Complexation of Cadmium, Copper and Methyl Mercury to Functional Groups in Natural Organic Matter

Studied by X-ray Absorption Spectroscopy and Binding Affinity Experiments

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Abstract

Due to various human activities contamination of soils, sediments and waters with trace metals like cadmium (Cd), copper (Cu) and mercury (Hg) are widespread. It is generally accepted that concentrations of specific metal species are of higher significance than total metal concentrations for the bioavailability and toxicity of trace metals. Thus, in order to make proper risk assessments of contaminated sites it is vital to know the speciation of the metals. Of special importance for the speciation are chemical interactions with colloidal and particle surfaces. The associations of trace metals with inorganic surfaces like minerals and metal oxyhydroxides are fairly well described, but less is known about metal associations with natural organic matter (NOM). In this thesis extended X-ray absorption fine structure (EXAFS) spectroscopy was used to determine the coordination chemistry of Cd and Cu in NOM and binding affinity experiments, using competitive complexation and ion selective electrodes (ISE), were used to determine the bonding strength of Cd and methyl mercury (CH$_3$Hg) to functional groups in soil organic matter (SOM).

Results from the EXAFS experiments revealed that reduced organic sulphur (S) ligands are involved in the complexation of Cd by NOM. In the first coordination shell, Cd is coordinated by 0.3–2.5 S atoms and 3.0–4.5 O/N atoms. A second shell of 1.7–6.0 C atoms was also found, providing direct evidence for inner-sphere complexation of Cd. Copper on the other hand is complexed to O and/or N containing functional groups. Four O/N atoms was found in the first coordination shell and analysis of the second shell showed that Cu form inner-sphere complexes consisting of one or two 5-membered chelate rings in NOM. Based on results from the EXAFS experiments, and earlier EXAFS studies on CH$_3$Hg, simple models in which Cd^{2+} and CH$_3$Hg$^+$ associate to thiol (RSH) groups in SOM were successfully used to describe the bonding at native and low metal concentrations. Determined stability constants were in accordance with stability constants for the bonding of these metals to reduced S in well-defined organic compounds. At Cd concentrations ranging from 500–54000 µg g^{-1}, experimental data was best described by a model consisting of one RSH group and one carboxylic group. Speciation calculations based on determined stability constants, in the concentration range 0.23–10000 µg Cd g^{-1}, showed that Cd complexed by SOM dominates the speciation and that the percentage free Cd^{2+} is only a few % of total Cd at pH values above 4.0. In an organic soil with native concentration of CH$_3$Hg (10 ng g^{-1}), speciation calculations showed that CH$_3$Hg complexed by SOM is the dominating CH$_3$Hg form in the pH range 3–7. Concentrations of free CH$_3$Hg$^+$ and its inorganic complexes, assumed to be the most bioavailable forms, are extremely low in comparison to CH$_3$Hg complexed by SOM.

Key words: cadmium, copper, methyl mercury, natural organic matter, thiol groups, stability constants, speciation, coordination chemistry, X-ray absorption spectroscopy.

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Contents

Appendix

Paper I-IV

This thesis is based on the following Papers, which will be referred to by their respective Roman numerals:

I.   Karlsson, T., Persson, P. and Skyllberg, U. 2005. Extended X-ray Absorption Fine Structure Spectroscopy Evidence for the Complexation of Cadmium by Reduced Sulfur Groups in Natural Organic Matter. *Environmental Science and Technology 39,* 3048-3055.

II. Karlsson, T., Persson, P. and Skyllberg, U. 2005. Coordination Chemistry of Copper(II) in Organic Soils and in Dissolved Natural Organic Matter - Studied by X-ray Absorption Spectroscopy. Manuscript.

III. Karlsson, T., Dalgren, K. E., Björn, E. and Skyllberg, U. 2005. Complexation of Cadmium to Functional Groups in Soil Organic Matter - Determined by Competitive Complexation and ISE Measurements. Manuscript.

IV. Karlsson, T. and Skyllberg, U. 2003. Bonding of ppb Levels of Methyl Mercury to Reduced Sulfur Groups in Soil Organic Matter. *Environmental Science and Technology 37,* 4912-4918.

Abbreviations

Cd	Cadmium
Cu	Copper
Hg	Mercury
CH_3Hg	Methyl mercury
NOM	Natural organic matter
SOM	Soil organic matter
DOM	Dissolved organic matter
Org-S_{red}	Reduced organic sulphur
RSH	Thiol groups
RCOOH	Carboxylic groups
EXAFS	Extended X-ray absorption fine structure spectroscopy
XANES	X-ray absorption near edge structure spectroscopy
ISE	Ion selective electrode
Å	Ångström (10^{-10} m)
$\mu g\ g^{-1}$	Concentration in relation to dry mass ($10^{-6}g\ g^{-1}$)

Introduction

The focus of this thesis has been to increase the understanding about the associations between trace metals and functional groups of natural organic matter (NOM). Increased knowledge about these associations is vital in order to get sufficient information to make proper risk assessments of contaminated soils and waters.

Trace Metals in the Environment

Trace metals are defined as metals that in natural materials are present at levels below 0.1% (Sparks, 1995). Hence, trace metals like cadmium (Cd), copper (Cu) and mercury (Hg) occur only at low concentrations in uncontaminated soils, 0.06–1.1 µg g^{-1}, 6–80 µg g^{-1} and 0.02–0.41 µg g^{-1}, respectively (McBride, 1994). However, due to various human activities contamination of soils, sediments and waters with trace metals are widespread. By long-range atmospheric transport, anthropogenic emissions have considerably increased the concentrations of Cd and Hg in the humus layer of Swedish forest soils during the 20th century (Johansson *et al.*, 2001). There are also a vast number of industrial sites in Sweden contaminated with Cd, Cu and Hg originating from local point sources like wood preservation facilities, smelters, mining and pulping industries. In 2004, the Swedish environmental protection agency estimated the total amount of sites contaminated with metals and/or organic pollutants in Sweden to more than 50000 (http://www.naturvardsverket.se/dokument/teknik/sanering/sanerdok/lage04.htm; 1-Sept-2005). Since these metals are toxic, they have caused and are causing severe environmental problems in soils, sediments and waters.

Cadmium is highly toxic to humans, animals and plants. It has partly similar properties as zinc (Zn), which is an essential micronutrient, is easily taken up by plants and bioaccumulates in the food web. Cadmium occurs in the divalent oxidation state [Cd(II)] in soils and waters and is thought to adsorb rather weakly in soils (McBride, 1994). The major route of exposure to Cd for the non-smoking general population is through intake of contaminated food and the kidney is considered the critical target organ. Cd is mainly used for electroplating, as electrode material in nickel-cadmium batteries, as stabilizers for poly-vinyl chloride (PVC), as pigments in plastics and glasses, and as a component of various alloys (Environmental Health Criteria 134, 1992). In agricultural soils, application of sewage sludge and phosphate fertilizers has led to elevated concentrations in crops. Phosphate fertilizers made from phosphate rock of sedimentary origin can have Cd concentrations as high as 75 µg g^{-1} (Loganathan *et al.*, 2003). This is a very high concentration since soils with Cd concentrations above 4 µg g^{-1} are considered to be heavily polluted (Gullbring and Östlund, 2002).

Copper is a micronutrient and essential to all organisms. Agricultural soils with less than 8 µg Cu g^{-1} may be deficient for crops (McBride, 1994). However, Cu is toxic at excessive concentrations and soils with concentrations above 1000 µg Cu g^{-1} are regarded as heavily polluted (Gullbring and Östlund, 2002). The

biochemical toxicity of Cu is derived from its effects on the structure and function of biomolecules such as DNA, membranes and proteins. For healthy, non-occupationally-exposed humans the major route of exposure to Cu is oral (Environmental Health Criteria 200, 1998). Copper occurs almost exclusively in the divalent oxidation state [Cu(II)] in soil solids and solutions and form strong complexes with organic matter (McBride, 1994). Since, Cu possesses high electrical and thermal conductivity and resists corrosion, it is widely used for electrical wiring and water pipes (Environmental Health Criteria 200, 1998). In Sweden wood preservatives consisting of copper, chromium and arsenic (CCA) has been broadly used resulting in contamination of soils and waters.

Mercury is one of the most toxic elements known to man. The toxicity is caused by the high affinity of Hg for sulphur (S). When Hg enters a cell and interacts with thiol containing molecules such as cysteine and glutathione it will impair various cellular functions (Bernard et $al.$, 2001). In soils the divalent oxidation state [Hg(II)] is most common. The reduced oxidation state [Hg(I)] has a limited stability range and the metallic elemental form [$Hg^{(0)}$] is somewhat volatile. Inorganic mercury can be methylated, forming organic mercury compounds of which methyl mercury (CH_3Hg) is the most common. It has been shown that sulphate-reducing bacteria are involved in the methylation process (Zillioux et $al.$, 1993; Benoit et $al.$, 1999) in sediments and soils showing seasonal fluctuations between reduced and oxidized conditions. The net effect of methylation and demethylation processes controls the concentration of CH_3Hg in soils and waters. Both, inorganic mercury (Hg^{2+}) and CH_3Hg^+ form stable complexes with NOM (Skyllberg et $al.$, 2000; Qian et $al.$, 2002). Due to an excessive release of $Hg^{(0)}$ into the atmosphere mainly as a consequence of fossil fuel combustion, Hg concentrations in fish in southern Swedish lakes are about five times higher than hundred years ago (Johansson et $al.$, 1991). Estimates suggests that over 40000 lakes in Sweden have pike ($Esox$ $lucius$) with a total Hg concentration that exceeds the guideline value for regular human consumption (0.5 mg kg^{-1} wet weight for 1 kg pike) (Håkanson, 1996). CH_3Hg constitutes only a small part of total-Hg in soils and waters, but it is highly toxic and bioaccumulates to a high degree (Lee et $al.$, 1994). In fish 80 to 99% of total-Hg is CH_3Hg (Downs et $al.$, 1998). For CH_3Hg, the most likely exposure route for humans is through fish consumption and CH_3Hg is known to be neurotoxic to humans (Environmental health criteria 101, 1990).

Natural Organic Matter

Natural organic matter (NOM), which is a summary term for organic substances in soils and aquatic systems, consist of a complex mixture of low molecular weight organic substances and macromolecules. Organic substances pertaining both to solid and aqueous phase are included in NOM. Since NOM acts as both a nutrient source and as an energy source for microorganisms it is a very important component in soils and waters as it. Due to the high specific surface area and cation exchange capacity, NOM also plays an important role in the adsorption/complexation of trace metals.

8

Soil organic matter (SOM) is a general term that includes all the organic compounds in soil, exclusive of undecayed plant and animal tissues, their partial decomposition products and soil biomass (Sparks, 1995; Essington, 2004). It originates mainly from plant and animal residues, root exudates and microorganisms and is often divided into two main groups of compounds, nonhumic substances and humic substances. Nonhumic substances consist of specific biomolecules with definite physical and chemical properties, like polysaccharides, carbohydrates and proteins. Humic substances consist of less well-defined, complex and resistant polymeric compounds formed by decomposition and synthesis of plant residues and constitute about 60–80% of SOM (Brady and Weil, 1996). On a weight basis, SOM contents range from 0.5 to 5% in surface horizons of mineral soils to 100% in pure organic soils like Histosols (peat soils). Soil organic matter improves structure, water holding capacity, aeration and aggregation in soils (Sparks, 1995). Both solid and dissolved organic matter (DOM) are included in SOM. Apart from organic matter content, the concentration of DOM in soils and surface waters is highly dependent on pH and ionic strength. At high pH and low ionic strength DOM often reach higher concentrations than at low pH and high ionic strength. This is due to the increased dissociation of functional groups at high pH and low ionic strength, resulting in increased water solubility. It is well known from a vast number of studies that DOM plays an important role for mobility as well for bioavailability and toxicity of trace metals (*e.g.* Richards *et al.,* 1999; Luider *et al.,* 2004).

In NOM a variety of different organic functional groups are involved in the adsorption/complexation of metals. Oxygen (O) containing groups like, carboxyls (COOH) and phenols (OH) are the most abundant (Essington, 2004), but less abundant functional groups like amine (RNH_2) and thiol (RSH) groups may still play a crucial role in the complexation of metals. Many functional groups act as acids and dissociates at certain pH values depending on the acidity constants (pK_a). Carboxyls in humic acids have pK_a values in the range 4–6, phenols 9–11 and thiols and amines 8.5–12.5. Since carboxyls are the most abundant functional group, the average pK_a of SOM is 4–4.5 (Tan, 2003). Figure 1 shows a proposed structure of a humic acid at pH below 5, where all groups are protonated.
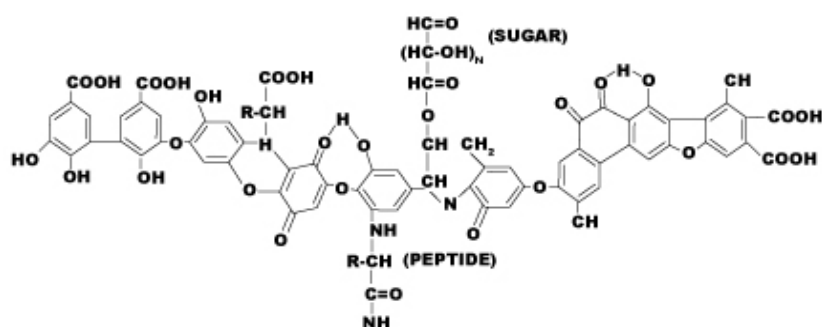

Figure 1. Hypothetical structure of humic acid (Stevenson, 1982).

Speciation of Trace Metals

Depending on the field of research, the term speciation is defined and used in different ways. The International Union for Pure and Applied Chemistry (IUPAC) has published guidelines for the definition of terms related to chemical speciation (Templeton *et al.*, 2000):

- *Chemical species.* Chemical elements: specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure.
- *Speciation analysis.* Analytical chemistry: analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample.
- *Speciation of an element; speciation.* Distribution of an element amongst defined chemical species in a system.

Thus, the speciation of a metal in soil or water is the particular chemical form in which the metal exists, a metal can be present as a simple hydrated ion, as a molecule, as a complex with another ion or molecule, and so forth. In addition to aqueous species, one can distinguish elements in different phases, for example, as aqueous species, as solid phases, or in adsorbed states (Stumm and Morgan, 1996).

It is generally accepted that mobility, toxicity and bioavailability of trace metals in soils and waters in most cases cannot be explained by total metal concentrations (*e.g.* Stumm and Morgan, 1996). Instead, these parameters are controlled by concentrations of specific metal species, *i.e.* the chemical speciation. Soluble metal species are considered to be bioavailable and the free metal ions (Cd^{2+}, Cu^{2+}, CH_3Hg^+) and soluble inorganic complexes are assumed to be the most toxic and bioavailable forms (*e.g.* Lund *et al.*, 1990). Thus, the potential toxicity of the metals depends on the concentration in solution. Of special importance for the speciation are chemical interactions with different colloidal and particle surfaces and trace metals binds to functional groups of natural organic matter (NOM), clay minerals and iron, manganese and aluminium oxyhydroxides (McBride, 1994). In contrast to associations with different inorganic surfaces such as minerals and metal oxyhydroxides, associations of Cd, Cu and CH_3Hg with NOM and its functional groups are not so well described, especially at low metal concentrations. To better understand the behaviour of these metals in soils and waters, the coordination chemistry and bonding strength to functional groups in NOM has to be determined. Knowing the speciation of a metal is vital in order to make accurate risk assessments of contaminated soils and waters.

Theory and Previous Related Studies

This thesis consists of two main parts. The first part deals with the coordination chemistry and the second part with the binding strength. X-ray absorption spectroscopy (XAS) was used to study the coordination chemistry of Cd and Cu(II) in NOM (Paper **I** and **II**) and binding affinity experiments, using competitive complexation and ISE measurements, were used to determine the

binding strength of Cd and CH₃Hg in SOM (Paper **III** and **IV**). Short theoretical descriptions of the methods used and short reviews of previous related studies are presented in this section of the thesis.

X-ray Absorption Spectroscopy

X-rays are light with energies ranging from ~ 500 eV to 500 keV, or wavelengths from ~ 25 Å to 0.25 Å. Light is absorbed by all matter through the photoelectric effect in this energy regime. When X-ray beams pass through a sample its intensity generally decreases due to absorption and scattering. There is a sharp increase in the absorption when the energy of the incident X-rays is equal to the binding energy of a core level electron, of the atom of interest (Figure 2). This is called the absorption edge. For Cd the K-edge is at 26.711 keV and for Cu the K-edge is at 8.979 keV. The term K-edge refers to excitation of electrons in the innermost (K) shell. X-ray absorption fine structure (XAFS) describes how X-rays are absorbed by an atom at energies near and above the core-level binding energies of that atom (Newville, 2004). Since all atoms have core level electrons, XAFS spectra can be measured for almost all elements in the periodic table. XAFS can be used to obtain information on the local chemical environment of metals in a variety of materials, including NOM. Samples analysed do not have to be crystalline, it is possible to analyse non-crystalline and highly disordered materials, including solutions.

   In the X-ray absorption spectrum two different regions can be distinguished: X-ray absorption near edge structure (XANES) spectroscopy and extended X-ray absorption fine-structure (EXAFS) spectroscopy (Newville, 2004). XANES provides information about oxidation state and coordination chemistry of the absorbing atom and applies to the energy region just below the absorption edge and approximately 50 eV past the edge. EXAFS is a result of back-scattered photoelectrons. It appears when atoms are in condensated state and does not occur for isolated atoms. At energies equal to or higher than the absorption edge, the core level electrons may be removed form its quantum level and any energy in excess of the electronic binding energy is given as kinetic energy to a photoelectron that is ejected from the atom. The EXAFS is a direct consequence of the wave nature of the outgoing and backscattered photoelectrons and their interference. The distance between the centre atom and backscattering atoms will determine how the phase varies with the wavelength of the photoelectron. EXAFS provides information about; type of neighbouring atom, bond distance and coordination number and typically applies to the region from 50 eV past the edge to 800 eV after the edge. Bond distances determined by EXAFS are reported in Ångström (Å), 10^{-10} m. Figure 2 shows a Cd K-edge XAFS spectrum, with the EXAFS and XANES regions given. More detailed theory of XAS can be found in Newville (2004) and Koningsberger and Prins (1988).
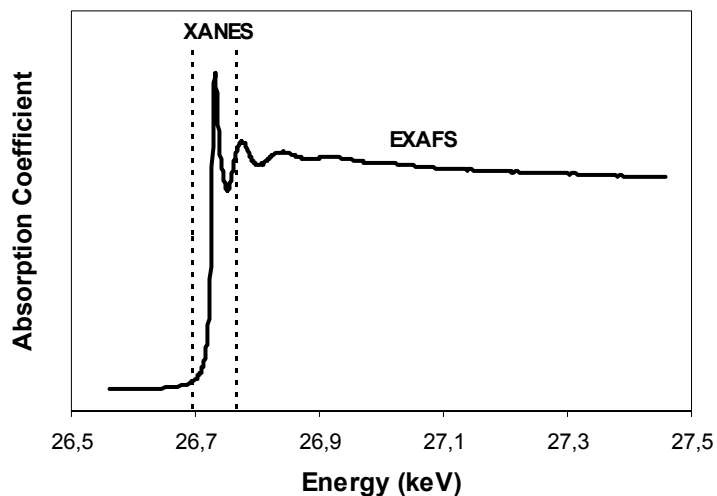
Figure 2. XAFS spectrum for a soil sample with a Cd concentration of 25000 μg g^{-1} at the Cd K-edge (26.711 keV).

Cadmium

For cadmium, EXAFS studies of well-defined compounds have shown that associations to O usually are 6-coordinated and associations to S usually are 4-coordinated in the first coordination shell (Block *et al.*, 1989; Holloway and Melník, 1995; Ramstedt *et al.*, 2004), with bonding distances of 2.21–2.50 Å and 2.43–2.62 Å, respectively. Because of the small difference in atomic mass, it is difficult to distinguish between O and nitrogen (N) atoms with EXAFS and therefore these associations will sometimes be reported as O/N. In the second coordination shell, Cd-C distances of 3.10–3.16 Å was reported for Cd in N-(phosphonomethyl)glycine (Ramstedt *et al.*, 2004). For small organic acids, like acetate, oxalate and citrate, substantially shorter Cd-C distances of 2.67–2.78 Å has been reported (Collins *et al.*, 1999; Boyanov *et al.*, 2003).

To my knowledge, there are no previous EXAFS studies published for Cd using organic soils and only two studies that cover the coordination chemistry of Cd in humic acids. Collins *et al.* (1999) reported Cd to be coordinated by 8.1 O atoms at a distance of 2.30 Å in the first shell and by 3 C atoms at a distance of 2.70 Å in the second shell. In this study the Cd loading to samples was large enough to more than saturate possible strong ligands such as thiols. In the other study, Liu *et al.* (2001) reported Cd to be complexed by 6.1–6.8 O atoms at a distance of 2.30 Å. However, data was not modelled in *k*-space, and therefore the contribution from other ligands like S with certainty cannot be ruled out. In a combined XANES and EDX (energy-dispersive X-ray analysis) study, Martinez *et al.* (2002) found that low solubility of Cd in a cultivated peat soil, despite high total Cd concentrations, was caused by associations of Cd to reduced organic or inorganic S. There are also some EXAFS studies on the coordination chemistry of Cd in plant tissues. Küpper *et al.* (2004) reported Cd to be complexed by both S (2.43–2.51 Å) and O/N (2.25–2.32 Å) atoms in the Cd/Zn hyperaccumulator *Thlaspi caerulescens* and

12

Salt *et al.* (1995) reported Cd to be coordinated by S atoms (2.53 Å) in root tissue and by O/N atoms (2.30 Å) in xylem sap of Indian mustard (*Brassica juncea* L.).

Based on these results, and given that Cd is a chalcophilic metal forming insoluble sulphide minerals in reducing environments (McBride, 1994), it is plausible that reduced organic S (Org-S_{red}) groups are involved in the complexation of Cd in NOM. Especially if the ratio of Cd to Org-S_{red} is kept to a minimum, to avoid complete saturation of strong complexing sites. EXAFS studies of other chalcophilic metals like Zn, Hg, and CH_3Hg have shown that these metals bind to Org-S_{red} in NOM (Xia *et al.*, 1997b; Xia *et al.*, 1999; Qian *et al.*, 2002).

Copper

Studies of well-defined compounds, using EXAFS, have shown that Cu(II) usually is 6 coordinated in the first coordination shell, with 4 O or N atoms at distances of 1.90–1.97 Å, and 2 axial O atoms at 2.15–2.78 Å (Åsbrink and Norrby, 1970; Korshin *et al.*, 1998; Nagy *et al.*, 2000; Sheals *et al.*, 2001). In the second coordination shell, Cu-C distances of 2.70–2.84 Å has been reported for well-defined organic molecules (Nagy *et al.*, 2000; Sheals *et al.*, 2001; Carrera *et al.*, 2004). As far as I know, there are no studies published using EXAFS on organic soils and only few studies cover the coordination chemistry of Cu(II) in humic substances and humic acids (*e.g.* Xia *et al.*, 1997a; Korshin *et al.*, 1998; Alacio *et al.*, 2001; Lee *et al.*, 2005). These studies were all conducted at quite high Cu/organic C ratios (>0,02) and showed that the first coordination shell of Cu(II) displays a Jahn-Teller distorted geometry, with four O atoms at a distance of 1.92–1.96 Å in the equatorial plane and two O in axial positions. There are only two EXAFS studies that present data for the second shell contributions in NOM. Xia *et al.* (1997a) found 4 C atoms at a distance of 3.11–3.16 Å in soil humic substances and Lee *et al.* (2005) found 2.9 C at a distance of 2.88 Å for Cu(II) complexed by an aquatic humic acid. Since Cu also is a chalcophilic metal, it is possible that Cu(II) binds to Org-S_{red} in NOM, at least at low Cu/Org-S_{red} ratios.

Methyl Mercury

Studies of well-defined compounds, using EXAFS, have shown that Hg in CH_3Hg forms a linear two coordination with the C from the methyl group at a distance of approximately 2.03–2.09 Å and S or O at a distance of approximately 2.4 Å and 2.1 Å, respectively (Qian *et al.*, 2002; Yoon *et al.*, 2005). For CH_3Hg, EXAFS studies have been conducted on organic soils, extracted humic substances from soils and on aquatic organic matter (Qian *et al.*, 2002; Yoon *et al.*, 2005). These studies clearly showed that CH_3Hg^+ preferentially forms complexes with Org-S_{red} groups, at a distance of 2.31–2.40 Å, through a mono-dentate coordination. By combining Hg EXAFS with S XANES results Qian *et al.* (2002) calculated that 24–37% of the Org-S_{red} groups were involved in the complexation of CH_3Hg by NOM.

Binding Affinity Experiments

Since concentrations of CH_3Hg and Cd in uncontaminated soils and waters are very low, metal has often been added to samples in order to determine the strength of associations to various constituents. This procedure may result in a saturation of the strongest bonding sites and subsequently weaker sites becomes involved in the bonding of the metals. The time needed for the added metal to find its highest affinity sites and reach equilibrium with these sites is in addition uncertain. Hence, determined binding strengths in spiked samples will probably not be representative of the binding strength at native metal concentrations. However, it is possible to overcome these problems. By using a competitive complexation (ligand exchange) method, very low concentrations of free metal ions (Cd^{2+}, CH_3Hg^+) can be determined. Competitive complexation is based on the competition for metals between natural organic ligands and added known ligands, and subsequent measurement of the concentration of the complexes with these known ligands. The concentration of free metal ions can then be determined by equilibrium calculations based on the stability constants for the complexes formed. Halides (Cl^-, Br^- and I^-) are suitable ligands since they form known complexes with CH_3Hg and Cd and don't bind to functional groups in NOM. A great advantage with this method is that the binding strength can be determined at native and low metal concentrations.

At higher metal concentrations, ion selective electrodes (ISE) can be used for a number different metals including Cd and Cu. These electrodes consist of a Cd/Cu sensing element, which in contact with free Cd^{2+} or Cu^{2+} ions develops an electrode potential that is related to the activity of Cd^{2+} or Cu^{2+} ions. By comparison with standard solutions with known concentrations of metal, the free metal concentration in a sample can be determined. The detection limit for these electrodes is approximately 10^{-6} M.

Cadmium

Literature data on Cd binding to NOM in fresh waters and soil solutions show no general consistency and a range of 0–100% of free Cd^{2+} in equilibrium with Cd-NOM is reported at slightly acid to neutral pH (*e.g.* Xue and Sigg, 1998; Sauve *et al.,* 2000a; Weng *et al.,* 2002; Krishnamurti and Naidu, 2003; Nolan *et al.,* 2003). This variation may be due to varying Cd/NOM ratios and use of different methods to determine free Cd^{2+}. Furthermore, the binding strength in soils is often determined as a solid-liquid partition coefficient (K_d). These K_d values are highly conditional and therefore displays a huge variation between different studies (Sauve *et al.,* 2000b). Xue and Sigg (1998) used a method based on ethylene diamine (EN) ligand exchange and differential pulse anodic striping voltammetry (DPASV) measurement to determine the binding of ambient nM concentrations of cadmium to organic ligands in freshwater. They reported conditional stability constants (log K) on the order of 8–11, suggesting a strong complexation to NOM functional groups. Hence, additional studies with more detailed and precise determinations of the bonding of Cd to functional groups in NOM are needed, at native and low metal concentrations, in order to better understand the behaviour of Cd in soils and waters.

14

Methyl Mercury

There are few studies in which the binding of CH_3Hg^+ to organic substances from soils and waters has been studied at CH_3Hg concentrations low enough to be realistic for natural conditions. Equilibrium dialysis is a method that has been used in several studies (*e.g.* Hintelmann *et al.*, 1997; Amirbahman *et al.*, 2002). Hintelmann *et al.* (1997) estimated the binding strength of CH_3Hg^+ to freshwater humic and fulvic acids and obtained conditional stability constants that only could be explained by complexation involving reduced S groups (log K = 12.15–14.48). Amirbahman *et al.* (2002) determined log K values in the range 10.4 to 14.8 for the bonding of CH_3Hg^+ to reduced S groups in extracted humic acids. In both studies, densities of binding sites (presumably RSH) were determined by an addition of CH_3Hg to their samples, and subsequently used as fitting parameters to data. Based on their model, Amirbahman and co-workers suggested that only 2% of the reduced S sites in their humic acids were involved in the complexation of CH_3Hg^+. Qian *et al.* (2002) used competitive complexation of CH_3Hg by halides to determine the average bonding strength of native concentrations of CH_3Hg to thiol groups in an organic soil. Stability constants (log K_{CH_3HgSR} = 16.7–17.1) were determined using a simple model (CH_3Hg^+ + RS^- ⇆ CH_3HgSR) in which the binding sites were independently determined by XANES. In their study, 24–37% of the Org-S_{red} groups in SOM were calculated to be involved in the complexation. Obviously additional studies, using other methods than dialysis, are needed in order to validate these results.

Objectives

The main objective of this thesis was to determine the coordination chemistry and binding strength of Cd, Cu and CH_3Hg in natural organic matter. The detailed objectives for the different Papers are as follows:

- To determine the coordination chemistry of Cd in natural organic matter and to investigate if reduced organic sulphur groups are involved in the complexation (Paper **I**).

- To determine the coordination chemistry of Cu in natural organic matter and to investigate if reduced organic sulphur groups are involved in the complexation (Paper **II**).

- To determine the bonding strength of Cd to functional groups in soil organic matter at native to highly polluted conditions (Paper **III**).

- To determine the bonding strength of CH_3Hg to reduced sulphur groups in soil organic matter at native metal concentrations (Paper **IV**).

Material and Methods

Sampling and General Chemical Analysis

Soil organic matter from several different locations was used in the different studies (Table 1). All samples listed in Table 1 were treated following protocols for clean sampling procedure sealed in double plastic bags and stored at 4 °C. Subsequently all samples, except the metal contaminated peat from Slagnäs, was freeze dried (Edwards Modulyo 4K Freeze Dryer) and homogenized by a tungsten carbide ball mill (Retsch, S2, Germany). The sample from Slagnäs was only homogenized by grinding in a mortar. For a more detailed description of the different SOM samples see Paper **I-IV**.

Three different types of dissolved organic matter were used in the EXAFS studies (Paper **I** and **II**); Suwannee River NOM (SRN) (International Humic Substance Society, IHSS) and two DOM extracts from a Spodosol (Soil Survey Staff, 1997), one from the O_h-horizon (DOH) and one from the B_{hs}-horizon (DBH) (Table 1). Suwannee River NOM was collected from the Suwannee River near Fargo, GA and concentrated by reverse osmosis. The DOM extracts were obtained using a modified method of Adams and Byrne (1989) and then freeze dried. For a detailed description of the method see Paper **I** and **II**.

Total sulphur in SOM and DOM samples was determined on a LECO sulphur analyzer (LECO Corp. MI, USA). Reduced organic S (Org-S_{red}) was determined either as the sum of sulphur species showing absorption peak maxima in the energy range 2472–2474 eV, using S K-edge XANES, according to the procedure of Xia *et al.* (1998) and Skyllberg *et al.* (2003), or as the sum of sulphur species with binding energy peak maxima in the range 162–164 eV, using X-ray photoelectron spectroscopy (XPS), in accordance with Urban *et al.* (1999). Soil organic carbon was determined by combustion on an elemental analyzer (Perkin-Elmer, 2400 CHN). The chemical composition of the different SOM and DOM samples used in the different studies are presented in Table 1.

The total concentration of Cd in the BFP (no. 6) soil used in Paper **III** was determined by microwave digestion in a mixture of HNO_3 and HCl followed by analysis with induced-coupled-plasma-mass-spectrometry (ICP-MS) (Perkin Elmer, Elan 6000). Total CH_3Hg in the BFP soils used in Paper **IV** was extracted according to the method described by Qian *et al.* (2000) followed by analysis with species-specific-isotope-dilution (SSID) gas-chromatography (GC)-ICP-MS in accordance with Lambertsson *et al.* (2001). The SSID method has the advantage of automatically correcting for possible losses of analyte, instrumental drift and matrix effects (Rodríguez-González *et al.*, 2005). For a detailed description of the methods see Paper **III** and **IV**.

Table 1. Chemical composition of the natural organic matter (NOM) samples used in the different studies. Soil organic matter (SOM), dissolved organic matter (DOM), organic carbon (Org-C), total nitrogen (N_{tot}), total sulphur (S_{tot}) and reduced organic sulphur (Org-S_{red})

NOM sample	[Org-C] (g kg^{-1})	[N_{tot}] (g kg^{-1})	[S_{tot}] (g kg^{-1})	Org-S_{red} (% of S_{tot})	Paper
Subalpine fen peat (SFP)[a]	410	23.3	20	75[c]	**I, II**
Boreal forest peat (BFP)[a], no. 1	492	13.4	10	78[c]	**IV**
Boreal forest peat (BFP)[a], no. 4	330	13.8	3.7	70[c]	**IV**
Boreal forest peat (BFP)[a], no. 5	303	12.3	4.2	71[c]	**IV**
Boreal forest peat (BFP)[a], no. 6	493	18.3	4.1	61[c]	**I-IV**
Boreal forest peat (BFP)[a], no. 7	469	18.2	5.0	71[c]	**IV**
Boreal forest peat (BFP)[a], no.11	197	7.9	0.9	50[c]	**IV**
Agricultural fen peat (AFP)[a]	427	26.0	8.8	61[c]	**I, II**
Slagnäs[a] (Cu contaminated peat)	338	27.5	nd[e]	nd[e]	**II**
Spodosol O_h-horizon (POH)[a]	565	10.5	3.0	60[d]	**I, II**
Spodosol B_h-horizon (PBH)[a]	7	nd[e]	0.98	50[d]	**I, II**
DOM extract from POH (DOH)[b]	431	19.1	3.0	26[d]	**I, II**
DOM extract from PBH (DBH)[b]	329	10.7	nd[e]	nd[e]	**I, II**
Suwannee River NOM (SRN)[b]	525	11.0	6.5	28[d]	**I, II**

[a] SOM. [b] DOM. [c] Determined by XANES. [d] Determined by XPS. [e] nd = not determined.

EXAFS Experiments

Data collection for the EXAFS experiments was performed at two different synchrotron radiation facilities. Cadmium data was collected at the European Synchrotron Radiation Facility (ESRF; Grenoble, France), at the undulator beam line ID26 with 6.0 GeV beam energy and 100–200 mA electron current (Paper **I**). Copper data was collected at Max-lab (Lund University, Sweden) at the super conducting multi-pole wiggler beam line i811 with 1.5 GeV beam energy and 100–200 mA electron current (Paper **II**).

For the EXAFS experiments, $Cd(NO_3)_2$ or $Cu(NO_3)_2$ dissolved in Milli-Q water were added to the different SOM and DOM samples to yield metal concentrations ranging from 990 to 25000 µg g^{-1} on a dry mass basis (in the following text and all Papers, concentrations given in µg g^{-1} are in relation to dry mass). This corresponds to metal (Me)/Org-S_{red} molar ratios of 0.02 to 2.21 and Me/organic C molar ratios of 0.0003 to 0.0065. To adjust the pH (2.8–6.6) and set the ionic strength, KOH (0.5 M) and $NaNO_3$ (10 mM) solutions were added to the soil suspensions. Excess water was evaporated from the suspensions and before EXAFS analysis the remaining wet paste was mounted in sample holders and sealed with Kapton tape (CHR-Furon). The ionic strength in the samples was calculated to be in the range of 20 to 200 mM, after correcting for losses of water

during evaporation. The total equilibrium time was more than 1 week for all samples.

Compounds obtained by addition of Cd and Cu to ion-exchange resins were considered to be relevant as model compounds for Cd/Cu-NOM complexes. A thiol resin (Amberlite GT-73, Rohm and Hass) was used to prepare a Cd/Cu-SR compound and a carboxylic resin (BioRex 70, Bio-Rad) to prepare a Cd/Cu-OOCR compound. $Cd(NO_3)_2$ or $Cu(NO_3)_2$ dissolved in Milli-Q water were added to the resins yielding metal concentrations of 2500–15000 µg g^{-1} on a dry mass basis. This corresponds to Me/SR molar ratios of 0.005 to 0.10 and Me/OOCR ratios of 0.0025 to 0.025. The pH was adjusted between 4.1 and 8.8. Before EXAFS analysis the resins were ground in a mortar, mounted into the sample holders, and sealed with Kapton tape.

For reduction and analysis of EXAFS data, the computer programme WinXAS97 (Ressler, 1997) was used. From each averaged spectrum a polynomial pre-edge function was subtracted and the data were normalized. Above the absorption edge a cubic spline fit was used to remove the background and the data were k^3-weighted to enhance the higher k-values. Theoretical EXAFS amplitudes and phase functions of mixed models for Cd/Cu-O, Cd-S and Cd/Cu-C associations, generated by FEFF 7.0 (Zabinsky et al., 1995), were fitted to the EXAFS data. These models were based on structural parameters from well-defined organic and inorganic compounds (Åsbrink and Norrby, 1970; Block et al., 1989; Holloway and Melník, 1995; Korshin et al., 1998; Sheals et al., 2001; Ramstedt et al., 2004). For a more detailed description of the EXAFS data collection and the reduction and analysis of data see Paper **I** and **II**.
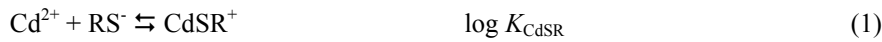
Binding Affinity Experiments

Competitive Complexation

The binding strength of Cd and CH$_3$Hg in SOM at native metal concentrations (0.23 µg Cd g^{-1} dry soil, 1.7–9.8 ng CH$_3$Hg g^{-1} dry soil) was determined by competitive complexation (ligand exchange) (Paper **III** and **IV**). Halides (Br, Cl and I) were used as competing ligands to the functional groups in SOM according to the method of Skyllberg et al. (2000) and Qian et al. (2002).

A series of experiments were performed to investigate the effect of equilibration time, pH, different halides, ionic strength and type of organic soil on the binding affinity. In these experiments, homogenized freeze-dried soil was weighed into 50 mL centrifuge tubes (Falcon polypropylene) and a KCl, KBr or KI solution was added. For each soil sample suspended in halide solution a reference soil sample suspended in KNO$_3$ was prepared to determine dissolved organically complexed metal. To adjust the pH (2.0–5.1), KOH was added and the suspensions were equilibrated on a rotary mixer. After equilibration the suspensions were centrifuged and the supernatants were transferred to new centrifuge tubes. Prior to analysis, a species-specific internal standard (^{106}Cd, CH$_3$^{200}Hg) was added to the supernatants.

Equilibrium concentrations of Cd were determined by SSID-ICP-MS. For CH_3Hg, equilibrium concentrations were determined by SSID-GC-ICP-MS in accordance with Lambertsson *et al.* (2001). For a detailed description of the methods see Paper **III** and **IV**.

Calculations of Stability Constants

Based on the results from the Cd EXAFS study (Paper **I**) and EXAFS studies on CH_3Hg (Qian *et al.*, 2002; Yoon *et al.*, 2005), showing that reduced S ligands are involved in the complexation of Cd and CH_3Hg by NOM, simple models were used to describe the bonding of Cd^{2+} and CH_3Hg^+ to thiol groups in SOM (reaction 1 and 2). In order to calculate the stability constant for the bonding of Cd^{2+} and CH_3Hg^+ to RSH (eq 3 and 4) the concentration of dissociated thiol groups (RS^-) is needed. Based on the findings by Qian *et al.* (2002) for CH_3Hg, 30% of Org-S_{red} (Table 1) determined by XANES, was considered to be RSH groups active in the bonding of both Cd^{2+} and CH_3Hg^+. For Cd, the pK_a value of mercaptoacetic acid (log K_{RSH} = 9.96; Hilton *et al.*, 1975) was used for the dissociation of RSH groups. For CH_3Hg two pK_a values were assumed, with 90% of the RSH groups having a pK_a of 9.95 and 10% a pK_a of 8.5. Conditional stability constants (K_{CdSR}, K_{CH_3HgSR}) were finally calculated according to eq 3 and 4. For a more detailed description of the calculations see Paper **III** and **IV**.

$$Cd^{2+} + RS^- \leftrightarrows CdSR^+ \qquad\qquad \log K_{CdSR} \qquad\qquad (1)$$

$$CH_3Hg^+ + RS^- \leftrightarrows CH_3HgSR \qquad\qquad \log K_{CH_3HgSR} \qquad\qquad (2)$$

$$K_{CdSR} = \frac{[CdSR^+]}{[RS^-]\{Cd^{2+}\}} \qquad\qquad (3)$$

$$K_{CH_3HgSR} = \frac{[CH_3HgSR]}{[RS^-]\{CH_3Hg^+\}} \qquad\qquad (4)$$

ISE Measurements

ISE measurements were performed using Orion 96-48 ionplus cadmium/cupric electrodes in conjunction with a Mettler Toledo SevenMulti in millivolt (mV) mode. The detection limit of the Cd and Cu electrode was 8.0×10^{-7} M and 7.8×10^{-7} M, respectively.

For all samples analysed by EXAFS (Paper **I** and **II**), the Cd^{2+} and Cu^{2+} activity in equilibrium with adsorbed metal was measured, in suspensions (1.5–2.0 mL of solution to 0.03–0.1 g dry mass of sample) with 10 mM $NaNO_3$. Based on these measurements, the percentage of free Cd^{2+} and Cu^{2+} in solution and their hydrolysis species ($CdOH^+ + Cd(OH)_2^0{}_{(aq)}$; $CuOH^+ + Cu(OH)_2^0{}_{(aq)}$) were calculated.

At Cd concentrations ranging from 500 to 54000 µg g^{-1} dry soil, the binding strength to SOM was determined by measuring the Cd^{2+} activity in equilibrium with adsorbed Cd (Paper **III**). A series of experiments was made to investigate the effect of equilibration time, pH and metal concentration on the binding. In these experiments, homogenized freeze-dried soil was weighed into ICP tubes (12 mL) and Cd(NO$_3$)$_2$ dissolved in Milli-Q water was added. To adjust the pH (2.9–7.3) and set the ionic strength, KOH (0.5 M) and NaNO$_3$ (10 mM) solutions were added to the soil suspensions. After that the samples were equilibrated on a shaker and the Cd^{2+} activity was measured.

Modelling of ISE Data

Data from ISE experiments were modelled using the speciation programme Visual MINTEQ 2.12 (http://www.lwr.kth.se/English/OurSoftware/Vminteq/index.htm; 21-Nov-2003). Models consisting of one RSH group (reaction 1) and one or two RCOOH groups (reactions 5 and 6) were tested in order to describe the binding to SOM. For the bonding of Cd to RS$^-$, results from the competitive complexation experiments at native metal concentrations were used. The pK_a value for the RCOOH groups was set to 4.5, reflecting the average pK_a of humic substances (Tan, 2003). The total amount of RCOOH groups active in the bonding of Cd^{2+} and log K_{CdOOCR} was determined by fitting a model with one RSH and one RCOOH group to the experimental data in the concentration range 500–54000 µg g^{-1}. Log $\beta_{Cd(OOCR)_2}$ was determined by fitting a model with one RSH and two RCOOH groups to the data in the same concentration range. No electrostatic effects were considered in the models.

$$Cd^{2+} + RCOO^- \leftrightarrows CdOOCR^+ \qquad\qquad \log K_{CdOOCR} \qquad\qquad (5)$$

$$Cd^{2+} + 2\ RCOO^- \leftrightarrows Cd(OOCR)_2 \qquad\qquad \log \beta_{Cd(OOCR)_2} \qquad\qquad (6)$$

Speciation Calculations

Equilibrium concentrations of different Cd species were calculated in the soil suspensions prepared for the competitive complexation and ISE experiments. For CH$_3$Hg, concentrations of different species were calculated in an organic soil with native CH$_3$Hg concentration (10 ng g^{-1}). The speciation programme Visual MINTEQ 2.12 (http://www.lwr.kth.se/English/OurSoftware/Vminteq/index.htm; 21-Nov-2003) was used for the calculations. The calculations were based on the determined stability constants for the association of Cd and CH$_3$Hg to S and O containing functional groups in SOM and stability constants taken from literature (Carty and Malone, 1979) and the MINTEQA2 (v4.0 U.S EPA) database. For a detailed description of the calculations see Paper **III** and **IV**.

Results and Discussion

EXAFS Experiments (Paper I and II)

Cadmium

Results from the EXAFS experiments showed that reduced S groups are involved in the complexation of Cd by NOM. In SOM samples (1000–25000 µg Cd g^{-1}, pH = 4.6–6.6), Cd was coordinated by 1.0 to 2.5 S atoms at an average distance of 2.52 Å and by 3.0 to 4.5 O/N atoms at an average distance of 2.23 Å in the first coordination shell. In DOM samples (1750–4250 µg Cd g^{-1}, pH = 5.4–6.3), Cd was coordinated by 0.3–1.8 S at an average distance of 2.54 Å and 3.6–4.5 O/N at an average distance of 2.24 Å. These distances are in accordance with data for first shell Cd-S and Cd-O/N associations in a variety of different inorganic and organic compounds as well as in plant tissues (Table 2). The Cd-O/N distance found is also in agreement with distances reported for humic acids (Table 2). In Figure 3 the Fourier transformed radial structure function (RSF) and the fit to the k^3-weighted EXAFS spectrum are shown for the SFP sample (c.f. Table 1), with a Cd concentration of 10000 µg g^{-1}. The first peak in the RSF represents O and S atoms in the first coordination shell. The RSF is not corrected for the scattering phase shift, which typically is around 0.5 Å.

Table 2. Determined coordination numbers (CN) and first and second shell bond distances (*R*) for Cd in soil organic matter (SOM) and dissolved organic matter (DOM). Compared to values reported for N-(phosphonomethyl)glycine (PMG), humic acids and different plant tissues

Compound	CN	Cd-O/N R (Å)	CN	Cd-S R (Å)	CN	Cd-C R (Å)	Ref.
SOM	3.0-4.5	2.22-2.25	1.0-2.5	2.49-2.55	1.7-5.2	3.06-3.18	[a]
DOM	3.6-4.5	2.23-2.26	0.3-1.8	2.51-2.56	3.0-6.0	3.08-3.14	[a]
PMG	6	2.28-2.29			3-6	3.10-3.16	[b]
Humic acid	6.1-6.8	2.30					[c]
Humic acid	8.1	2.30			3	2.70	[d]
Xylem sap	6	2.30					[e]
Root tissue			4	2.53			[e]
Plant tissue	2.9-5.7	2.25-2.32	0.3-3.1	2.43-2.51			[f]

[a] Results from the EXAFS experiments. [b] Ramstedt *et al.* (2004). [c] Liu *et al.* (2001). [d] Collins *et al.* (1999). [e] Salt *et al.* (1995). [f] Küpper *et al.* (2004).
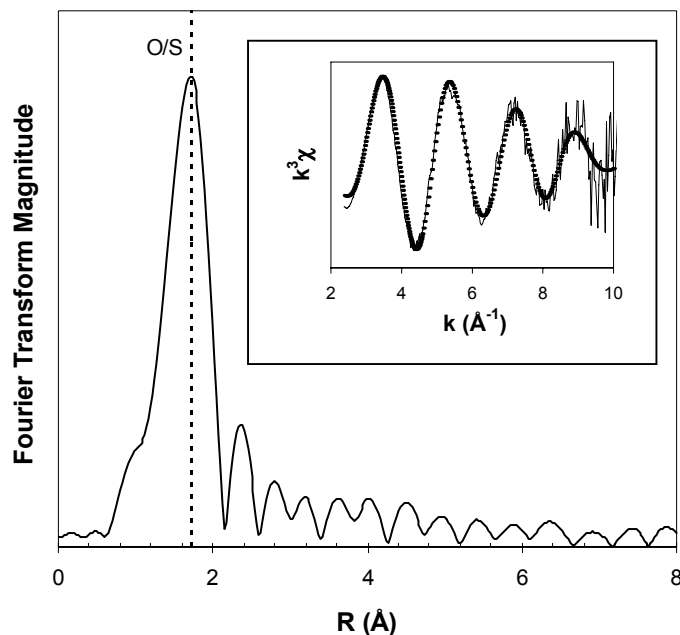
Figure 3. Fourier transform (not corrected for phase shifts) and k^3-weigthed EXAFS spectrum for the SFP sample with a Cd concentration of 10000 µg g^{-1}. Experimental spectrum (solid line) and spectrum derived form fitting parameters (dotted line). The vertical dashed line indicates O/S atoms in the first coordination shell.

In both SOM and DOM samples, a second coordination shell of 1.7 to 6.0 carbon atoms was found at a distance of 3.06–3.18 Å (Table 2). This provides direct evidence for inner-sphere complexation of Cd by functional groups in NOM. Furthermore, ISE measurements showed that less than 1% of total Cd was in the form of free Cd^{2+} in all SOM and DOM samples. The Cd–C distance is in agreement with the Cd-C distance in N-(phosphonomethyl)glycine (Table 2), but substantially longer than Cd–C distances of 2.67–2.70 Å reported for acetate (Boyanov *et al.*, 2003), 2.78 Å for oxalate and 2.70 for citrate (Collins *et al.*, 1999). In the latter study a Cd–C distance of 2.70 Å was reported for humic acid. It was possible to fit a Cd-C distance of approximately 2.7 Å to our NOM data, but the fit was always better at the longer distance of approximately 3.1 Å. More C atoms were found in the second shell of DOM than in SOM. Because DOM in average consists of smaller and more flexible (less sterically hindered) organic molecules, it is reasonable that a greater number of organic functional groups (and consequently less water molecules) could be involved in the complexation of Cd in DOM, as compared to SOM. Bond distances and coordination numbers suggest that Cd complexed in SOM and DOM is a mixture of a 4-coordination, with S (thiols) and water molecules, and a 6-coordination with O/N ligands alone. A proposed structure for Cd complexed by S in NOM is shown in Figure 4. EXAFS data for the SFP and BFP (no.6) samples (Table 1) showed a decrease in the relative contribution from S ligands (CN_S / [CN_S + $CN_{O/N}$]) with increasing

22

Cd/Org-S_{red} ratio. The relative S contribution decreased from 41–45% at 1000 µg g^{-1} to 23–25% at 10000 µg g^{-1}. Given a 4-coordinated structure with 2 water molecules (Figure 4), this indicates that almost 100% of the Cd was associated to S at 1000 µg g^{-1} and approximately 50% at 10000 µg g^{-1}. Since Cd-S associations in average are stronger than Cd-O/N associations, our results strongly indicates that reduced S ligands are involved in the complexation of Cd by NOM also at native concentrations of metal in oxidized organic rich soils and in humic streams. The EXAFS results presented here are the first published showing a clear contribution from S ligands in the complexation of Cd by SOM and DOM. The reason for this is probably the low Cd/Org-S_{red} ratios used in the study.
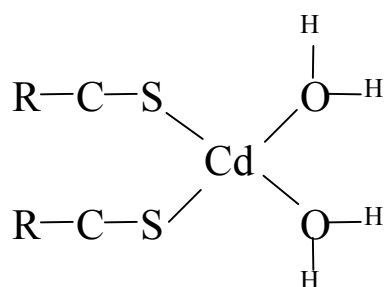

Figure 4. Proposed structure for 4-coordinated Cd in NOM. Cadmium complexed by two monodentate S ligands and two water molecules in tetrahedral configuration.

Copper

Results from the Cu EXAFS experiments showed that Cu is complexed by O and or N atoms in the first coordination shell in NOM. In both SOM and DOM samples (990–11000 µg Cu g^{-1}, pH = 2.8–6.3), Cu was coordinated by 4 O/N atoms at an average distance of 1.94 Å in the first shell (Table 3). These four atoms are most likely positioned in the equatorial plane of a Jahn-Teller distorted elongated octahedron. The contribution to the EXAFS signal from the more distant axial O is expected to be small (Sheals *et al.*, 2001) and there was no significant improvement in the fits of the first coordination shell when axial oxygens were included, compared to a model with only equatorial O/N. The O/N distances found are in accordance with data for Cu coordinated by 4 equatorial O/N in a variety of different inorganic and organic molecules as well as for data reported for different types of NOM (Table 3). There are no indications for S associations, not even in samples with Cu/Org-S_{red} molar ratios as low as 0.03 to 0.10. In Figure 5 the RSF and the final fit to the k^3-weighted EXAFS spectrum are shown for the DBH sample (DOM extract from PBH, Table 1) with a Cu concentration of 10000 µg g^{-1}. The first peak in the RSF represents O/N atoms in the first coordination shell and the second peak C atoms in the second shell.

Table 3. Determined coordination numbers (CN) and first and second shell bond distances (*R*) for Cu in soil organic matter (SOM) and dissolved organic matter (DOM). Compared to values reported for $Cu(H_2O)_6^+$, N-(phosphonomethyl)glycine (PMG) and humic acids

Compound	CN	Cu-O/N$_{eq}$ R (Å)	CN	Cu-C R (Å)	Ref.
SOM	4	1.92-1.95	2-4	2.76-2.84	[a]
DOM	4	1.92-1.96	4	2.82-2.86	[a]
$Cu(H_2O)_6^{2+}$	4	1.97			[b]
PMG	4	1.96	3	2.81	[c]
Aquatic humic acid	4.4	1.94	2.9	2.88	[d]
Humic substance	4	1.93-1.94	4	3.11-3.16	[e]
Soil humic acid	5.41	1.94			[f]

[a] Results from the EXAFS experiments. [b] Korshin *et al.* (1998). [c] Sheals *et al.* (2001). [d] Lee *et al.* (2005). [e] Xia *et al.* (1997a). [f] Alacio *et al.* (2001).
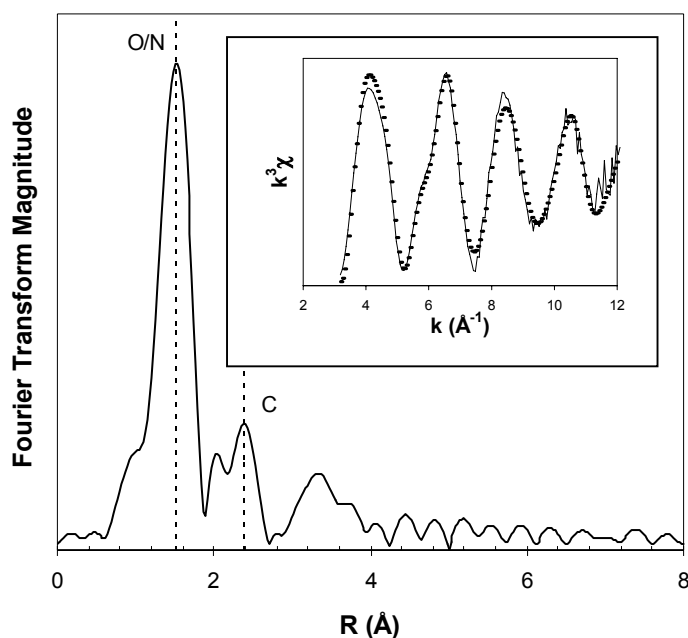

Figure 5. Fourier transform (not corrected for phase shifts) and k^3-weigthed EXAFS spectrum for the DBH sample with a Cu concentration of 10000 µg g^{-1}. Experimental spectrum (solid line) and spectrum derived form fitting parameters (dotted line). The vertical dashed lines indicates O/N and C atoms in the first and second coordination shell, respectively

In SOM and DOM samples with a pH above 4.8 a second coordination shell consisting of 2 or 4 C atoms was found at an average distance of 2.83 Å (Table 3). The Cu-C distance is in accordance with Cu-C distances found in N-(phosphonomethyl)glycine and with data presented by Lee *et al.* (2005) for aquatic humic acids (Table 3). A third coordination shell was also found in SOM and DOM samples with 2 or 4 O/C at an average distance of 3.69 Å, with an average multiple scattering distances of 4.19 Å. The second and third shell

24

contributions provides direct evidence for inner-sphere complexation and shows that Cu is complexed by one or two 5-membered chelate rings. A proposed model for Cu complexed by two 5-membered chelate rings in NOM is shown in Figure 6. There seems to be a difference in the complexation of Cu between SOM and DOM. All DOM samples have a structure consisting of two chelate rings and two water molecules while all SOM samples, except one, have a structure with one chelate ring and four water molecules. As for Cd, it is reasonable that a greater number of organic functional groups (and less water molecules) are involved in the complexation of Cu in DOM. Furthermore, ion activity measurements showed that less than 0.2% of total Cu was in the form of free Cu^{2+} in samples with a pH above 4.8. In the metal contaminated peat from Slagnäs (6200 µg Cu g^{-1}) 9% was free Cu^{2+} in solution, despite a pH of 4.1. This is probably due to high concentrations of Zn (~20000 µg g^{-1}) and Pb (~4000 µg g^{-1}), resulting in competition for binding sites between the metals. At pH 2.8 in the BFP (no. 6) sample (Table 1), free Cu^{2+} constituted approximately 50% of total Cu. The absence of second shell contributions in these two samples suggests a formation of outer-sphere complexes and/or a very distorted average geometry. Hence, there is a possibility of a mixture of outer- and inner-sphere complexes and free Cu^{2+} in these samples.
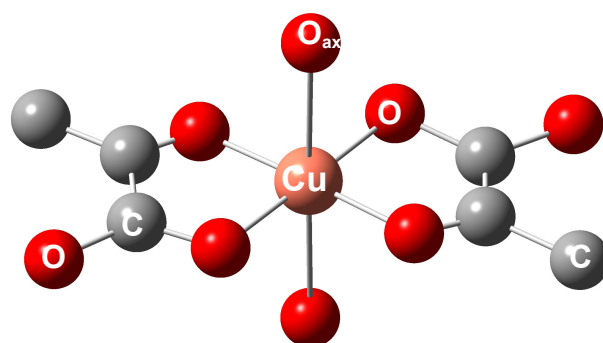

Figure 6. Proposed model for Cu complexed by two 5-membered chelate rings in NOM. The more distant axial oxygens (O_{ax}) are not included in the final fits to the EXAFS data.

Binding Affinity Experiments (Paper III and IV)

Competitive Complexation

Cadmium

Determined log K_{CdSR} values, calculated by eq 3, showed a range of 11.1–11.4 and remained quite constant when different halides (Br, Cl) were used as competing ligands (Table 4). The relatively small variation in log K_{CdSR} indicates that the method used give reproducible results. In the soil used for these experiments (BFP

no. 6, Table 1) the amount Org-S_{red} is in large excess compared to Cd, resulting in an Org-S_{red}/Cd molar ratio of 3.8×10^4. The activity of Cd^{2+} in equilibrium with Cd bonded to RSH groups is 1.45×10^{-9}–1.58×10^{-10} in the pH range 3.1–4.6 (Table 4).

Table 4. Log K_{CdSR} values (at I = 0) determined at pH 3.1 to 4.6 and a total Cd concentration of 0.23 µg g^{-1}, calculated by eq 3. Different halides (Br, Cl) were used at an ionic strength of 0.1 M and the pK_a for the RSH groups was set to 9.96. Dissociated reduced sulphur groups (RS$^-$), Cd bonded to reduced sulphur groups (CdSR$^+$) and activity of Cd^{2+} ($\{Cd^{2+}\}$)

pH	Halide	[RS$^-$] (mol kg^{-1} C)	[CdSR$^+$] (mol kg^{-1} C)	$\{Cd^{2+}\}$	log K_{CdSR}
3.09	Br	6.40×10^{-9}	2.49×10^{-6}	1.45×10^{-9}	11.4
3.54	Br	1.80×10^{-8}	2.96×10^{-6}	1.05×10^{-9}	11.2
4.53	Br	1.76×10^{-7}	4.00×10^{-6}	1.58×10^{-10}	11.2
4.56	Cl	1.89×10^{-7}	4.04×10^{-6}	1.73×10^{-10}	11.1

Determined log K_{CdSR} values are in fair agreement with stability constants reported for the association between Cd and reduced S groups in well-defined complexes. In a review of data supposedly relevant for soils and waters, Smith *et al.* (2002) reported log K values in the range 8.5 ± 2.0 (Table 5). The lower values in the given range probably represent inorganic and organic sulphides and the higher values thiols in proteins. Li and Manning (1955) reported a log K_1 value of 10.5 for Cd associated to glutathione. Based on the pK_a value given for glutathione (Table 5), our data results in log K_{CdSR} values in the range 9.9–10.2. Furthermore, based on the pK_a value for bisulphide, our data results in log K_{CdSR} values of 7.9–8.1, well in agreement with reported constants for Cd-bisulphide complexes (Table 5). These results are in agreement with results from the EXAFS experiments that showed a contribution from S ligands in the complexation of Cd by NOM.

Table 5. Stability constants and pK_a values reported for well-defined Cd complexes

Compound	pK_a	log K_1	log β_2	Ref.
Sulphidic-thiolate groups (4)[a]	8+	8.5 ± 2.0		[d]
Glutathione	8.75	10.5		[e]
Bisulphide	6.67	8.4	11.4	[f]
Carboxyl groups (8)[a]	2-6	2.24 ± 1.39		[d]
Malonate	2.83[b], 5.69[c]	3.2	4.0	[g]
Phthalate	2.95[b], 5.41[c]	3.4	4.0	[g]

[a] Functional groups expected in natural environments and bio-organic matter. With number of groups included in parentheses. [b] pK_{a1}. [c] pK_{a2}. [d] Smith *et al.* (2002). [e] Li and Manning (1955). [f] Al-Farawati and van den Berg (1999). [g] Stumm and Morgan (1996).

Methyl Mercury

For CH$_3$Hg, determined log K_{CH_3HgSR} values for SOM and DOM, using two pK_a values for the RSH groups (8.5 and 9.95), showed a range of 15.6–17.1 for all experiments (pH range 2.0–5.1). Despite large differences in affinities between Cl, Br and I for CH$_3$Hg$^+$, determined constants were independent on type and concentration of halide used in the experiments (log K_{CH_3HgSR} = 16.1–16.7 at pH 3.5–3.6). For six different organic soils (BFP no. 1, 4–7, 11; Table 1) log K_{CH_3HgSR} varied between 16.2 and 16.7 for SOM (Table 6) and between 16.0 and 16.7 for DOM. The variation in log K_{CH_3HgSR} is small despite varying concentrations of CH$_3$Hg and Org-S$_{red}$ in the soils. The amount of Org-S$_{red}$ is in large excess as compared to CH$_3$Hg in all soils, resulting in Org-S$_{red}$/CH$_3$Hg molar ratios of 1.7 x 10^6–1.7 x 10^7. The activity of CH$_3$Hg$^+$ in equilibrium with CH$_3$Hg bonded to RSH groups is extremely low in organic soils with native CH$_3$Hg concentrations (Table 6).

Table 6. Log K_{CH_3HgSR} values (at I = 0) for SOM in 6 boreal forest peat soils, calculated by eq 4. Determined at pH 3.5 to 3.7 and total CH$_3$Hg concentrations of 1.7-9.8 ng g^{-1}. Two pK_a values were used for the RSH groups (8.5 and 9.95). Dissociated reduced sulphur groups (RS$^-$), CH$_3$Hg bonded to reduced sulphur groups (CH$_3$HgSR) and activity of CH$_3$Hg$^+$ ({CH$_3$Hg$^+$})

Soil no.	pH	[RS$^-$] (mol kg^{-1} C)	[CH$_3$HgSR] (mol kg^{-1} C)	{CH$_3$Hg$^+$}	log K_{CH_3HgSR}
1	3.5	1.96 x 10^{-7}	2.88 x 10^{-8}	7.39 x 10^{-18}	16.3
4	3.6	1.22 x 10^{-7}	1.45 x 10^{-7}	6.28 x 10^{-17}	16.3
5	3.7	1.92 x 10^{-7}	1.50 x 10^{-7}	1.78 x 10^{-17}	16.6
6	3.5	6.26 x 10^{-8}	2.60 x 10^{-8}	2.46 x 10^{-17}	16.2
7	3.5	9.34 x 10^{-8}	4.58 x 10^{-8}	9.60 x 10^{-18}	16.7
11	3.6	3.55 x 10^{-8}	4.03 x 10^{-8}	4.35 x 10^{-17}	16.4

Determined log K_{CH_3HgSR} values, calculated by eq 4, are in fair agreement with identically defined stability constants determined for the association between CH$_3$Hg$^+$ and thiol groups in cysteine and glutathione (Table 7). Based on the pK_a values used by Simpson (1961), our data result in log K_{CH_3HgSR} values of 15.2−16.8 and 14.8−16.4, respectively. Qian *et al.* (2002) reported log K_{CH_3HgSR} values in the range 16.7–17.1, using eq 4 with one RSH group having a pK_a of 9.96, with a slight increase in log K_{CH_3HgSR} from pH 3.0 to 4.3. Using a pK_a value of 9.96, we obtained log K_{CH_3HgSR} values of 16.2–17.7 in the pH range 2.0–5.1. Compared to Qian *et al.* (2002), we have performed a more detailed study. In our study 6 different soils and three different halides were used (Cl, Br, I). Furthermore, we varied the ionic strength and used a wider pH range. Based on our results we conclude that the concentration of free CH$_3$Hg$^+$ is controlled by associations to RSH groups at both native and slightly polluted conditions in organic rich soils and humic streams.

Table 7. Stability constants and pK_a values reported for well
-defined CH$_3$Hg complexes and CH$_3$Hg complexed by thiol
groups (RSH) in an organic soil

Compound	pK_a	log K_1	Ref.
Glutathione	9.0	15.9	[a]
Cysteine	8.6	15.7	[a]
SOM (RSH)	9.96	16.7-17.1	[b]
Acetic acid	4.76	3.20	[c]
Methylamine	10.43	7.57	[d]

[a] Simpson (1961). [b] Qian *et al.* (2002). [c] Jawaid and Ingman
(1978). [d] Rabenstein *et al.* (1974).

ISE Measurements

In the concentration range 500–54000 µg Cd g^{-1} (pH 4.6) a model consisting of one RSH and one RCOOH group (reaction 1 and 5) gave the best fit to the complete range of experimental data (Figure 7). The model with only RSH functional groups, used to describe the binding at native metal concentrations, gave a good fit up to a Cd concentration of approximately 2500 µg g^{-1}. This is reasonable since the ratio between Cd and RSH groups considered to be active in the bonding (30% of Org-S$_{red}$) is 0.9 at 2500 µg Cd g^{-1}. At higher Cd concentrations the RSH groups become saturated. The Cd/RSH ratio is 3.6 at 10000 µg Cd g^{-1} and 19.6 at 54000 µg Cd g^{-1}. This is in agreement with results from the EXAFS experiments, showing a decrease in the relative contribution from S ligands with increased Cd concentration. A model with one RSH group and two RCOOH groups was also tested (reaction 1 and 6). This model gave a good fit up to a total Cd concentration of approximately 26000 µg g^{-1} (Figure 7). The stability constants (log K_{CdOOCR} = 3.2; log $\beta_{Cd(OOCR)_2}$ = 4.6) for Cd associated to RCOOH groups, obtained by fitting models consisting of one RSH and one or two RCOOH groups to the experimental data, are in fair accordance with stability constants determined for the association between Cd and carboxylic groups in well-defined Cd complexes (Table 5).
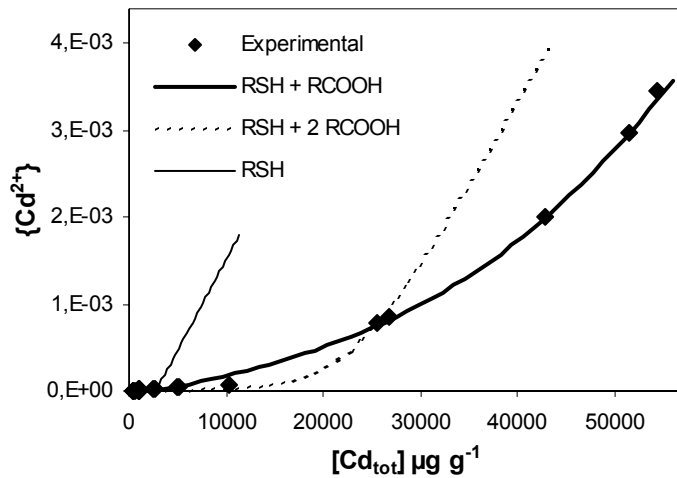
Figure 7. Effect of total Cd concentration on the free Cd^{2+} activity in equilibrium with Cd adsorbed to SOM at pH 4.6. Models consisting of 1 RSH group, 1 RSH group + 1 RCOOH group and 1 RSH group + 2 RCOOH groups are fitted to the experimental data.

In the pH range 2.9–7.3 and a total Cd concentration of 10000 µg g^{-1}, the best fit to the experimental data was obtained with a model consisting of one RSH group and two RCOOH groups (Figure 8). This is in accordance with the concentration study were this model gave a good fit up to 26000 µg Cd g^{-1} at pH 4.6 (Figure 7). There is a sharp increase in the percentage of free Cd^{2+} at pH below 4 (Figure 8). At pH 3.0 the percentage of free Cd^{2+} is approximately 50%. This suggests that Cd is quite soluble in acidic organic soils that are heavily polluted.
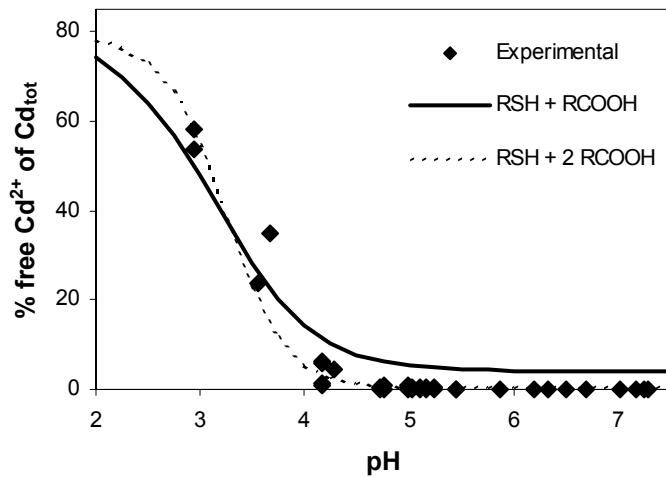

Figure 8. Effect of pH on the percentage free Cd^{2+} in equilibrium with Cd adsorbed to SOM at a total Cd concentration of 10000 µg g^{-1}. Models consisting of 1 RSH group + 1 RCOOH group and 1 RSH group + 2 RCOOH groups are fitted to the experimental data.

Speciation Calculations

Speciation calculations based on determined stability constants, at Cd concentrations ranging from 0.23–10000 $\mu g\ g^{-1}$, show that Cd complexed by SOM totally dominates the speciation and that the percentage free Cd^{2+} is only a few % of total Cd at pH values above 4.0. At native Cd concentrations (0.23 $\mu g\ g^{-1}$), Cd complexed by thiol (RSH) groups in SOM dominates the speciation in the pH range 3–5. The percentage of Cd complexed by RSH, at a Cd concentration of 10000 $\mu g\ g^{-1}$, is rather constant at approximately 24% in the pH range 3–7, while the percentage of Cd complexed by RCOOH increases from around 25% at pH 3 to a maximum of 75% at pH above 4.5. These results are in contrast to the general impression from the scientific field of soil science that Cd is highly soluble and adsorbs rather weakly in soils.

In an organic soil with native concentration of CH_3Hg (10 ng g^{-1}) speciation calculations show that CH_3Hg complexed by RSH in SOM is the dominating CH_3Hg form in the pH range 3-7. Concentrations of free CH_3Hg^+ and inorganic CH_3Hg complexes, generally assumed to be the most mobile and bioavailable forms, are extremely low in comparison to CH_3Hg complexed by RSH. In absence of substantial concentrations of inorganic sulphides, neutral chloro-complexes (CH_3HgCl) and free CH_3Hg^+ reach concentrations on the order of 10^{-17}–10^{-18} M at pH 5 in soil solutions with 3×10^{-5} M of chloride.

Environmental Implications

Results from the EXAFS and binding affinity experiments, for organic soils and DOM, show that Cd, Cu and CH_3Hg form strong inner-sphere complexes with both SOM and DOM. Furthermore, our results imply that at pH values relevant for most organic soils and humic surface waters, the speciation of these metals is determined by the strong complexation with NOM functional groups. By lowering the concentrations of free metal ions and soluble inorganic complexes, NOM controls biouptake, toxicity and transport in these systems. Since Cd and CH_3Hg form stronger complexes with S than with O/N containing functional groups, the speciation at pH values relevant for most soils and surface waters will be totally dominated by Cd and CH_3Hg bonded to RSH groups until these groups are saturated. In the BFP (no. 6) sample (Table 1) saturation was reached at approximately 2500 μg Cd g^{-1}. Thus, in a mineral soil with 1% organic carbon, where the organic matter have the same concentration of reduced organic sulphur (Org-S_{red}) as the BFP (no. 6) sample, the RSH groups would be saturated at a Cd concentration of approximately 50 $\mu g\ g^{-1}$. Soils with Cd concentrations above 4 $\mu g\ g^{-1}$ are considered to be heavily polluted. This indicates that there is enough RSH groups also in polluted mineral soils, with low SOM content, to control the speciation of Cd. This should also be valid for CH_3Hg, since CH_3Hg form stronger complexes with RSH groups and generally occurs at much lower concentrations. So, in order to make an accurate speciation of Cd and CH_3Hg in soils and waters it is necessary to determine the total amount of both Org-S_{red} and metal.

The results presented in this thesis should be of great importance as a basis for further in depth studies and modelling of speciation, toxicity, mobility and

bioavailability of Cd, Cu and CH$_3$Hg in soils and waters. An increased understanding of the behaviour of these metals in soils and waters is important in order to get sufficient information to make proper risk assessments of contaminated sites.

Major Conclusions

- Cd, Cu and CH$_3$Hg form strong inner-sphere complexes with functional groups in natural organic matter.

- Reduced sulphur groups are involved in the complexation of Cd and CH$_3$Hg by solid and dissolved natural organic matter and therefore determines the speciation of these metals in organic rich soils.

- The stability constants determined for the bonding of Cd and CH$_3$Hg to reduced sulphur groups in soil organic matter are in accordance with constants reported for the association between these metals and reduced S groups in well-defined organic molecules.

- Cu binds to O/N containing functional groups in solid and dissolved natural organic matter. There are no indications of S associations.

- Modelling of EXAFS data suggests that Cu form complexes consisting of one or two five-membered chelate rings.

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