

# Binding of metals to macromolecular organic acids in natural waters

Does Organic Matter?

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Cover: Experimental setup for the isolation of DOM fractions  
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## Abstract

Trace metal speciation and bioavailability have become keys to current day toxicity and risk assessments. For many metals, macromolecular organic acids constitute the major ligand in fresh water and soil solution. Therefore, understanding their characteristics and behaviour is necessary for understanding trace metal behaviour. This study comprises investigations of the proton- and copper-binding properties of hydrophobic and hydrophilic dissolved organic matter fractions, and competition effects of iron(III) and aluminium. The solutions studied were a forest floor solution and a municipal solid waste incinerator bottom ash leachate. Two geochemical models (SHM and NICA-Donnan) were tested and calibrated against the experimental data. A structural analysis of the binding mode of iron(III) to fulvic acid in acid aqueous solutions was made using extended X-ray absorption fine structure (EXAFS) spectroscopy. Dissolved organic carbon (DOC) in the bottom ash leachate had fulvic acid-like properties and was dominated by the hydrophilic acid fraction. Three organic fractions (hydrophobic, transphilic and hydrophilic) were isolated from the forest floor solution using an XAD-8/XAD-4 tandem. All fractions were characterised by distinct but differing proton-binding properties, suggesting a more acidic character than 'generic' fulvic acid. The copper-binding isotherms were very similar for all three fractions and suggested strong copper binding to a small number of sites. In general, both models tested could be adjusted to obtain good fits to data on both proton- and copper-binding, but iron(III) and aluminium competition was better predicted by the SHM than the NICA-Donnan model. Only mononuclear iron(III) complexes were included in the model calculations, as the EXAFS study showed that these dominated in the aqueous phase. Studies on untreated soil solution indicated that the three isolated fractions were the only contributors to the observed copper binding and together constitute the 'active' DOC fraction. Thus, combination of Leenheer fractionation data with the model parameters derived in this study is recommended for improved predictions of trace metal speciation in soil solutions. However, further studies along this research line, including other samples and trace metals, are highly recommended.

*Keywords:* trace metal, humic substance, DOC, EXAFS, copper, heavy metal, hydrophobic, hydrophilic, MSWI, metal toxicity.

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*There's no metal like heavy metal!*

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## List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

- I van Schaik, J.W.J., Persson, I., Kleja, D.B., Gustafsson, J.P. (2008). EXAFS study on the reactions between iron and fulvic acid in acid aqueous solutions. *Environmental Science & Technology* 42(7), 2367-2373.
- II Olsson, S., van Schaik, J.W.J., Gustafsson, J.P., Kleja, D.B., van Hees, P.A.W. (2007). Copper(II) binding to dissolved organic matter fractions in municipal solid waste incinerator bottom ash leachate. *Environmental Science & Technology* 41(12), 4286-4291.
- III van Schaik, J.W.J., Kleja, D.B., Gustafsson, J.P. Acid-Base and copper-binding properties of three organic matter fractions isolated from a forest floor soil solution (manuscript).
- IV van Schaik, J.W.J., Olsson, S., Kleja, D.B., Gustafsson, J.P. Copper-binding properties of total and hydrophilic organic matter in a forest floor soil solution (manuscript).

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## Abbreviations

BA	Bottom ash
CE	Cation exchanged
DDL	Diffuse double layer
DGT	Diffusive gradient thin-film
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
EXAFS	Extended X-ray absorption fine structure
FA	Fulvic acids
FIAM	Free ion activity model
HiA	Hydrophilic acids
HiB	Hydrophilic bases
HiC	Hydrophilic compounds
HiF	Hydrophilic fraction
HiN	Hydrophilic neutrals
HoA	Hydrophobic acids
HoN	Hydrophobic neutrals
HPI	Hydrophilic
HS	Humic substances
LMWOA	Low molecular weight organic acids
MSWI	Municipal solid waste incinerator
MWCO	Molecular weight cut-off
NICA	Non-ideal competitive adsorption
NOM	Natural organic matter
SHM	Stockholm humic model
TiA	Transphilic acids
XANES	X-ray absorption near edge structure

# 1 Introduction

'Heavy metal' is a term that may provoke quite different reactions, depending on the recipient. Some people may stick out a hand with two fingers spread out wide, start banging their head violently and reach for the beer can. Others may get an indifferent look in their eyes and mumble something incoherent along the lines of 'all metals are heavy'. Still others may jump to attention and engage in a discussion concerning environment, pollution and scientific research. For me personally, any of the above could happen, depending on mood, who is asking, surroundings, etc.

Obviously the term 'heavy metal' can be interpreted in several ways. In fact, it was called 'meaningless and misleading' in an IUPAC (International Union of Pure and Applied Chemistry) technical report, due to its contradictory definitions and lack of a coherent scientific basis (Duffus, 2002). Hodson (2004) presented several alternatives in a paper dedicated specifically to the term, referring to heavy metals as 'bogey men'. In this thesis, the focus is on heavy metal from a soil chemical and scientific point of view. Rather than engaging in a continuous struggle with terminology, the term 'trace metals' will be adopted from this point onward:

*Trace metals - Chromium, cobalt, copper, iron, manganese, magnesium, molybdenum, selenium, zinc and other elements that occur in very small amounts (usually less than 1 to 10 parts per million) as constituents of living organisms, and are necessary for their growth, development and health. Whereas the shortage of trace elements in the body may result in stunted growth or even death, their presence in larger amounts is also harmful (Business Dictionary, Retrieved: August 27, 2008).*

Trace metals are naturally present in most soil systems, due to weathering of parent material (Ross, 1994). Whereas small quantities of trace metals are considered essential for optimal functioning of biological processes and organs in humans and other living beings, ingestion of excess amounts can result in a variety of symptoms and diseases (e.g. Fergusson, 1990; Merian & Clarkson, 1991). Examples can be nausea, vomiting, diarrhoea or memory

and concentration problems (copper), seizures, dizziness or impotence (zinc) and asthma, anxiety, angina or other cardiac symptoms (nickel and cobalt) (Roth, 2008). Due to this potential toxicity, trace metal contamination is a global problem (Siegel, 2002).

Various strategies have been developed for making risk assessments of contaminated and polluted areas. The common feature of most of these strategies is that they consider total soil metal contents as criteria. However, awareness has increased that the relevant variable in risk assessment is not the total content, but rather the distribution of these contents over various chemical forms (Souren, 2006). In simple words, there is no need to worry just because a soil contains elevated amounts of trace metals. It is when those metals become mobile and/or accessible that problems may arise, as they find their way into food webs via plant, animal and microorganism uptake, and into natural waters via leaching to groundwater and subsequent transport.

This is where *natural macromolecular acids* come into the picture. A macromolecule is defined as a very large molecule, such as a polymer, consisting of many smaller structural units linked together. Natural macromolecular acids are the most active and relevant part of natural organic matter (NOM), a collective term assigned to broken down organic matter originating from plants, animals and microorganisms, in terms of proton buffering and trace metal binding. Since macromolecular acids have the potential to dissolve under natural conditions, formation of soluble organic complexes is an important mechanism for the mobilisation of many trace metals in soils (Bergkvist *et al.*, 1989; Berggren, 1992b; Lundström, 1993).

Dissolution of organic matter and the formation of organic metal complexes are controlled to a large degree by soil acidity (Sposito *et al.*, 1978; Cabaniss & Shuman, 1988; McBride, 1994). Changes in soil acidity will therefore have major effects on the behaviour of trace metals. Acid rain is a well-known and actual phenomenon that results in soil acidification. Another cause of soil acidification can be the termination of lime application and conversion of arable lands to forests, pasture, heathlands or wetlands (Johnston *et al.*, 1986; Römkens & De Vries, 1995). In addition, plantation of forest on former agricultural soils (afforestation) results in accumulation of organic material in the topsoil, which in turn can increase dissolved organic matter (DOM) concentrations in the soil solution (Andersen *et al.*, 2002).

In short, increasing our understanding of the fate and behaviour of metal contaminations in soil systems is of major importance for improving risk assessments of contaminated areas.

## 2 Background

### 2.1 Trace metals in the environment

#### 2.1.1 How did they get there?

The origin of trace metals in our soils and environment is twofold. Firstly, trace metals can be present in the parent material, in the form of constituent and replacement elements in rock and soil minerals. *In situ* chemical weathering of such rock minerals results in a local accumulation of trace metals in soils; the ease and speed of weathering, as well as the trace metal contents of the parent material, are the main factors determining the rate of trace metal release. Secondly, trace metals are released into the environment due to anthropogenic (human) activities and sources, such as industry, atmospheric deposition, agriculture, waste disposal on land and metalliferous mining and smelting (Ross, 1994; Alloway, 1995).

Due to the large differences in parent material trace metal contents, trace metal concentrations in soils can vary widely. In contrast to this natural variation, the input from anthropogenic sources is determined by the location of a soil or site relative to metal pollution sources and, inherently, human activity. In general, however, the quantity of trace metals originating from anthropogenic sources exceeds that from natural sources by several orders of magnitude (Campbell *et al.*, 1983). Once metals enter the soil system, the options are limited; *i*) metals are sorbed onto various solid components and retained in the soil (e.g. Mighall *et al.*, 2002), or *ii*) they are dissolved and consequently susceptible to leaching and plant uptake. Although anthropogenic inputs are site-specific, the fate of trace metals once they enter the soil system is soil-specific; actual soil properties determine the distribution of the trace metals between the solid and solution phase.

In general, the mobility of metals in soil systems is fairly low, which results in accumulation and consequently elevated trace metal levels,

particularly at sites with high anthropogenic inputs (e.g. Brinkmann, 1994; Hamon *et al.*, 1998; Cicek & Koparal, 2004). Unfortunately, although major efforts are – and have been – made to reduce these anthropogenic inputs, many soils are already contaminated beyond ‘acceptable’ levels.

### 2.1.2 Total or biologically available?

In order to be able to speak of ‘acceptable’ levels, it is essential that critical toxicity guidelines and limits for trace metals are established. As a well-known saying goes, ‘wisdom comes with the years’; during the past decade, the emphasis in assessing heavy metal toxicity and critical limits has shifted from total metal contents to soluble and bio-available concentrations (e.g. Salbu, 1991; Ashmore *et al.*, 2000). Morel (1983) introduced the free ion activity model (FIAM), which proposes that metal toxicity is related to uptake of specific metal species at the organism–solution interface. Thus, the concentration of the free metal ion in solution is supposed to be a better predictor of toxicity than the total (dissolved) metal content or concentration.

An essential concept in defining bio-availability is the chemical speciation of a trace metal. Metal speciation has been defined as determination of the individual concentrations of the various physico–chemical forms of a metal that together make up the total concentration of that metal in a sample (Florence, 1986). Knowledge of the speciation of a metal is essential in order to assess *i*) the bioavailability or toxicity of the metal or *ii*) the mechanism controlling the solubility of the metal.

Many researchers have found evidence in support of the ‘free metal ion hypothesis’, where the toxicity or bioavailability of any metal ion is assumed to be directly related to the activity of the free, hydrated form of the metal (Bingham *et al.*, 1984; Brümmer *et al.*, 1986; Hue *et al.*, 1986; Sauve *et al.*, 1998). However, dissolved organic matter has been found to be an important factor in the uptake of trace metal ions by plants, although no consensus has been reached concerning its exact role (e.g. Jones & Darrah, 1992; Parker *et al.*, 2001; Molas & Baran, 2004; Inaba & Takenaka, 2005). Stumm & Morgan (1996) reported that the bioavailability of metal ions, and their toxicity to soil organisms, is lowered by complex binding to dissolved organic matter. Others have reported increased plant metal uptake in soils with increased DOM levels (Pietz *et al.*, 1989; Clemensson-Lindell, 1992). These discrepancies might be explained by the dynamic nature of organic metal complexes. Zhang *et al.* (2001), for example, suggested that the presence of (labile) metal–organic complexes might indirectly increase metal bio-availability by acting as a buffer for metal ions in the soil solution. In a

similar fashion, breakdown of DOM into low molecular weight organic acids (LMWOA) might further increase metal solubility and availability to plants (Parker *et al.*, 2001; Inaba & Takenaka, 2005).

Several studies have reported good correlations between ‘effective’ metal concentrations in soil solution, determined by a technique called DGT (diffusive gradients in thin films), and plant metal contents (Zhang & Davison, 2000; Zhang *et al.*, 2001; Tye *et al.*, 2004; e.g. Zhang *et al.*, 2004; Hough *et al.*, 2005; Nolan *et al.*, 2005; Zhang *et al.*, 2006). The effective concentrations have been reported to incorporate both the free metal ion concentrations in solution and a labile complex fraction. Thus, the correlations found give further weight to the importance of organic metal complexes.

## 2.2 Organic matter

### 2.2.1 Does it matter?

Natural organic matter is a general collective term for organic molecules formed by decomposition of plants, animal products and microbial material. Due to the large variety in original material and decomposition stage, NOM is far from a ‘simple molecule’, as it lacks an unique structure or composition, cannot be crystallised and is extremely difficult to characterise. Instead, NOM consists of a heterogeneous mixture of complex molecules, with basic structures created from cellulose, tannin, cutin, and lignin, along with other various proteins, lipids, and sugars. The chemically most significant fraction consists of the humic substances, which are generally of an acidic character (Swift, 1989).

Due to their origin, humic substances are abundant in the environment; due to their character, they interact with a variety of solutes that may be present. Consequently, humic substances (HS) are factors in a range of environmental issues (Table 1), and thus give rise to a worldwide interest in attempting to understand their characteristics and environmental behaviour. Of particular interest within the scope of this thesis is the role of humic substances in controlling solubility and mobility of trace metals (Schnitzer & Skinner, 1963; Schnitzer & Skinner, 1964; Schnitzer & Skinner, 1965; Kerndorff & Schnitzer, 1980; Perdue & Lytle, 1983; Weber, 1988). For many metal ions such as aluminium(III), iron(III), copper(II), lead(II) and mercury(II), macromolecular organic acids constitute the major ligand in fresh water and the soil solution (Tipping, 2002).

Table 1. *Environmental issues involving humic substances (adapted from Tipping, (2002))*

Issue	Role of humic substances
Carbon cycling	Major carbon pool, transformations, transport and accumulation
Light penetration into waters	Absorption and attenuation of light by humic chromophores
Soil warming	Absorption of solar radiation by soil humic matter
Soil and water acidification	Binding of protons, aluminium and base cations in soils and waters
Nutrient source	Reservoir of carbon, nitrogen, phosphorus and sulphur
Nutrient control	Binding of iron and phosphate
Microbial metabolism	Substrate for microbes
Weathering	Enhancement of mineral dissolution rates
Soil formation (podsolisation)	Translocation of dissolved humic substances and associated metals (aluminium, iron)
Properties of fine sediments	Adsorption at surfaces and alteration of colloidal properties
Soil structure	Aggregating effect on soil mineral solids
Photochemistry	Mediation of light-driven reactions
<b>Trace metals</b>	<b>Binding, transport, influence on bioavailability, redox reactions</b>
Pesticides, xenobiotics	Binding, transport, influence on bioavailability
Radioactive waste disposal	Binding and transport of radionuclide ions in groundwater
Ecosystem buffering	Control of proton and metal ion concentrations, persistence

### 2.2.2 'Natural' macromolecular acids

Humic substances have traditionally been divided into three main groups; *i*) humic acids – soluble in bases, insoluble in acids, *ii*) fulvic acids – soluble in both bases and acids, and *iii*) humin –soluble in neither bases nor acids. In general, fulvic acids have a lower molecular weight, degree of polymerisation and carbon/oxygen (C/O) ratio than humic acids, but a higher exchange acidity (Figure 1). Solubility is related to the size-relative charge of the humic molecules, which in turn is related to pH – the higher the pH, the more acidic groups will be deprotonated and carry a negative charge. Adding one and one, this implies that fulvic acids generally carry a higher size-relative charge than humic acids and, even more so, humin. As a

result of their surface charge, part of the humic substances may be present in the aqueous phase under natural conditions. This dissolved organic matter (DOM) maintains many of the characteristics of its solid precursor, and therefore plays an important role in bioavailability and transport of trace metals (Bergkvist *et al.*, 1989; Berggren, 1992b; Berggren, 1992a).

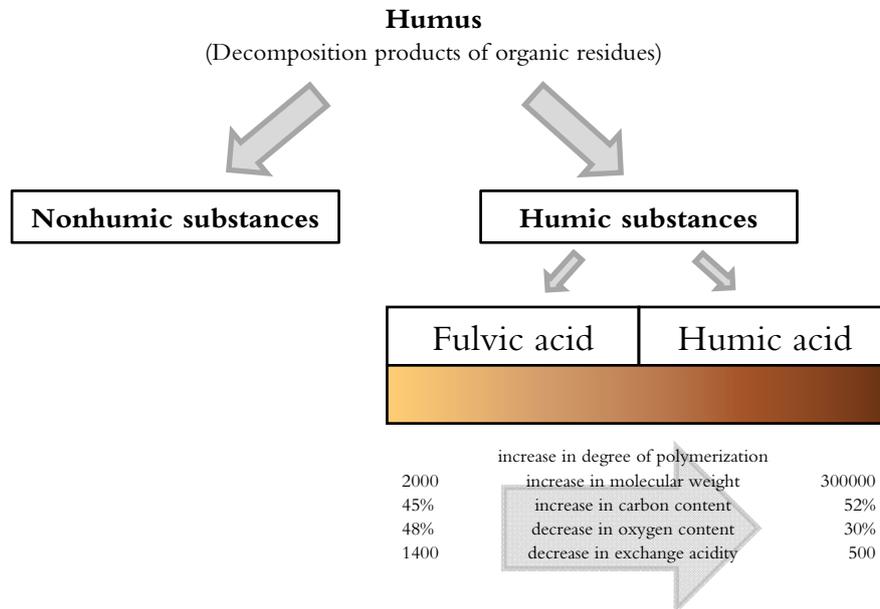


Figure 1. Classification and observed chemical properties of humic substances (adapted from Scheffer and Ulrich, (1960).

The majority of macromolecular acids in ecosystems have a natural origin, being the products of NOM decomposition. However, there are additional sources which have a more anthropogenic character, such as municipal solid waste incinerator (MSWI) bottom ash and sewage sludge. MSWI bottom ash is used for various purposes, such as raw material for cement (Polettini *et al.*, 2001; Aubert *et al.*, 2006; Pan *et al.*, 2008), road construction (Schreurs *et al.*, 2000; Åberg *et al.*, 2006; Hjelmar *et al.*, 2007; Izquierdo *et al.*, 2007), and as a drainage layer in landfills (Palmer *et al.*, 2000). Sewage sludge is often used as a soil amendment and fertiliser, and has been used extensively to stimulate re-vegetation of mine tailings (Bergholm & Steen, 1989; Pichtel *et al.*, 1994; Voeller *et al.*, 1998). Consequently, organic acids that are present in the ash or sludge have the

potential to dissolve and leach into the soil system. Several studies have shown that organic acids derived from these sources, like their natural siblings, have pronounced proton- and metal-binding properties (e.g. Sposito & Holtzclaw, 1977; Sposito *et al.*, 1977; Sposito *et al.*, 1979; da Silva & Oliveira, 2002; Plaza *et al.*, 2005; Plaza *et al.*, 2006). However, very little is known with respect to the intrinsic properties of the hydrophilic fraction of these anthropogenically introduced humic substances.

### 2.2.3 Fractionate, isolate, investigate

Procedures for fractionation and isolation of the different fractions from soils and waters have been developed and published, providing a good basis and foothold for continued research (Aiken *et al.*, 1985; Stevenson, 1994). The major difference between fractionation and isolation is that the fractionation procedure is qualitative, whereas the isolation procedure is quantitative. In other words, the purpose of a fractionation procedure is to accurately determine the exact distribution of organic carbon over the different fractions, whereas the purpose of an isolation procedure is to obtain useable amounts of the major fractions.

An extensive, well-established fractionation scheme for aqueous samples was published by Leenheer (1981). According to this scheme, aqueous macromolecular acids are divided into hydrophobic and hydrophilic acids based on how they react with an Amberlite™ XAD-8 resin (Leenheer & Huffman Jr., 1976; Leenheer, 1981). XAD resins are non-ionic, macroporous copolymers that possess large surface areas, allowing for high recoveries of organic compounds from water. Hydrophobic acids are defined as the fraction of dissolved organic matter that - following a uniform standard procedure - is retained by a XAD-8 resin column, and desorbs on (back-)flushing the column with sodium hydroxide. For solutions, the compounds isolated in this way are also referred to as (aquatic) fulvic acids (Aiken *et al.*, 1985).

*‘DOM is a complex mixture of aromatic and aliphatic hydrocarbon structures that have attached functional groups’  
(Leenheer & Croue, 2003)*

The compounds that pass through the XAD-8 resin column and are consequently retained by a strongly basic anion exchanger are defined as hydrophilic acids. Table 2 gives an overview of the general composition of hydrophobic and hydrophilic compounds. An analytical procedure for the isolation of major organic fractions from forest floor leachate was presented by Vance & David (1991, Figure 2). In soil solution and most other natural waters, the hydrophobic acids constitute about 50% and the hydrophilic

acids about 30% of total DOC (Leenheer & Huffman Jr., 1976; Aiken *et al.*, 1992; Malcolm & MacCarthy, 1992).

Table 2. *General composition of DOM fractions (adapted from Zech & Guggenberger, (1996)*

Fraction	Composition
Hydrophobic acids	Polyelectrolytic aliphatic and aromatic acids, highly degraded lignin- and lignocellulose-degradation products
Hydrophobic neutrals	Aliphatic compounds (fatty acids, waxes), less degraded lignin- and lignocellulose-degradation products
Hydrophilic acids	Polyelectrolytic aliphatic and aromatic acids, very highly degraded lignin- and lignocellulose-degradation products, more oxidized than hydrophobic acids
Hydrophilic neutrals	Mainly carbohydrates, polyfunctional alcohols
Hydrophilic bases	Amino acids, amphoteric proteins, amino sugars

Despite the fact that hydrophilic acids make up a significant part of the total DOC, the vast majority of research on metal binding studies has been done on isolated humic and fulvic acids (Tipping, 1993b; Pinheiro *et al.*, 1999; Pinheiro *et al.*, 2000; Christl *et al.*, 2001; Milne *et al.*, 2003). The hydrophilic fraction remains relatively unexplored with respect to its metal binding properties (Peuravuori *et al.*, 2001).

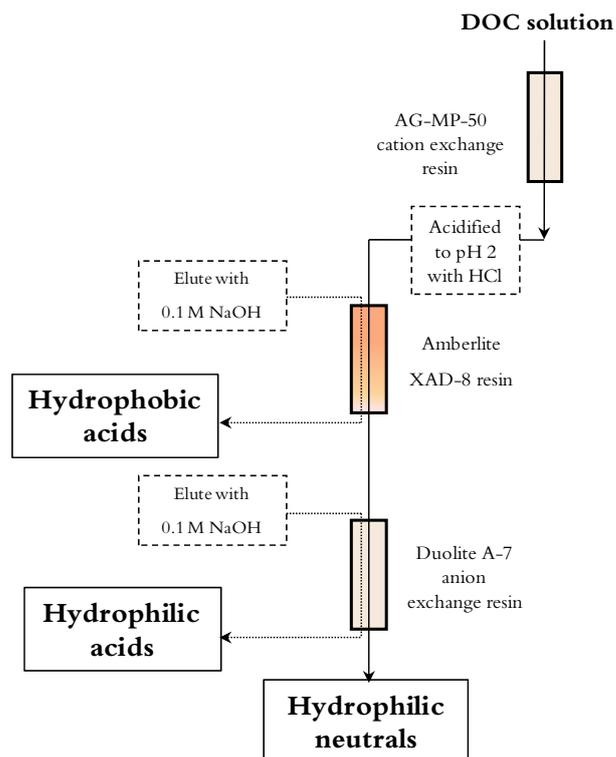


Figure 2. Analytical procedure for dissolved organic carbon isolation (adapted from Vance & David, (1991).

#### 2.2.4 Practical limitations

Why have so few studies been published on the hydrophilic fraction? Practical difficulties, time and money are the simple and short answers to this question. As illustrated in a recent publication, the costs of performing a fractionation of a sample were about \$300 in 2000 (Leenheer & Croue, 2003, Figure 3).

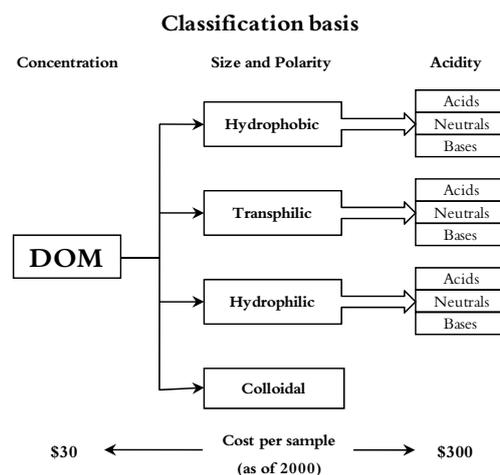


Figure 3. Classification of DOM. Resolving the detailed components of dissolved organic matter can be difficult and expensive (adapted from Leenheer & Croue, (2003).

However, these are the costs for an analytical-scale DOC fractionation. To acquire pure samples of the actual fractions, a more extensive isolation procedure needs to be followed. In addition, much larger solution volumes must be processed in order to obtain adequate amounts of isolates for follow-up experiments and analyses. The hydrophobic fraction usually makes up the largest proportion of the total DOC, and is isolated in the first step of the procedure. The hydrophilic fraction makes up a smaller part of the total DOC and requires a second column and desorption step in an isolation procedure. For example:

*Hydrophobic and hydrophilic acid fractions of natural water containing 15 mg L<sup>-1</sup> DOC are to be isolated. Using an XAD-8 resin column with a total volume of 100 mL, the volume of sample to pass through is calculated to 2750 mL. Assuming that the sample contains 60% hydrophobic acids and 25% hydrophilic acids, this means that the maximum amount of material isolated (100% recovery) equals approximately 25 and 10 mg for the respective fractions. Considering actual recovery losses, the final amount of hydrophilic acids obtained would thus perhaps be 8-9 mg, after about one full week of work and treatment of nearly 3 L of solution.*

In the example above, the isolation followed the procedure by Vance & David (1991) described in Figure 2. An alternative isolation procedure has been suggested in which the anion exchange column is replaced by an

Amberlite XAD-4 column. Thus, a second split on hydrophobicity is made, and a third, intermediate, fraction is introduced. Malcolm & MacCarthy (1992), who first introduced the XAD-8/XAD-4 tandem isolation procedure, labelled the fraction retained on the XAD-4 column as 'XAD-4 acids'. Croué *et al.* (2003) studied the fraction retained on and consequently desorbed from the XAD-4 resin by elution with 0.1 M NaOH, and introduced the term 'transphilic acids' for this fraction. This term was considered more appropriate and has therefore been adopted in this thesis.

## 2.3 Geochemical models

### 2.3.1 General background

During recent years, sophisticated models have been developed that are able to describe the acid-base chemistry and metal complexation properties of isolated humic and fulvic acids in a general way. The underlying principle is that metal speciation can ideally be predicted from relatively easily obtainable variables, such as total contents or concentrations of metals, major cations and anions, organic carbon and pH. Such models can prove vital in the prediction of possible effects of land use changes on accumulated metals, for example in areas exposed to diffuse metal pollution. The most frequently applied models include Model VI (Tipping, 1998), Non-Ideal Competitive Adsorption Donnan (NICA-Donnan) (Kinniburgh *et al.*, 1999a) and the Stockholm Humic Model (SHM) (Gustafsson, 2001).

The general approach in these models is that the binding of cations occurs through the processes of specific and non-specific binding. Specific binding relates to specific interactions between the cation and negatively charged surface groups (inner-sphere complex); non-specific binding relates to coulombic interactions due to any residual negative charge (counterion accumulation). The models use different approaches and assumptions to account for both of these binding types.

### 2.3.2 Applicability

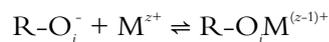
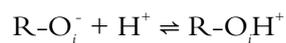
Model VI and its precursor Model V (Tipping & Hurley, 1992) have been applied to a range of metals, including the alkaline earths, aluminium and transition metals (Tipping & Hurley, 1992; Tipping, 1993a; Tipping, 1993b; Tipping, 1998). It was found in applying these models to proton and metal binding data that there were distinct differences between fulvic and humic acids in terms of binding affinity and site density. However, great similarities were observed between the numerous datasets on fulvic and humic acids,

indicating that there was no consistent dependence on the source of the two materials – soil, water, etc. (Tipping, 1998). Thus, it seems to be possible to define reasonable, consistent, general (average) binding characteristics for each of the two groups, something that is crucial for a wider application of models on natural waters.

Following these findings, Milne *et al.* (2001; 2003) developed a set of ‘generic’ NICA-Donnan parameters for proton and metal binding to humic and fulvic acids. The proton-binding parameters were derived from a total of 49 datasets, consisting of literature and experimental data. Derivation of metal-ion binding parameters comprised a total of 171 datasets, also consisting of literature and experimental data. A similar set of generic proton-binding parameters was developed for the SHM, using a total of 18 datasets, divided evenly over fulvic and humic acids (Gustafsson, 2001); this was later extended with additional data sets used by Milne *et al.* (2001), to arrive at the current version of the generic proton-binding parameters as used in the Visual MINTEQ software (Gustafsson, 2008). Similarly, the generic metal-binding parameters of SHM were derived from data sets used by Milne *et al.* (2003). Good results were obtained in applying these parameters in recent studies on proton and metal binding (Oste *et al.*, 2002; Weng *et al.*, 2002b; Gustafsson *et al.*, 2003; Gustafsson & van Schaik, 2003; Ge *et al.*, 2005).

### 2.3.3 NICA-Donnan model

The NICA model was initially developed to describe ion-specific interactions on heterogeneous surfaces, such as humic substances (Koopal *et al.*, 1994; Benedetti *et al.*, 1995; Benedetti *et al.*, 1996). In a later stage, the model was further developed to incorporate non-specific binding, by means of the Donnan model (Kinniburgh *et al.*, 1996; Kinniburgh *et al.*, 1999a). One of the main assumptions underlying the model is a bimodal, continuous distribution of site affinities, accounting for a pool of low (carboxylic) and high (phenolic) proton affinity binding sites. As a basis for the NICA-Donnan model, proton and metal ion binding to a negatively charged site of type R-O<sub>i</sub><sup>-</sup> are described according to:



where  $R-O_i^-$  represents a binding site of carboxylic or phenolic type, and  $M^{z^+}$  is a cation with charge  $z^+$ . These reactions assume monodentate binding to all the sites.

In a mono-component system, the amount of protons bound ( $Q_H$ ) is given by the Langmuir-Freundlich equation

$$Q_H = Q_{\max,1} \frac{(\tilde{K}_{H,1} c_{D,H})^{m_1}}{1 + (\tilde{K}_{H,1} c_{D,H})^{m_1}} + Q_{\max,2} \frac{(\tilde{K}_{H,2} c_{D,H})^{m_2}}{1 + (\tilde{K}_{H,2} c_{D,H})^{m_2}} \quad (\text{Eq. 1})$$

where  $Q_{\max}$  is the overall site density,  $\tilde{K}$  represents the median proton affinity constant,  $m$  defines the width of the proton affinity distributions and  $c_{D,H}$  is the concentration of protons in the so-called Donnan layer, explained in the following. Subscripts 1 and 2 refer to sites of type 1 (carboxylic) and 2 (phenolic), respectively.

When using the Donnan model for describing non-specific binding due to electrostatics, it is assumed that the total net charge on the humic particle,  $q$ , is at all times completely neutralised by counter-ions within the Donnan volume. The overall electroneutrality is then given by the basic equation:

$$\frac{q}{V_D} + \sum z_i (c_{i,D} - c_i) = 0 \quad (\text{Eq. 2})$$

where  $c_{D,i}$  is the concentration of component  $i$  with charge  $z_i$  (including sign) in the Donnan phase and  $c_i$  is its concentration in the external solution. The concentration of a component in the Donnan layer is related to the concentration in solution, according to:

$$c_{D,j} = c_j \exp\left(-\frac{z_j F \psi_D}{kT}\right) \quad (\text{Eq. 3})$$

where  $F$  is the Faraday constant,  $\psi_D$  is the (uniform) Donnan potential,  $k$  is the Boltzmann constant and  $T$  is the absolute temperature.

Contrary to the diffuse double layer (DDL) model, in which the electrostatic potential varies with the distance from the humic particle (McBride, 1994; Fawcett & Smagala, 2006), the Donnan model assumes that the potential is distributed uniformly within the Donnan volume or 'phase'. This volume is given by the relationship:

$$\log V_D = b(1 - \log I) - 1 \quad (\text{Eq. 4})$$

where  $b$  is an empirically adjustable coefficient, varying with the type and source of humic substance; it can also be analytically determined as the slope of a plot of  $\log V_D$  vs.  $\log I$  (Milne et al., 2001).

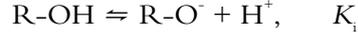
For a multi-component system containing one or more metal ion species, the width of the site distribution,  $m$ , is split up into an ion-specific non-ideality parameter,  $n$ , and a humic substance-specific intrinsic chemical heterogeneity parameter,  $p$ . The total bound amount of component  $i$  is given by:

$$Q_i = Q_{\max 1,H} \cdot \frac{n_i}{n_H} \cdot \frac{(\tilde{K}_{i,1} c_i)^{n_{i,1}}}{\sum_i (\tilde{K}_{i,1} c_i)^{n_{i,1}}} \cdot \frac{\left[ \sum_i (\tilde{K}_{i,1} c_i)^{n_{i,1}} \right]^{p_1}}{1 + \left[ \sum_i (\tilde{K}_{i,1} c_i)^{n_{i,1}} \right]^{p_1}} + Q_{\max 2,H} \cdot \frac{n_i}{n_H} \cdot \frac{(\tilde{K}_{i,2} c_i)^{n_{i,2}}}{\sum_i (\tilde{K}_{i,2} c_i)^{n_{i,2}}} \cdot \frac{\left[ \sum_i (\tilde{K}_{i,2} c_i)^{n_{i,2}} \right]^{p_2}}{1 + \left[ \sum_i (\tilde{K}_{i,2} c_i)^{n_{i,2}} \right]^{p_2}} \quad (\text{Eq. 5})$$

where  $n_H$  and  $n_i$  reflect the non-ideal behaviour of protons and the ion  $i$ , respectively.

#### 2.3.4 Stockholm Humic Model

Contrary to the NICA-Donnan model, the SHM uses a discrete site distribution assumption to describe proton and metal binding by HS (Figure 4). The underlying assumptions and nomenclature regarding active functional groups were adopted from Model VI (Tipping, 1998). The HS dissociation reaction can be written as:



where  $\text{R-O}^-$  represents a functional group and  $K_i$  is an intrinsic dissociation constant, including an electrostatic correction term. The SHM uses an empirically adjusted version of the Basic Stern Model to describe salt dependence. For proton binding, the intrinsic dissociation constant is defined as:

$$K_i = \frac{\{RO^-\}\{H^+\}}{\{ROH\}} \cdot e^{-g_f \frac{F\psi_0}{RT}}, \quad (\text{Eq. 6})$$

where  $F$  is the Faraday constant,  $\psi_0$  is the potential in the  $0$ -plane (i.e. at the surface),  $R$  is the gas constant,  $T$  is the absolute temperature, and  $g_f$  is the ‘gel fraction’ parameter, which is a measure of the proportion of HS that is aggregated in gel-like structures, and has a value between 0 and 1. As follows from equation (2), the gel fraction parameter determines the exact value of the stoichiometry of the electrostatic correction terms. For metal binding, equation (2) takes a more complex form, as corrections need to be made for binding in the  $d$ -plane as well; for more detailed information, see Gustafsson (2001).

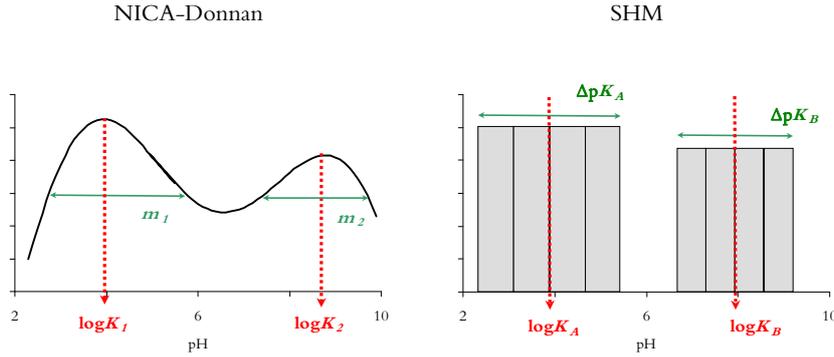


Figure 4. Simplified, schematic overview of a continuous (NICA-Donnan, left) and discrete (SHM, right) distribution of sites;  $\log K_i$  gives the median value of the affinity distribution of sites of type  $i$  for protons (NICA-Donnan) or the intrinsic proton dissociation constant for type  $i$  sites (SHM);  $m$  defines the width of the proton affinity distributions and  $\Delta p K_i$  is the distribution term that modifies  $\log K_i$ .

In the SHM, a total of eight R-O sites are used, each with a unique acidity; thus, eight  $K_i$  values are defined. These eight sites are divided between two groups A and B, which are commonly assumed to represent carboxylic and phenolic acid sites. The carboxylic group contains the strongest acid sites (numbers 1-4), and the phenolic group contains the remaining sites (numbers 5-8). The eight  $K_i$  values are defined by four constants ( $\log K_A$ ,  $\log K_B$ ,  $\Delta p K_A$ ,  $\Delta p K_B$ ), according to:

$$\begin{aligned} i = 1 - 4 : \quad \log K_i &= \log K_A - \frac{(2i - 5)}{6} \Delta p K_A, \\ i = 5 - 8 : \quad \log K_i &= \log K_B - \frac{(2i - 13)}{6} \Delta p K_B. \end{aligned} \quad (\text{Eq. 7})$$

Sites 1–4 and sites 5–8 are present in equal amounts in their respective groups; the total amount of all proton-dissociating sites,  $n$  ( $\text{mol g}^{-1}$ ), is equal to the sum of all sites in both groups. The distribution of total sites between carboxylic and phenolic type sites is a variable that depends on the HS studied. The SHM uses default values as follows: the average total amount of phenolic type sites is 30% of the total amount of carboxylic type sites for fulvic acids, and 50% for humic acids (Gustafsson, 2001); however, these values can be changed by the user when proton-binding data are available.

## 2.4 Complex competition

An important factor controlling the speciation and bioavailability of trace metals is competition for binding sites by other cations. Simply stated, the amount of available binding sites is limited by the prevailing conditions, which means that competition for sites occurs if the demand for sites exceeds their availability. Iron(III) and aluminium are abundant elements in natural systems, and have been reported to compete strongly for binding sites on organic ligands (Schnitzer & Skinner, 1964; Senesi *et al.*, 1977; Kerndorff & Schnitzer, 1980). Ge *et al.* (2005) emphasised the importance of estimates or measurements of iron(III) and aluminium concentrations in order to provide reasonable estimates of trace element speciation. In addition to this, competition data are particularly important since they provide a stringent test to geochemical models (Hering & Morel, 1988).

The binding of iron(III) and aluminium to humic and fulvic acids has been described in several recent papers (e.g. Tipping, 1998; Kinniburgh *et al.*, 1999a; Pinheiro *et al.*, 2000), and modelling efforts have provided generic parameters for the complex formation of iron(II) and iron(III) with humic and fulvic acids (Tipping *et al.*, 2002; Milne *et al.*, 2003). However, experimental data on the competitive effect of iron(III) and aluminium on the binding of trace metals to humic substances are scarce, while no data at all could be found regarding the interaction of iron(III) and aluminium with hydrophilic humic substances.

## 2.5 EXAFS – A closer look

Geochemical models, such as those described in detail in the previous sections, are excellent tools in interpreting experimental metal-humic substance binding data. However, although the models can be used to describe and predict trace metal speciation in the presence of humic substances, they do not provide any structural information. Therefore, assumptions have to be made about the functional groups involved in the

binding process, e.g. the chemical identity of the coordinating atoms of the ligand, whether the binding is mono- or bidentate or both, or whether complexes formed are inner- or outer-sphere.

The rapid development of synchrotron-based X-ray absorption spectroscopy (XAS) during the past two decades has provided the means to retrieve answers to the questions posed above (e.g. Xia *et al.*, 1997; Bochatay & Persson, 2000; McNear *et al.*, 2005). The techniques used for the structural analysis of metal ions to humic substances in soils and dilute solutions are EXAFS (extended X-ray absorption fine structure) and XANES (X-ray absorption near edge structure). In addition to the information mentioned, these techniques also allow for determination of the redox state of the element under study. This can be of particular interest for metals such as iron and molybdenum, which under natural conditions can be present in different redox states that differ considerably in their chemical behaviour.

Several EXAFS studies on metal-humic substance interactions have been published during recent years. Sarret *et al.* (1997) reported that zinc formed inner-sphere complexes in both octahedral and tetrahedral coordination with HS at low zinc concentrations. At higher zinc concentrations, however, the majority of the zinc ions formed outer-sphere complexes instead. Several studies have reported that sulphur groups are involved in the binding of cadmium, zinc and mercury to HS (Karlsson *et al.*, 2005; Skyllberg *et al.*, 2006; Karlsson & Skyllberg, 2007), and copper has been found to form inner-sphere complexes with either one or two five-membered chelate rings, involving possible combinations of amino, carboxyl, or carbonyl functional groups (Karlsson *et al.*, 2006).

The information obtained from such spectroscopic studies can be used to constrain geochemical models. For example, Gustafsson *et al.* (2008) studied iron(III) binding to organic mor layers from forest soils and found that the major fraction of the organically complexed iron was hydrolysed, most likely in a mixture of dimeric and trimeric complexes. The results were successfully used to constrain metal ion-humic complexation in the SHM, considering one dimeric hydrolysed iron(III) humic complex. In particular, models for metal complexation can be improved by considering the actual coordination environment of the formed metal-ligand complex (Peacock & Sherman, 2004a; Peacock & Sherman, 2004b). This should lead to better simulations of metal binding and competition effects in soils and natural waters.

### 3 Objectives of this study

The overall aim of this study was to improve understanding of the characteristics and behaviour of macromolecular organic acids in natural waters, in particular the seldom-studied hydrophilic acid fractions, with the main focus on proton- and metal-binding properties. In order to achieve this goal, specific objectives were formulated as follows:

- \* To produce consistent datasets on binding of proton and copper by hydrophobic and hydrophilic acids of natural origin at environmentally relevant concentrations.
- \* To quantify the competitive effect of aluminium and iron(III) ions on the binding of copper by hydrophobic and hydrophilic acids.
- \* To obtain a molecular understanding of the mechanisms involved in the binding of iron(III) by fulvic acids.
- \* To assess the copper-binding properties of organic acids in a municipal solid waste incinerator (MSWI) bottom ash leachate.
- \* To calibrate and test the SHM and NICA-Donnan models on systems containing natural and man-made macromolecular acids.

## 4 Experimental work

### 4.1.1 Study site & sampling of soil solutions

Asa Experimental Forest and Research Station, which was established in 1988, is located 37 km north of Växjö (57°10'N, 14°47'E), in the village of Asa. Approximately 80% of the total area is forest land, divided between Norway spruce (*Picea abies* (L.) Karst., approximately 50%), Norway spruce/Scots pine (*Pinus silvestris* L.) mixed coniferous forest (approximately 25%) and mixed coniferous/broadleaf forest (remainder).

The soil at the experimental plot used for this study is classified as a Haplic Podsol (Typic Haplorthod) (Soil Survey Staff, 2006). The plot was clear-cut in 1966 and planted in 1967 with 4-year old Norway spruce seedlings. Properties of the site are summarised in Table 3.

Table 3. Site characteristics (adapted from Berggren *et al.*, (2004))

Latitude	57°08'N
Longitude	14°45'E
Altitude (m.a.s.l.)	190–200
Mean annual air temperature (°C) <sup>1)</sup>	5.5
Mean length of growing season (days) <sup>2)</sup>	190
Mean annual precipitation (mm) <sup>1)</sup>	688
Nitrogen deposition (kg ha <sup>-1</sup> yr <sup>-1</sup> )	8.8
Sulphur deposition (kg ha <sup>-1</sup> yr <sup>-1</sup> )	4.9
Major tree species	Norway spruce
Stand age in 2005 (years)	42
Soil type according to FAO (1990)	Podsols
Vegetation zone	Boreo-nemoral

<sup>1)</sup> Long-term mean values (1961–1990) from nearest meteorological station (Berg); <sup>2)</sup> Days with average temperature >5 °C

In this project, we used zero-tension lysimeters to collect soil solution from the O horizon, which comprises a mor-type organic layer with a typical thickness of 3–10 cm. Samples were taken on two occasions (May 2005 and August 2005), filtered ( $<0.2 \mu\text{m}$ , Supor 200) and stored in glass bottles at  $+2 \text{ }^\circ\text{C}$ . Dissolved organic matter in the O horizon solution from this site has previously been characterised in detail (Fröberg *et al.*, 2003). Following the Leenheer fractionation scheme, Fröberg *et al.* (2003) found that the two major fractions, the hydrophobic and hydrophilic acids, made up 57.5 and 30% of the total DOC, respectively (Figure 5).

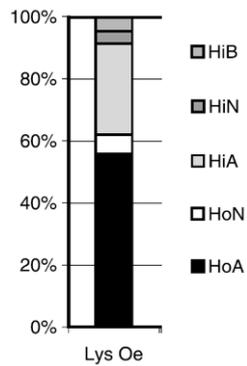


Figure 5. Overview of average ( $n=2$ ) contents of hydrophobic acids (HoA) and neutrals (HoN), hydrophilic acids (HiA), neutrals (HiN) and bases (HiB) in lysimeter water (adapted from Fröberg *et al.*, (2003).

#### 4.1.2 MSWI Bottom ash

A four-month-old MSWI bottom ash (BA) sample from Uppsala municipality, central Sweden, was collected from outdoor heaps in March 2004 and was stored for a further four months at room temperature (around  $20^\circ\text{C}$ ) with free access to air (Figure 6). At this point, carbonation of the ash sample was nearly complete, as indicated by the pH value of an extract (Meima & Comans, 1999).



Figure 6. Uppsala municipality MSWI bottom ash sample after several months of storage.

Some pre-treatment was required to produce ash leachate that could be used for fractionation and titration studies. First, metal parts, unburned material and particles  $>10$  mm were removed. Second, the sample was 'leached' with ultrapure  $\text{H}_2\text{O}$  in an end-over-end shaker, using a liquid-to-solid ratio of 5 (i.e. 5 litres of  $\text{H}_2\text{O}$  per kg of ash). Third, the slurry was centrifuged, after which the supernatant was filtered through a sterilised membrane ( $0.2 \mu\text{M}$ ). The leachate thus obtained was analysed for pH, cations, anions, DOC and LMWOAs (Paper II). A sub-sample was fractionated according to Leenheer (1981) for determination of the composition of the humic substances.

#### 4.1.3 Total versus hydrophilic NOM

For Papers II and IV, we used a simplified fractionation scheme, as the aim was to compare the copper-binding properties of the total organic fraction with those of the hydrophilic part of the solution. We studied leachates from both the MSWI bottom ash (BA) sample from Uppsala and the soil solution from Asa. To achieve this, the entire solution was passed through an  $\text{H}^+$ -saturated AG-MP-50 column and no further purification was done. Part of

the sample was used as such, and part was passed through an XAD-8 column, using a similar volume-to-resin ratio as in the isolation procedure. The solutions obtained this way are referred to as 'BA-CE', 'BA-HPI', 'Asa-CE' and 'Asa-HPI', where CE and HPI are short for cation-exchanged and hydrophilic, respectively. Acid-base and copper-titrations were made on the untreated, the cation-exchanged, and the hydrophilic bottom ash leachate (Paper II), and on the cation-exchanged and the hydrophilic Asa sample (Paper IV).

#### 4.1.4 Isolation of organic matter fractions

In this study we used an Amberlite® XAD-8/XAD-4 two-column array, linking both columns in series, for isolation of the main organic matter fractions in the Asa forest floor soil solution (Malcolm & MacCarthy, 1992). Following this procedure we isolated three main fractions, as illustrated in Figure 7. As mentioned previously, we adopted the terminology as introduced by Croué *et al.* (2003). Thus, the fraction retained by and desorbed from the XAD-8 column was labelled 'hydrophobic acids', the fraction retained by and desorbed from the XAD-4 column was labelled 'transphilic acids' and the fraction which passed through both columns and remained after dialysis was labelled 'hydrophilic compounds'. Note the use of the word 'compounds' rather than 'acids', which was chosen *i*) because the hydrophilic fraction might also contain neutral molecules, and *ii*) to avoid confusion with the hydrophilic acid fraction in a Leenheer fractionation.

Prior to isolation, the filtered soil solution was passed through a H<sup>+</sup>-saturated Bio-Rad AG-MP-50® cation-exchange column, to remove trace metals and major cations (Vance & David, 1991). Samples were diluted to 20 mg L<sup>-1</sup> DOC prior to the isolation procedure, to ensure that the hydrophobic/hydrophilic split was independent of the total DOC concentration (Qualls & Haines, 1991), and acidified to pH 2 using concentrated nitric acid (HNO<sub>3</sub>). Hydrophobic acids and hydrophilic acids were desorbed from the XAD-8 and XAD-4 column using a small volume 0.1 M NaOH solution. The desorbed eluent was then passed directly through another H<sup>+</sup>-saturated AG-MP-50 column, so that excess sodium ions were exchanged for protons. Thus, the final product was a highly concentrated organic solution, containing little or no impurities (salts or trace metals). These solutions were frozen and freeze-dried to remove water and to obtain the pure organic material.

The organic solutes remaining in the solution after passage through both XAD columns were very dilute, with concentrations as low as 4-5 mg L<sup>-1</sup>

DOC. In addition to this, the solution contained an elevated concentration of nitrate ions, as a result of the acidification step. To overcome these obstacles, the solution was rotor-evaporated to increase the organic solute concentration, then dialysed (molecular weight cut-off 500) to remove excess salts and low molecular weight organic compounds. For further details concerning the isolation procedure, see Paper III.

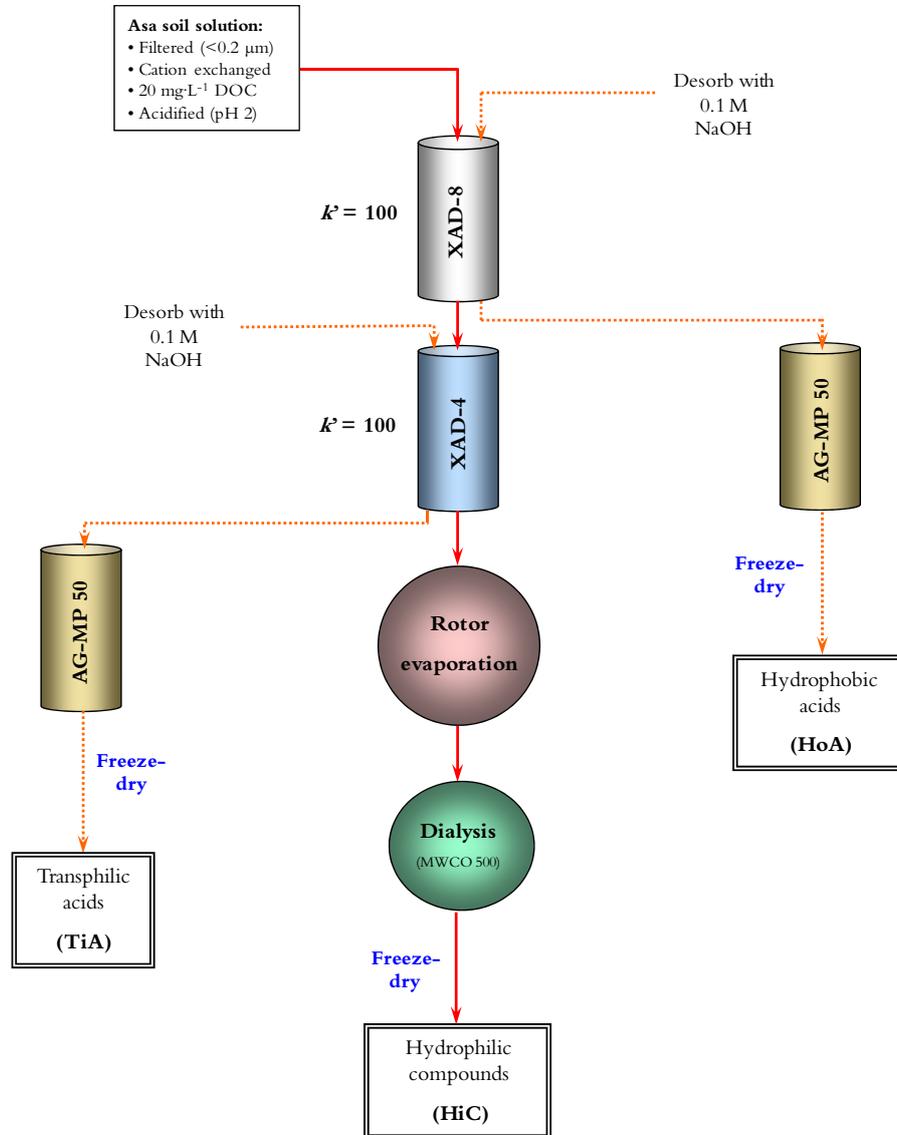


Figure 7. Flowchart for fractionation and isolation of hydrophobic, transphilic and hydrophilic fractions of dissolved organic matter.

#### 4.1.5 Proton and copper binding

In Papers II, III and IV, proton- and copper-binding characteristics were determined by titration experiments. Initial titrations were fully manual; additions of NaOH solution were made with a digital burette and pH was recorded after every addition. Due to the time consumed by the manual titrations, the laboratory strategy needed to be modified. Adapting the equipment readily at hand, proton titrations were automated using a TIM900 Titration Manager coupled with an ABU900 Autoburette (Figure 8). A custom programme was defined and tested against the results of the manual titrations for validation of the procedure and the equipment used. A minor disadvantage of this automated procedure was that the results were available as printouts only, meaning that the titration data still had to be digitalised manually.

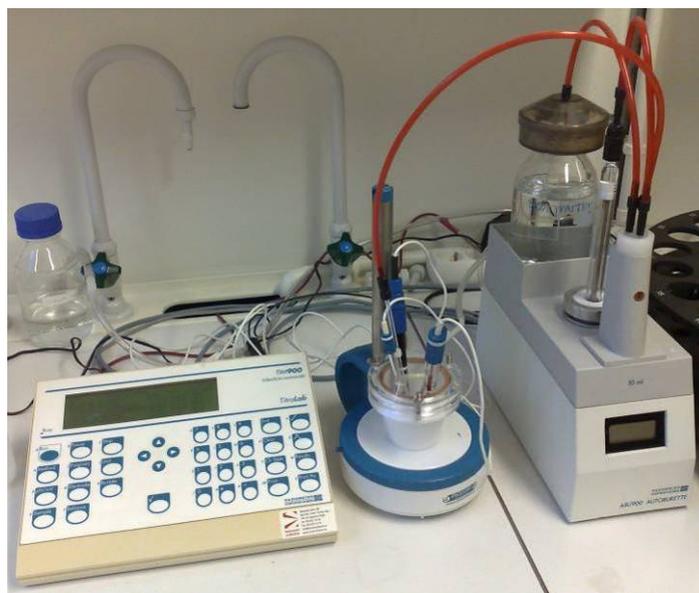


Figure 8. Laboratory setup of TIM900 Titration Manager coupled with the ABU900 Autoburette. Sample was contained in a custom-made polyethylene vial, which was securely covered with a polyethylene top with pre-drilled holes to fit electrodes and burette tip.

Copper-binding characteristics were studied by means of copper titrations of two different types; *i*) alkalimetric copper titrations (Papers II & IV), and *ii*) pH-stat copper titrations (Papers II-IV). During alkalimetric copper titrations, the total amount of copper was kept constant throughout the entire titration, while pH was increased manually from a value of around 3

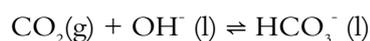
up to a value of around 10. During pH-stat copper titrations, on the other hand, total amount of copper was increased, but the pH was kept constant. Copper(II) activities were recorded with a copper selective electrode. The electrode was successfully calibrated against a copper-ethylene diamine solution, using Visual MINTEQ and the incorporated thermodynamic database for speciation calculations (Gustafsson, 2008). Free copper(II) ion activities were measured in the range  $pCu=4$  to  $pCu=12$ .

The pH-stat copper titrations were performed at pH values of 4, 6 and 9. Initially, these titrations were performed on the same equipment as the proton titrations (Papers II & IV). However, the pH-stat titrations on the isolated fractions were performed on the newly purchased, more modern and advanced TIM960 Titration Manager (Figure 9). The TIM960 was controlled through a personal computer running the Titramaster 85 programme software (Radiometer-Analytical, 2007).



Figure 9. Laboratory setup of TIM960 Titration Manager coupled with PC and Titramaster 85 Titration Software. Sample was contained in an uncovered polyethylene vial; partial cover was provided by the electrode holder.

The major problem with all titrations was the carbon dioxide present in the air. This carbon dioxide can enter the solution and disturb the titrations, through the following reaction:



Thus, if no efforts are made to eliminate the interference of carbon dioxide, the titration results will be obscured by carbon dioxide buffering. To avoid this problem, we stirred and purged the sample solution continuously, using CO<sub>2</sub>-free nitrogen (N<sub>2</sub>). This N<sub>2</sub> bubbling caused small drops of sample solution to gather on electrode, stirrer, titrator and beaker surfaces and, with the TIM900 setup, on the inside of the polyethylene top cover, which could be a problem. However, this was found not to have any significant influence on the titration results.

#### 4.1.6 Modelling approaches

We tested the ability of the SHM and NICA-Donnan model to describe the experimental data. In Paper II, assumptions were made regarding the active fraction of DOC, based on results from the fractionation experiments. As generic parameters were derived for natural humic substances, model parameters were calibrated for the bottom ash leachate where needed, in order to increase the model performance. In Paper III, proton- and copper-binding model parameters were fitted to the experimental data on the isolated organic fractions. For the SHM, a manual procedure using trial-and-error methodology was used to minimise the root mean square error (rmse) whilst retaining realistic parameter values, whereas the NICA-Donnan model was optimised using the FIT package (Kinniburgh & Tang, 1999), following the procedure described in detail by Benedetti *et al.* (1995) and Kinniburgh *et al.* (1996). Model calculations in Paper IV are a culmination of the experiences and results of Papers II and III. Three model assumptions were made in an attempt to describe proton binding and copper binding data on the total and hydrophilic humic fractions of a boreal forest floor soil solution.

#### 4.1.7 EXAFS

Extended X-ray absorption fine structure (EXAFS), first reported by Sayers *et al.* (1971), is a technique that is much used in various areas of chemistry and biology (Gurman, 1995). Its use in soil chemistry studies has increased rapidly during recent years (Brown & Sturchio, 2002; McNear *et al.*, 2005). Core electrons in the sample are excited, as the sample is subjected to high-energy X-rays (photoelectrons). This creates an electromagnetic wave, which is backscattered by neighbouring atoms. The properties of the

electromagnetic wave are determined by the host element in the sample, while the properties of the backscattered waves are dependent on the backscattering neighbouring atoms. Important information, such as coordination number, neighbouring atoms, oxidation state and structure geometry, can be extracted from the EXAFS spectra (Jalilehvand, 2000). The measurements are element-specific and can be performed on any physical state of sample, and information can be obtained from dilute samples.

EXAFS was used to study the binding mechanism of iron to fulvic acid on a molecular level (Paper I). EXAFS measurements were made on aqueous solutions, initially containing iron(III) and dissolved fulvic acid. Samples were stored for an extended period of time and measurements were made on several occasions so that the kinetics could be studied. Extraction and refinement of the EXAFS data was performed using two different software packages: EXAFSPAK (including FEFF7) (George & Pickering, 1993; Zabinsky *et al.*, 1995) and GNXAS (Filipponi & Di Cicco, 2000).

## 5 Results and Discussion

### 5.1 Iron(III) under the microscope (Paper I)

As mentioned earlier, iron(III) is an abundant and important element in natural systems, as it forms strong complexes with humic substances and thereby influences trace metal speciation. In this paper, the binding of iron(III) to a natural fulvic acid (IHSS Soil Fulvic Acid Standard II - 1S102F) was studied at a molecular level at pH values of 2 and 4.

#### 5.1.1 Binding chemistry of iron(III) to aqueous fulvic acid

Analysis of the EXAFS data revealed that iron(III) formed complexes with fulvic acid in the aqueous phase within 15 minutes after the solutions were prepared. This followed from the observation of Fe···C single scattering and Fe-O-C three-leg scattering in the respective spectra. The complexation occurred through 3-6 carboxylate and phenolate groups, with iron(III) coordinated octahedrally by oxygen atoms. Iron(III) was reduced to iron(II) to varying degrees, depending on sample pH and age of the sample. No complex formation with iron was observed after it reduced to iron(II), which indicated that iron(II) was mainly present as the hydrated ion.

Contrary to the results found in a study on iron(III) binding in organic mor layers from forest soils (Gustafsson *et al.*, 2007), iron(III)-fulvic acid complexes were mononuclear in the aqueous phase at both pH 2 and 4. An interesting observation, however, was that part of the iron had precipitated from the pH 2 sample after 34 months. The EXAFS spectrum recorded for the solid precipitate revealed traces of iron trimers,  $O(FeO-R)_3$ , such as observed for the organic soils. In addition to this, iron in the precipitate was present in a trivalent redox state only, which suggested that iron(III) was stabilised in the particulate phase through formation of trimeric complexes.

### 5.1.2 Influence of pH on iron redox state

The aqueous sample at pH 2 showed signs of reduction of iron(III) to iron(II) after 24 hours, as the main peak indicating the Fe-O bond started to shift to a longer distance (Figure 10, left), indicative of an octahedral iron(II) coordination (D'Angelo & Benfatto, 2004; Lundberg *et al.*, 2007). The reduction continued with time, and was near complete at the time of the final measurements (34 months). The sample at pH 4 only showed a minor amount of reduction, and even after 28 months the majority of the iron remained in trivalent state (Figure 10, right).

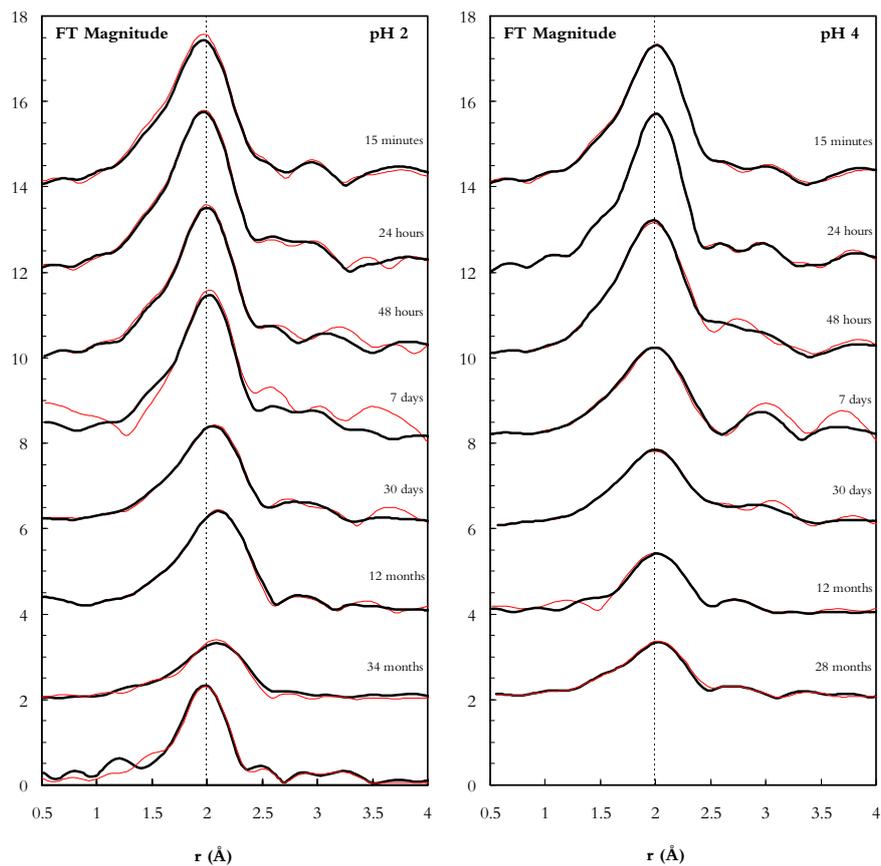


Figure 10. Phase-corrected Fourier transforms of EXAFS data for the pH 2 (left) and pH 4 samples (right) as a function of storage time (thick line); thin lines are model fits; the dotted line at 2.0 Å is given for easier comparison of the Fourier transforms. Reduction of iron(III) to iron(II) is indicated by a shift of the main peak from 2.0 Å to 2.1 Å.

The findings clearly indicate that the reduction of iron(III) is favoured by a low pH value. After the final EXAFS spectra were recorded, chemical analyses were performed to test for iron(II) and these confirmed that close to all iron was present in divalent state at pH 2, whereas more than 75% of total iron remained in its trivalent state at pH 4. The observed pH dependence of iron(III) reduction in the presence of humic substances was consistent with expected results based on thermodynamic calculations for model ligands (see Figure 3 in Paper I).

### 5.1.3 Implications

Following their findings, Gustafsson *et al.* (2007) introduced a dimeric hydrolysed iron(III) humic complex to the SHM database. With this complex the model successfully described both experimental iron(III) binding data at low pH and the competition effect on trace metals. However, the study of iron(III) to fulvic acid in acid aqueous solution showed that no dimeric complexes were formed under those conditions. Therefore, it was critical that the dimeric iron(III) humic complex was omitted during model calculations on dissolved humic substance fractions in later stages of this project. Instead, refined parameters for mononuclear iron(III) complexes were used.

## 5.2 Isolation work (Papers II-IV)

The separation of hydrophobic and hydrophilic organic solutes is dependent on solute polarity and the ratio of solution to resin (Leenheer, 1981; Thurman & Malcolm, 1981). Leenheer operationally defined the hydrophobic/hydrophilic solute split using a distribution coefficient,  $k'$ , defined according to:

$$V_E = V_0(1 + k') \quad (\text{Eq. 8})$$

where  $V_0$  is the void volume (i.e. porosity x bed volume) and  $V_E$  is the volume of sample passing through the column. In other words,  $k'$  is a measure of the relationship between the sample volume passing through the column and the resin volume. Another parameter, which is closely related to  $k'$ , is  $k'_{0.5r}$ , also called column capacity factor. The latter is defined as:

$$V_{0.5r} = 2V_0(1 + k'_{0.5r}) \quad (\text{Eq. 9})$$

where  $V_{0.5r}$  is the effluent volume at which 50% of the total DOC which passed through the column is retained. Figure 11 illustrates the implementation of  $k'$  and  $k'_{0.5r}$  on a hypothetical breakthrough curve, i.e. a curve obtained by plotting the measured DOC concentration in the column effluent, versus the volume of effluent which has passed through the column. In other words, using  $k'$  as the criterion yields the effluent volume at which the DOC concentration in the effluent is 50% of the total DOC concentration in the sample; using  $k'_{0.5r}$  as the criterion yields the effluent volume at which the total amount of DOC that has passed through the column equals the amount that is retained on it. Graphically this means that at  $k'_{0.5r}$  the surface area above and below the breakthrough curve are equal.

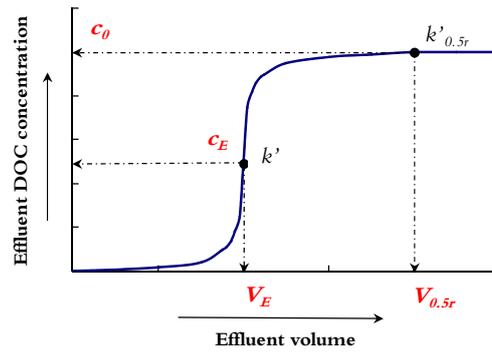


Figure 11. Theoretical break-through curve of a DOC sample being passed through an XAD-8 column;  $V_E$ =effluent volume defined by  $k'$ ,  $V_{0.5r}$ =effluent volume defined by  $k'_{0.5r}$ ,  $c_0$ =total sample DOC concentration,  $c_E$ =effluent DOC concentration at  $k'$ ,  $c_E=0.5 \times c_0$ ,  $k'$  and  $k'_{0.5r}$  are defined in the text.

Aiken *et al.* (1979) determined the  $k'$  value for fulvic acid on several XAD resins; for XAD-8 resin, a value of approximately 600 was found, and referred to as  $k'_{cutoff}$ . Thus,  $k'$  needs to be chosen such that the solutes of interest will be 100% retained on the resin. The problem, however, is that the separation of retained and non-retained solutes is not sharp, and part of the solutes with  $k' < k'_{cutoff}$  will also be retained on the column. Thus, in order to standardise a fractionation or isolation procedure, it is of utmost importance that a fixed value for  $k'$  is used in all studies, so that it is possible to compare results of different studies. For the isolations performed in this thesis we used a value of  $k' = 100$ , similar to that used in several other studies (Aiken *et al.*, 1979; Thurman & Malcolm, 1981; Guggenberger &

Zech, 1994; Croué *et al.*, 2003). Given (Eq. 8), this implies that a total sample volume corresponding to 101 void volumes was passed through the columns.

Several pilot experiments were conducted in order to obtain a better understanding of the function of the various resins, the importance of the initial DOC concentration and the impact of the distribution factor  $k'$ . In one experiment, small effluent samples were collected and analysed for DOC concentration and SUVA (Figure 12). For this experiment, the bed volume of the XAD-8 resin was 2.4 mL. Using (Eq. 8), the effluent volume is calculated by  $V_E = 0.65 \times 2.4 \times (1 + 100) \approx 160$  mL. Thus, the fraction of DOC that is retained after passing 160 mL of sample solution through the column is defined as the hydrophobic fraction. From the figure (left) it becomes apparent that using a standardised value for  $k'$  is essential for standardisation purposes, as any given lower value of  $k'$  would have resulted in a decreased retention of DOC, and hence a smaller hydrophobic fraction. Likewise, the opposite would probably have been the case if a larger value of  $k'$  had been chosen, as the effluent DOC concentration increases until it equals the starting concentration of the sample (as the column is 100% saturated).

Another interesting observation was that the specific ultraviolet absorbance (SUVA) of the effluent increased more or less gradually, as the effluent volume increased. SUVA is a parameter that indicates the nature or quality of DOC in a given sample and has been used as a surrogate measurement for DOC aromaticity (Chin *et al.*, 1994; Weishaar *et al.*, 2003). This puts extra emphasises on the previous statement that the boundary between hydrophobic and hydrophilic organic substance is not a sharp and distinct one.

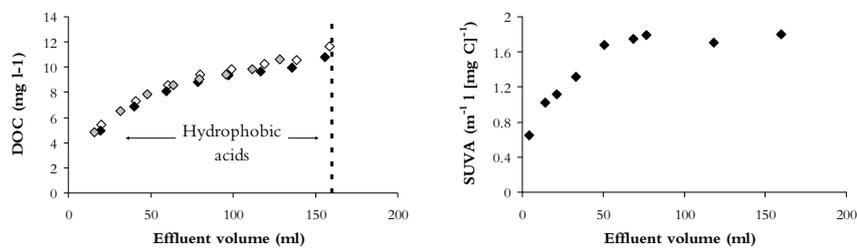


Figure 12. Results of a pilot experiment to study the dynamics of DOC breakthrough (left) and SUVA (measured at 254 nm) of the effluent solution (right).

Because of practical considerations, the isolation setup design used in this study was controlled by the maximum sample volume that could be treated per run (5 litres). Thus the required bed volume of the XAD-8 and XAD-4 columns was calculated using (Eq. 8), with a  $k'$  value of 100 and porosity of 0.65 for both the XAD-8 and the XAD-4 resin. This yielded resin bed volumes of 76 mL. The experimental setup is illustrated on the cover of this thesis. Isolated hydrophobic and transphilic acid material is illustrated in Figure 13.

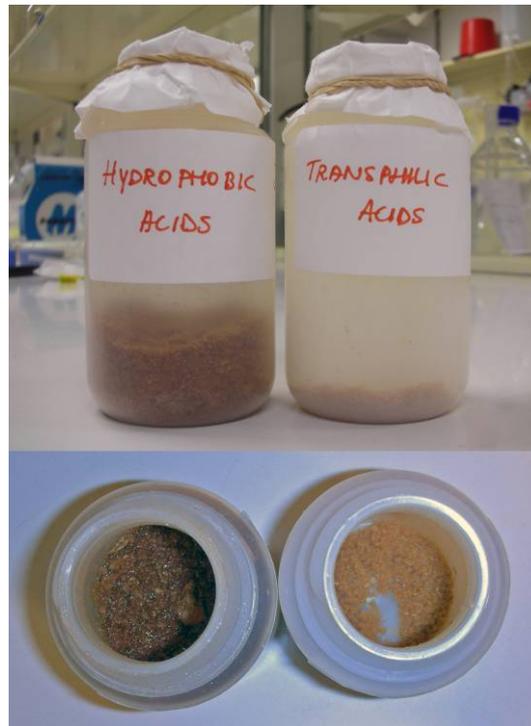


Figure 13. Isolated and freeze-dried hydrophobic and transphilic acid fractions.

### 5.3 Hydrophilic acids unmasked (Paper II)

The use of MSWI bottom ash as a construction material saves resources in the form of natural aggregates and decreases the burden on landfill. However, due to potential contaminant leaching from the ash, its use is

restricted in many countries (Olsson *et al.*, 2006). MSWI bottom ash has a high carbon content, which can potentially dissolve and thus leach from the ash material. Although natural DOM and its affinity for the complex binding of trace metals have been reasonably well documented (e.g. Kinniburgh *et al.*, 1999b; Lu & Allen, 2002; Croué *et al.*, 2003), studies on the intrinsic properties of MSWI bottom ash are scarce. This paper focused on the copper-binding properties of an MSWI bottom ash leachate and its hydrophilic fraction.

### 5.3.1 Composition of MSWI bottom ash DOC

Chemical analyses and fractionation of the MSWI bottom ash leachate revealed that a significant amount of organic material had dissolved and remained in solution during the extraction procedure (Table 4). Contrary to what is found in most natural waters, hydrophilic acids constituted the majority of the DOC. Concentrations of LMWOAs (malonate, oxalate, acetate and formate) were negligible.

Table 4. Overview of fractionation results (Leenheer, 1981) for the MSWI bottom ash sample

Total DOC	Hydrophobic acids	Hydrophobic neutrals	Hydrophilic acids	Hydrophilic neutrals	Hydrophilic bases
mg L <sup>-1</sup>			% of total DOC		
28	16.5	8.9	57.7	16.0	0.9

Similarly to natural organic acids, proton titrations of the BA-CE sample revealed a large distribution of proton affinity constants, as indicated by the gradual change in organic charge with increasing pH (Figure 14). However, the bottom ash DOC was characterised by a larger amount of carboxylic binding sites than commonly found for natural fulvic acids, whereas the opposite was the case for phenolic sites. Attempts were made to describe the experimental data using the SHM and NICA-Donnan model with generic parameters (Figure 14).

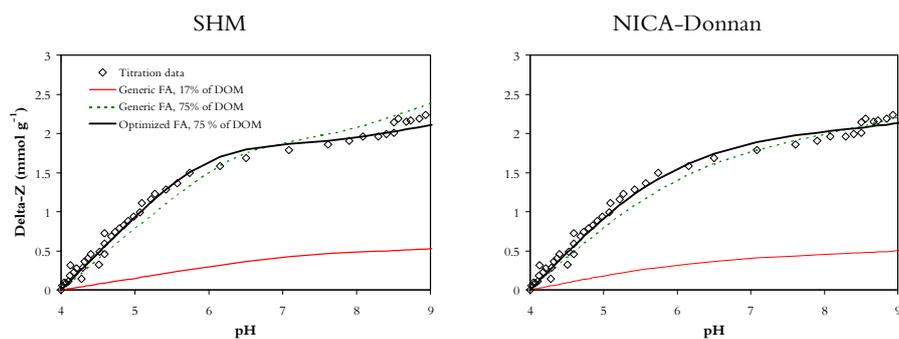


Figure 14. Acid-base titration of cation-exchanged MSWI bottom ash leachate (BA-CE). Lines show model predictions with the generic parameters at 17% and 75% FA of DOM and the optimised proton-binding parameters at 75% FA of DOM. Left: SHM; Right: NICA-Donnan.

The model simulations required the inclusion of the hydrophilic acid fraction as part of the total active DOC in order to obtain reasonable results. The model predictions could be further improved by modifying the acid-base parameters accounting for the number of different types of sites (see Table 1 in Paper II).

### 5.3.2 Copper binding

The majority (>95%) of copper in the MSWI bottom ash leachate was found as organic complexes. The alkalimetric titrations (see Figure 3 in Paper II) clearly illustrated the effect of proton competition, as free copper(II) ion concentrations decreased rapidly with increasing pH (and thus decreasing proton competition). Interestingly, the titrations on the hydrophilic fraction showed a close resemblance to those on the cation-exchanged solution, which suggests that part of the hydrophilic fraction had a high affinity for copper complexation. This was further confirmed by the pH-stat titrations, which yielded near linear copper binding isotherms (Figure 15) for both samples. These observations were in line with the results reported for natural waters (Vance & David, 1991; Croué *et al.*, 2003) and for the boreal forest floor solution studied in this thesis (Papers III and IV).

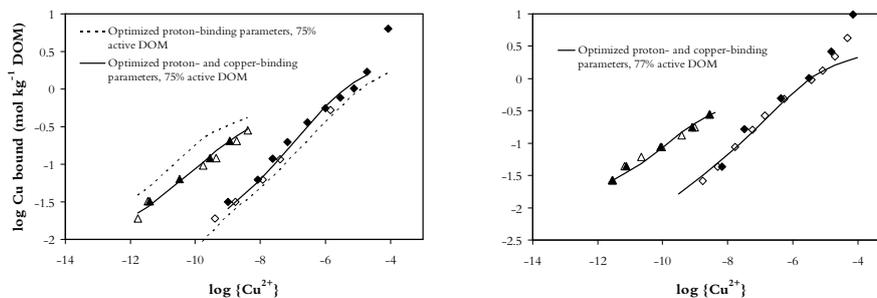


Figure 15. Copper titration of cation-exchanged MSWI bottom ash leachate (BA-CE, left) and its hydrophilic fraction (BA-HPI, right) at pH 6 (diamonds, two separate titrations) and pH 9 (triangles, two separate titrations). Lines show SHM predictions.

Again, the SHM and NICA-Donnan model were tested against the experimental data. Following the observation that the hydrophilic fraction had marked copper binding properties, model simulations assumed 75 and 77% active DOC for the cation-exchanged and hydrophilic sample, respectively. These numbers were determined as the sum of hydrophobic and hydrophilic acids (BA-CE), and as the percentage of hydrophilic acids of total hydrophilic DOC (BA-HPI), see Table 4. Both the SHM and the NICA-Donnan model described the copper binding very well using the previously calibrated proton-binding parameters, and after lowering the respective constants for complex formation with phenolic sites (pH 9).

One important finding was that the pH-stat titration data on the untreated (non-cation-exchanged) ash leachate (BA-0) could not be satisfactorily described by either model. Possible reasons for this could be that competition by other cations is poorly described by the models (relatively high calcium concentrations may result in underestimation of copper complexation), or that the aluminium present in the samples did not actively compete for complexation sites (see Figure 4 in Paper II).

#### 5.4 Isolated fractions – alone but by no means lonely (Paper III)

As discussed in the previous section, the bulk hydrophilic fraction of MSWI bottom ash leachate was characterised by fulvic acid-like proton- and copper-binding properties. Thus it was found that the hydrophilic (acid) fraction has to be considered when assessing copper speciation. This paper focused on the proton- and copper-binding properties of purified, isolated hydrophobic and hydrophilic acid fractions of a boreal forest floor soil solution.

### 5.4.1 Composition

Isolation of the different fractions proved to be a time-consuming and, particularly in the case of hydrophilic acids, relatively unrewarding process: each complete isolation run, including cleaning and preparation of resin columns, required approximately one full week. Rotor evaporation, dialysis and freeze-drying added to this in a later stage. A total of 12 successful isolation runs were performed, which yielded total amounts of 1100, 180 and 100 *milligrams* of hydrophilic acids (HoA), transphilic acids (TiA) and hydrophilic compound (HiC), respectively.

In contrast to the BA leachate, the Asa soil solution was dominated by the HoA fraction (Table 5). However, this was in line with values previously reported for natural waters (e.g. Qualls & Haines, 1991; Vance & David, 1991; Malcolm & MacCarthy, 1992). The TiA and HiC fraction constituted 9% and 18% of total DOC, respectively. Approximately 9% of total DOC was retained on the XAD-4 column and not eluted by 0.1 M NaOH. The sum of the TiA, the HiC and the retained fraction was considered to equal the hydrophilic acid fraction as found by performing a Leenheer fractionation.

Table 5. *Elemental analyses of organic components in the hydrophobic acid (HoA), transphilic acid (TiA) and hydrophilic compound (HiC) fractions (element analyses on fractions corrected for inorganic contents)*

	% of total DOC <sup>a)</sup>	C	N	O	S	H	P	Inorg. <sup>b)</sup>	$\Sigma^c)$	C/O
		% (w/w)								Mass ratio
HoA	58.8	46.2	0.8	41.1	0.3	4.1	≤0.5	1.7	94.7	1.1
TiA	9.3	34.5	0.8	38.2	0.4	3.7	≤0.5	1.9	80.0	0.9
HiC <sup>d)</sup>	13.8	27.5	1.0	36.9	0.5	3.5	≤0.5	17.7	87.6	0.7

<sup>a)</sup> Average values (n=12); <sup>b)</sup> inorganic residue was determined by dissolving 100 mg L<sup>-1</sup> DOM and calculating the sum of major cations and anions (Na, Al, Fe, Cl, SO<sub>4</sub>, PO<sub>4</sub>); <sup>c)</sup> total recovery, sum of organic and inorganic components; <sup>d)</sup> recovery after dialysis

### 5.4.2 Proton binding

All three fractions showed distinct proton-binding properties and binding site heterogeneity, as evident from the gradual increase in the organic charge against pH curve (Figure 16). The effect of electrostatics was also evident, as proton titrations at high and low ionic strength yielded a marked difference

in organic charge density. In summary, the total organic charge increased with a decrease in hydrophobic character. This was in line with the observed trends in SUVA and carbon/oxygen ratios, which suggested a decrease in aromaticity and an increase in carboxylic contents in the order HoA → TiA → HiC.

Comparison with model calculations using generic fulvic acid parameter values indicated that the Asa soil solution HoA fraction had a lower amount of carboxylic and phenolic binding sites than the generic fulvic acid. Overall, the carboxylic binding sites had a more acidic character than the average fulvic acid, which may be explained by the source of the material. The majority of datasets that were used for derivation of the generic fulvic acid parameters considered surface waters and soil extracts, whereas this study focused on DOC in a soil solution. DOM dissolution kinetics and the fact that the DOC was dissolved under *in situ* conditions could therefore be a plausible reason for the more acidic character: the stronger the acid, the higher its charge at a given soil pH and the higher its solubility.

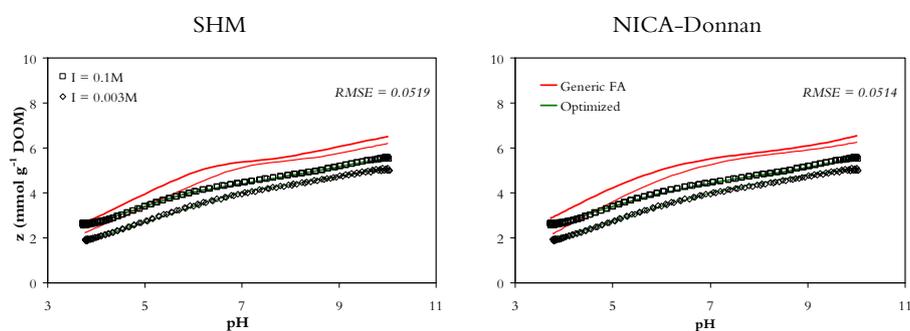


Figure 16. Acid-base titration curves for the HoA fraction. Red and green lines represent SHM (left) and NICA-Donnan (right) model predictions with generic and optimised acid-base parameters.

Good model predictions could be obtained for titration data in a pH range of 4 to 9 (10 for HoA fraction) by optimisation of the acid-base parameters (see Figure 3 in Paper III). However, neither model managed to capture the observed proton dissociation of the TiA nor HiC fraction at higher pH values, which suggests that a third type of functional group should perhaps be taken into consideration. Croué *et al.* (2003) found evidence of the presence of nitrogenous binding sites, deprotonating only at  $\text{pH} > 9$ , in the transphilic neutral fraction (retained on XAD-4 and not eluted by 0.1 M NaOH) of a surface water sample. In this study, however,

nitrogen contents of the TiA and HiC fraction did not differ significantly from the HoA fraction (Table 5).

The HiC fraction contained trace amounts of iron(III) and aluminium, despite the early stage cation-exchange. For the model calculations, it was therefore important to account for iron(III) and aluminium complex formation and hydrolysis, as this affects the observed charge increase.

### 5.4.3 Copper binding

Similarly to the BA leachate experiments, the majority of the dissolved copper ions were complex-bound to the organic fraction at pH values of 6 and 9. At pH 4, however, the free ionic species was dominant at higher total copper concentrations ( $pCu > 5.5$ ). In accordance with the observations in the MSWI bottom ash study, the pH-stat titrations revealed that all three fractions had distinct and comparable copper binding properties, although at a first glance the HiC fraction showed weaker binding at pH 4 and 6 (Figure 17, left). However, the presence of iron and aluminium may be the reason for the observed discrepancy. To test this, model calculations simulating the copper-binding in the absence of both iron(III) and aluminium were performed. Following this approach, the predicted copper binding increased significantly and became comparable to the isotherms for the HoA and TiA fractions (Figure 17, right).

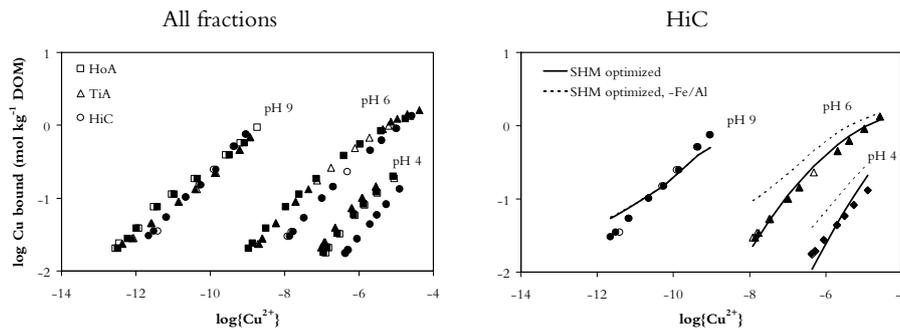


Figure 17. Comparison of Cu-binding isotherms of the HoA fraction at pH 4, 6 and 9 (left); and effect of Fe and Al on the Cu-binding isotherm of the HiC fraction (right). Open and closed symbols represent duplicate titration data. Lines represent the optimised model fit for a system including or excluding Fe and Al.

Model simulations by both the SHM and the NICA-Donnan model showed that the copper-binding of the isolated fractions was generally overestimated by the generic parameters. However, good agreement was

achieved between model predictions and experimental data for both models after optimisation of the copper binding parameters (see Figure 5 in Paper III).

#### 5.4.4 Competition and model performance

Both iron(III) and aluminium were found to compete strongly with copper for binding to the organic fractions, thereby effectively lowering the percentage of copper bound and increasing the concentration of its free ionic species. For the SHM simulations, iron(III) complex binding was constrained to monomeric complexes only, as evidenced by the EXAFS study on iron(III) binding to fulvic acids (Paper I). The SHM described the competition of both iron(III) and aluminium very well at pH 4, but slightly overestimated it at pH 6. The NICA-Donnan model, on the other hand, did not manage well at either pH 4 or 6. At pH 4, some competition was predicted, but not as much as was observed in the experiments. At pH 6, however, the model did not predict any noticeable competition at all.

The observed iron(III) and aluminium competition was in line with previous findings (Tipping *et al.*, 2002; Weng *et al.*, 2002a; Tipping, 2005; Weber *et al.*, 2006). This implies that both metals *must* be considered when using a model to simulate speciation. In order to do so properly, model parameters for iron(III) and aluminium binding need to be improved and validated by more datasets.

Another important finding was that the SHM and the NICA-Donnan model responded very differently to changes in acid-base properties during simulation of copper binding. For the SHM, the predicted copper binding was improved by applying the optimised acid-base properties. For the NICA-Donnan model, on the other hand, the opposite was true (Figure 18, green lines). This prohibits the use of the NICA-Donnan model with ‘partly’ optimised parameters; instead, proton- and metal-binding parameters should be optimised simultaneously. Use of the generic FA parameters, however, appeared to be a reasonable alternative.

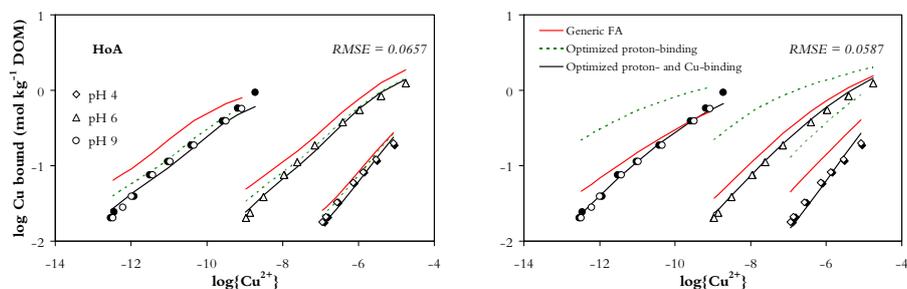


Figure 18. Copper titration isotherms for the HoA, TiA and HiC fractions at pH 4, 6 and 9. Red and green lines represent model predictions with default and optimised proton-binding parameters; black lines represent model predictions with optimised proton- and copper-binding parameters. Reported *mse* values are for the fully optimised model predictions.

## 5.5 Theory put to the test (Paper IV)

The studies discussed in the previous sections clearly showed that the hydrophilic fraction of dissolved humic substances, whether dissolved from MSWI bottom ash or natural organic matter, must be taken into consideration for assessment of proton buffering and copper speciation (Papers II & III). This can be done in several ways, depending on the assumptions made. The ability of the SHM to describe experimental proton- and copper-binding was tested in Paper IV, using three different model approaches.

### 5.5.1 The theory

One of the absolute requirements for model-based speciation calculations is that total DOC concentrations are known. However, not this entire DOC is actively involved in proton- and copper-binding. The assumptions that were made with respect to active DOC fraction, and choice of model parameters, are presented in Table 6.

Table 6. Summary of the three model approaches; see Paper IV for a detailed description of each approach

Asa-CE			Asa-HPI		
Fractions	% of total DOC	Model parameters	Fractions	% of total DOC	Model parameters
Model approach 1					
HoA	58	HoA	TiA	50.1	TiA
TiA	18	TiA	HiC	37.8	HiC
HiC	14	HiC			
Model approach 2					
HoA*	57.5	Generic FA	HiA*	86.7	Generic FA
HiA*	30	Generic FA			
Model approach 3					
HoA*	57.5	HoA	HiA*	86.7	TiA
HiA*	30	TiA			

### 5.5.2 The test

The model calculations were tested against experimental data, obtained through acid-base, alkalimetric (not shown) and pH-stat copper titrations. Both the cation-exchanged Asa soil solution sample (Asa-CE) and its hydrophilic fraction (Asa-HPI) were considered.

Model approaches 1 and 3 resulted in very comparable charge density and copper-binding curves in both samples, predicting the experimental data satisfactorily (Figure 19). Model approach 2 gave poor predictions, particularly for copper binding at pH values of 6 and 9. At these values, the copper binding was strongly overestimated by the generic fulvic acid parameters.

### 5.5.3 The verdict

As observed in Paper III, the three isolated fractions showed marked differences in proton-binding characteristics. Copper-binding characteristics, on the other hand, were found to be comparable. These findings were reflected in the model approaches tested in this study. Whereas the approaches yielded noticeably different results for the charge against pH curves, the predicted copper-binding isotherms varied little between the three approaches, except at pH 9 (Figure 19).

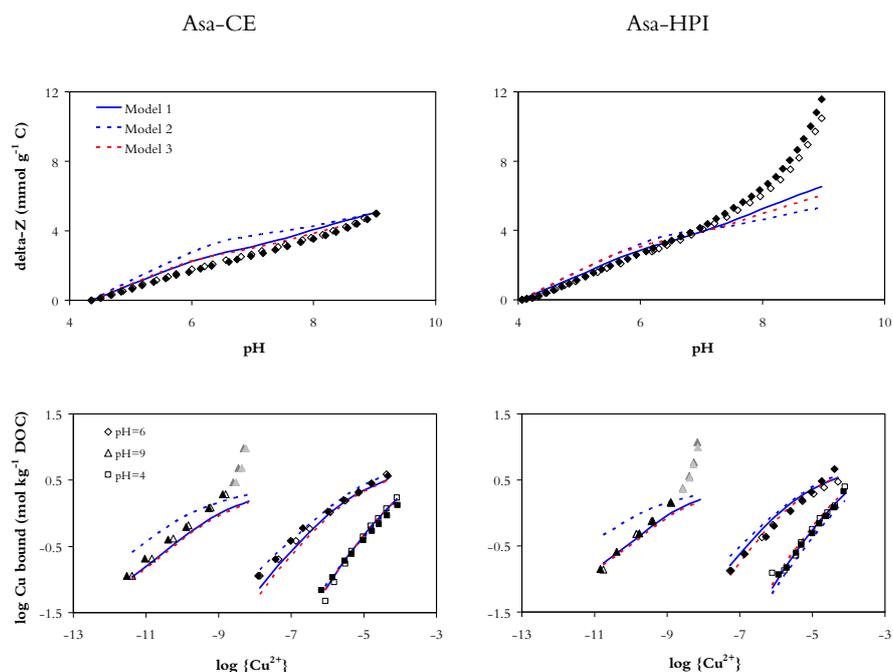


Figure 19. Acid-base and pH-stat copper titrations of cation-exchanged Asa soil solution (Asa-CE, left) and its hydrophilic fraction (Asa-HPI, right) (symbols represent separate titrations). Lines show model predictions (SHM) using three different assumptions (Table 6).

A good prediction of the copper-binding properties of both sample solutions was obtained by defining the active DOM as the sum of the HoA, TiA and HiC fractions, in combination with previously derived optimised parameter values for each fraction. This suggests that copper binding in particular could be attributed to the HoA, TiA and HiC fractions as the only contributors. However, the simplified procedure used in model approach 3 yielded nearly as good predictions of experimental data. Thus, satisfactory predictions can be obtained by taking analytical DOC fractionation data, determined with a Leenheer fractionation, as input for 'active' DOM, instead of model fits. However, this study only contained data for one metal and one soil solution sample, and more research and data are essential to further test and validate this possibility.

## 6 Conclusions

- ★ Three organic matter fractions were successfully isolated from a boreal forest floor solution, using previously described standardised procedures and guidelines. These fractions were labelled hydrophobic acids (HoA), transphilic acids (TiA) and hydrophilic compounds (HiC). Despite differences in proton-binding characteristics, the transphilic and hydrophilic fractions had very similar copper-binding properties compared to the hydrophobic fraction. The observed differences were small, especially in relation to the variation observed in properties of fulvic acids isolated from different environments. Thus, while inclusion of the transphilic and hydrophilic fractions in the model calculations appears to be critical, it may not be necessary to distinguish between the fractions.
- ★ Two geochemical models, the SHM and the NICA-Donnan model, were tested against the experimental data. Use of generic parameters resulted in noticeable discrepancies between experimental data and model predictions. However, both models described the data satisfactorily after calibration of relevant parameters.
- ★ EXAFS spectroscopy, a technique becoming increasingly important in unravelling coordination modes of trace metals to humic substances, was used to study the binding of iron(III) to fulvic acids in acid aqueous solutions. Iron formed mononuclear iron(III) complexes in solution, whereas hydrolysed polynuclear iron(III) complexes dominated in the solid phase (pH 2). Complex formation occurred within 15 minutes of sample preparation. At pH 2, reduction of iron(III) to iron(II) was evidenced within 48 hours, and practically complete after 12 months. Reduction was much less distinct at pH 4, where only a minor fraction of total iron(III) was reduced to iron(II) after 34 months. These observations were consistent with expected results based on thermodynamic calculations for model ligands.

- ★ Iron(III) and aluminium were found to compete strongly with copper for binding to all three isolated fractions at the pH values of 4 and 6. The competition was captured reasonably well with the SHM, but the NICA-Donnan model significantly underestimated the competition effect, particularly at pH 6. Consideration of iron(III) and aluminium competition is essential for model simulation of trace metal speciation.
- ★ A proportion of MSWI bottom ash organic matter dissolved readily upon extraction with H<sub>2</sub>O. Contrary to most natural waters, the DOC in the ash leachate was dominated by the hydrophilic acid fraction. Proton- and copper-binding characteristics of both the hydrophobic and hydrophilic fraction showed a resemblance to those of natural fulvic acids, but carboxylic contents were higher for the ash leachate DOM.
- ★ Copper binding to DOM in the forest floor solution could be predicted by the three individually isolated fractions (HoA, TiA and HiC) and their optimised proton- and copper-binding parameters. Very comparable results were obtained using a simplified approach, in which it was assumed that the hydrophobic and hydrophilic acid fractions, determined with a Leenheer fractionation, constitute the total 'active' DOM. In this approach, optimised HoA and TiA parameters were used for describing proton- and copper-binding to the hydrophobic and hydrophilic acid fractions, respectively. Poor results were obtained when generic fulvic acid parameters were used.
- ★ Yes, organic matters!

## 7 Implications & future research

Current day risk assessments are creating increasing demand for methods to estimate or predict free metal ion activity in the soil solution, utilising common and easily measurable variables as a basis. Several geochemical models have shown promise to this end, but a shared disadvantage is that model parameters need to be restrained by means of experimental data. This thesis provided a 'look-behind-the-curtains' of a natural soil solution and an MSWI bottom ash leachate, examining proton- and copper-binding properties of hydrophobic and hydrophilic organic matter fractions. The individual papers can be considered as building blocks of a LEGO structure, albeit one without building instructions and lacking a number of pieces.

The EXAFS study yielded specifics regarding the binding mode of iron to DOM, whereas quantitative data on the competition effect of iron(III) and aluminium were obtained from the titration experiments. Both iron(III) and aluminium were shown to compete strongly for binding sites with Cu, implicating the importance of inclusion of both metals in geochemical speciation calculations; in the presence of either or both, free Cu ion concentrations in solution will be higher than if no competition occurs. This may have major consequences for the toxicity and bioavailability of Cu for microorganisms and plants.

While the proton- and Cu-binding properties of hydrophobic acids have been discussed and quantified in detail, this thesis presented evidence that hydrophilic acids in both soil solution and an MSWI bottom ash leachate behave in a very similar fashion. With this information in mind, geochemical model calculations were made with the SHM, in an attempt to predict proton and Cu binding behaviour of an untreated soil solution. Good predictions of experimental data could be obtained by defining 'active' DOM as the sum of the hydrophobic and hydrophilic acid fractions, describing proton and Cu binding with parameters derived for the soil solution DOM isolated fractions. This method is preferable over estimation

or optimisation of 'active' DOM, and the required variables can be obtained through relatively cheap and fast laboratory analyses.

Although the findings described in this work may help in increasing our understanding of the characteristics and behaviour of macromolecular organic acids under natural conditions, it would be safe to state that no more than the tip of the iceberg has been uncovered. Thus, the use of the sum of hydrophobic and hydrophilic acids as 'active' DOM should be explored further, possibly using different parameter setups for the two fractions. To this end, it would be very beneficial to develop quicker and easier ways to determine the fractions of hydrophobic and hydrophilic acid relative to total DOM. In addition, data sets should be produced for samples of different origins. Follow-up research is also required to broaden the horizon and include other trace metals, such as zinc, cobalt, cadmium and lead. Another interesting metal that has been relatively poorly studied is molybdenum, present as an anion ( $\text{MoO}_4^{2-}$ ) in aerobic environments. Little is known about the way this anionic species interacts with humic substances. Consistent datasets on the binding properties of hydrophobic and hydrophilic acids with respect to these metals would prove invaluable for improvement of existing geochemical models, such as the SHM and the NICA-Donnan model. Quantification of the competition effect of iron(III) and aluminium on the binding of these metals to organic ligands would help to further improve our understanding of the complex mechanisms involved in trace metal speciation. In the scope of this thesis work, pilot experiments considering copper, lead, cadmium, cobalt and silver were conducted. No sensitive ion selective electrodes are available for measuring free ion concentrations of these metals. Development of new, sensitive speciation techniques allowing for analysis on small sample volumes would be valuable.

As another aspect of this thesis, EXAFS spectroscopy was shown to be a very efficient technique for assessment of coordination modes of trace metals to organic ligands. Several papers detailing binding modes of trace metals to organic matter have been published recently (e.g. Denecke *et al.*, 1999; Bochatay & Persson, 2000; Sarret *et al.*, 2004; Karlsson *et al.*, 2006; Gustafsson *et al.*, 2007; Karlsson & Skyllberg, 2007; Karlsson *et al.*, 2008). Continued structural analysis of trace metal-organic matter complexes, including complexes involving sulphurous and nitrogenous binding sites, could further constrain geochemical models.

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