendix D.14. This experiment was beyond the scope of this thesis as the instrumental set-up was not readily available. However if it is of interest to determine why MMA-nitramine did not function as an IS a post column infusion experiment can be conducted. If an isotope-labelled IS is used the retention time will be almost identical to that of the analyte, and this problem will not occur.

Instrumental variation larger than 65 % was observed for water based calibration solutions during an analysis sequence, indicating fundamental issues with the analysis method. MMA-nitramine was added as IS but did not vary similarly to MEA-nitramine in the samples, and could therefore not be used to correct for the instrumental variation. Use of MMA-nitramine as IS for MEA-nitramine in soil water based samples is therefore not an option. The large instrumental variations observed indicates that the analysis method on LC-MS/MS was not stable and that the method should be re-evaluated.

4.2.13 Re-analysis of Sorption to Soil with 5.01 mg/L MEA-nitramine and with Matrix Matched Soil Water based Calibration Curves, after Changes in MS Settings

As it now was clear that the instrument was not stable with the method employed and the author did not have the opportunity to spend any more time in the lab, colleges Dr. L. Zhu and MSc. C. B. Gundersen (University of Oslo) started investigating the stability of the analysis method on the LC-MS/MS for their own work. Their investigations led to the conclusion that the electrospray was not stable, probably due to accumulation of MEA-nitramine on the H-ESI capillary (charged electrospray capillary, see Figure 4). The accumulation of MEA-nitramine in the instrument system is illustrated in Figure 34. This figure shows the measured signal abundance for MEA-nitramine when the LC is reconnected after a direct infusion experiment[14], i.e. with only MP passing through the system and no injection of analyte. A gradual decrease in the MEA-nitramine signal (background) is seen over a long time period ($\sim$ 28 h), MEA-nitramine is obviously slowly released

re-analysis.

[14]Direct infusion: continuous direct injection directly into the MS, i.e. no separation (LC) is employed.

from surfaces in the MS instrumentation. The voltage potential was reduced and the MP flow, axillary spray pressure and capillary temperature increased to ensure a stable electrospray. New method details are provided in Appendix D.12.

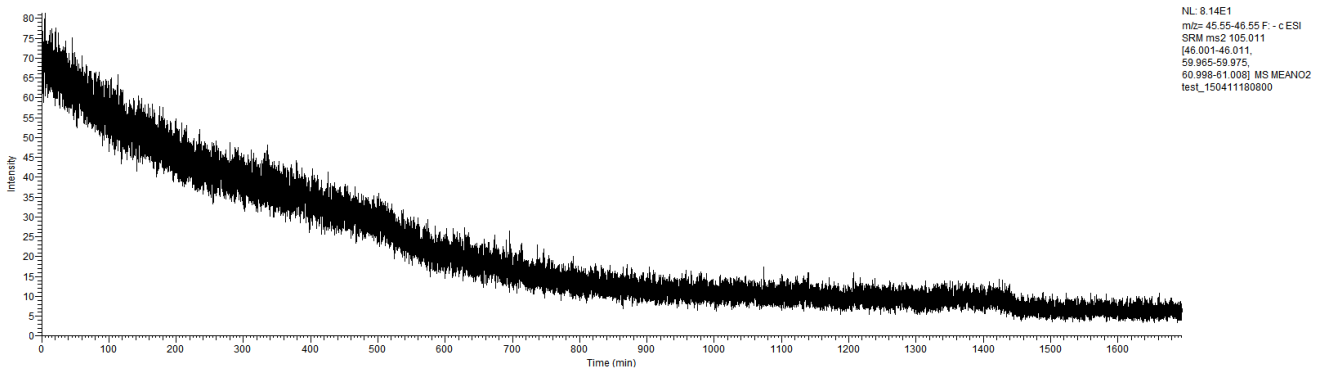

Figure 34 – Decrease in MEA-nitramine signal over $\sim$ 28 hours. Note that this is *after* a direct infusion experiment has been *stopped*, there is no injection of MEA-nitramine, only a continuous MP flow. There should therefore be no MEA-nitramine in the system.

The solutions from the sorption experiment described in Chapter 4.2.12 were re-analysed applying the new method. The new method was optimized for MEA-nitramine. The signal intensity of the acetic acid (the MP additive) throughout the analysis was used to monitor the stability of the electrospray. The RSD for the signal intensities of the acetic acid was 9 %. The RSD for the repeated measurement (approximately ever 10th injection) of the 3.01 mg/L MEA-nitramine calibration solution in water was 6 %. The RSD of the measurement replicates (n=3) for the soil water based samples and the matrix matched soil water based calibration solutions varied between 0 and 7 %. The relative error of the control solution was calculated to be 11 % which indicates the stability of MEA-nitramine in the aqueous phase is OK and no significant absorption to the glass walls occur. Note that the LC-MS vials had been stored (in the dark at 4 ℃) for almost a month before this re-analysis was performed. The good linearity seen in the soil water calibration curves (curves are provided in Appendix D.13) thus indicates that MEA-nitramine is stable in solution and not significantly sorbed to the glass walls. In the experiment described in Chapter 4.2.9 the uncertainty in the sample replicates were 10 % or lower, as the stability of the instrument was better in the present experiment the uncertainty related to each soil can be assumed to be no higher than 10 %. The total method uncertainty can then be estimated to be less than $\pm$ 11 %. The stability of the method should therefore be better than in

previous experiments. In addition the use of matrix matched soil water based calibration curves should reduce the uncertainty further, as the calibration solutions for a given soil are measured right after the soil water based sample for that soil, i.e. the time between measurements for one soil, was short while the variations in instrument stability occur on a larger time scale (see Figure 48 in Appendix D.13).

K_d was calculated for the different soil samples using each soils matrix matched soil water based calibration curve. In order to assess if the trends observed in the sorption experiment described in Chapter 4.2.9 could be confirmed, the same four samples were assessed first. Looking at the four samples that was analysed in the sorption experiment described in Chapter 4.2.9, similar relationships between K_d with OM content (LOI) (r= 0.9970), total aluminium content (r=-0.9957), total iron content (r=-0.8217) and ionic strength (conductivity) (r=-0.6969) were observed (Figure 35). All the correlation coefficients indicate strong associations between the variables.

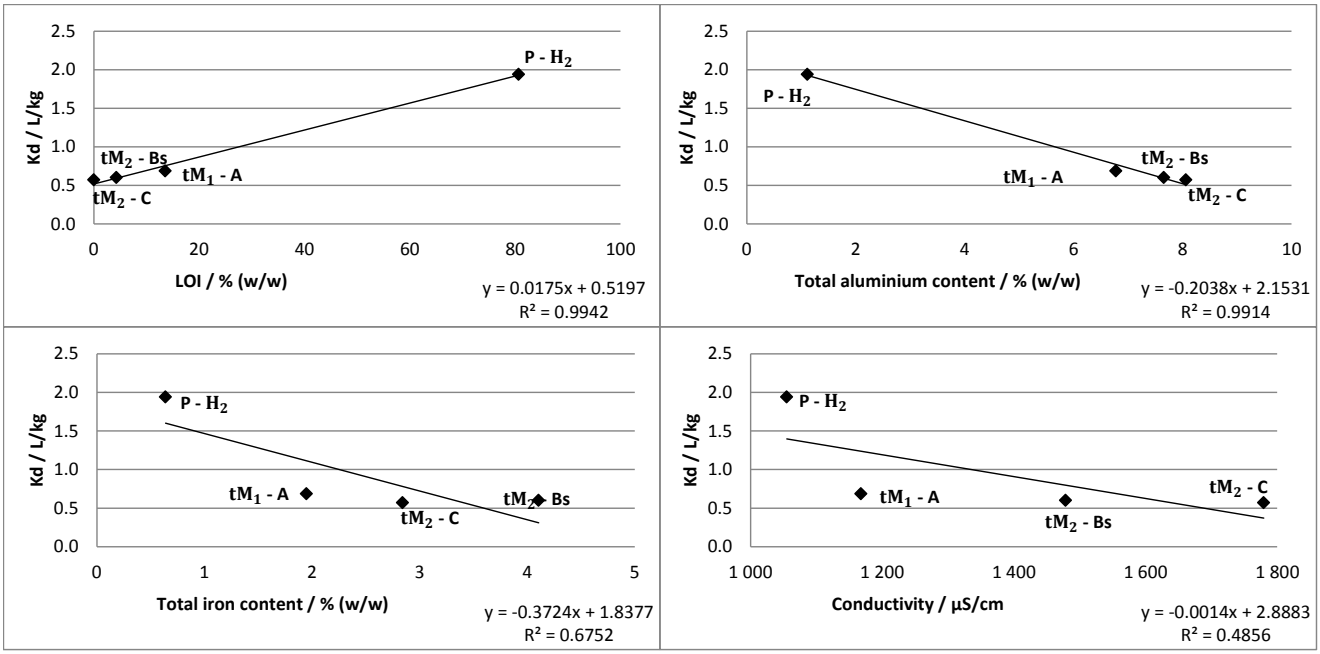

Figure 35 – Relationship between K_d and OM content (LOI) in the samples (top, left), total aluminium content in the samples (top, right), total iron content in the samples (bottom, left) and ionic strength (conductivity) in the samples (bottom, right).

If the fifth sample (tM_1-O) is included, the trends are not that clear (Figure 36).

The correlation coefficients are now 0.7880, -0.7537, -0.7702 and -0.4342 for the associations between K_d and LOI, total aluminium content, total iron content and conductivity, respectively. These correlation coefficients still indicate strong associations, except between K_d and conductivity. The correlation between K_d and iron content is a bit lower than what was seen with four samples (both in this experiment and the experiment described in Chapter 4.2.9), but it is no longer so dependent on the one peat soil sample (P-H_2), implying that the correlation might actually be there. K_d values and measured values for pH and conductivity is provided in Appendix D.13.

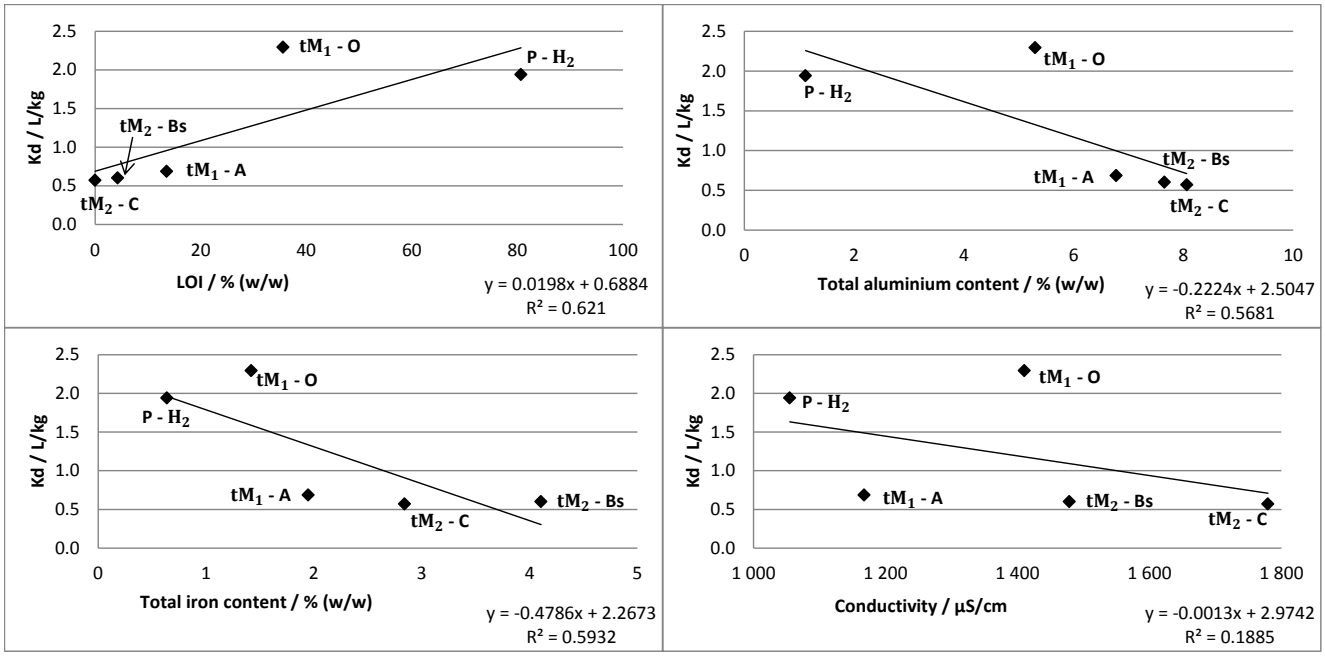

Figure 36 – Relationship between K_d and OM content (LOI) in the samples (top, left), total aluminium content in the samples (top, right), total iron content in the samples (bottom, left) and ionic strength (conductivity) in the samples (bottom, right).

However the P-H_2 sample is now dominating the relationships between K_d and the soils OM (LOI) content and aluminium content. As mention above the OM in sample P-H_2 is likely to have different physiochemical properties compared to the others. If this sample is removed, clear exponential associations between K_d and the soils OM content (LOI) and K_d and the soils aluminium content are seen (Figure 37).

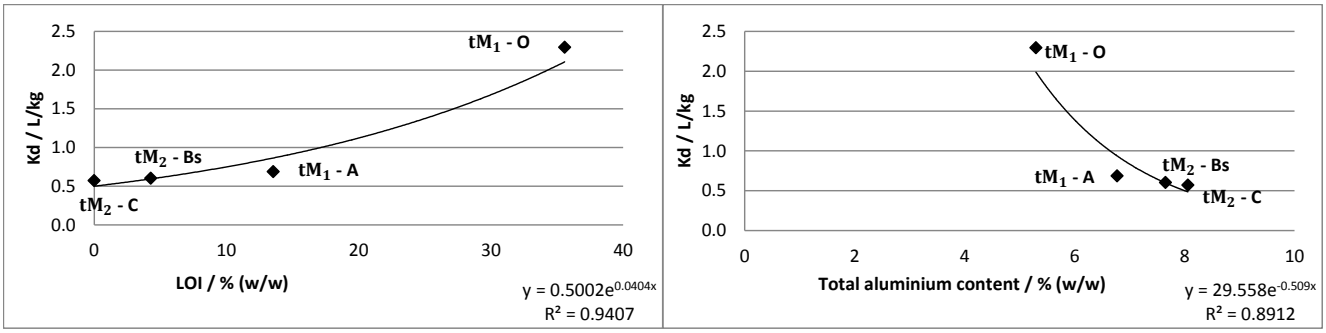

Figure 37 – Relationship between K_d and OM content (LOI) in the samples, P-H_2 not included, (left). K_d and total aluminium content in the samples, P-H_2 not included, (right).

That different relationships were seen with and without the peat sample, implies that the assumption about the differences in OM physiochemical properties are accurate. It should therefore be noted that soil parameters that are not accounted for, e.g. physiochemical characteristics of OM and DNOM, could play vital roles in explaining the sorption of MEA-nitramine to soil. The aluminium content is still almost perfectly correlated with OM content (Figure 38), and thus follows the same trend. The correlation coefficient between OM content and iron and aluminium content in the soils for all five samples are -08345 and -0.9985, respectively (Figure 38).

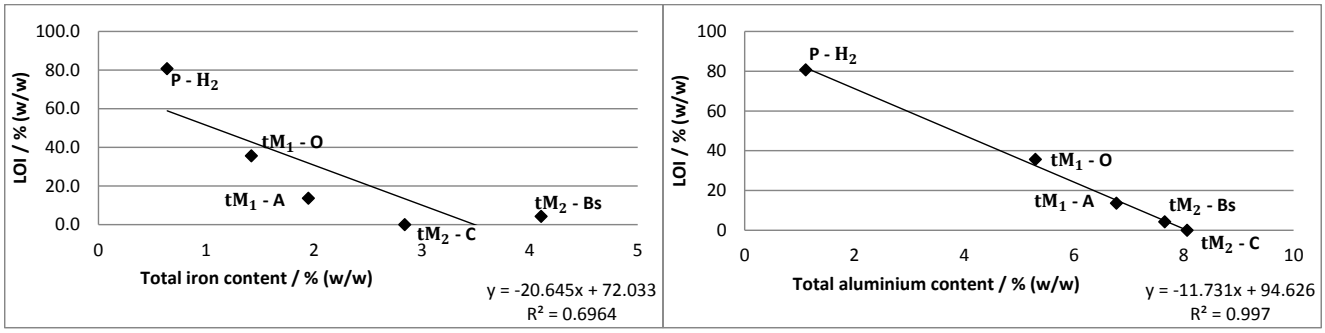

Figure 38 – Relationship between OM content (LOI) and total iron and aluminium content in all five soil samples.

The same correlations deduced in the experiment described in Chapter 4.2.9 is also deduced in this experiment, where the added concentration of MEA-nitramine is 2 orders of magnitude higher. Sorption of MEA-nitramine correlates strongly with the OM content in the soils as well as the soils iron and aluminium content. That

the correlations can be reproduced at different concentrations of MEA-nitramine increase their credibility. The OM in the peat soil sample (P-H_2) seem to have different physiochemical properties than the OM in the other samples, thus it relates differently to the sorption of MEA-nitramine. The K_d values are different than the ones calculated in the experiment described in Chapter 4.2.9, especially for the peat soil sample (P-H_2), which can be due to the uncertainties in the methods and/or due to the difference in added MEA-nitramine concentration, i.e. the sorption isotherms are not linear for the considered concentration range.

5 Conclusions

With the possible implementation of amine based PCC plants worldwide, the fate of nitramines, as one of the degradation products from emitted amines, is of environmental concern. The nitramines ability to sorb to soil will determine if they accumulate in the soil column or are transported to groundwater and freshwater rivers and lakes. The main goal of this thesis was therefore to assess the soil physiochemical characteristics that govern the ability of soil to sorb nitramines. This was examined by sorption experiments. A soils ability to sorb MEA-nitramine (K_d) seem to be mainly related to its OM content, as indicated by strong correlations between K_d and OM content. The highest K_d values occur therefore in the top soil horizons (H and O), however these K_d values are smaller than 5 L/kg. In the mineral A, Bs and C soil horizons the K_d is especially low. With K_d's < 1 the concentration of free MEA-nitramine in the aqueous phase is higher than what is sorbed to soil. MEA-nitramine can thus be expected to be quite mobile in these soil horizons. In addition will sorption to DNOM also enhance the mobility of MEA-nitramine in the environment.

Accurate determination of free MEA-nitramine in soil water was a challenge due to the effects of constituents in the soil water matrices and the properties of the analyte itself. Both ion suppression/enhancement effects and sorption to DNOM are suspected to play important roles. In addition large variations in the analyte signal on the LC-MS/MS analysis method were observed. This poor accuracy and precision seem to be related to both traditional instrumental variations (injection volume, ionization potential etc.) as well as a build-up of MEA-nitramine in the instrument system.

MMA-nitramine was tested as IS to correct for instrumental variation. The initial results were promising, though sorption of MMA-nitramine to DNOM in the sample matrix meant that matrix matched calibration curves had to be employed. The loss of MMA-nitramine signal correlated strongly with the DNOM concentration measured in soil supernatants. Separation of analyte and matrix with SPE with a C_{18} sorbent was tested and rejected due to breakthrough in the SPE column during sample application. The analysis of the collected SPE flow-thorough, did however indicate that MMA-nitramine mainly sorbs to the hydrophilic fractions of the DNOM. Further experiments showed that the analytical signal of MMA-nitramine did not vary in the same manner as MEA-nitramines signal, and thus could not be used to correct for instrumental variation.

Experimental data from the sorption experiments were therefore processed without an IS. A completely developed accurate analytical method for determination of

nitramines in soil water is thus still pending. It has been determined that matrix matched calibration curves must be employed and that an IS should be used. In addition was sorption to DNOM observed. This means that the reported sorption in the pilot study by Mohr and Vogt [19] could largely be due to matrix effects on ionization and sorption to DNOM, as matrix matched calibration curves were not employed. This was further confirmed by re-analysis of a soil sample from the pilot study [19] which showed large differences in the calculated sorption when water based and matrix matched calibration curves were employed (35 % and -5 %, respectively).

This study confirms that sorption of nitramines to soil is dominated by the OM content in the samples. Sorption to the top soil horizons is therefore dominating. As deposition of nitramines will be to the soil surface this can serve to hinder the mobility of nitramines. Sorption to the DNOM is also observed, which would enhance mobility. However, the analytical determination of nitramine concentrations in a soil water matrix have been a large challenge, and the analysed sample population (n=5) is therefore small. Repetition of these experiments with a larger sample population and a validated analytical method for determining the concentration of nitramines in soil water is therefore required. To draw clear conclusions regarding the environmental fate of nitramines is therefore dubious based on the present data. Still it is possible to conclude that a fraction of nitramines will likely be sorbed to organic soil horizons, a fraction will be sorbed to DNOM and some will remain as free nitramine in the soils pore water.

5.1 Future Research

In order to acquire accurate sorption data the analytical method for determination of nitramine concentrations in soil water must be improved. Employing isotope-labelled nitramines is recommended for any further research on sorption of nitramines to soil using LC-MS/MS for determination of nitramine concentrations. Matrix matching the calibration solutions should be considered a must for further work unless proper sample pretreatment (matrix removing) methods are used. Standard addition instead of matrix matched calibration curves is an option. Though as the analyte is added to the samples, standard addition and matrix matching are very similar in this case. A possible alternative to both isotope-labelled IS and matrix matching might be matrix effect compensation through use of multi component post column infusion internal standards, as recently described for small molecules in urine samples on LC-TOF (time of flight) by Gonzlez et al. [63].

Investigation of sorption to DNOM should be conducted to access the mobility of the nitramines. In addition should further investigation into the role of different physiochemical properties of the DNOM and OM in the soil samples be conducted to check if certain properties are governing the sorption capacity of the OM and DNOM.

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Appendix

A Nitramines

The CAS numbers for the nitramines used in the thesis are provided in Table 7.

Table 7 – Nitramines with CAS number.

Name	Abbrevation	CAS number
N-nitroethanloamine	MEA-nitramine	74386-82-6
N-nitromethylamine	MMA-nitramine	598-57-2

B Sample collection

Sample collection information is shown in Table 8, 9 and 10. Note that in the horizon designations Bh/Bhs the *h* stands for accumulation of organic matter (humic material) and the *s* for illuvial accumulation of iron and aluminium sesquioxides [64]. Horizon designation, nature of lower horizon boundary, and number and size of roots are defined according to ISO 25177:2008 [64], which uses the FAO [65] system for horizon designation.

Table 8 – Sample Collection Details, part 1.

Sample	Sample Site	Horizon Designation	Horizon order	Depth (cm, from - to)	Nature of lower horizon boundary	Roots (no. , size)
tM_1-O	tM_1	O	1	0 - 2	Wavy	Common very fine
tM_1-A	tM_1	A	2	2 - 10	Wavy	Few very fine, many fine, few medium
tM_1-B	tM_1	B	3	10 - 50	Wavy	Few very fine, few fine
tM_2-O	tM_2	O	1	0 - 5	Diffuse and wavy	Common fine
tM_2-A	tM_2	A	2	5 - 30	Diffuse and wavy	Very few fine, very few medium
tM_2-Bhs	tM_2	Bhs	3	30 - 50	Diffuse and wavy	Few medium
tM_2-Bs	tM_2	Bs	4	50 - 85	Diffuse and wavy	None
tM_2-Bh	tM_2	Bh	5	85 - 100	Diffuse and wavy	None
tM_2-C	tM_2	C	6	100 - Sample taken to 180	Diffuse and wavy	None
TM_1-O/A	TM_1	O/A	2	5 - 15	Smooth	Common very fine, few fine
TM_1-B	TM_1	B	3	15 - sample taken to 25	Smooth	Few very fine
TM_1-O	TM_1	O	1	0 - 5	Smooth	Many very fine, many fine, very few medium
TM_2-H_1	TM_2	H_1	1	0 - 70	Diffuse	None
TM_2-H_1/H_2	TM_2	H_1/H_2	2	80 - sample taken to 120	Diffuse	None
P-H_1	P	H_1	1	10 - 60	Diffuse	None
P-H_2	P	H_2	2	60 - sample taken to 120	Diffuse	None

Table 9 – Sample Collection Details, part 2.

Sample Site	Date	Time	X - Coordinate	Y - Coordinate	Altitude (m)	Slope (°)	Slope (direction)	Weather
tM_1	27.03.2014	11.45	N6055'16.5"	E0510'46.3"	289	40 - 50	NW	Sunny, blue sky
tM_2	27.03.2014	13.45	N6055'06.9"	E0510'34.5"	211	40 - 50	W - NW	Sunny, blue sky
TM_1	27.03.2014	14.30	N6055'17.4"	E0510'21.6"	125	0	N/A	Sunny, blue sky
TM_2	27.03.2014	15.30	N6055'47.5"	E0510'15.3"	61	0	N/A	Sunny, blue sky
P	27.03.2014	16.30	N6055'42.5'	E0510'58.4"	51	0	N/A	Sunny, clouds

Table 10 – Sample Collection Details, part 3.

Sample	Vegetation	Soil Wetness	Comments	Time until dry (days)
tM_1-O	Birch, sphagnum moss, sedges, common juniper, cranberry heath	Slightly moist	Blocky soil	30
tM_1-A	Birch, sphagnum moss, sedges, common juniper, cranberry heath	Slightly moist		30
tM_1-B	Birch, sphagnum moss, sedges, common juniper, cranberry heath	Slightly moist		10
tM_2-O	Planted spruce wood, sample taken in road cut on cart road	Slightly moist		30
tM_2-A	Planted spruce wood, sample taken in road cut on cart road	Slightly moist	Much stones, blocky soil	10
tM_2-Bhs	Planted spruce wood, sample taken in road cut on cart road	Slightly moist	Much stones	30
tM_2-Bs	Planted spruce wood, sample taken in road cut on cart road	Slightly moist		30
tM_2-Bh	Planted spruce wood, sample taken in road cut on cart road	Very wet	Large stones	30
tM_2-C	Planted spruce wood, sample taken in road cut on cart road	Slightly moist	Large stones	10
TM_1-O/A	Grass, common juniper, moss	Slightly moist	Next to riverbed	30
TM_1-B	Grass, common juniper, moss	Slightly moist	Next to riverbed	10
TM_1-O	Grass, common juniper, moss	Slightly moist	Next to riverbed	30
TM_2-H_1	Grass, planted	Slightly moist	Boggy pasture	30
TM_2-H_1/H_2	Grass, planted	Moist	Boggy pasture	30
P-H_1	Bog	Moist	10 cm sphagnum moss on top	30
P-H_2	Bog	Very wet	10 cm sphagnum moss on top	30

C Determination of Soil Properties, Raw data and Method and Instrument Details

C.1 pH: Calculation of Averages, STD and RSD

As pH is a logarithmic value, averages, STD and RSD must be calculated via H^+ concentration. Assuming the activity coefficient (γ) for H^+ ions are 1, the definition of pH can be written as shown in Equation 8, with the concentration of H^+ ions given in mol/ dm^3 [52].

$$pH = -log[H^+] \tag{8}$$

The measured pH data for the samples with 3 replicates are shown in Table 11, together with calculated concentration, average, STD and RSD for H^+ as well as the corresponding average pH. Note that significant number of digits is not used, as calculation were done on the raw data.

Table 11 – pH measurements for the samples, with all reported decimal places.

Sample	Temp. T(℃)	pH	$[H^+]$ (mol/ dm^3)	Average (mol/ dm^3)	STD (mol/ dm^3)	RSD (%)	Corresponding average pH
tM_2-C - rep.1	22.5	5.43	3.72E-06				
tM_2-C - rep.2	22.6	5.39	4.07E-06				
tM_2-C - rep.3	22.6	5.41	3.89E-06				
tM_2-C				3.89E-06	1.79E-07	4.6	5.41
TM_1-O/A - rep.1	22.3	4.53	2.95E-05				
TM_1-O/A - rep.2	22.3	4.52	3.02E-05				
TM_1-O/A - rep.3	22.4	4.54	2.88E-05				
TM_1-O/A				2.95E-05	6.80E-07	2.3	4.53
P-H_2 - rep.1	22.2	4.23	5.89E-05				
P-H_2 - rep.2	22.2	4.24	5.75E-05				
P-H_2 - rep.3	22.3	4.24	5.75E-05				
P-H_2				5.80E-05	7.74E-07	1.3	4.24

C.2 Dry Matter and Organic Matter Content

The dry matter content measured in the soil samples are provided in Table 12.

Table 12 – Dry matter content in the soil samples.

Sample	Dry matter content (% w/w)
tM_1-O	95.8
tM_1-A	97.9
tM_1-B	96.3
tM_2-O	92.2
tM_2-A	96.2
tM_2-Bhs	96.2
tM_2-Bs	95.5
tM_2-Bh	94.0
tM_2-C	99.6
TM_1-O/A	96.9
TM_1-B	98.9
TM_1-O	94.9
TM_2-H_1	91.0
TM_2-H_1/H_2	90.2
P-H_1	91.2
P-H_2	90.6

The LOI values for the mineral soil samples needed to be corrected for clay content. The correction was done by subtracting a correction number, depending on clay content, form the percentage LOI value, according to Krogstad [47]. The correction numbers for different clay contents are provided in Table 13.

Table 13 – Clay content correction number for LOI.

Clay content % (w/w)	Correction no.
5-9	1
10-24	2
25-39	2.5
40-59	3.5
> 59	4.5

The correction number of the mineral that was not analysed, sample tM_2-Bh, was approximated by looking at the % (w/w) clay in horizons from same sample site. The horizons, in descending order, for sample site tM_2 and their corresponding correction numbers are seen in Table 14.

The tM_2-Bh correction number was therefore approximated to 1.5. The LOI values, clay content, corresponding correction number and corrected LOI value can

Table 14 – Sample site tM_2 horizons and corresponding correction number.

Horizon in descending order	Correction no.
O	Organic soil
A	2
Bhs	1
Bs	2
Bh	Not analysed

be seen in Table 15.

Table 15 – LOI data before and after correction

Sample	LOI % (w/w)	Clay content % (w/w)	Correction number	Corrected LOI % (w/w)
tM_2-C	0.7	2	1	0*
TM_1-B	3.1	5	1	2.1
tM_2-Bs	6.3	11	2	4.3
tM_1-B average	8.5	8	1	7.5
tM_2-A	8.7	10	2	6.7
tM_2-Bh	9.0	not analysed	1.5**	7.5
tM_2-Bhs	9.4	9	1	8.4
tM_1-A	14.5	3	1	13.5
TM_1-O/A	15.4	4	1	14.4
TM_1-O	31.6			31.6
tM_2-O	32.6			32.6
tM_1-O	35.6			35.6
TM_2-H_1	69.6			69.6
P-H_1	71.5			71.5
TM_2-H_1/H_2	78.3			78.3
P-H_2	80.7			80.7

*Clay corrected LOI value that became a negative number was set to 0.
**Approximated number.

C.3 Dissolved Natural Organic Matter Concentration

The soil was mixed with water containing 0.001 M $CaCl_2$ in same ratios as for the sorption experiment. A control solution with only Type 1 water and 0.001 M $CaCl_2$, no soil, was treated in the same way as the samples. The samples were rotated for 24 hours on an end-over-end turner and the liquid was decanted. The decanted liquid was filtered with 25 mL syringe filters (VWR, Radnor, PA, USA) with 0.45 µm pore size. All samples were prefiltered with 0.7 µm filters (Whatman^{TM} 1825-025, Sigma-Aldrich, St. Louis, MO, USA). Sample tM_1-A and TM_1-O/A were pre-prefiltered with 11 µm pore size filters (Whatman^{TM} 1001-090, Sigma-Aldrich, St. Louis, MO, USA).

After filtration UV absorbance of the soil water at 254 and 400 nm was measured in 10 mm quartz cuvettes. By using the relationship established between UV absorbance at 254 nm and measured DOC from the EUTROPIA project (pers. comm., C. W. Mohr, 2015), expected DOC concentration could be calculated from the linear equation made by the regression line, Equation 9 and Figure 39.

$$DOC\ (mg\ C/L) = 18.7 \times abs_{254\ nm} + 2.7 \tag{9}$$

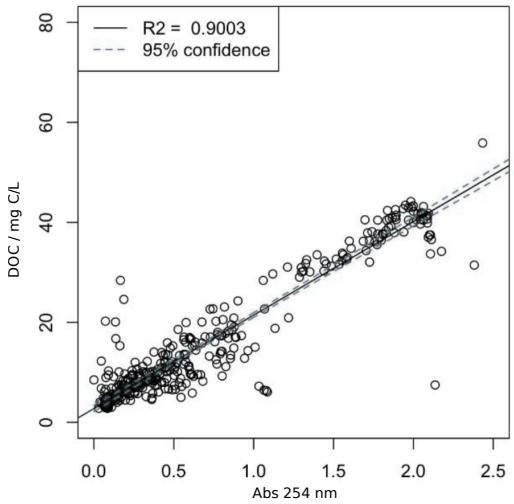

Figure 39 – Relationship between DOC and UV absorbance at 254 nm (pers. comm. C. W. Mohr, 2015).

Knowing approximate concentrations allowed dilution of samples with high expected concentration so the results, hopefully, would be within range of the prepared standards.

A 1000 mg C/L stock solution was prepared by dissolving 2.125 g of potassium hydrogen phthalate (p.a. grade, Merck Millipore, Billerica, MA, US) in 1000 mL of Type I water according to NS-EN 1484:1997 [48]. A diluted working solution of 100 mg C/L was used to make standards with 1, 3, 5, 7, 10, 15, 20, 25, 30, 35 and 40 mg C/L. The average calibration curve for calibration standards measured before and after samples are provided in Figure 40.

Sample details are summarized in Table 16.

Sample tM_1-A and TM_1-O/A were diluted 1:1 with Type 1 water as the estimated concentrations showed that they might be outside the range of the standards. The

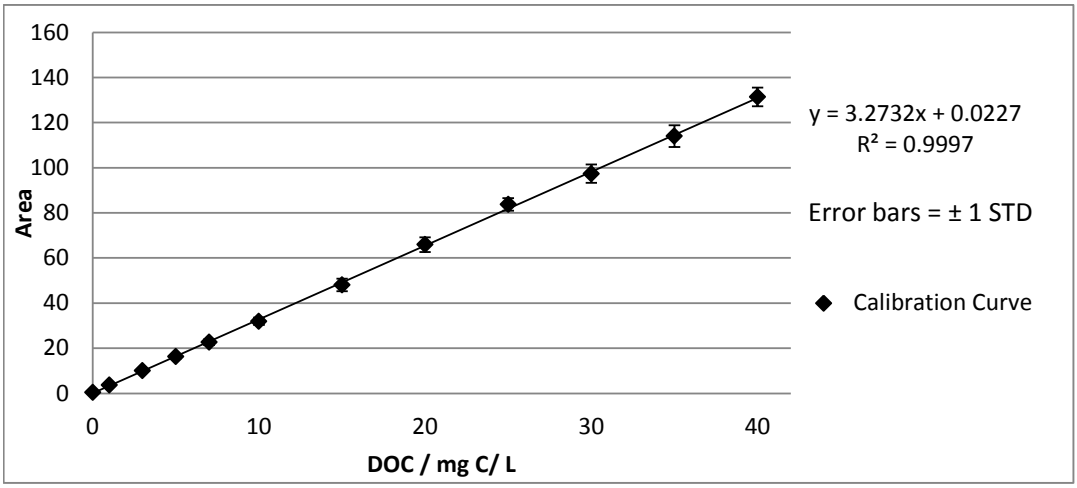

Figure 40 – Average calibration curve for DOC measurements. Error bars represent the STD of the measurement replicates (n=3-5).

instrumental measurements showed that the estimated concentrations were to low and all samples except for control, tM_2-Bs, tM_2-Bh and tM_2-C was diluted, on line, 1:10 with Type 1 water.

The instrument measured each sample 3-5 times; until the STD of the signal area was less than 0.1 or the RSD was less than 2 %, or 5 measurements had been conducted. The average concentrations (from measurement replicates) were corrected for dilution and subtracted the blank solution concentration. The DOC results are summarized in Table 17.

Table 16 – Summary of sample details for DOC analysis.

Sample	Soil mass (m)*	Soil:water ratio (L/kg)	Liquid volume (mL)	$UV_{254\ nm}$ **	$UV_{400\ nm}$ **	Expected DOC conc. (mg C/L)	Temperature (℃)	Conductivity (μS/cm)	pH
tM_1-O	9.0080	10	90	1.342	0.100	28	21.6	375	4.5
tM_1-A	35.1301	2	70	2.490	0.188	49	22.5	439	4.3
tM_1-B average	34.9742	2	70	0.752	0.042	17	23.1	328	4.3
tM_2-O	9.0048	10	90	0.494	0.020	12	23.4	309	4.7
tM_2-A	35.0047	2	70	0.343	0.011	9	23.4	313	4.8
tM_2-Bhs	34.9894	2	70	0.158	0.005	6	23.5	286	4.7
tM_2-Bs	35.0113	2	70	0.103	0.005	5	23.6	275	4.7
tM_2-Bh	35.0070	2	70	0.076	0.003	4	23.5	277	4.8
tM_2-C	35.0069	2	70	0.037	0.002	3	23.5	268	5.0
TM_1-O/A	34.9668	2	70	1.185	0.064	25	23.6	367	4.5
TM_1-B	35.0148	2	70	0.335	0.016	9	23.8	294	4.3
TM_1-O	9.0000	10	90	0.972	0.067	21	23.9	312	4.6
TM_2-H_1	8.9919	10	90	1.158	0.093	24	23.9	370	5.2
TM_2-H_1/H_2	9.0025	10	90	1.046	0.076	22	24.0	378	5.1
P-H_1	9.0032	10	90	0.590	0.039	14	24.0	325	4.7
P-H_2	9.0061	10	90	1.353	0.094	28	24.1	390	4.0
Control**	-	10	90	0.005	0.001	3	23.5	263	5.0

*Soil mass is corrected for moisture content.

** UV absorbance at 254 nm and 400 nm is corrected for absorbance in control solution.

Table 17 – DOC concentration in the decanted soil water samples.

Sample	Average concentration (mg C/L)	STD (mg C/L)	RSD (%)
Control (blank)	2	0.1	3.4
tM_2-C	3	0.1	3.9
tM_2-Bh	11	0.2	2.1
tM_2-Bs	17	0.3	1.6
tM_2-Bhs	44	1.3	2.9
TM_1-B	62	0.8	1.3
tM_2-O	73	0.7	0.9
TM_2-H_1/H_2	75	0.1	0.1
TM_2-H_1	75	0.3	0.4
tM_2-A	85	2.5	3.0
P-H_1	90	1.1	1.2
tM_1-B	109	2.8	2.5
TM_1-O	112	1.5	1.3
tM_1-O	133	1.4	1.1
P-H_2	145	2.6	1.8
TM_1-O/A	239	5.0	2.1
tM_1-A	276	5.0	1.8

C.4 Particle Size Distribution

Samples were analysed by S.A. Rodriguez at Eurofins Agro Testing Norway. Results are provided in Table 18 and 19. Results were reported with a relative standard deviation of 10 %.

Table 18 – PSD Analysis Results, part 1.

Parameter	Unit	tM_1-A	tM_1-B	tM_2-A	tM_2-Bhs
Coarse sand (0.6 - 2.0 mm)	% (w/w)	5	9	18	6
Medium sand (0.2 - 0.6 mm)	% (w/w)	25	23	34	25
Fine sand (0.06 - 0.2 mm)	% (w/w)	31	31	18	26
Coarse silt (0.02 - 0.06 mm)	% (w/w)	29	17	15	22
Medium silt (0.006 - 0.02 mm)	% (w/w)	4	8	3	8
Fine silt (0.002 - 0.006 mm)	% (w/w)	3	3	3	4
Clay (< 0.002 mm)	% (w/w)	3	8	10	9
Total percentage	% (w/w)	100	99	101	100

Table 19 – PSD Analysis Results, part 2.

Parameter	Unit	tM_2-Bs	tM_2-C	TM_1-O/A	TM_1-B
Coarse sand (0.6 - 2.0 mm)	% (w/w)	5	30	7	10
Medium sand (0.2 - 0.6 mm)	% (w/w)	23	21	31	37
Fine sand (0.06 - 0.2 mm)	% (w/w)	23	18	23	25
Coarse silt (0.02 - 0.06 mm)	% (w/w)	28	24	30	15
Medium silt (0.006 - 0.02 mm)	% (w/w)	6	3	2	4
Fine silt (0.002 - 0.006 mm)	% (w/w)	3	2	2	3
Clay (< 0.002 mm)	% (w/w)	11	2	4	5
Total percentage	% (w/w)	99	100	99	99

C.5 XRD

C.5.1 Instrumentation and Determination Principle

Production of X-rays for XRD occurs in a vacuum tube through electron bombardment of a metal target [52]. A monochromator is used to select a single wavelength that is beamed at the sample. The sample is placed in the centre of the goniometer circle that holds both the radiation source and detector, so different angles of incident rays can be measured. Diffraction is only detected for mineral planes that are coplanar with the focal plane [58]. It is therefore essential that the minerals in the sample have a random orientation so an even representation of all crystal

planes can be achieved [58].

Diffracted beams form a diffraction pattern due to the spacing between atom layers. Each crystalline material have a distinct distance, d, called d-spacing, between the crystalline planes and will therefore give a specific diffraction pattern. Bragg's law describes how XRD can be used to determine the d-spacing when wavelength, λ, and diffraction angle, θ, is known. The instrument output is a diffractogram, a plot of diffracted beam intensity versus diffraction angle.

$$Bragg's\ Law:\ n\lambda = 2dsin\theta \tag{10}$$

Where the diffraction order, n, is an integer. A schematic presentation of diffraction is shown in Figure 41.

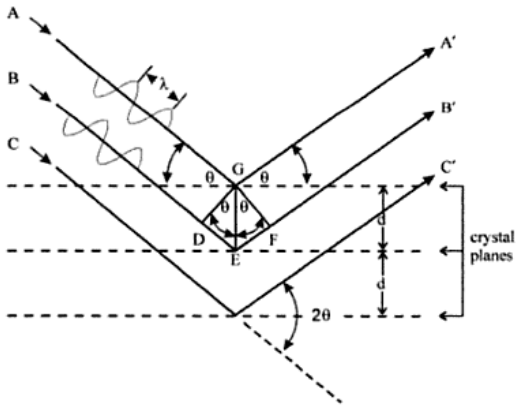

Figure 41 – Schematic presentation of diffraction. The incident X-rays, A, B and C, are refracted by the three shown planes of a crystal. When the distances DE + EF are equal to nλ the diffracted beams are in phase and constructive interference occur giving a peak in the diffractogram. Retrieved from [58].

C.5.2 Analysis Specific Details

The estimated standard deviation for the different phases in each sample varied between 0.03 - 2.99 % (w/w), a cut of value of 3 % (w/w) was therefore chosen. However, some mineral phases have been include as they clearly are present in the sample, though their values are lower than 3 % (w/w). A scenario such as this is illustrated by the diffractogram for sample tM_2-Bh in Figure 42. The highlighted

mineral phase, Clinochlore, is the only phase that will give a peak fitting the diffraction pattern shown in the red circle. The blue line is the measured diffraction, the red line partially overlaying the blue is the total fit of all the included mineral phases, the purple line is the contribution of Clinochlore to the fit and the bottom grey line shows measured minus fitted, i.e. for a perfect fit it should be a flat line.

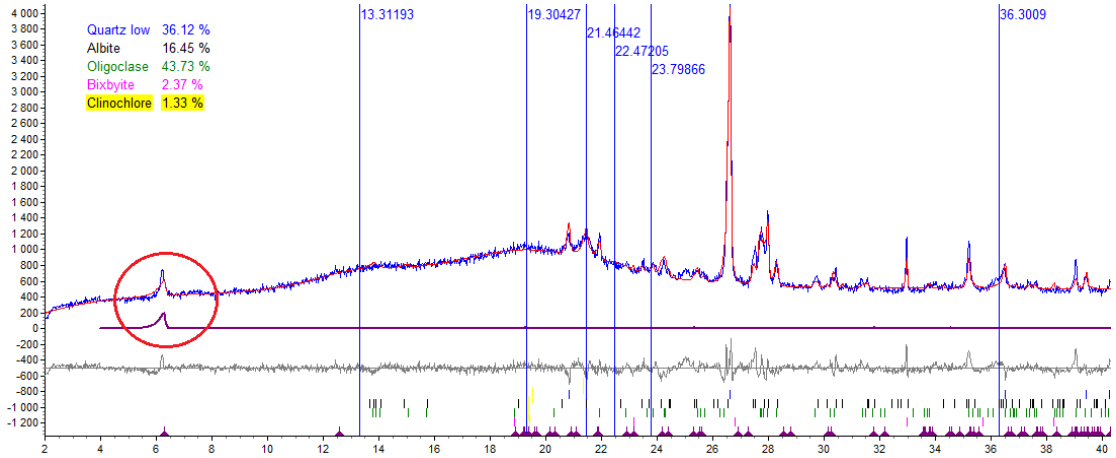

Figure 42 – Example of mineral phase included, though its value is lower than the highest estimated uncertainty. Only part of the diffractogram is shown, it originally extended to $2\theta = 70$ degrees. The y-axis is intensity and the x-axis 2θ degrees.

The quantitative amounts, % (w/w), of each mineral phase present in each sample is provided in Table 20.

Table 20 – Mineralogy of samples.

Sample	Quartz low % (w/w)	Albite % (w/w)	Oligoclase % (w/w)	Other % (w/w), (name)	Position of unidentified phase(s) in sample
tM_1-O	30	18	38	13 (Bytownite)	Peak at $2\theta = 46$ and 68.5
tM_1-A	22	9	49	13 (Microline), 7 (Labradorite)	Peak at $2\theta = 46$, 50.5 and 67.5
tM_1-B	31	16	44	9 (Microline)	Peak at $2\theta = 45.5$
tM_2-O	34	12	23	31 (Labradorite)	
tM_2-A	22		51	18 (Labradorite), 10 (Kalifersite)	Peak at $2\theta = 28.5$ and 43
tM_2-Bhs	39	41	20		
tM_2-Bs	48		52		Peak at $2\theta = 33.5$, 42.5 and 51.5
tM_2-Bh	36	16	44	2 (Bixbyite), 1 (Clinochlore)	
tM_2-C	48	6	42	4 (Microline)	Peak at $2\theta = 27$, 27.5, 35.5, 36, 45, 51.5
TM_1-O/A	44	9	46		Peak at $2\theta = 67.5$
TM_1-B	37	11	50	1 (Kalifersite), 1 (Sklodowskite), 0.3 (Algodonite)	Peak at $2\theta = 27.5$, 36.5, 47.5, 50, 57.5 and 59
TM_1-O	31	15	47	7 (Diopside)	
TM_2-H_1	25		75		Peak at $2\theta = 35.5$
TM_2-H_1/H_2	31		69		Peak at $2\theta =$ at 67.5
P-H_1	23	54	22		
P-H_2	30	70			

C.6 XRF

C.6.1 Instrumentation and Determination Principle

High energy X-rays for XRF are produced in an X-ray tube. They are absorbed by the sample producing electronically excited ions. The excited ions can return to ground state through several different transitions. The transition between states with same spin quantum number is called (X-ray) fluorescence. The fluorescence intensity is normally detected by a solid-state detector, a scintillation counter and/or a proportional counter depending on which XRF system is used and which elements are detected. The wavelengths of the peaks are element specific while their net intensities correspond to the concentration of the respective element.

A wavelength dispersive XRF system (WDXRF) was employed in this analysis, a WDXRF can detect elements from beryllium to uranium [53]. In WDXRF an analysing crystal with known d-spacing is used to diffract the incoming energy in different directions depending on the incoming wavelength. As in XRD in-phase wavelengths (n is an integer) constructively interfere and are detected. The detector is placed on a goniometer and the placement of the detector determines which wavelength is registered depending on which obeys Bragg's Law (Equation 10, Figure 41). The WDXRF can also be used to look for specific emission lines at a given angel, when the lines and wavelengths of a specific element is known for a given matrix. Calibration is then based on standards with similar matrix. This last method was employed for the analysis in this thesis.

The sample matrix can interfere with the quantitative determination of the elements. This occurs when other elements in the matrix absorb the incident or emitted beam stronger or weaker than the matrix in the standards [66]. Matrix matching the sample and standards is therefore very important for achieving as accurate quantitative measurements as possible.

C.6.2 Analysis Specific Details

The powdered samples were baked in a Carbolite CWF 12/13(Carbolite, Derbyshire, UK) chamber furnace with the temperature program provided in Table 21.
A few minutes after being removed from the furnace the samples were placed in a desiccator to cool. 0.6 g cooled sample was mixed with 6 g of flux, lithiumtetraborate 66.5 % / lithiummetaborate 33.5 % (Fluxana, Bedburg-Hau, Germany). The flux-sample mix was melted to a glass bead in an Eagon 2 (PANalytical, Almelo,

Table 21 – Temperature program for baking of soil samples for XRF.

Temperature (℃)	Time (min)
350 - 550	0-60
550	60-90
550 - 1050	90-150
1050	150-210

Netherlands) XRF furnace fusion system.

The XRF raw-data are provided in Table 22, together with $LOI_{1050\ ^{\circ}C}$ values. Note that while the amount of several of the elements are below the uncertainty of the method (0. 1-0.3 % (w/w)) for uncorrected values), if a measured value are there, the element is present, but the amount is very low and trace elemental analysis must be conducted for accurate quantitative determination (pers. comm., M. Aerts, Department of Geosciences, University of Oslo).

The data reported in the result section was corrected for $LOI_{1050^{\circ}C}$ and reported as total elemental content in % (w/w) of total sample mass.

Table 22 – Elemental composition of analysed samples, raw data reported as metal oxides. Not rounded to significant figures.

% (w/w) of:	tM_1-O	tM_1-A	tM_2-Bs	tM_2-C	P-H_2
$LOI_{1050℃}$	33.000	15.000	7.000	1.000	91.000
SiO_2	69.699	70.099	67.164	67.440	50.208
TiO_2	0.896	0.879	0.964	0.568	1.747
Al_2O_3	14.942	15.060	15.557	15.387	23.380
Fe_2O_3	3.030	3.275	6.313	4.104	10.145
MgO	0.404	0.310	1.129	2.265	1.289
CaO	3.142	2.872	3.010	3.755	5.065
Na_2 O	4.000	4.003	3.585	3.671	3.267
K_2 O	2.951	2.995	1.765	2.136	2.128
P_2 O_5	0.162	0.081	0.098	0.172	1.787
SO_3	0.008	0.000	0.001	0.001	0.015
V_2O_5	0.013	0.015	0.017	0.011	0.026
Cr_2O_3	0.000	-0.002	0.004	0.003	0.004
SrO	0.041	0.042	0.029	0.038	0.075
ZrO_2	0.058	0.052	0.035	0.032	0.036
BaO	0.116	0.116	0.064	0.064	0.172
NiO	-0.002	-0.001	0.001	0.003	0.005
CuO	0.000	0.000	0.001	0.002	0.004
ZnO	0.006	0.002	0.002	0.006	0.025
PbO	0.017	0.013	0.008	0.008	0.023
HfO_2	-0.001	-0.001	0.001	-0.003	-0.001

D Sorption Experiments and Determination of MEA-nitramine on LC-MS/MS

D.1 General Analysis Sequence on the LC-MS/MS

A typical sequence on the LC-MS/MS would have the following sequence of samples. All solutions had 3 consecutive injections when analysed.

- Calibration solutions, from low to high concentrations
- Control
- Sample 1:
    1. Spiked soil blank
    2. Soil Blank

3. Soil water based sample replicate(s)
4. Matrix matched soil water based calibration solutions (if employed)

- Wash
- Sample 2 (same set-up as for sample 1)
- Wash
- Calibration solutions, from low to high concentrations
- Control
- Sample 3 (same set-up as for sample 1)
- Wash
- Sample 4 (same set-up as for sample 1)
- Wash
- Calibration solutions, from low to high concentrations
- Control

Water blanks (Type II water) would be injected between all samples, except between consecutive injections of the same sample and between calibration solutions.

D.2 Instrumental Settings for LC and MS/MS

The instrumental settings for the LC are summarized in Table 23.

Table 23 – LC method settings and adjustable parameters.

Column oven Temp. Control	on
Colmn oven temperature, nominal (℃)	22.0
Column A. Active column	no
Column B. Active column	no
Sampler temperature control	on
Pressure, lower limit (bar)	50
Pressure, upper limit (bar)	450
Maximum flow ramp, down (ml/min^2)	0.300
Maximum flow ramp, up (ml/min^2)	0.300
A. Equate	"H_2O"
B. Equate	"2mM AcA in H_2O"
C. Equate	"2mM AcA in MeOH"
D. Equate	"MeOH"
Draw speed (µL/s)	1.000
Draw delay (ms)	3000
Dispense speed (µL/s)	1.000
Dispense delay (ms)	0
Waste speed (µL/s)	2.000
Sample height (mm)	2.000
Inject wash	Both
Wash volume (µL)	10.000
Wash speed (µL/s)	2.000
Loop wash factor	0.000
Puncture offset (mm)	0.0
Pump device	Pump
Inject mode	Normal
Pump pressure, step (s)	0.01
Pump pressure, average	off
Sampler temperature, nominal (℃)	4.0
Sampler temperature, lower limit (℃)	4.0
Sampler temperature, upper limit (℃)	45.0
Sampler, ready temp. delta (℃)	1.0
Flow (mL/min)	0.050
B (%)	70.0
C (%)	30.0
D (%)	0.0
Curve	5

The instrumental settings for the MS are summarized in Table 24 and 25.

Table 24 – MS method settings and adjustable parameters.

MS method settings	
Method type	EZ method
MS run time (min)	5.00
Experiment type	SRM
Chrom filter peak with (s)	10.0
Collision gas pressure (mTorr)	1.1
Used tuned S-lense value	no
Q1 peak width (FWHM)	0.70
Q3 peak width (FWHM)	0.70
Display time range for SRM table	yes
Cycle time (s)	0.500
DCV (V)	not used
Adjustable parameters (tune method)	
Capillary temperature (℃)	270.0
Vaporizer temperature (℃)	325.0
Sheat gas pressure (a.u)	40.0
Ion sweep gas pressure (a.u)	0.0
Aux Valve flow (a.u)	5
Spray voltage	Postitive polarity: 3500.0, Negative polarity: -3000.0
Discharge current	Positive polarity; 4.0, Negative polarity: - 4.0
Divert valve	not used

Table 25 – Selected Reaction Monitoring (SRM) information on precursor and product ion with MS instrumental settings.

Precursor	Product	CE	Start	Stop	S-Lens	Polarity	Trigger	Reference	Name
75.069	31.047	18	0.00	5.00	22	-	1.000 E+05	no	(empty)
75.069	44.954	5	0.00	5.00	22	-	1.000 E+05	no	(empty)
75.069	59.948	21	0.00	5.00	22	-	1.000 E+05	no	(empty)
105.054	43.169	8	0.00	5.00	36	-	1.000 E+05	no	(empty)
105.054	46.056	23	0.00	5.00	36	-	1.000 E+05	no	(empty)
105.054	56.939	7	0.00	5.00	36	-	1.000 E+05	no	(empty)
105.054	61.192	14	0.00	5.00	36	-	1.000 E+05	no	(empty)

The elution time of the mobile phase and unretained compounds can be calculated from Equation 11, assuming the column packing material has a standard porosity of 0.6 (ϵ).

$$t_m = \frac{Volume_{column} \times porosity}{Flow\ rate} = \frac{\pi r^2 L \times \epsilon}{F} \tag{11}$$

Where L is column length (cm), r is column radius (cm) and F is flow rate (mL/min). The length of the column is 15 cm and the diameter 0.1 cm, the flow rate employed was 0.05 mL/min. t_m is then calculated to be 1.4 minutes.

D.3 Optimised Liquid to Soil Ratio

Choosing an optimal soil/solution ratio for a sorption experiment is, according to OECD guidlines 106 [44], done by using the relationship between the degree of desired sorption and the estimated distribution coefficient, K_d. The distribution coefficient must therefore either be known from preliminary studies or be estimated, examples of estimation techniques are given in the guidelines [44]. Soil samples from the pilot study by Mohr and Vogt [19] was still available and K_d was estimated for this soil in the pilot study. Note that the concentration of MEA-nitramine added was 0.5 mg/L in the pilot study, while 5 mg/L was added in this test. As the distribution coefficient can be concentration dependent this is not ideal. The relationship between K_d, desired sorption and soil to solution ratio is calculated by assuming linear sorption, the resulting figure used for determination of soil/solution ratio is shown in Figure 43.

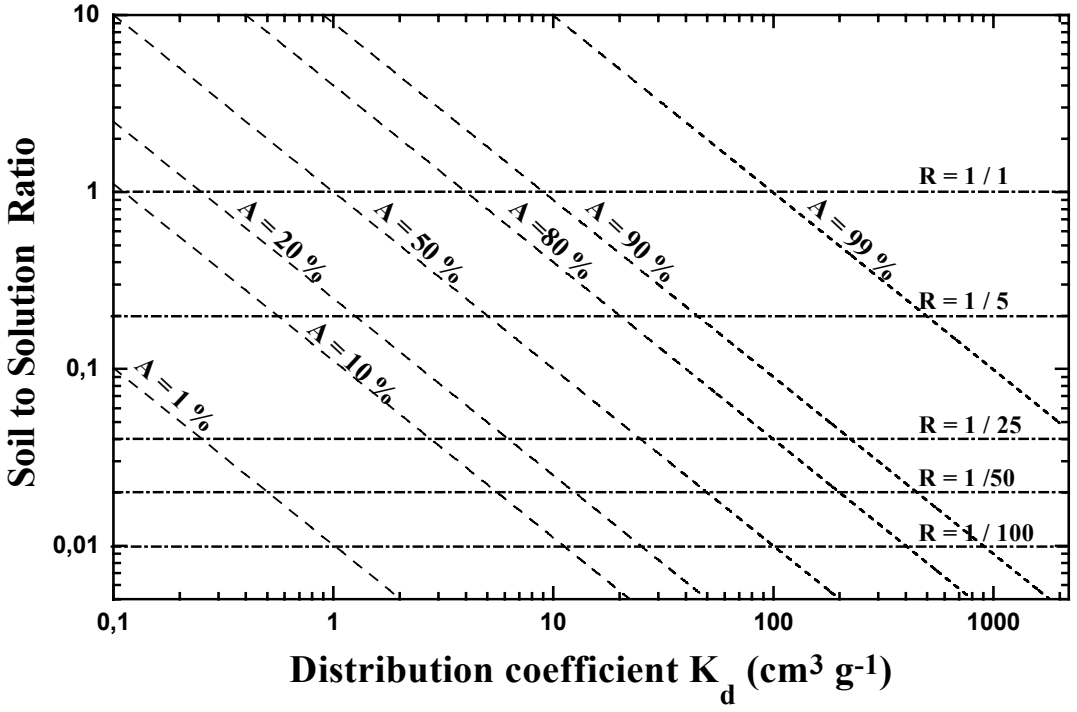

Figure 43 – Soil to solution ratios as a function of K_d at different sorption percentages. Retrieved from [44].

The chosen soil, 005-1, from the pilot study [19] has the reported distribution coefficient; $K_d = 14$. As the stability of the analysis method was uncertain, a desired sorption of 50 % was chosen, to ensure a measurable change in concentration.

D.4 Spiked Soil Blanks

The spiked soil blanks were prepared in two ways:

For a resulting concentration of 0.05 mg/L MEA-nitramine:

- 780 µL of the blank soil water was transferred to a LC-MS vial containing 220 µL 0.00025 g/L MEA-nitramine and 100 µL of 11 mg/L MMA-nitramine. Mixed on a whirlimixer.

For a resulting concentration of 5 mg/L MEA-nitramine:

- 800 µL of the blank soil water was transferred to a LC-MS vial containing 200 µL 0.025 g/L MEA-nitramine. 100 µL of 11 mg/L MMA-nitramine was added to the vials, i.e. this diluted the concentrations of the vials, this was corrected for in calculations. Mixed on a whirlimixer.

D.5 Quantification

Determination of MEA-nitramine in the samples was done in three different ways in the different sorption experiments:

- *Water based calibration solutions* were employed when matrix matched soil water based calibration solutions were not made. They were analysed two or three times, before, in the middle of and at the end of an analysis sequence. A linear regression line of the average was used to calculate MEA-concentration.

- *Water based calibration solutions and spiked soil blanks* were used together to quantify the amount of MEA-nitramine lost from the aqueous phase to soil. The calculated concentration of the spiked soil blank, when using the linear regression line for the water based calibration solution, was used as "starting" concentration for the sample. It was then assumed that the water based and matrix matched soil water based calibration curves are parallel for the concentration range of interest. This concept is illustrated in Figure 20.

- Matrix matched soil water based calibration curves were used to determine MEA-nitramine concentration in the samples, when employed.

Internal standard were used together with these quantification methods in some of the tests.

D.6 Control Solutions and Test Experiment with 10 L/kg Liquid to Soil Ratio

The measured signal area and corresponding concentration for each control solution measurement replicate and what pretreatment it was subjected to is provided in Table 26.

Table 26 – Control solution signal areas and pretreatments.

Centrifuged	Shaken for 24 h	Measurement replicate no.	Area	Corresponding MEA-nitramine concentration (mg/L)	Sample average ± STD (mg/L)
	√	1	125535	4.8	
	√	2	127601	4.9	
	√	3	129992	5.0	4.9 ± 0.1
		1	123460	4.8	
		2	127358	4.9	
		3	129315	5.0	4.9 ± 0.1
√	√	1	125433	4.8	
√	√	2	126192	4.9	
√	√	3	122997	4.8	4.8 ± 0.1
√		1	126861	4.9	
√		2	127172	4.9	
√		3	125699	4.9	4.89 ± 0.03

The average integrated peak area for the water based calibration solutions (n=3), with STD and RSD is provided in Table 27.

Table 27 – Peak area for the calibration solution measurements (n=3).

Calibration solution no.	MEA-nitramine (mg/L)	Average	STD	RSD (%)
1	0.99	23864	2780	12
2	1.99	50072	2827	6
3	2.98	75042	2747	4
4	3.98	103739	6424	6
5	4.97	130870	7563	6
6	5.97	153072	5512	4

The control solution and sample concentration are calculated from the linear equation given by the calibration curve shown in Figure 44. The water based calibration solutions were analysed three times during the analysis sequence to correct for any instrument variations in time, once before the control solutions and twice after (Figure 44).

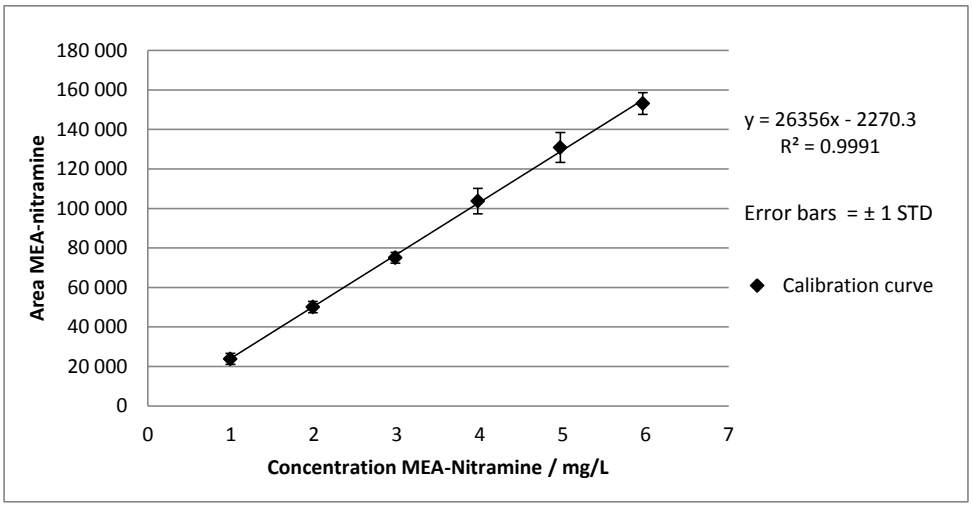

Figure 44 – Average water based calibration curve. Error bars represent ± 1 STD from the measurement replicates (n=3).

The area for the sample replicates and corresponding concentration using the average water based calibration curve is shown in Table 28.

Table 28 – Integrated area for the sorption sample replicates (n=3), with corresponding MEA-nitramine concentration employing the average water based calibration curve.

Sample	Area ± STD	Conc. using average water based cal.curve (mg/L)	STD (mg/L)	RSD (%)
Sorption sample	71675 ± 2713	2.8	0.1	4

D.7 Raw Data for Test Experiment with 25 L/kg Liquid to Soil Ratio

The average integrated peak area for the measurement replicates of the water based calibration solutions (measured three times; before and twice after the set of samples) (n=9), with STD and RSD are provided in Table 29.

Table 29 – Peak area for the calibration solution measurements (n=3).

Calibration solution no.	MEA-nitramine (mg/L)	Average area	STD	RSD (%)
1	1.00	27086	5767	21
2	2.01	44574	11091	25
3	3.01	69304	12031	17
4	4.01	90617	14337	16
5	5.02	110894	8524	8
6	6.02	125440	11467	9

The average water based calibration curve is shown in Figure 45.

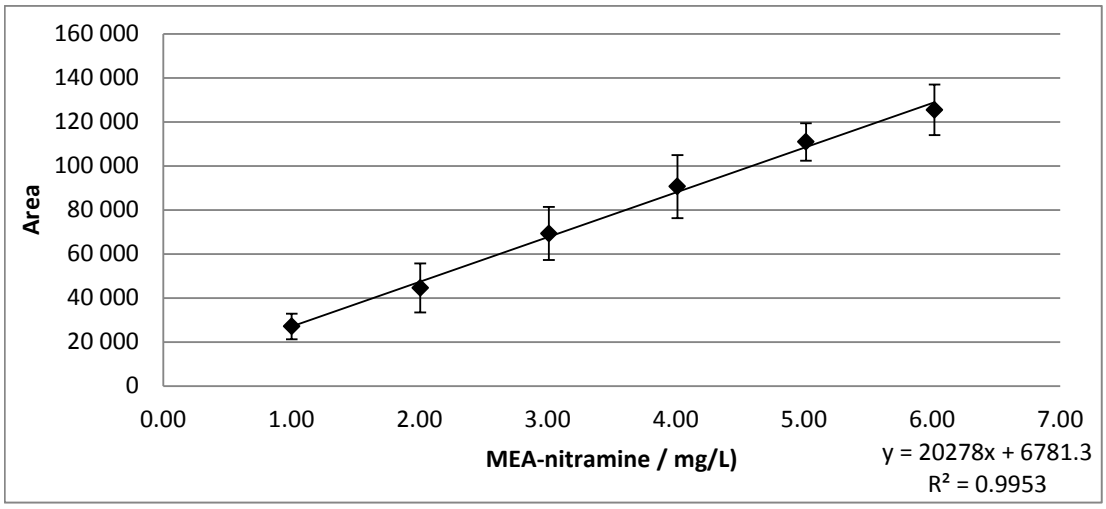

Figure 45 – Average calibration curves for water based calibration solutions. The error bars represent ± 1 STD calculated from the measurement replicates of the water based calibration solutions (n=9).

The area for the control solution and sample replicates (average) with corresponding concentration, using the average water based calibration curve, is shown in Table 30.

Table 30 – Integrated area for the sorption sample replicate average (n=3) and control, with corresponding MEA-nitramine concentration employing the average water based calibration curve.

Sample	Area ± STD	MEA-nitramine conc. (mg/L)	STD (mg/L)	RSD (%)
Sorption sample	122330 ± 28070	6	1	24
Control sample	86823 ± 5680	3.9	0.3	7

D.8 Raw Data for Test Experiment with 0.05 mg/L MEA-nitramine and Matrix Matched Calibration Curves

The average integrated peak area for the water based calibration solutions (measured three times; before, in the middle of and after the samples) (n=3), with STD and RSD is provided in Table 31.

Table 31 – Peak area for the average calibration solution measurements (n=3).

Calibration solution no.	MEA-nitramine (mg/L)	Average area	STD	RSD (%)
1	0.010	181	19	11
2	0.021	408	50	12
3	0.031	765	52	7
4	0.041	1000	135	14
5	0.052	1520	127	8
6	0.062	1699	143	8

The integrated peak area for the matrix matched soil water based calibration solutions (n=1) is provided in Table 32.

Table 32 – Peak area for the matrix matched soil water based calibration solution measurements (n=1).

Calibration solution no.	MEA-nitramine (mg/L)	Area
2	0.021	287
3	0.031	403
4	0.041	604
5	0.052	820
6	0.062	991

The area for the average of the sample replicates and corresponding concentration, using the matrix matched soil water based calibration curve or the average water based calibration curve, is shown in Table 33.

Table 33 – Integrated area for the sorption sample replicates (n=3) and control measurement replicates (n=6), with corresponding MEA-nitramine concentration employing the matrix matched soil water based calibration curve or the average water based calibration curve.

Sample	Area ± STD	Conc. using soil based cal. Curve (mg/L)	STD (mg/L)	RSD (%)	Conc. using average water based cal.curve (mg/L)	STD (mg/L)	RSD (%)
Sorption sample average	882 ± 102	0.054	0.006	10	0.034	0.003	10
Control average	1398 ± 88	0.050	0.003	6	0.050	0.003	6

D.9 Raw Data for Sorption to Soil with 0.05 mg/L MEA-nitramine

Raw data for spiked soil blank concentrations is provided in Table 34

Table 34 – Average concentration of the spiked soil blank for each soil with STD and RSD based on an IS corrected water based calibration curve.

Spiked soil blank average for:	MEA-nit. area ± STD	MMA-nit. area ± STD	Ratio	STD	Conc. using IS corrected water based cal.curve (mg/L)	STD (mg/L)	RSD (%)
tM_2-Bs	396 ± 40	721 ± 100	0.55	0.06	0.059	0.005	9
tM_2-C	403 ± 28	994 ± 67	0.41	0.02	0.046	0.002	4
tM_1-A	228 ± 13	395 ± 26	0.58	0.02	0.062	0.002	3
P-H_2	540 ± 28	621 ± 19	0.87	0.04	0.087	0.003	4

The calculated deviation of MEA-nitramine concentration in the spiked soil blanks from the added concentration (Table 35), using the equation for the linear regression line in the IS corrected calibration curve (Figure 22).

Table 35 – The concentration of MEA nitramine calculated from the water based calibration curve corrected by IS and its deviation from added concentration.

Spiked soil blank from sample:	Added MEA-nitramine (mg/L)	MEA-nitramine (mg/L) calculated from measurements	Deviation from (%) added concentration (%) *
tM_2-C	0.050	0.046	7
tM_2-Bs	0.050	0.059	18
P-H_2	0.050	0.087	73
tM_1-A	0.050	0.062	23

*Calculated using non-rounded values

Scatter plots with linear regression for relationships between or K_d and LOI, total aluminium content, total iron content and conductivity, respectively, without sample P-H_2 (Figure 46).

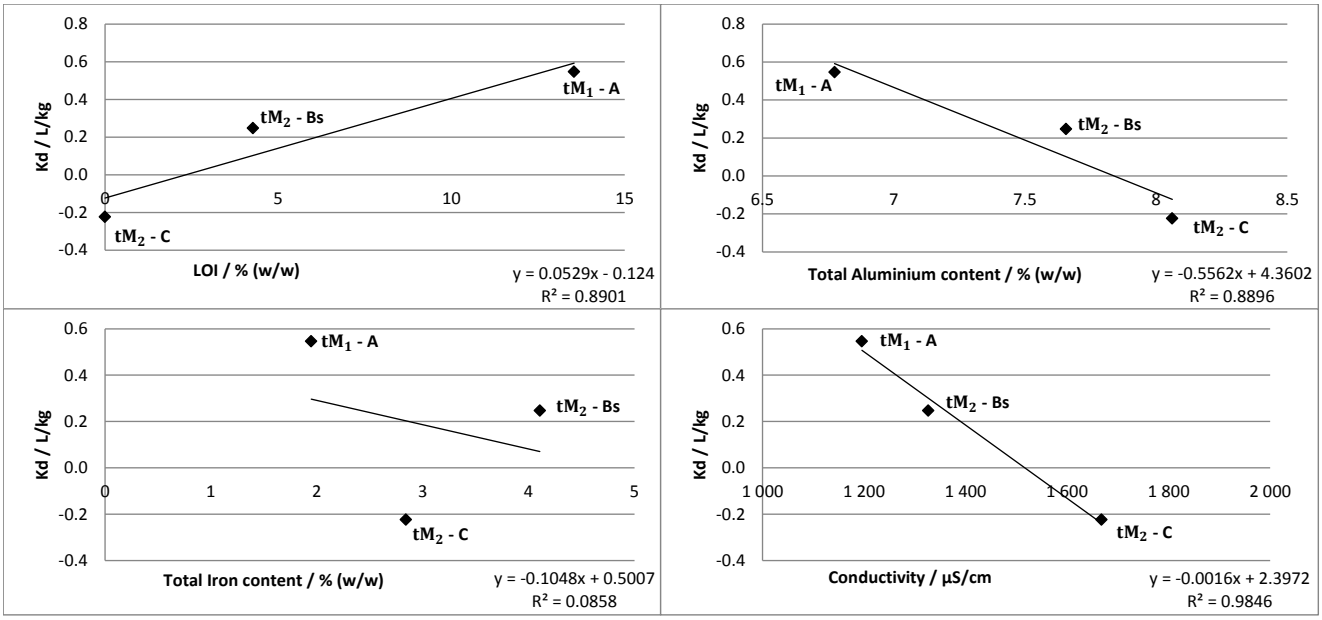

Figure 46 – Relationship between K_d and OM content (LOI) in three of the samples (top, left), total aluminium content in the samples (top, right), total iron content in the samples (bottom, left) and ionic strength (conductivity) in the samples (bottom, right).

Calculated K_d and measured pH and conductivity in the sorption experiment samples/supernatant is provided in Table 36.

Table 36 – Calculated K_d and measured pH and conductivity in the sorption experiment samples/supernatant.

Sample	K_d	Conductivety (µS/cm)	pH
P-H_2	4.6	1110	4.5
tM_1-A	0.5	1196	4.5
tM_2-Bs	0.2	1327	5.2
tM_2-C	-0.2	1668	5.6

D.10 Raw Data for Ivestigation into Reduction in Loss of MEA-nitramine to Soil Water Matrix

Filter and centrifuge specifications are provided in Table 37.

Table 37 – Filter and centrifuge specifications.

Manufacturer	Name	Membrane	Pore size (µm)	rpm/g	Time (min)
VWR	25 mm Syringe filter	PES*	0.45		
Alltech	True 30 mm Syringe filter	RC**	0.45		
Sartorius	Minisart 25 mm Syringe filters	RC**	0.34		
Eppendorf	Centrifuge 5415R			13200/16100	60

*PES = polyethersulfone, **RC = regenerated cellulose.

The signal area for MMA-nitramine with different sample pretreatment in the three different soil samples and the control is provided in Table 38. The STD and RSD of the measurement replicates are provided in Table 39.

Table 38 – Signal area for MMA-nitramine with different sample pretreatment in the three different soil samples and the control. Three measurement replicates (n=3) were used for each sample (the average, STD and RSD is reported in Table 39). The total signal average with STD and RSD for each sample is included.

	Signal area for MMA-nitramine			
Sample	Control	tM_2-O	tM_2-C	TM_2-H_1
Centrifuged, measurement rep. 1	829	618	857	550
Centrifuged, measurement rep. 2	842	537	826	561
Centrifuged, measurement rep. 3	764	567	823	579
PES filter, measurment rep. 1	707	540	852	585
PES filter, measurment rep. 2	842	661	964	519
PES filter, measurment rep. 3	908	564	713	511
RC filter 1, measurment rep. 1	825	606	761	593
RC filter 1, measurment rep. 2	908	568	801	599
RC filter 1, measurment rep. 3	845	612	738	606
RC filter 2, measurment rep. 1	800	568	864	574
RC filter 2, measurment rep. 2	775	606	850	556
RC filter 2, measurment rep. 3	841	539	807	551
Blank, measurement rep. 1	856			
Blank, measurement rep. 2	839			
Blank, measurement rep. 3	786			
Average	824	582	821	565
Std	52	38	66	30
RSD (%)	6	7	8	5

Table 39 – Average signal area, STD and RSD for the measurement replicates (n=3) for each pretreatment of each sample.

Pretreatment	Average	STD	RSD (%)
Control			
Centrifuged	812	42	5
PES	819	102	13
RC1	859	43	5
RC2	805	33	4
Blank	827	37	4
tM_2-O			
Centrifuged	574	41	7
PES	588	64	11
RC1	595	24	4
RC2	571	34	6
tM_2-C			
Centrifuged	835	19	2
PES	843	126	15
RC1	767	32	4
RC2	840	30	4
TM_2-H_1			
Centrifuged	563	15	3
PES	538	41	8
RC1	599	7	1
RC2	560	12	2

D.11 SPE

The method for solid phase extraction (SPE) was adapted from a method provided by PhD candidate Tore Vehus (pers. com., Bioanalytical Chemistry, University of Oslo).
Method:

- Activate column with 1 mL 100 % methanol (MeOH) with 2 mM acetic acid (AcA)
- Activate column with 3 × 1 mL 2 % (v/v) MeOH with 2 mM AcA
- Apply sample, collect flow-through
- Wash with 2 % (v/v) MeOH with 2 mM AcA, collect flow-through
- Elute with 5 % (v/v) MeOH with 2 mM AcA, collect flow-through

- Elute with 10 % (v/v) MeOH with 2 mM AcA, collect flow-through
- Elute with 90 % (v/v) MeOH with 2 mM AcA, collect flow-through

The eluate containing 90 % (v/v) MeOH was evaporated and re-dissolved in HPLC grade water. MeOH, HPLC water and AcA were the same as used for mobile phase.

The measurements of MEA- and MMA-nitramine area and the average, STD and RSD for the MMA-nitramine measurement replicates are provided in Table 40.

Table 40 – MEA- and MMA-nitramine integrated area for the collected SPE flow-throughs. The average values with STD and RSD for each flow-through is reported for MMA-nitramine.

Sample	Measurement replicate no.	MEA-nit. area	MMA-nit. area	Average MMA-nit area.	STD MMA-nit. area	RSD (%) MMA-nit. area
SPE sample application	1	61	170			
SPE sample application	2	61	237			
SPE sample application	3	109	223	210	35	16
SPE wash	1	49	631			
SPE wash	2	37	714			
SPE wash	3	69	696	680	44	6
SPE eluate 1	1	0	646			
SPE eluate 1	2	0	670			
SPE eluate 1	3	0	715	677	35	5
SPE eluate 2	1	0	686			
SPE eluate 2	2	0	775			
SPE eluate 2	3	0	646	702	66	9
SPE eluate 3	1	0	920			
SPE eluate 3	2	0	691			
SPE eluate 3	3	0	620	744	157	21

D.12 New Instrument Settings after Changes to Analysis Method on LC-MS/MS

The only changes to the LC settings, was a slight increase in column oven temperature to 30 ℃ instead of 22 ℃, and an increase in the flow rate to 0.100 ml/min instead of 0.05 ml/min.

The instrumental setting of the MS, after changes to analysis method by colleges Dr. L. Zhu and MSc. C. B. Gundersen (pers. comm.), is provided in Table 41 and 42. Note that acetic acid (MP additive) is added to the list of compounds to be analysed (precursor with m/z 59.050), as it can be used to monitor the stability of the electrospray. Also note that the product ion with m/z 31.047 for MMA-nitramine has been removed completely as it did not give a stable signal.

Table 41 – New MS method settings and adjustable parameters, changes compared to method in Table 24 is in **bold text**.

MS method settings	
Method type	EZ method
MS run time (min)	**8.00**
Experiment type	SRM
Chrom filter peak with (s)	10.0
Collision gas pressure (mTorr)	1.1
Used tuned S-lense value	No
Q1 peak width (FWHM)	0.70
Q3 peak width (FWHM)	0.70
Display time range for SRM table	yes
Cycle time (s)	**0.100**
DCV (V)	not used
Adjustable parameters (tune method)	
Capillary temperature (℃)	**350.0**
Vaporizer temperature (℃)	325.0
Sheat gas pressure (a.u)	40.0
Ion sweep gas pressure (a.u)	0.0
Aux Valve flow (a.u)	**10**
Spray voltage	Postitive polarity: 3500.0, Negative polarity: **-2500.0**
Discharge current	Positive polarity; 4.0, Negative polarity: - 4.0
Divert valve	not used

Table 42 – New SRM information on precursor and product ion with the new MS instrumental settings, changes compared to method in Table 25 is in **bold text**.

Precursor	Product	CE	Start	Stop	S-Lens	Polarity	Trigger	Reference
59.050	**59.050**	**3**	**0.00**	**8.00**	**36**	**-**	**1.000 E+05**	**no**
75.069	44.954	5	0.00	**8.00**	22	-	1.000 E+05	no
75.069	**59.950**	21	0.00	**8.00**	22	-	1.000 E+05	no
105.054	**43.100**	8	0.00	**8.00**	36	-	1.000 E+05	no
105.054	**46.000**	**27**	0.00	**8.00**	36	-	1.000 E+05	no
105.054	**59.935**	**30**	0.00	**8.00**	36	-	1.000 E+05	no
105.054	**61.050**	**19**	0.00	**8.00**	36	-	1.000 E+05	no

D.13 Raw Data for Re-analysis of Sorption to Soil with 5.01 mg/L MEA-nitramine Solutions after Changes in MS Settings

The integrated MEA-nitramine peak areas for soil water samples and soil water calibration solutions, as well as calculated concentration for the soil water samples

based on the equation for the linear regression line for each soils matrix matched calibration curve is provided in Table 43.

Table 43 – MEA-nitramine area for soil water samples and soil water calibration solutions, and corresponding concentration for the soil water samples, part 1.

Sample	Average MEA nit. area	STD	RSD (%)	Average MEA-nit. conc. (calculated based on soil water cal.curve) ± STD
tM_2-Bs				
Soil water sample, sorption experiment	18415	319	2	3.85 ± 0.06
Soil water calibration solution 1.00 mg/L	3943	7	0	
Soil water calibration solution 3.01 mg/L	12901	210	2	
Soil water calibration solution 6.02 mg/L	30282	397	1	
tM_2-C				
Soil water sample, sorption experiment	14835	223	2	3.90 ± 0.05
Soil water calibration solution 1.00 mg/L	3059	130	4	
Soil water calibration solution 3.01 mg/L	10173	318	3	
Soil water calibration solution 6.02 mg/L	24159	175	1	
tM_1-A				
Soil water sample, sorption experiment	12585	306	2	3.73 ± 0.08
Soil water calibration solution 1.00 mg/L	2928	127	4	
Soil water calibration solution 3.01 mg/L	9383	180	2	
Soil water calibration solution 6.02 mg/L	21168	267	1	
P-H_2				
Soil water sample, sorption expermient	20523	236	1	4.20 ± 0.05
Soil water calibration solution 1.00 mg/L	4642	336	7	
Soil water calibration solution 3.01 mg/L	14565	97	1	
Soil water calibration solution 6.02 mg/L	29582	807	3	
tM_1-O				
Soil water sample, sorption experiment	12253	310	3	3.87 ± 0.09
Soil water calibration solution 1.00 mg/L	2387	76	3	
Soil water calibration solution 3.01 mg/L	8283	213	3	
Soil water calibration solution 6.02 mg/L	20349	320	2	

The resulting calibration curves for the water based and soil water based calibration solution are shown in Figure 47.

Calculated K_d and measured pH and conductivity in the sorption experiment samples/supernatant is provided in Table 44.

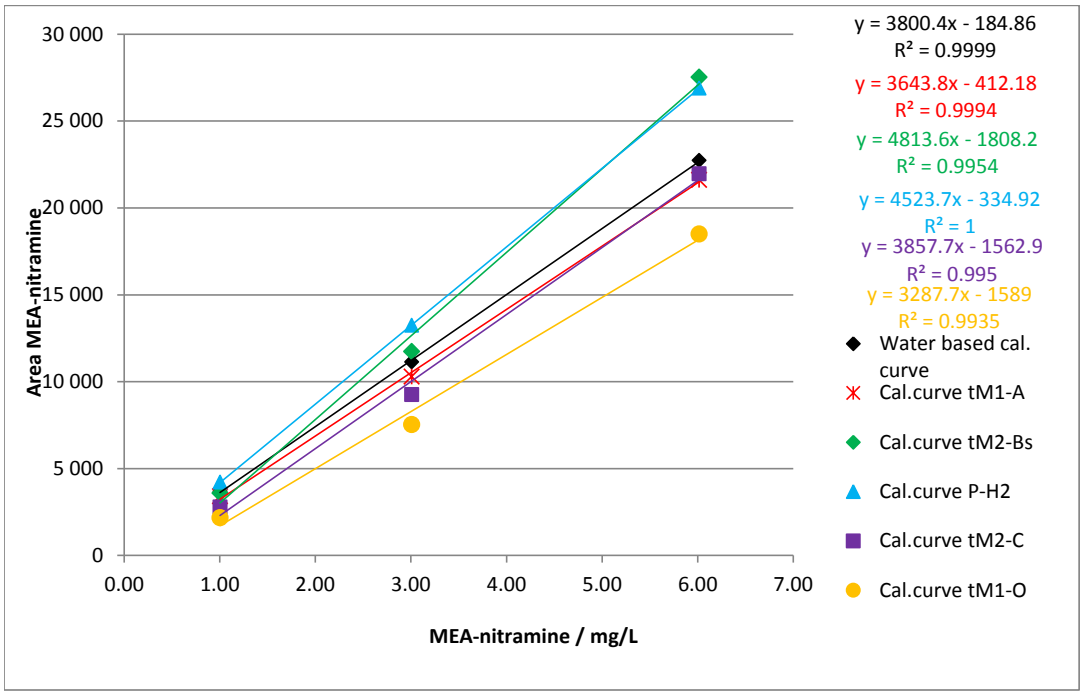

Figure 47 – Calibration curves for the water based and soil water based calibration solutions.

The stability of the electrospray was controlled by monitoring the the intensity of the acetic acid (MP additive) (Figure 48). The intensity of the acetic acid was measured for each injection during the analysis, i.e. for every solution.

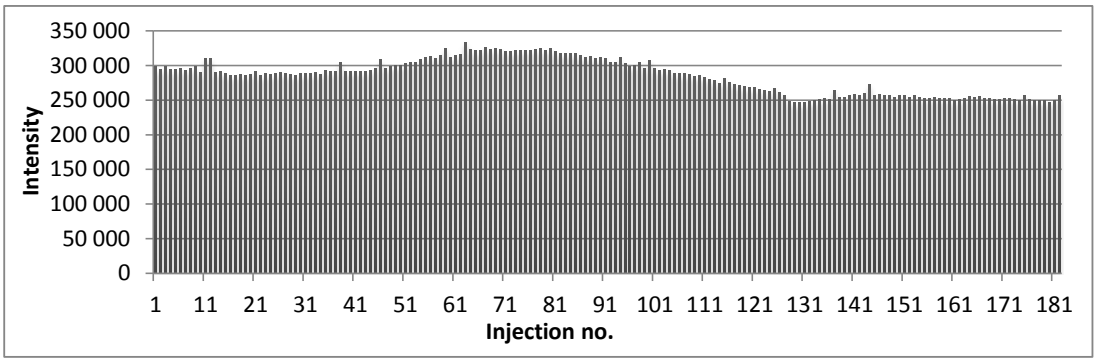

Figure 48 – Intensity of acetic acid as a proxy for the stability of the MS electrospray.

Table 44 – Calculated K_d and measured pH and conductivity in the sorption experiment samples/supernatant.

Sample	K_d	Conductivety (µS/cm)	pH
P-H_2	1.9	1055	4.7
tM_1-A	0.7	1168	4.8
tM_2-Bs	0.6	1479	5.2
tM_2-C	0.6	1780	5.6
tM_1-O	2.3	1411	4.9

D.14 Post Column Infusion Experiment

A post column infusion experiment can be used to check if analyte(s) and IS(s) are affected differently by the matrix. An injected blank sample (matrix) is connected through a T-piece to a standard solution of analyte and the mixed solutions is analysed on an MS (Figure 49). The blank sample goes through the LC-column and the components in the matrix are separated as in any other LC-analysis.

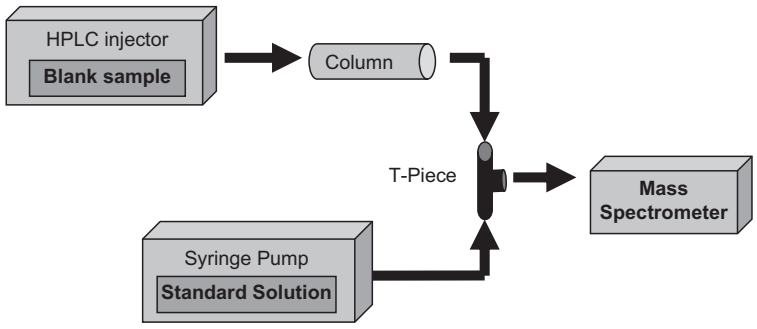

Figure 49 – Principle of post column infusion experiment. Retrieved from [29].

If the matrix causes ion suppression/enhancement this will be registered as a loss/increase of signal. If these results are overlaid with the peaks of the analyte and IS from a normal LC-MS(/MS) analysis, the effect of the matrix on the signals can be estimated. The results of this type of test where the IS and analyte are affected differently by the ion suppression is illustrated in Figure 50. Here a 9 second difference in retention time causes a 32 % difference in signal response due to different effect of ion suppression.

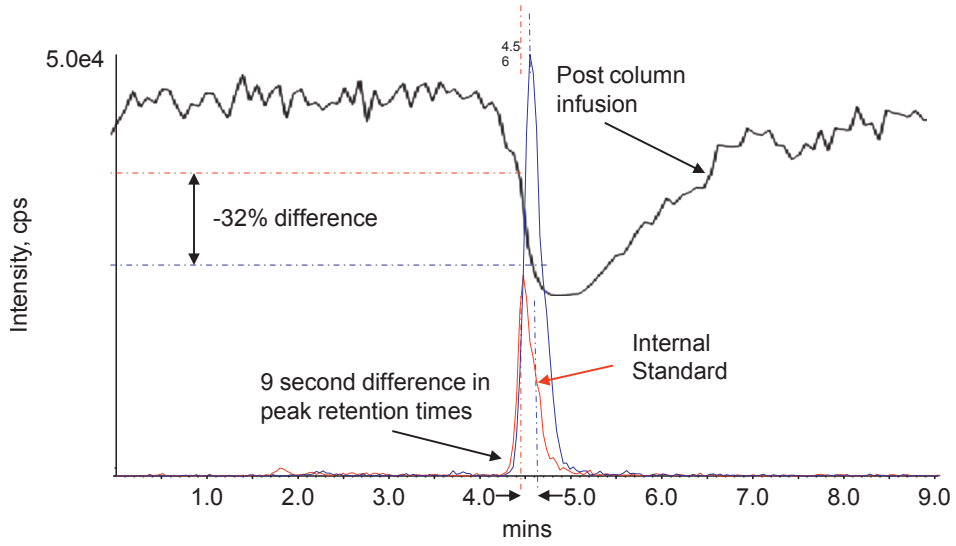

Figure 50 – Illustration of different effect on IS and analyte from matrix ion suppression. Retrieved from [29].