Characterization of coexisting dissolved organic carbon and heavy metals in surface waters

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Marcus Wengel
Geboren am 25.09.1973 in Zwickau / Sachsen

Gutachter:

1. PD Dr. Gerd Gleixner
2. Prof. Klaus Heide

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Ich sah manche Wahrheit siegen, aber stets durch die wohlwollende Unterstützung von hundert Irrtümern

<<NIETZSCHE>>

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Diese Arbeit umfasst 108 Seite, 42 Abbildungen, 13 Tabellen, 109 Literaturangaben und einen Anhang mit 50 Seiten

Marcus Wengel

Max-Planck-Institut für Biogeochemie Jena und
Friedrich-Schiller-Universität Jena,
Chemisch-Geowissenschaftliche Fakultät

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Ausführliche Zusammenfassung in deutscher Sprache

Gelöster organischer Kohlenstoff (DOC) ist allgegenwärtig in terrestrischen und aquatischen Systemen. Neben der direkten Beeinflussung unserer Umwelt durch eine Vielzahl organischer Substanzen hat DOC ebenfalls einen starken indirekten Einfluss auf Vorgänge in unserer Umwelt. Solche indirekten Einflüsse sind die Komplexierung von Schwermetallen und der Transport von Schadstoffen. Der Einfluss auf die Mobilität von Schwermetallen und weiteren Schadstoffen richtet sich nach den chemischen Eigenschaften der organischen Komponente, besonders nach den chemisch reaktiven Gruppen. Funktionelle Phenol-, Carboxyl- oder Hydroxid Gruppen sind neben Heteroatomen wie Stickstoff, Schwefel und Phosphor besonders für die Komplexbildung geeignet. Eine Zusammenlagerung von DOC besteht meist aus einer Vielzahl organischer Moleküle und enthält somit ebenfalls mehrere chemisch reaktive Gruppen. Folglich kann eine solche Zusammenlagerung eine Vielzahl von Schwermetallen in aquatischen Systemen binden und transportieren. Konzentration, Löslichkeit und Oxidationsstufe der Schwermetalle sowie Redox-Potential, pH-Wert, elektrische Leitfähigkeit und Temperatur der Gewässer sind neben den physikochemischen Eigenschaften des DOC stark beeinflussende Größen für die Mobilität organischer Schwermetallkomplexe. Erste Modelle zur Berechnung von Schwermetallkonzentrationen in organischen Komplexen sowie erste strukturelle Beschreibungen solcher Komplexe wurden bereits angefertigt. Solche Untersuchungen werden jedoch meist an definierten, vereinfachten und standardisierten Matrices unter Laborbedingungen durchgeführt.

Daher wurde in der vorliegenden Arbeit besonders darauf geachtet, analytische Verfahren und Methoden einzusetzen bzw. weiterzuentwickeln, welche die natürliche Matrix minimal beeinflussen und verändern. Die Arbeit beschäftigte sich hauptsächlich mit der Abtrennung und Charakterisierung organischer Schwermetallkomplexe sowie mit der Beschreibung von Wechselwirkungen zwischen gelösten organischen Komponenten und gelösten Schwermetallen in Oberflächengewässern. Um Eigenschaften wie DOC Konzentrationen, Schwermetallkonzentrationen, Herkunft der organischen Liganden sowie Mobilität der entstandenen organischen Schwermetallkomplexe in Oberflächengewässern divers untersuchen zu können, wurde ein Untersuchungsgebiet mit möglichst unterschiedlichen Oberflächengewässern gesucht. Ein solches Untersuchungsgebiet stellte das ehemalige Uranbergbaugebiet Ronneburg dar.

Quelle für saure Haldensickerwässer (AMD) ist die Nordhalde, auf der pyrithaltiges Gestein aus Untertagebau und Tagebau abgelagert wurde. Am Fuße der Nordhalde fließen der Badergraben und der Gessenbach. Aus der Stadt Ronneburg kommend und Haushaltsabwässern führend, fließt der Gessenbach ca. 1300 m entlang des Fußes der Nordhalde und dann weiter in Richtung Gera. Der Badergraben entspringt in der Gemeinde Kauern südwestlich der Nordhalde und enthält in seinem Oberlauf ebenfalls ungeklärte Haushaltsabwässer. In seinem weiteren Lauf nimmt der Badergraben zahlreiche diffus austretende saure Haldensickerwässer der Nordhalde auf und mündet nach ca. 1500 m in den Gessenbach. Das Auftreten von sauren Haldensickerwässern neben ungeklärten Haushaltsabwässern ermöglichte somit die Untersuchung von organischen Schwermetallverbindungen in Haushaltsabwässern mit erhöhten DOC- und niedrigen Schwermetallkonzentrationen. Weiterhin konnten Haldensickerwässern mit niedrigen DOC und erhöhten Schwermetallkonzentrationen sowie Mischwässer mit moderaten DOC- und Schwermetallkonzentrationen untersucht werden. Neben DOC, Eisen, Mangan, Nickel, Kupfer und Uran wurden ebenfalls Silizium, Chlorid und Sulfat Konzentrationen der Oberflächenwässern analysiert.

Durch Rohwasseruntersuchungen des Haushaltsabwassers, Haldensickerwassers und Mischwassers wurden verschiedene Mobilitäten der untersuchten Schwermetalle festgestellt. Hierbei wurden Eisen und Mangan als wenig mobil charakterisiert, wohingegen Nickel und Kupfer aufgrund ihrer besseren Löslichkeiten als mobilere Schwermetalle charakterisiert wurden. Die präparative Aufkonzentrierung der Proben mit Hilfe einer Nanofiltrationsanlage führte zur selektiven Anreicherung der organischen Schwermetallkomplexe. Hierbei wurden 50 l Rohwasserproben auf etwa 6 l aufkonzentriert. Schwierigkeiten bei der Anreicherung bereiteten Schwermetallausfällungen in den Haldensickerwässern aufgrund hoher Eisenkonzentrationen. Diese setzten teilweise die Nanofiltrationsmembran zu und verhinderten somit die ausreichende Abtrennung anorganischer Spezies.

Organische Schwermetallkomplexe aus den angereicherten Proben wurden anschließend durch Hochleistungsgrößenausschlußchromatographie (HPSEC) isoliert. Die Proben wurden nach der Trennung auf einer Gelsäule entsprechend ihren Molekülgrößen in 11 Fraktionen geteilt. Als mobile Phase wurde deionisiertes Wasser eingesetzt. Auf herkömmliche, der mobilen Phase zugesetzte Additive wie Phosphatpuffer oder EDTA-Salze wurde verzichtet, da diese unerwünschte Fällungsreaktionen und Komplexierungen hervorgerufen hätten. Zur Detektion der organischen Schwermetallkomplexe sowie zur

Validierung der Methode wurden UV Spektren im Bereich 190 nm bis 350 nm verwendet. Anorganische Standardlösungen wurden bei einer Wellenlänge von 200 nm bzw. 220 nm detektiert wohingegen organische Standards zusätzlich bei 254 nm detektiert wurden. Natürliche organische Schwermetallkomplexe wurden hauptsächlich bei der Wellenlänge 254 nm detektiert. Entsprechend ihrer Retentionszeit wurden charakteristische Chromatogramme und korrespondierende Schwermetallkonzentrationen der organischen Komponente aufgetragen. Neben erhöhten Schwermetall- und DOC Konzentrationen wurden in isolierten Fraktionen ebenfalls Silizium, Chlorid und Sulfat gefunden. Die hauptsächlich organische Schwermetallkomplexe enthaltenden Fraktionen wurden anschließend gefriergetrocknet. Die Gefriertrocknung ermöglichte eine Stabilisierung der Komplexe und stellte festes Probenmaterial für weitere Untersuchungen zur Verfügung.

Weitere Charakterisierungen der gefriergetrockneten Proben fand einerseits durch massenspektrometrische Untersuchungen und andererseits durch $^{12}C/^{13}C$ Isotopenverhältnisuntersuchungen statt. Die volatilen Bestandteile der Probe wurden mit Hilfe eines Curie-Punkt Pyrolysators freigesetzt und durch einen Gas-Chromatographen in einzelne Komponenten aufgetrennt. Nach der Trennung wurden 10% des Eluates im Massenspektrometer identifiziert und das restliche Eluat wurde nach on-line Oxidation dem Isotopenverhältnis-Massenspektrometer zugeführt um dort die $^{12}C/^{13}C$ Isotopie einzelner Komponenten zu bestimmen. Ziel dieser Methode war es, die in den Gewässern gefundenen gelösten organischen Substanzen mit Hilfe ihrer isotopischen und massenspektrometrischen Signale bestimmten Quellen zuzuordnen und ihre Strukturen und Mobilität zu untersuchen. Diese Untersuchungen zeigten, dass organische Schwermetallkomplexe in Haushaltsabwässern sich durch eine hohe Variabilität der organischen Liganden auszeichnen. Diese Vielfalt an organischem Material spiegelte sich in den Mischwasserproben wieder. Organische Schwermetallkomplexe aus sauren Haldensickerwässern wurden als äußerst stabil und mobil charakterisiert. Diese Verbindungen wurden neben den Haldensickerwässern in allen untersuchten Mischwasserproben flussabwärts wieder gefunden. Pyrolysebruchstücke in Haushaltsabwässern und Mischwasserproben konnten DOC Komponenten aus Abwässern oder Kläranlagen zugeordnet werden. Anorganische Salze in den gefriergetrockneten Haldensickerwässern erschwerten die Pyrolyse und verhinderten eine umfassende Charakterisierung organischer Komponenten.

Als potentielle Quelle für DOC in Haldensickerwässern wurde Schwarzschiefer des Haldenmaterials identifiziert. Ein Laborexperiment konnte den biogenen Abbau von Schwarzschiefer nachweisen. Dieser Abbau von festem organischem Haldenmaterial ging einher mit der Freisetzung von Schwermetallen. Folglich konnten gelöste organische Schwermetallkomplexe als Abbauprodukte identifiziert werden.

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Abstract

In this thesis attempts were made to investigate dissolved organic carbon (DOC) and coexisting heavy metals in an area highly polluted with organic waste from municipal sewage and heavy metals from acid mine drainage. An earlier established method was applied for sampling and separation of dissolved organic carbon and heavy metals from thesepolluted surface waters (Wengel 2001). This method was validated and further developed. The improved method was finally applied to isolate dissolved organic carbon and heavy metals from surface waters like acid mine drainage (AMD) and municipal waste water (MWW). Isolated organic heavy metal complexes were further characterized. In the former uranium mining area of Ronneburg remaining spoil piles were sources of acid mine drainage. These acidic waters hold elevated concentrations of heavy metals and low concentrations of dissolved organic carbon. The study focused on the heavy metals iron, manganese, nickel, copper and uranium as these heavy metals appeared in elevated concentrations and are environmentally significant. Additional to heavy metals, silicon, chloride and sulfate concentrations were determined. The acid mine drainage entered the creeks Gessenbach and Badergraben which consisted mainly of untreated house hold sewages from the surrounding villages Ronneburg and Kauern. The two creeks passed the spoil pile before their unification. These surface waters hold low concentrations of heavy metals and elevated concentrations of dissolved organic carbon. Dissolved components smaller than 450 nm and larger than 1 nm were concentrated by nanofiltration. The nanofiltration procedure enriched organic heavy metal complexes by simultaneous separation of inorganic salts. Organic heavy metal complexes were isolated from this solution by high performance size exclusion chromatography (HPSEC). Different size classes of molecules were collected from the column effluent, depending on their elution time, and were characterized by UV spectroscopy, inductively coupled plasma-optical emission spectrometry (ICP-OES) and dissolved organic carbon measurements. Identified organic heavy metal complexes were isolated and freeze dried to allow further investigations on constant sample material.

Freeze dried samples were separated by pyrolysis-gas chromatography (Py-GC) and characterized by atomic emission spectroscopy (AES), mass spectrometry (MS) and isotopic ratio mass spectrometry (IRMS). All analytical investigations were validated by standard samples. Therefore significant different physicochemical conditions between acid mine drainage and municipal waste water samples were simulated by investigating weak and strong organic heavy metal complexes under changing pH values and inorganic salt concentrations.

Different affinities of heavy metals to different size classes of dissolved organic carbon were found in separated organic heavy metal fractions. This affinity of the heavy metals differed with size and origin of the dissolved organic carbon. Municipal waste water samples hold higher concentrations of dissolved organic carbon than acid mine drainage samples and resulted therefore in a broader range of potential ligands for heavy metals. This was mirrored in UV investigations as a broad range of weak and strong organic heavy metal complexes in municipal waste water and mixed samples were detected. UV investigations further resulted in the detection of similar organic heavy metal species in acid mine drainage samples and in all downstream samples. These complexes could only partly be characterized by pyrolitical investigations because of the difficult matrix of organic heavy metal fractions. Traces of silicon in organic heavy metal fractions and corresponding silicon detections in mass spectrometry gave first advice on dissolved compartments of biofilms and on amorphous silica which may have an affinity to heavy metals and could therefore influence the mobility of heavy metals in surface waters.

It was found that heavy metals iron and manganese were less mobile than the heavy metals nickel and copper because of different redox potential and solubility in this specific system. Changing biological and physicochemical parameters of the surface waters influenced the mobility of heavy metals and organic heavy metal complexes significant.

Finally, mobility of heavy metals in the investigated surface waters depended first on biogenic and physicochemical conditions which mainly influence the appearance and concentration of the ligand dissolved organic carbon. Second the mobility of heavy metals was strongly influenced by the composition of the organic ligands. Sources of organic ligands from surface waters were investigated in a laboratory study. This study focused on the biological degradation of solid organic matter from spoil pile material. It was found that biological degradation of grained black shale by the wood-rotting fungus *Schizophyllum commune* was accompanied by the release of heavy metals. Therefore biological degradation of spoil pile material into dissolved organic carbon most likely results in the formation of organic heavy metal complexes which are available for aqueous paths in the environment.

1 Introduction

Dissolved organic carbon (DOC) is ubiquitous in the terrestric and aquatic environment and represents one of the largest active organic carbon reservoirs in the biosphere (Amon and Benner 1996; Hertkorn, Claus et al. 2002). As a vital recourse (Wetzel, Hatcher et al. 1995; Sun, Perdue et al. 1997) DOC affects the environment directly by increase or decrease of organisms or indirect by mechanisms like metal complexation and transport of contaminants (McIntyre, McRae et al. 2002; Han and Thompson 2003; McDonald, Bishop et al. 2004). DOC is operationally defined as the fraction of organic matter that passes through a 450 nm filter. The impact of DOC on complexation and transport mechanisms depends strongly on its chemical structure and the arrangement of the various functional groups (Schäfer, Mastrup et al. 2002). Branched and cyclic aliphatic structures, such as terpenoids and steroids, are resistant to aerobic degradation processes (Spitzy A. 1991). Highly substituted aromatic ring structures, which were found e.g. in tannin and lignin residues, are also resistant to biological degradation. Beside resistance of the chemical structure, functionality plays a major role in the formation of DOC complexes. Most important for complexation are heteroatoms like nitrogen, sulfur and phosphor additional to functional groups like phenolic and carboxylic or hydroxide groups (Antelo, Arce et al. 2000; Lu, X. Q., Hanna et al. 2000; Pompe, Schmeide et al. 2000; Davis, Volesky et al. 2003; Rose and Waite 2003). An agglomeration of DOC may contain several different heteroatoms and chemical reactive groups (Sigg and Stumm 1996; Piccolo 2001; Simpson, Kingery et al. 2002) and is therefore able to bind various heavy metals in stable aqueous complexes (Burba, Jakubowski et al. 2000; Burba, Beer et al. 2001). This has been demonstrated e.g. for iron, manganese, nickel, copper, uranium, cadmium or zinc (Sedlak, Phinney et al. 1997; Vogl and Heumann 1997; Bedsworth and Sedlak 1999; Gu and Chen 2003; Rose and Waite 2003). As DOC has a high potential to complex heavy metals it is an important carrier of heavy metals in surface water and also from surface water into the sediment (Ran, Fu et al. 2000; Croue, Benedetti et al. 2003; Parthasarathy, Pelletier et al. 2004). The formation of organic heavy metal complexes depends on the properties of the metal ion (Christensen, Jensen et al. 1996; Lu, Y. F. and Allen 2002) and on the composition and size of DOC (Christensen, Jensen et al. 1996; Kracht 2001; Martino, Turner et al. 2003). The stability of the complex depends on factors such as redox potential, pH value, concentration of the heavy metals, or competition effects between the heavy metals (Sternbeck, Sohlenius et al. 2000; Bryan, Tipping et al. 2002; Lu, Y. F. and Allen 2002; Twardowska and Kyziol 2003; Alvarez-Puebla, Valenzuela-Calahorro et al. 2004). First models of heavy metal concentrations in organic complexes exist (Gustafsson, Pechova et al. 2003) as well as first descriptions of the chemical structure (Christensen and Christensen 1999; Buerge-Weirich, Hari et al. 2002; Nachtegaal and Sparks 2003). However, most investigations were only possible by simplifying the natural matrix to laboratory conditions or by using artificial matrixes. These procedures included e.g. changes of DOC because of extraction or treatment with strong acids or bases. This study on natural field samples focused on a minimum of modification.

Most investigations on naturally occurring organic heavy metal compounds focused on only one aqueous system like bog waters, or waters from specific rivers or lakes. Very few studies were available of aqueous systems with significant different chemical and biological properties. Therefore this research was set up for two contrary waters of the same field site which were mixed and changed their physicochemical and biogenic composition frequently.

The former uranium mining area of Ronneburg hold spoil piles leaving acid mine drainage. DOC from acid mine drainage resulted most likely from weathered solid organic matter of black shales. Such weathering processes are known to release degraded organic matter as DOC in the environment (Schmidt 2005). In addition to abiotic degradation, microbial activity plays a major role in degradation processes (Robinson, Mansingh et al. 1999; Gadd 2000; Jenisch-Anton, Adam et al. 2000; Petsch, Eglinton et al. 2001; Petsch, Edwards et al. 2003). Therefore a further focus of this study was set on the biological degradation of such solid organic matter by the ubiquitous fungi *Schizophyllum commune*. As the degradation of organic matter from shales in natural systems is usually accompanied by the release of heavy metals (Littke, Klussmann et al. 1991) the resulting formation of organic heavy metal complexes was proved in a laboratory experiment (Wengel, Schmidt et al. 2005) which results are presented in this thesis.

Insufficient environmental protection during the mining period resulted in the entry of AMD into surface waters from creeks passing the spoil pile. These creeks consisted mainly of untreated household waters. Therefore the field site Gessental holds surface water samples of acid mine drainage, municipal waste water and mixed samples which were strongly influenced by each other. Organic heavy metals could be studied in the initial samples and in downstream samples varying in physicochemical and biogenic conditions. In contrast to other investigations which dealt with non streaming water bodies this investigation analyzed water samples from streaming water bodies. Therefore the research focused beside characterization of organic heavy metal compounds also on the mobility of these species. Mobility, stability and complexation behavior of naturally occurring dissolved organic heavy metal complexes cannot simply be simulated studying artificial samples (Van den Bergh, Jakubowski et al. 2001). As this study focused on natural field samples the complex matrices of the acid mine drainage and the municipal waste waters complicated the investigations. With the development of new analytical methods and the conformation of existing methods a minimum of modification of the natural matrix could be guaranteed during the preparative enrichment and fractionation process. Dissolved components smaller than 450 nm and larger than 1 nm resulting from municipal waste waters, AMD and mixed samples were concentrated by nanofiltration to obtain preparative analytical investigations. To ensure minimum modification of organic heavy metal complexes during isolation no additives to the mobile phase were used at high performance size exclusion chromatography (HPSEC) (De Nobili and Chen 1999; Wengel and Gleixner 2005). Different size classes of organic heavy metal complexes were collected from the column effluent, depending on its elution time. Characterizations of these different organic heavy metal agglomerations from MWW, AMD and mixed samples were done by on line UV spectroscopy, ICP-OES, ICP-MS and DOC measurements. Differences were mainly based on the concentration and variety of DOC ligands. Characteristic UV detection was applied at wavelengths between 190 nm and 400 nm where detections at the wavelength 254 nm indicated DOC which is able to complex heavy metals (Vogl and Heumann 1997; Specht and Frimmel 2000; Perminova, Frimmel et al. 2003). As the shape of organic heavy metal complexes is yet not clearly identified most investigations on molecular size used aliphatic molecules as standard solutions. In this study molecular weight approximations of the identified organic heavy metal complexes was done with straight-chain aliphatic and spherical organic standard material to determine an upper and a lower size edge (Wells, Koopal et al. 2000).

Minimum modification of the aqueous samples could only be realized by extensive validation of the method. Therefore standard solutions holding organic heavy metal complexes under different physicochemical conditions were inserted to validate the concentration procedure and the HPSEC-UV investigations (Berthon 1995). Physico chemical conditions were selected with focus on naturally occurring parameters like pH value, salinity and complex stability (Herrero, Barrado et al. 2000; Collins, Onisko et al.

2001; Wu and Tanoue 2001). A further key aspect of minimum modification was fast preparative work. Fast isolation and separation could assure the rapid transfer of the analyte into a stable composition. This stable composition was achieved by freeze drying. Beside inorganic heavy metal salts and dissolved organic carbon low to moderate concentrations of silicon were analyzed in the municipal waste water and moderate to elevated concentrations in the acid mine drainage (Geletneky 2002). 25% of the mass of the earth crust consists of silicon mostly as inorganic salt of silicic acid (silicates) (Hollemann and Wiberg 1995). In the aqueous phase predominantly silicic acid or derivatives of silicic acid occur. These amorphous, dissolved silicates result mainly of degradation processes of solid silicates (Hollemann and Wiberg 1995). In addition to these inorganic species, dissolved silicon organic materials in aqueous systems are known (Varaprath and Cao 2000; Dixit and Van Cappellen 2002; Michalopoulos and Aller 2004). Laboratory experiments proved the adsorption of DOC and heavy metals on such dissolved silicon organic materials (Cazacu, Marcu et al. 2004; Czerwinski, Ostrowska-Gumkowska et al. 2004). Latest investigations concerned with chemical properties and surface characteristics of biogenic silica suggested the possibility of heavy metal transport by these species (Dixit and Van Cappellen 2002; Gendron-Badou, Coradin et al. 2003; Michalopoulos and Aller 2004). Therefore biogenic silica depends on inorganic and organic silica species in combination with DOC as the silica surface is covered with biofilms. As these biofilms hold excellent ligands for heavy metals like fatty acids and lipids these compounds are able to transport heavy metals (Hamers 1997). Based on this knowledge, the role of biogenic silica as compound of the organic heavy metal matrix was also considered in this study. Further characterization of organic heavy metal complexes was therefore achieved by gas chromatographic investigations. Freeze dried samples were analyzed by pyrolysis (Py)-gas chromatography (GC)-mass spectrometry (MS) and isotopic ratio mass spectrometry (IRMS) (Gleixner and Schmidt 1998; Schulten and Gleixner 1999; Kracht and Gleixner 2000; Olivella, del Rio et al. 2002).

The main objectives of the investigation can be summarized as follows:

- Investigations on representative raw water samples from surface waters;
- Characterization of molecular properties and specificities of organic ligands to heavy metals and other elements by HPSEC-UV, ICP-OES, ICP-MS, IC-UV and DOC measurements;

- Characterization of pyrolysis fragments from separated organic heavy metal fractions by mass spectrometry and isotopic carbon ratios mass spectrometry;
- Validation of the developed preparative analytical method by standard samples;
- Investigations on the biogenic degradation of solid organic carbon resulting in the release of organic heavy metal complexes in the aqueous environment

2 Materials and Methods

2.1 Characteristics of dissolved organic carbon (DOC) and important interactions of DOC with other natural occurring components

The term Dissolved Organic Carbon (DOC) is used to describe thousands of dissolved compounds found in aqueous systems that derive from organic materials. The heterogeneity of DOC results in a range of different properties. These facts make it difficult to assign a proper and common chemical definition or structure (Figure: 2.1).

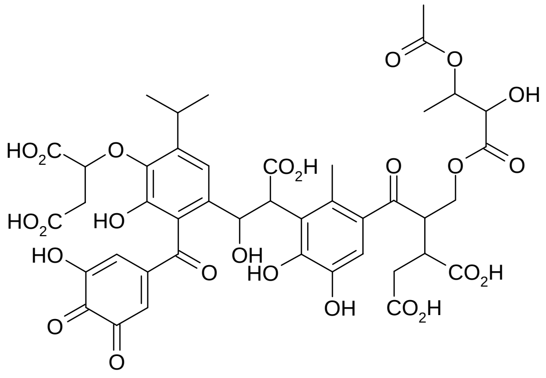
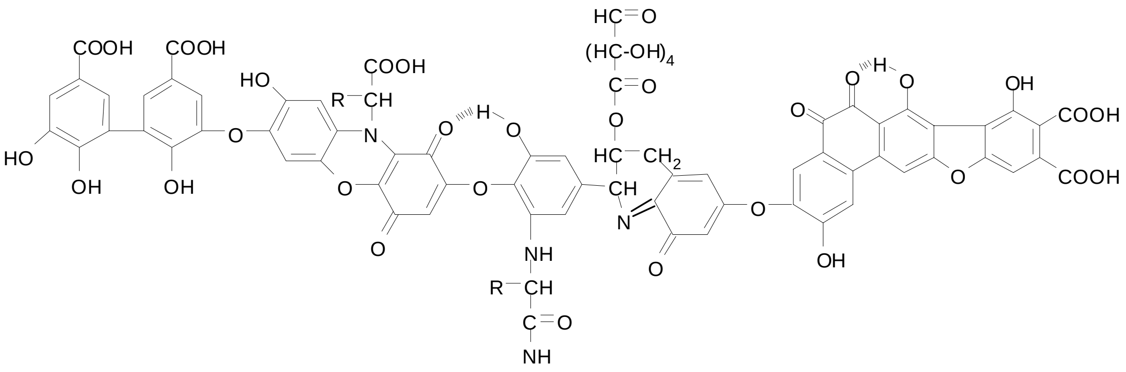


Figure 2.1: Hypothetical structures of subunits from dissolved organic carbon (DOC). Molecular structures present chemical active ether, aromatic, carboxylic and hydroxide groups as well as heteroatoms.

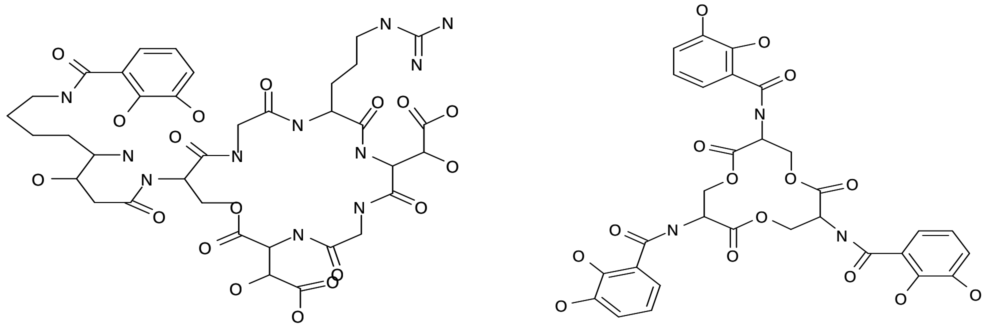
(MacCarthy 2001)



(Stevenson 1994)

A more constructive approach is to characterize chemical active parts of DOC depending on its effects in the environment. Such chemical active parts are e.g. long chain aliphates which were analyzed in DOC with high surface activity in contrast to DOC with low surface activity which was composed of aromatic rings substituted to a high content of carboxyl groups and a low content of ether groups (Piccolo 2001). Further main structural subunits of DOC may be fatty acids, peptides or sugars (Hayes 1998; Jandl, Schulten et al. 2002). The DOC structure affects the way aqueous ecosystems work as well as the bioavailability of organic substances in the environment (Piccolo 2001). Further investigations characterized DOC as agglomerations of instable polymers. Such a supra molecular association of relatively small heterogeneous molecules is held together by weak dispersive forces. The addition of organic acids alters such instable conformations through the formation of energy rich hydrogen bonds (Piccolo 2001). The variability of DOC composition and concentration in surface waters results from its sources, transport and reactivity. DOC can derive from terrestrial and aquatic environments as well as from anthropogenic sources such as agricultural or industrial activities (Sachse, Babenzien et al. 2001). Therefore DOC in aquatic systems is regionally and seasonally variable (Sachse, Babenzien et al. 2001). In the investigated field site a potential DOC source was refractory organic carbon from black shales. These shales were formed in an abiotic, geogenic long time process and are composed of clay minerals, quartz, sulfides and organic matter. This carbon source was part of the spoil pile material and its biological degradation was investigated in a laboratory study (Gatzweiler, Paul et al. 1997; Schmidt 2005; Wengel, Schmidt et al. 2005). The results proving the degradation of solid organic matter as dissolved organic heavy metal complexes are presented in this theses.

The complexing potential of DOC was confirmed by diverse studies which found that DOC acts as complexing agent to nutrients, metals and other organic substances such as pollutants (Sachse, Babenzien et al. 2001) because of its various heterogenic and chemical reactive sub units. Figure 2.2 gives a short overview of possible molecular subunits of DOC including heteroatoms which are able to complex heavy metals.



Alterobactin A ***Enterobactin***

Protoporphyrin ***Ethyleneteraamineacetic acid***

Figure 2.2: Possible DOC subunits which might act as organic ligand for heavy metals in aqueous systems. These compounds having a variety of functional groups that include carboxylic acids, amines, hydroxides and ring systems are known to form organic heavy metal complexes with high stability constants (Luther 2001).

Naturally occurring organic heavy metal complexes with DOC ligands were found to have complex stability constants (log k values) between 7 and 22 (Luther 2001; Wu and Tanoue 2001). Such DOC formations are mainly responsible for heavy metal complexes in surface waters and play a crucial role in the mobilization and transport of heavy metals in the aquatic environment (Wu and Tanoue 2001). Examples for ligands found in nature and having a high complexing potential are porphyrines and organic acids which mostly result from breakdown of solid terrestrial matter (Fitch 1994). This study found that DOC is only one part of organic heavy metal complexes found in surface waters. Nevertheless, with view to the mobility, structure and transport, highly biochemical active DOC is the major influencing compound of organic heavy metal complexes in surface waters. Beside DOC and the complexed heavy metals also inorganic salt compounds were analyzed in these agglomerations. Dissolved agglomerations analyzed in this investigation which hold stable and dominant organic ligands included also moderate concentrations of silicon. Such organic silicon compounds may play a role in heavy metal transport and mobility.

Dissolved agglomerations of organic ligands, biogenic silica and inorganic salts can not simply be classified as inorganic or organic species and are therefore often named different. In this study these compounds are named “organic heavy metal complexes”. An other name may be colloid (Kimball, Callender et al. 1995; Hofmann 2004) which is rarely defined as a compound with a size between 10^{-7} to 10^{-5} cm or 10^{3} to 10^{8} atoms (Falbe J. 1996-1999). As DOC is defined as compounds with a size smaller $4.5 \bullet 10^{-5}$ cm the name colloid may be correct. Colloidal systems may further be defined as a system containing at least two compounds: a continuous dispersing medium and a disperse phase. More detailed definitions focus on the chemistry like on methods of formation and stability of those substances that form colloid systems (Cross 1979). However, definitions and characterizations of colloids shall not be discussed in detail in this investigation. The question if the stability of such an agglomeration results from ionic bonds, van der waals forces, electrostatic forces or other adsorption forces is very difficult to answer. Therefore the name complex defined as a combination of molecules at the Römpp lexicon is also just an approximation. Nevertheless, the name dissolved organic heavy metal complexes reflected in this work mainly heavy metals bound to various kinds of DOC with traces of inorganic salts. Consequently the properties of the organic species dominated the properties of the complexes. Such properties are e.g. mobility, stability and size of the dissolved organic heavy metal complexes.

2.1 Introduction to the field site of Ronneburg

The sampling area was located in the former uranium mining area Ronneburg, Thuringia, Germany (Lange 1995). Research was achieved in the valley Gessental and on the spoil pile Nordhalde (Figure: 2.3).

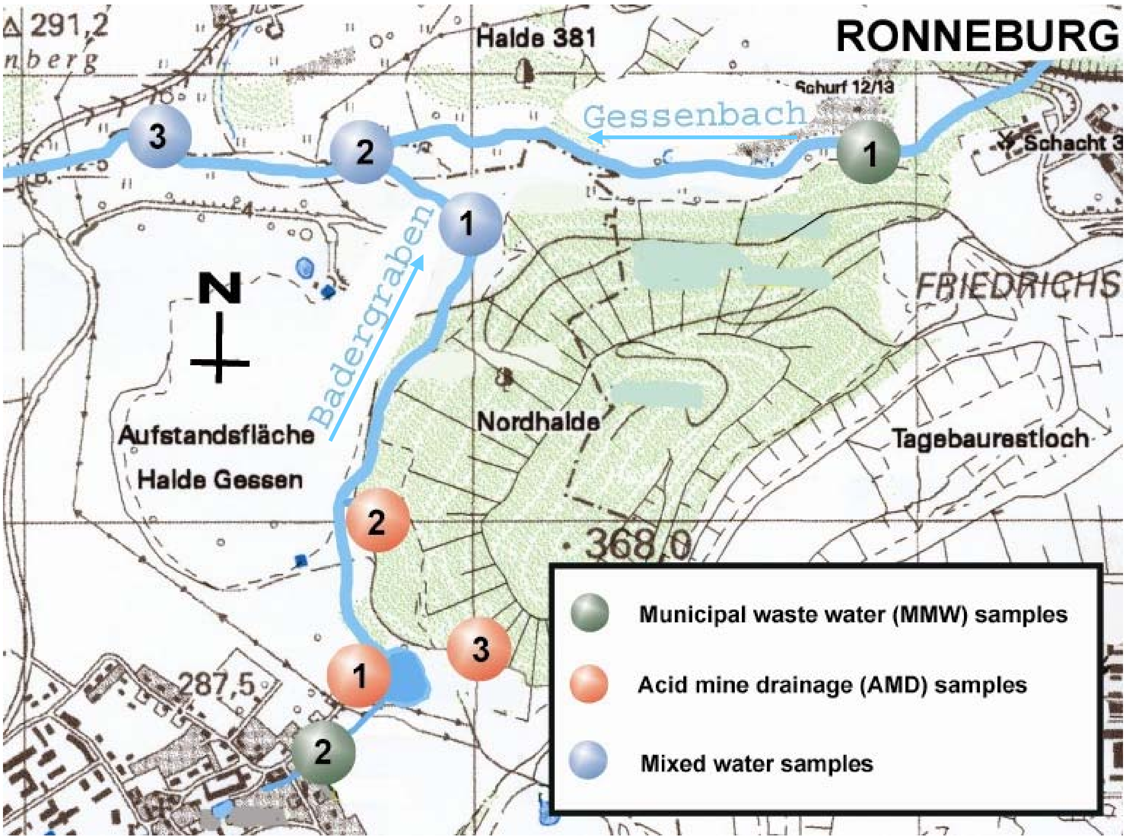


Figure 2.3: Map of the field site Ronneburg with the spoil pile Nordhalde, the creeks Badergraben and Gessenbach and the sampling points for municipal waste water, acid mine drainage and mixed samples

The spoil pile was passed by the two creeks Badergraben and Gessenbach which merged and continued as Gessenbach. The Badergraben contained mainly untreated municipal waste waters from the village Kauern. This small creek had, depending on seasonal and raining events a low to no flow. The creek Gessenbach contained untreated municipal waste water from the town Ronneburg. It had a higher flow than the creek Badergraben. The Badergraben passed the spoil pile closely where numerous diffuse runnels of AMD entered the creek. The spoil pile consisted mainly of black shale (appr. 68%) (Gatzweiler, Paul et al. 1997). A major compound of these black shales was pyrite causing acid mine drainage and corresponding release of dissolved heavy metals (Evangelou 1998). As shales are fine-grained sedimentary rocks it is further composed of clay minerals, quartz, sulfides, and a small percentage of organic matter (6% TOC). Most shales are deposited under marine environments, and derive organic matter principally from marine algae (Petsch, Berner et al. 2000). Thus the OM will comprise mainly long chain alky units (saturated hydrocarbons). These kerogenes are less soluble in water. Weathering of shales gets the organic matter fraction in the aqueous phase (Schmidt 2005). Biological degradation and therefore alteration of alkanes to carboxylic acids released them also in the aqueous phase. Therefore the municipal waste waters from the village Kauern got contaminated by AMD of the spoil pile. After passing the spoil pile the highly contaminated municipal waste water entered the less contaminated creek Gessenbach. More detailed information on the field site can be found elsewhere (Geletneky 2002).

This constellation of the spoil pile and the two creeks existed till 2001. Continuous removing of spoil pile material resulted during 2001/2002 in removing of numerous escapes of AMD. Most runnels of AMD were than merged in closed acid mine drainage collectors. Further environmental protection was done by separating the creek Badergraben from diffuse AMD runnels. For this purpose the creek bed was moved away from the spoil pile and a barrier consisting of gravel was built up. Therefore contamination of the Badergraben by AMD and heavy metals especially less mobile species like iron salts decreased strongly between 2001 and end 2002. The composition of the municipal waste waters changed only slightly during the sampling period 2001 till 2004 whereas the impact of the acid mine drainage to the creeks Gessenbach and Badergraben and therefore to the environment decreased significant.

Samples were taken from municipal waste water, acid mine drainage and mixed downstream waters (Figure: 2.3). Municipal waste water (MWW) 1 and 2, acid mine drainage (AMD) 1, 2, 3 and mixed samples (Mix) 1, 2 and 3 were shortly characterized as follows. More detailed characterization was given elsewhere (Wengel 2001; Geletneky 2002).

- MWW 1 (=B3 at Geletneky; =e-415 at Wismut corporation) is located at the creek Gessenbach south of the spoil pile Nordhalde. This water contained elevated DOC concentrations (up to 130 mg/l) resulting from municipal waste water of the town Ronneburg. Heavy metal concentrations are low because of less contamination of dissolved spoil pile material.
- MWW 2 (=G13 at Geletneky; = e-461 at Wismut corporation) is located at upper reaches of the creek Badergraben. These samples were characterized by elevated DOC concentrations (up to 185 mg/l). The location beside of the spoil pile resulted also in low contamination and therefore low heavy metal concentrations.

- AMD 1(= pond Pohlteich; =e-412 at Wismut corporation) is an acid mine drainage collector located between the village Kauern and the spoil pile. This water was characterized by low DOC concentrations (1.4 mg/l – 7.4 mg/l) and elevated heavy metal concentrations. Characteristic for these samples is its brown color resulting from iron and manganese precipitates.
- AMD 2 (= Q4 at Geletneky; e-443 at Wismut corporation; from 2002 = Sammelbecken 1 at Wismut corporation) was till 2002 a spring of diffuse runnels from the spoil pile. From 2002 to 2004 samples were taken from an AMD collector merging numerous AMD runnels. Low DOC concentrations and elevated heavy metal concentrations were also characteristic for AMD 2.
- AMD 3 (= e-353/4 at Wismut corporation) was mainly sampled for HPSEC investigations on raw water samples. This collector could not be sampled continuously because it was used as temporary storage for AMD and therefore not filled any time. Spot tests on DOC and heavy metal concentrations resulted in similar values like AMD 1 and AMD 2.
- Mix 1 (=G7 at Geletneky; =e-414 at Wismut corporation) was the first downstream sample containing municipal waste waters from the village Kauern and AMD from diffuse runnels. Up to 2001/2002 this brownish samples were also characterized by high iron precipitates. Further on these samples held moderate DOC concentrations and moderate heavy metal concentrations.
- Mix 2 (=G5 at Geletneky; =e-416 at Wismut corporation) was located further downstream after the creek Badergraben entered the creek Gessenbach. These samples held also moderate DOC and heavy metal concentrations.
- Mix 3 (=G6 at Geletneky) was the furthest downstream sample located on the creek Gessenbach. Moderate DOC and heavy metal concentrations were also characteristic for that sample.

2.2 Investigations of raw water samples

Sampling was achieved between April 2001 and January 2004 at sampling points MWW 1, MWW 2, AMD 1, AMD 2, Mix 1, Mix 2 and Mix 3 where Mix 3 was not sampled in 2001. Preparative investigations were focused on samples December 2001, July 2002 and June 2003. DOC measurements were performed with high TOC, (elementar analytic, Hanau, Germany). DOC concentrations were determined as total inorganic carbon (TIC) and non purge able organic carbon (NPOC) (Wengel 2001). Concentrations were analyzed at undiluted samples. Calibration between 0 mg/l and 500 mg/l was done with stock solutions holding 4412.2 mg/l sodium carbonate and 1260.7 mg/l trishydroxymethylaminomethan. External standard was Super-02 (National Water Research, Canada). Results were performed by double and triple determinations. Determination of the elements Fe, Mn, Ni, Cu and Si was done by ICP-OES (Optima 3300 DV, Perkin-Elmer, Wellesley, USA). AMD samples were diluted 1:10 where the MWW samples and the Mix samples were analyzed undiluted. Calibration between 0 mg/l and 2.5 mg/l was achieved with the multi element standard Super Certi Prep (Metuchen, USA). External standards were SPS-WW 1, SPS-WW 2 and SPS-SW 1 (Spectrapure, Oslo, Sweden). Concentrations were analyzed by triple determination at wavelengths 259.939 nm for iron, 257.610 for manganese, 231,604 for nickel, 324.752 nm for copper and 251.611 nm for silicon. Uranium concentrations were analyzed by ICP-MS (PQ3 S, ThermoElemental, Winsford, UK). Samples were diluted 1:10. Calibration was achieved between 0 mg/l and 2 mg/l with the multi element standard CLMS-2 (Spese, Grasbrunn, Germany). Internal standards were rhenium in 5% nitric acid (Merck, Darmstadt, Germany) and ruthenium as ruthenium(III)chloride in 8% hydrochloric acid (Merck, Darmstadt, Germany). Concentrations were analyzed by triple determination. Anions Cl^- and SO_4^{2-} were determined by ion chromatography with conductivity detection (Dionex 500 system, Idstein, Germany). The flow of the system was 1.2 ml/min by injection volumes of 25 µl. For separation a Ion Pac AS14 column was used. Eluent was an aqueous solution holding 3.5 mmol/l Na_2CO_3 and 1.0 mmol/l $NaHCO_3$ from Fluka. Calibration between 0.5 mg/l and 80 mg/l was done with Multielement Ion Chromatography Standard solution I and II and Nitrite Ion Standard solution (Fluka). Additional the standard QCP 51 from Dionex was used.

Beside laboratory analyses the physicochemical parameters electrical conductivity and pH value were measured on site (MULTI LINE F, WTW, Weilheim, Germany). Further on the water flux was measures volumetric. Therefore the water was dammed up and selective collected in 1.5 l and 10 l beakers (Geletneky 2002). The collection time was measured by stopwatch. The measurement was repeated three times. The minimization of the flux by water loss was estimated by 10%. Values for redox potential were inherited from Geletneky 2002. As this investigation focused mainly on analytical procedures than on monitoring aspects the reproducibility of the sampling and the sampling data needed to be guaranteed. Therefore chemical and physicochemical data achieved in sampling procedures of this study were compared with data from the Wismut database and data from Geletneky (Geletneky 2002). This comparison confirmed the reproducibility of the sampling data (Figures: 3.1-3.4).

2.3 Prefiltration and nanofiltration of raw water samples and standard solutions

50 l water of each sample was prefiltered to 0.45 µm and filled into stainless steel barrels (BLEFA, Kreuztal, Germany). The prefiltration system combined four filter units of 50 µm, 20 µm, 0.9 µm and 0.45 µm. The 50 µm and the 20 µm units were stainless steel filters (BRAUKMANN, Germany), whereas the 0.9 µm and 0.45 µm unit were polyethylene filters (AQUA FLOW, Pall Corporation, Dreieich, Germany). The 50 l barrels were completely filled with prefiltered water in order to avoid oxidation processes. After each prefiltration the system was washed for 30 min with 0.005 mol/l HCl in order to prohibit heavy metal contamination from stainless steel. Heavy metal contamination was also controlled by ICP-OES measurements of the raw samples. Control samples were transported in PET bottles. There was no significant difference in heavy metal concentration of the samples transported in stainless steel barrels and the samples transported in PET bottles.

To prohibit oxidation processes and precipitation of oxides in the AMD sample, the field samples were concentrated immediately after prefiltration. The nanofiltration system was a cross flow system (WERNER, Leverkusen, Germany). Continuous recirculation of the concentrate and purging of the filtrate resulted in the concentration of the samples. The prefiltered samples were nanofiltrated at a maximum pressure of 8 bar. Two NF 50 membrane filters (WERNER, Leverkusen, Germany) with an active area of 2.1 m^2 and a pore size of approximately 1 nm were used for the concentration. The generation of a filter cake was prevented by a 3:1 ratio of the concentrate flow parallel to the membrane and the filtrate flow orthogonal to the membrane. Conductivity was detected continuously in the filtrate and in the concentrate. The complete nanofiltration system was cleaned after each sample. The cleaning procedure included 15 min flushing with 0.005 molar HNO_3, 15 min flushing with 0.005 molar HCl, 15 min flushing with 0.005 molar NaOH and finally 30 min flushing with deionized water.

The retention capacity of the nanofiltration membrane was determined concentrating 20 l of $3.8 * 10^{-4}$ molar Ni-EDTA solution, 20 l of a solution containing $3.0 * 10^{-5}$ mol/l

$Ni(NO_3)_2$•6 H_2O and 30 mg/l humic acid (Fluka) as well as 50 l of the alkali- and earth alkali salt solutions containing 200 mg/l $CaCl_2$•2 H_2O, 280 mg/l $MgCl_2$•6 H_2O, 882 mg/l Na_2SO_4, 50 mg/l Na_2CO_3 and 80 mg/l NaCl respectively. The dependency of molecular size of the ions on their loss was specified by calculating the water loss [vol%] and the ion recovery [%].

A second concentration procedure focused more on the retention capacity of weak and strong organic heavy metal complexes and on aqueous complexes overlapped with corresponding inorganic salts. Therefore 50 l samples of EDTA-Ni, citric acid monohydrate-copper (Cit-Cu), citric acid monohydrate-iron (Cit-Fe), desferrioxamine (DFOM)-Fe and a mixed sample were concentrated to final volumes of 8 l. Organic ligands had DOC concentrations about roughly 20 mg/l. Equimolar complexes were synthesized from 53.4 mg/l citric acid anhydrous and 45.0 mg/l $FeCl_3$, 45.1 mg/l citric acid monohydrate and 69.8 mg/l $CuSO_4 \cdot 5\ H_20$, 42 mg/l Desferrioxamine (DFOM) and 10.8 mg/l $FeCl_3$ and from 61.8 mg/l Na_2-EDTA and 48.32 mg/l $Ni(NO_3)_2$•6 H_2O. Additional a mixed sample was synthesized by unifying similar concentrations of Cit-Fe, Cit-Cu and DFOM-Fe. EDTA-Ni complexes with ligand: donor ratios 1:2 and 1:3 were synthesized using 61.8 mg/ Na_2-EDTA with 96.6 mg/l $Ni(NO_3)_2$•6 H_2O and 144.8 mg/l $Ni(NO_3)_2$•6 H_2O respectively. A 1:3 Cit-Cu complex was synthesized with 45.1 mg/l citric acid anhydrous and 209.4 mg/l $CuSO_4 \cdot 5\ H_20$. Concentrations of DOC and heavy metals from all samples before and after the concentration process were analyzed by ICP-OES and DOC measurements for mass balances. Additional investigations on the complexation behavior were achieved by HPSEC-UV from Cit-Cu, Cit-Fe, DFOM-Fe and the mixed samples before and after the concentration.

2.4 High Performance Size Exclusion Chromatography with UV detection (HPSEC-UV) of concentrated field samples and standard solutions

2.4.1 Set up of the HPSEC-UV system

The SEC system consisted of a pump (P580, DIONEX), an auto sampler (ASI 100, DIONEX), a column oven (STH 585, DIONEX), a guard column (TSK-GEL PWKL, TOSO HAAS), a column (TSK-GEL PW 3000 XL, TOSO HAAS), an UV-VIS detector (PDA-100, DIONEX) and a multi valve (CSD 12 UW, VALCO VICI) to collect fractions after given elution times. Deionized water (Milli Q gradient A10, Millipore) was pumped with a constant flow of 0.6 ml/min. Blank runs accompanied with purging of the syringe were processed all 5 injections. After each sequence the whole system was cleaned by flushing it with 0.01 molar HNO_3 for 1 hour followed by deionized water for 1 hour and H_3SO_4 for another hour. After final flushing by deionized water for 1 hour the next sequence was run. The column oven was held at 20 °C. The exclusion limit and total permeation limit of the column were determined injecting 10 µl and 250 µl of polyethyleneoxide solution ($7.7 * 10^{-7}$ g/mol; $M=9.2 * 10^5$ g/mol) and 10 µl and 250 µl of 0.01 molar NaCl and $Ni(NO_3)_2$•6 H_2O solutions (Wengel 2001). These limits were independent from the injected amount and volume of the salts. The detector effluent was collected as a function of the elution time between the exclusion limit and total permeation limit. A multi port valve was able to separate 11 fractions into 50 ml glass tubes. To collect 11 fractions of 50 ml, approximately 100 runs of 20 minutes each were needed. Chromatograms at the wavelength 200 nm were used to analyze standard components whereas the wavelength 254 nm was mainly used to determine organic heavy metal complexes in the field samples. Additionally, UV-spectra were recorded continuously at wavelengths between 190 nm and 400 nm.

2.4.2 Validation of the HPSEC-UV system by standard complexes

First impressions of the separation of organic heavy metal complexes by HPSEC were achieved by analyzing a synthetic nickel (Ni)-humic acid (HA) complex. The HA was kindly provided from the Forschungszentrum Rossendorf (FZR). 15 mg/l HA and 23.4 mg/l $Ni(NO_3)_2$•6 H_2O were mixed for 48 hours. The solution with a pH value of 6 was filtered to 450 nm. An additional Ni-HA solution was adjusted to pH 3 and also filtered to 450 nm. The injection volume of both samples was 50 µl and 250 µl which resulted in equal chromatograms.

To simulate the influence of different physicochemical parameters on the stationary phase of the column 22 synthetic standardized organic heavy metal complexes were analyzed (Table: 2.1). The complexes can be classified in weak complexes (citric acid (cit)-Ni, citric acid (cit)-Cu) and strong complexes (protoporphyrine-Fe, protoporphyrine-Ni, desferrioxamine-Ni, desferrioxamine-Cu, apoferritine-Fe, apoferritine-Ni, EDTA-Ni, EDTA-Cu and EDTA-Fe). These 1:1 complexes were overlapped with 30% donor element to ensure total complexation of the organic ligand. Additionally standard complexes were synthesized by overlapping the 1:1 complex by its inorganic donor atom (EDTA-Ni 1:2, EDTA-Ni 1:3 and EDTA-Cu 1:3). These solutions simulated field samples with elevated inorganic salt concentrations. Further on different pH values of field samples were simulated by standard compounds (EDTA-Ni pH 2, EDTA-Ni pH 5, EDTA-Ni pH 8, EDTA-Cu pH 3, EDTA-Cu pH 6, EDTA-Fe pH 3 and EDTA-Fe pH 6). Initial concentrations and educts of the 22 standards are given in table 2.1. Injection volumes of all samples were 50 µl and 250 µl which resulted in similar UV traces.

Table 2.1: Concentrations of initial compounds for the preparation of 22 weak and strong organic heavy metal standard complexes. Additionally, specific acids and bases for with pH adjustment are given.

final complex		initial compounds				acid/base
	stab. const. [log k]	Ligand	[mg/l]	Donor	[mg/l]	for pH adjustment
Prot-Fe	22	Disodium Protoporphyrine IX	22.2	$FeCl_3$	10.6	
Prot-Ni	n.k.	Disodium Protoporphyrine IX	22.2	$Ni(NO_3)_2 \bullet 6\ H_2O$	18.8	
DFOM-Ni	n.k.	Desferrioxamine Mesylate	42.0	$Ni(NO_3)_2 \bullet 6\ H_2O$	18.6	
DFOM-Cu	n.k.	Desferrioxamine Mesylate	42.0	$CuSO_4 \bullet 5\ H_2O$	15.8	
Apo-Ni	n.k.	Apoferritine	1.5	$Ni(NO_3)_2 \bullet 6\ H_2O$	1.0	
Apo-Fe	22	Apoferritine	30.0	$FeCl_3$	20.0	
EDTA-Ni pH 2	32	Disodium EDTA	61.8	$Ni(NO_3)_2 \bullet 6\ H_2O$	64.7	HNO_3
EDTA-Ni pH 5	25	Disodium EDTA	61.8	$Ni(NO_3)_2 \bullet 6\ H_2O$	64.7	
EDTA-Ni pH 8	21	Disodium EDTA	61.8	$Ni(NO_3)_2 \bullet 6\ H_2O$	64.7	NaOH
EDTA-Ni (1:1)	25	Disodium EDTA	61.8	$Ni(NO_3)_2 \bullet 6\ H_2O$	48.0	
EDTA-Ni (1:2)	25	Disodium EDTA	61.8	$Ni(NO_3)_2 \bullet 6\ H_2O$	96.0	
EDTA-Ni (1:3)	25	Disodium EDTA	61.8	$Ni(NO_3)_2 \bullet 6\ H_2O$	144.2	
EDTA-Cu pH 3	29	Disodium EDTA	61.8	$CuSO_4 \bullet 5\ H_2O$	55.6	
EDTA-Cu pH 6	24	Disodium EDTA	61.8	$CuSO_4 \bullet 5\ H_2O$	55.6	NaOH
Cit-Cu (1:1)	25	Disodium EDTA	61.8	$CuSO_4 \bullet 5\ H_2O$	55.6	
Cit-Cu (1:3)	25	Disodium EDTA	61.8	$CuSO_4 \bullet 5\ H_2O$	55.6	
EDTA-Fe pH 3	36	Disodium EDTA	61.8	$FeCl_3$	36.0	
EDTA-Fe pH 6	30	Disodium EDTA	61.8	$FeCl_3$	36.0	NaOH
Cit-Cu pH 2	9	Citric acid Monohydrate	53.4	$CuSO_4 \bullet 5\ H_2O$	69.8	H_2SO_4
Cit-Cu pH 5	6	Citric acid Monohydrate	53.4	$CuSO_4 \bullet 5\ H_2O$	69.8	NaOH
Cit-Cu pH 8	5	Citric acid Monohydrate	53.4	$CuSO_4 \bullet 5\ H_2O$	69.8	NaOH
Cit-Ni	6	Citric acid Monohydrate	53.4	$Ni(NO_3)_2 \bullet 6\ H_2O$	81.2	

n.k. = not known

composition by DOC measurement, ICP-OES, ICP-MS and IC-CD. The resulting common maxima of UV chromatograms and heavy metal concentrations evidenced the detection of organic heavy metal complexes. After initial analyses of organic heavy metal complexes a second fractionation resulted in separation of approximately 300 ml – 400 ml of the dissolved complexes. Subsequently all liquid fractions were freeze dried (Christ Gamma 1-20, Osterode, Germany).

2.5 Pyrolysis-GC-AED analyses on freeze dried fractions

Freeze dried samples were analyzed on its contents of carbon, oxygen, nitrogen, sulfur and silicon by pyrolysis-gas chromatography with atomic emission detection (Py-GC-AED). Dry samples of weights between 5.9 mg/l and 6.3 mg/l were transferred into a nickel alloy beaker for pyrolyses. The ferromagnetic beaker was inserted into a glass liner of the Curie-point pyrolyzer (Fischer 0316, Meckenheim, Germany). Samples were flushed with helium for 30 seconds to remove oxygen. The used alloy had a Curie-point of 500°C which was reached by magnetic fluxes induced into the conductor surrounding the alloy beaker. The Curie-point was hold for 9.9 s before the volatile pyrolysis products were transferred to the injection chamber of the GC. Attention was given to avoid contamination by left over sample material from one injection to the next. Therefore the sample beaker was positioned into an additional quartz glass liner which collected pyrolysis precipitates. This glass liner was renewed for each sample. The sample beaker and the injection needle were cleaned after each run by washing with a mixture of chloroform and methanol (1:1) in an ultrasonic bath. The sample beaker was cleaned of residues in a base solution in the ultrasonic bath. Weighing before and after analysis confirmed proper removal of the residue.

Pyrolysis products were separated by gas chromatography (GC) (Hewlett Packard 5890, Waldbronn, Germany). The needle of the pyrolysis chamber was inserted into a glass liner of the injection chamber of the GC. The injector temperature of the GC system was set to 280°C. To get enough volatile compounds on the GC column a splitless method was used. The helium gas flow was 2.0 ml/min. A 60 m long BPX5 (5% phenyl polysilphenylene-siloxane) (SGE, Weiterstadt, Germany) column with a film thickness of 1 µm and an inner diameter of 0.32 µm was used for separation. A 1 m retention gap was inserted between injector and GC column. An initial oven temperature of 50°C was hold for 5 minutes followed by a heating rate of 15°C/min up to a final temperature of 320°C which was hold for 12 minutes. The BPX5 column used for this investigation is a durable multi purpose column that is suitable for trace analysis of multiple substances, polar and non-polar. A major characteristic of the column is its high operating temperature of up to 370°C.

The GC system was online coupled to an atomic emission detector (Hewlett Packard AED G2350 A) where traces of carbon were detected at wavelengths of 193 nm and 179 nm, traces of sulfur at 181 nm, nitrogen 174 nm, oxygen 171 nm and traces of silicon at 252 nm. Well characterized cellulose standards were injected after each 6th sample to ensure the cleanliness of the system and reproducibility of the method (Appendix: II Figure: II-18).

2.6 Pyrolyses-GC/MS-IRMS investigations on freeze dried fractions

Py-GC-AED yield results of carbon, oxygen, nitrogen, sulfur and silicon content in the separated and freeze dried fractions from field samples. With mass spectrometry (MS) and isotopic ratio mass spectrometry (IRMS) pyrolysis products were characterized depending on its chemical structure. The method for pyrolysis and separation of pyrolysis products by gas chromatography in Py-GC/MS-IRMS was identical to Py-GC-AED. In this instrumental set up the effluent from GC was analyzed by two mass spectrometers. An ion trap mass spectrometer (Finnigan GCQ, Bremen , Germany) was used for the identification of pyrolysis products. Additionally an IRMS (DeltaPlusXL, Finnigan, Bremen, Germany) detected carbon isotopes for the calculation of δ^{13}C values of each compound. The GC column effluent was split where 1/9th was transferred into the ion trap and 8/9th into the IRMS (Figure: 2.4).

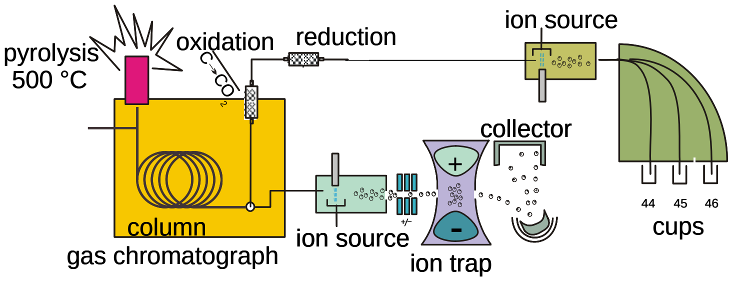


Figure 2.4: Scheme of the Py-GC/MS-IRMS system as it was set up for the present study.

The ion trap MS was connected to an X-piece of the GC by a heated transfer line. The temperature of the transfer line was set to 270°C to prohibit condensation of the volatile GC effluent. The separated pyrolysis fragments entered the ion source (temperature set to 180°C) and were ionized by positive-ion electron impact (EI) with 70 eV ionization energy. Mass spectra were detected by scanning masses from m/z 27 to m/z 500. The scan time was 0.5 sec/scan. Evaluation and interpretation of the mass spectra was done with Finnigan GCQ software and library data from spectral data base Wiley 6.0 and the data base from the National Institute of Standards and Technology. Consistency of carbon signals was proved by comparing chromatograms resulting from GC-MS with corresponding chromatograms from GC-AED.

In contrast to the ion trap MS, the IRMS was featured by simultaneous collection of relevant carbon ion beams in a multiple Faraday collector system. For measuring $\delta^{13}C$ values of the separated pyrolysis products, on line combustion of the GC effluent to CO_2 was necessary. Therefore the system was equipped with a GC-Combusting Interface III (Finnigan, Bremen, Germany). This unit ensured quantitative conversion of C to CO_2 without isotopic fractionation. The oxidation oven was set to 960°C and was equipped with the catalytic materials CuO, NiO and Pt. After combustion the CO_2 was transferred in the helium stream to the ion source. $^{13}C/^{12}C$ ratios were calculated by transforming pyrolyzed organic carbon into CO_2 which was detected depending on its m/z values. Detection was achieved of m/z values 44, 45 and 46 corresponding to $^{12}C^{16}O_2$, $^{13}C^{16}O_2$ and $^{12}C^{16}O^{18}O$. The proportion of $^{12}C^{17}O^{16}O$ (m/z 45) was corrected automatically after Craig (Craig 1957). For calculation and determination of $\delta^{13}C$ values ISODAT 2.0 software was used.

Samples were analyzed 3 to 5 times depending on the amount of sample material. Each run included a sequence of 2-3 reference gas peaks at the beginning and at the end of the analysis. The $\delta^{13}C$ value of the reference gas was -38.16‰. One of the reference gas peaks at the beginning and at the end was set on this value to enable automatic data calculation. Generally the isotopic ratio is expressed in δ-values. It was introduced for the first time by McKinney et al. 1950 (Craig 1957) as the deviation of the isotopic ratio relative to a standard. For carbon the international standard is based on V-PDB (Vienna-Pee Dee Belemnite $CaCO_3$). When R_{samp} is the isotopic ratio $^{13}C/^{12}C$ of a sample and R_{stand} is the $^{13}C/^{12}C$ ratio of PDB (=0.0112372) the $\delta^{13}C$ value of a sample can be calculated as followed:

$$\delta^{13}C\ [‰] = (R_{samp} - R_{stand}) / R_{stand} \times 1000$$

Drift correction was done by analyzing the external standard caffein before and after each series. Reproducibility of system conditions was assured by injecting cellulose each 6th run (Appendix I: Figure: I-19).

3 Results

3.1 Investigations on raw water samples

3.1.1 Chemical and physicochemical parameters of raw water samples

The spoil pile Nordhalde as source for elevated heavy metal concentrations and acid mine drainage was passed by the surface waters Gessenbach and Badergraben. The two creeks were strongly affected from untreated household sewage. For investigating DOC and heavy metal mobility as well as formation of organic heavy metal complexes 7 sampling points in the valley Gessental were sampled and analyzed from 2001 to 2004. As this field site unified municipal waste waters and acid mine drainage as well as mixed surface waters it was excellent appropriated for investigations on interactions of these oppositional surface waters. Predominant attention was given to dissolved organic carbon concentrations, concentrations of the heavy metals iron, manganese, nickel, copper and uranium. Further on concentrations of silicon, chloride and sulfate were determined. To prove the reproducibility of the sampling data of this study, comparison with data from the Wismut Corporation and the data set from Geletneky (Figure: 3.1-3.4 and Appendix II: Table II-1) was done. Beside, these parameters pH value and conductivity were measured.

Figures 3.1-3.4 confirmed the reproducibility of the analyzed data. Correlation of elevated DOC concentrations with low or moderate heavy metal concentrations on one hand and correlation of low DOC concentrations with moderate to high heavy metal concentrations on the other hand were characteristic properties for the investigated field samples. This resulted from the two contrary surface water sources municipal waste water (DOC between 7.5 mg/l and 130.0 mg/l, iron <0.9 mg/l, manganese <0.7 mg/l, nickel <1.9 mg/l, uranium <5.3 µg/l) and acid mine drainage (DOC <10.0 mg/l, iron <1663 mg/l, manganese <253 mg/l, nickel <48 mg/l, uranium <6.5 mg/l). Therefore nearly constant and low heavy metal concentrations characterized the municipal waste water whereas elevated heavy metal concentrations were characteristic for the AMD samples. Dominant heavy metal of the AMD was iron.

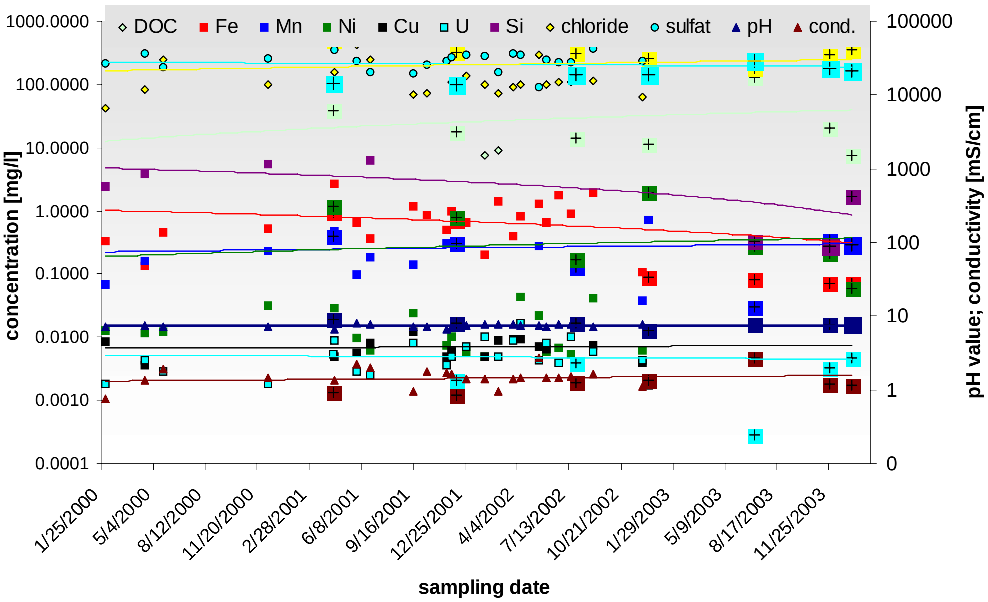


Figure 3.1: DOC, Fe, Mn, Ni, Cu, U, Si, chloride, sulfate concentrations, pH value and electrical conductivity from raw waters of sampling point municipal waste water 1 (MWW 1). Plotted samples were taken between the years 2000 and 2004. Data points labeled with "+" represent data of this study, other values were analyzed by the Wismut Corporation and Geletneky.

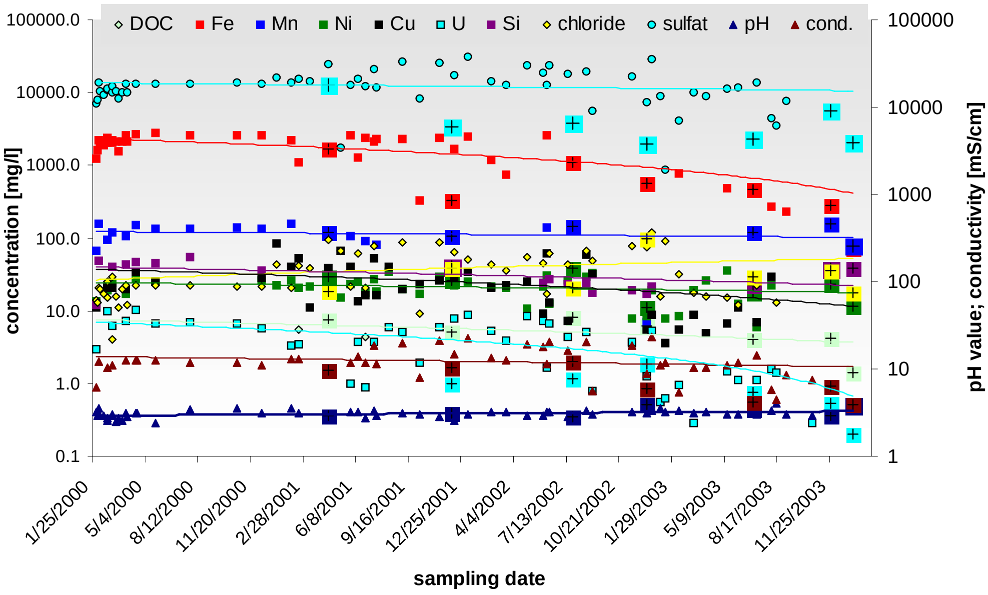


Figure 3.2: DOC, Fe, Mn, Ni, Cu, U, Si, chloride, sulfate concentrations, pH value and electrical conductivity from raw waters of sampling point acid mine drainage 2 (AMD 2). Plotted samples were taken between the years 2000 and 2004. Data points labeled with "+" represent data of this study, other values were analyzed by the Wismut Corporation and Geletneky.

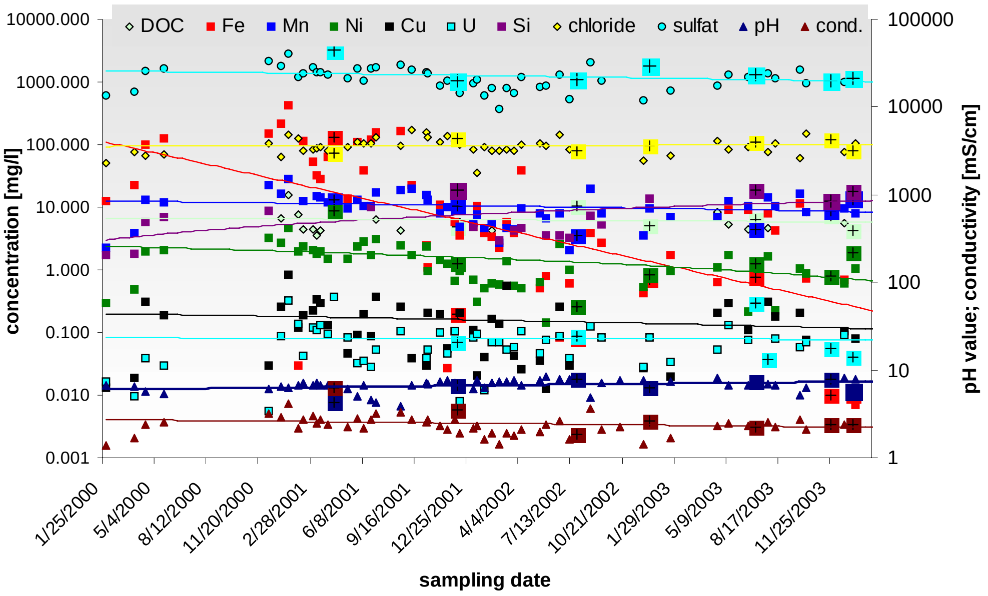


Figure 3.3: DOC, Fe, Mn, Ni, Cu, U, Si, chloride, sulfate concentrations, pH value and electrical conductivity from raw waters of sampling point Mix 1. Plotted samples were taken between the years 2000 and 2004. Data points labeled with "+" represent data of this study, other values were analyzed by the Wismut Corporation and Geletneky.

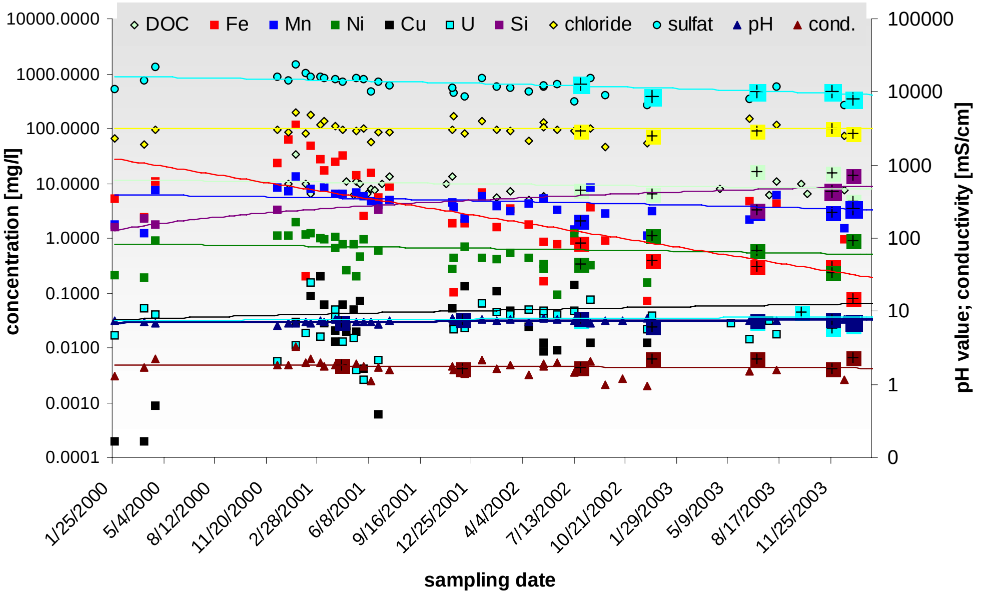


Figure 3.4: DOC, Fe, Mn, Ni, Cu, U, Si, chloride, sulfate concentrations, pH value and electrical conductivity from raw waters of sampling point Mix 2. Plotted samples were taken between the years 2000 and 2004. Data points labeled with "+" represent data of this study, other values were analyzed by the Wismut Corporation and Geletneky.

Significant lower concentrations were found for manganese, nickel, copper and uranium. Mixed downstream samples held lower heavy metal concentrations than AMD samples but significant higher concentrations than municipal waste water. In contrast to AMD samples where iron concentrations were always highest, the dominance in downstream samples changed from 2001 to 2004 from iron to manganese. Further on heavy metal concentrations in downstream samples were more similar. These close concentrations resulted from different mobility of the analyzed heavy metals in the mixed samples. The major influence on concentration is the distance of the heavy metals to their source. Therefore elevated concentrations of less mobile metals iron and manganese in AMD resulted in similar concentrations to more mobile metals nickel and copper in the downstream samples.

Highest silicon values were detected in acid mine drainage samples (Si <67 mg/l) (Appendix II: Table: II-1). Downstream samples hold lower silicon concentration (Si <19 mg/l) whereas lowest concentrations were measured in municipal waste waters (Si <2.5 mg/l). Highest concentrations of chloride were detected in MWW (chloride <511 mg/l) and lowest in AMD (chloride <98 mg/l) samples. Contrary ratios were found for sulfate where highest concentrations were found in AMD (sulfate <23 g/l) samples resulting from pyrite oxidation.

Municipal waste waters had pH values between 6.3 (December 2002) and 9.0 (April 2001) where AMD samples had pH values between 2.5 (December 2001) and 3.9 (December 2002) (Table: 3.1). Downstream samples showed pH values between 4.3 (April 2001) and 8.0 (July 2002). Conductivity in MWW samples differed between 0.8 mS/cm (December 2001) and 3.2 mS/cm (June 2003), in AMD samples between 4.2 mS/cm (June 2003) and 21.5 mS/cm (December 2002) and in downstream samples between 1.0 mS/cm (December 2002) and 6.4 mS/cm (April 2001) (Table: 3.1). By comparing these values with other data sets they can also be assumed as representative.

Municipal waste waters had slightly higher pH values than downstream samples where acid mine drainage samples showed significant lower pH values. After the displacement of the creek Badergraben lower concentrations of AMD were detected in the mixed downstream samples. This fact was mirrored by increasing pH values of downstream samples during April 2001 and July 2002 especially in sample Mix 1 as it had the strongest influence from AMD. Similar results were represented by the interpretation of conductivity values. MWW predominantly showed lower conductivity than mixed water samples whereas AMD samples holding higher ion concentrations showed higher conductivity. The displacement of the creek Badergraben resulted also in conductivity decrease in downstream samples especially in Mix 1. Further on, as activities to remove the spoil pile resulted in moving of AMD collector 1 conductivity of AMD 1 increased significant in December 2002.

Table 3.1: pH values and conductivity of municipal waste water, acid mine drainage and mixed samples from the Ronneburg field site. Samples were taken between April 2001 and January 2004.

pH value	MWW 1	MWW 2	AMD 1	AMD 2	Mix 1	Mix 2	Mix 3
Apr. 2001	9.0	7.8	2.9	2.8	4.3	7.0	n.a.
Dec. 2001	8.0	7.5	3.1	2.5	6.6	7.9	n.a.
Jul. 2002	8.1	7.4	2.8	2.9	7.9	8.0	7.7
Dec. 2002	6.4	6.3	3.9	2.7	6.3	6.1	7.1
Jun. 2003	7.7	7.6	3.4	2.8	7.4	7.5	7.7
Nov. 2003	7.7	7.6	2.9	3.0	8.0	7.8	7.6
Jan. 2004	7.6	6.9	3.8	3.6	5.7	7.2	6.9
Conductivity [mS/cm]							
Apr. 2001	0.9	1.7	9.6	18.9	6.4	1.8	n.a.
Dec. 2001	0.8	1.6	10.2	16.4	3.6	1.7	n.a.
Jul. 2002	1.3	1.6	12.2	11.3	1.9	1.7	2.1
Dec. 2002	1.3	1.3	5.9	21.5	2.6	2.2	1.0
Jun. 2003	2.7	3.2	4.2	19.8	2.2	2.3	1.5
Nov. 2003	1.2	1.6	6.3	17.8	2.4	1.6	1.7
Jan. 2004	1.2	1.8	3.9	18.4	2.4	2.3	1.9

n.a. – not analyzed

3.1.2 Heavy Metal concentrations downstream from AMD 2 to Mix 3

As the spoil pile was major source for heavy metals entering the surface water focus was set on changes in heavy metal concentrations of AMD 2 and the three downstream raw water samples. Comparison of relative intensities of the heavy metals iron, manganese, nickel, copper and uranium suggested differences in mobility and solubility of the heavy metals. Therefore absolute heavy metal concentrations of samples from summer 2001, 2002 and 2003 were converted into relative concentrations. With respect to different flows at the four sampling points (Table: 3.2) relative concentrations were converted into relative heavy metal fluxes (Figure: 3.5). As this investigation had not sufficient sampling date from summer 2001, for this period literature data was used (Geletneky 2002). Within the scope of anthropogenic remediation activities the creek bed of the Badergraben was displaced between sampling procedure 2001 and 2002. These activities caused lower contamination of the creek Badergraben by diffuse AMD runnels and therefore lower heavy metal mobility from AMD to the mixed samples. Especially iron mobility decreased significant.

Table 3.2: Flows of acid mine drainage and mixed samples in summer 2001, 2002 and 2003

flow [l/s]	AMD 1	Mix 1	Mix 2	Mix 3
June 2001	0.3	1.1	1.5	0.5
July 2002	0.2	0.6	0.9	0.7
June 2003	0.2	0.4	0.5	0.5

Interpretation of these relative fluxes given in figure 3.5 suggested less mobility of iron and manganese species compared to nickel and copper species. In 2001 before the displacement of the creek Badergraben relative iron concentrations decreased from AMD (100%) to Mix 1 only to 22% and further to 0.1% in Mix 2. In the following years maximum iron concentrations after the barrier were detected with only 0.4%. Therefore the displacement of the creek resulted in a barrier separating iron precipitates. This observance can be explained by the strong oxidizing conditions of the surface water. As strong oxidizing conditions from AMD to Mix 1 (Eh between 300 and 700 mV) shifted the equilibrium between Fe^{2+} and Fe^{3+} to the side of Fe^{3+}, precipitation of Fe^{3+} species resulted in high iron decrease between AMD and Mix 1. Less oxidizing conditions and higher pH values downstream resulted in nearly constant fluxes of iron and manganese. As most inorganic nickel and copper species are soluble, changing oxidation conditions affected nickel and copper fluxes less than iron fluxes. Nevertheless co-precipitations of nickel and copper caused by iron precipitations were observed. As manganese showed similar relative fluxes over the three years (Figure: 3.5) concentrations of this element were also less affected by the displacement of the creek. This may resulted from lower total concentrations of manganese in the AMD. Contrary to iron, nickel and copper as well as uranium fluxes were less influenced by the displacement of the creek bed and depended less on redox potential and anthropogenic barriers. Uranium fluxes differed significant in the three years and between the sampling points. This was probably caused by the variety of soluble and insoluble uranium species and their sensitivity on changing physicochemical parameters.

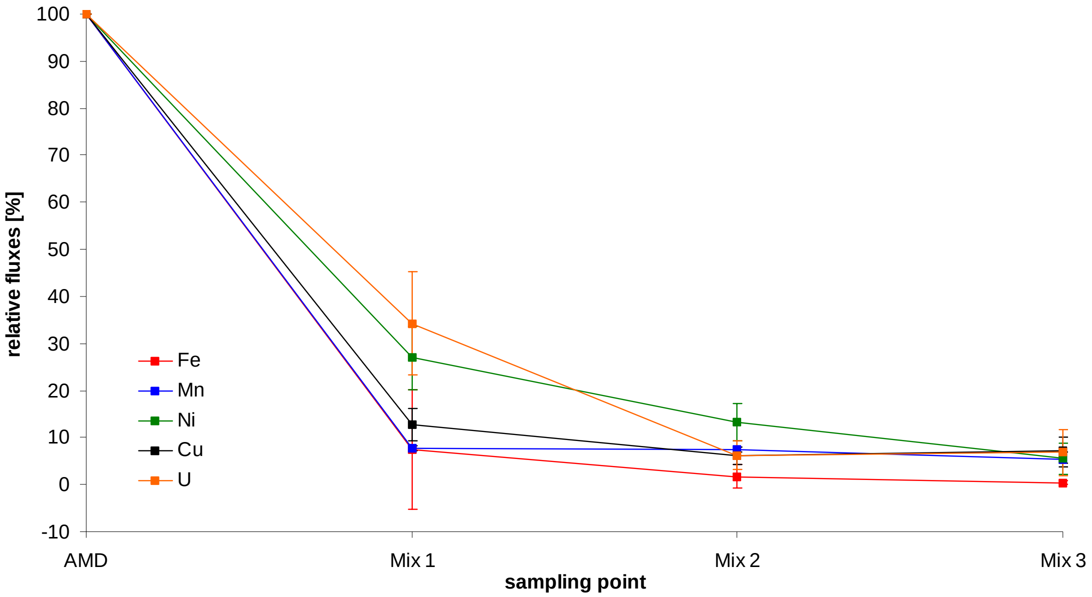


Figure 3.5: Relative fluxes representing the mobility of heavy metals iron, manganese, nickel, copper and uranium downstream from sample AMD 2 to sample Mix 3. Presented values are mean values from sampling procedures in 2001, 2002 and 2003

As physicochemical factors controlled the mobility of heavy metals upstream of Mix 1, further influencing factors on mobility downstream from Mix 1 were elevated DOC concentrations. These elevated DOC concentrations were mirrored in an elevated complexing potential of DOC and therefore on governed heavy metal mobility by DOC. DOC concentrations and therefore the mobility of the heavy metals were influenced by seasonal climate changes. With few to the influence of redox potential and seasonal climate changes the heavy metals can be divided in less mobile (Fe, Mn) and more mobile (Ni, Cu, U) elements.

3.2 Concentration of field samples and standard solutions by nanofiltration

Enrichment of organic heavy metal complexes from surface waters was performed by nanofiltration. Separation of non dissolved compounds from dissolved compound was achieved by prefiltration of the field samples to 450 nm. As the nanofiltration membrane cut off was approximately 1 nm molecular sizes of concentrated field samples were between 1 nm and 450 nm. The impact of the concentration process on the composition of organic heavy metal complexes was determined by concentrating standard solutions.

3.2.1 Nanofiltration of standard solutions

To guarantee the enrichment of organic heavy metal complexes from surface waters standard solutions under different physicochemical conditions were concentrated. The retention capacity of the nanofiltration membrane was defined by concentrating inorganic salts and organic heavy metal standards. Two nanofiltration procedures were done to simulate field site conditions. The first focused mainly on the retention of inorganic salts where the second procedure focused on the retention and stability of organic heavy metal complexes (Table: 3.3 and 3.4). Initial sample volumes of 50 l for inorganic salts and 20 l for organic heavy metal complexes resulted in the first nanofiltration procedure in final volumes between 4.5 l and 6.5 l. This equaled water losses between 71% (EDTA-Ni) and 91% (Na_2CO_3) (Table: 3.3). Nanofiltration of inorganic salts resulted in lower recoveries than nanofiltration of organic heavy metal complexes. Recoveries between 57% (Na_2CO_3) and 65% ($CaCl_2$) mirrored an inorganic ion loss of approximately 1/3. Significant lower loss was analyzed for the larger molecules EDTA-Ni and humic acid (HA)-Ni. Relatively low recovery of HA (69%) resulted from non complexed small HA units which were separated by nanofiltration. Larger HA units were bound to Ni^{2+} ions which were recovered to 96%. The higher efficiency for removal of inorganic salt ions and low efficiency for ions from organic heavy metal complexes confirmed higher loss of small molecules or ions relative to larger heavy metals and organic compounds. Consequently, heavy metal complexes with organic ligands were selectively enriched in the solutions, whereas inorganic salts were removed by nanofiltration at the same time.

Table 3.3: Water loss, cation and anion concentrations before and after nanofiltration and recovery of inorganic salts and Ni-EDTA during the nanofiltration procedure.

	water loss	before nanofiltration		after nanofiltration		% recovery	
substance	vol [%]	cation [mg]	anion [mg]	cation [mg]	anion [mg]	cation [mg]	anion [mg]
$CaCl_2$	88	56	99	36	65	65	65
$MgCl_2$	90	34	99	22	60	65	60
NaCl	89	32	49	19	28	58	57
Na_2SO_4	87	1426	2976	1155	2411	81	81
Na_2CO_3	91	100	26	59	15	59	57
HA-Ni	78	35	600	34	414	96	69
EDTA-Ni	72	446	912	424	876	95	96

As the main focus of the nanofiltration procedure was set on the selective enrichment of organic heavy metal complexes, the second procedure concentrated exclusively on organic heavy metal complexes (Table: 3.4). To evidence the separation of inorganic salts at simultaneous enrichment of organic heavy metal complexes, Ni-EDTA and Cit-Cu solutions were overlapped with the inorganic salts $Ni(NO_3)_2 \cdot 6H_2O$ and $CuSO_4 \cdot 5H_2O$. Concentrating strong and weak organic heavy metal complexes simulated different stabilities of organic heavy metal compounds under changing physicochemical conditions. Comparability of the two procedures was achieved by concentrating Ni-EDTA in both experiments. In contrast to the first procedure the initial volume of all samples in the second experiment was set to 50 l and the final volume was set to 8 l (Table: 3.4). Recovery of organic ligands was between 75% for Citrate (Cit) in the Cit-Fe complex and 98% for EDTA in an EDTA-Ni complex.

Table 3.4: Water loss, cation and anion concentrations before and after nanofiltration, recovery and complex stability constants of organic heavy metal standard solutions during the nanofiltration procedure.

	water loss	before nanofiltration		after nanofiltration		% recovery		
substance	vol [%]	DOC [mg]	metal [mg]	DOC [mg]	metal [mg]	DOC [mg]	metal [mg]	log K
EDTA-Ni 1:1	84	997	493	963	466	97	95	25
EDTA-Ni 1:2	84	996	971	957	731	96	75	25
EDTA-Ni 1:3	84	999	1465	977	793	98	54	25
Cit-Cu 1:1	84	999	886	849	376	85	42	6
Cit-Cu 1:3	84	997	2658	873	622	88	23	6
DFOM-Fe	84	369	73	326	63	88	86	22
Cit-Fe	84	999	779	748	579	75	74	13
Mix: Cit	84	1998		1625		80		
Mix: DFOM	84	42						
Mix: Cu	84		886		703		79	
Mix: Fe	84		881		786		89	

Corresponding heavy metal recoveries in the equimolar complexes were between 42% for copper in its citrate complex and 95% for nickel in its EDTA complex. Beside the investigation on potential separation of single complexes, a mixture of iron and copper salts together with DFOM and citrate ligands was concentrated. The enrichment of this solution resulted in recoveries between 79% and 89% for heavy metals and 80% for DOC ligands. These recoveries proved also the high enrichment potential of the nanofiltration method. The lower recovery of the citrate complexes corresponded with its stability constants (Figure: 3.6).

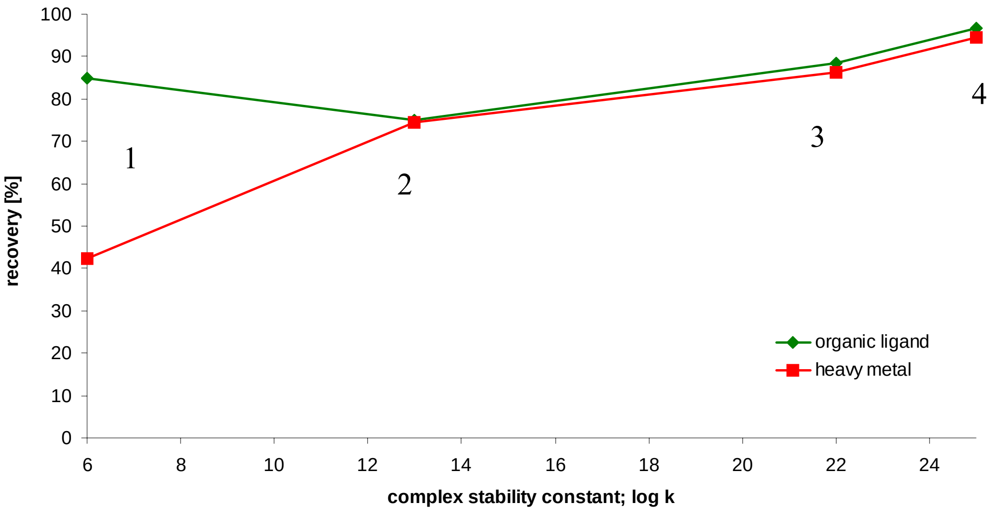


Figure 3.6: Recoveries of the organic complexes Citrate-Cu (1), Citrate-Fe (2), DFOM-Fe (3) and EDTA-Ni (4) depending on their complex stability constants. Recovery of organic ligand and corresponding heavy metal were analyzed independent.

A complex stability constant of log k=6 represents under norm conditions weak complexes. The weak complex Cit-Cu was partly dissociated under the nanofiltration conditions which is shown in Figure 3.6. Therefore the smaller copper ion was stronger separated (recovery = 42%) in contrast to the larger citrate ligand (recovery = 85%). The Cit-Fe complex with log k=13 was not dissociated under these conditions. Donor and ligand had similar recoveries (Cit 75%, Fe 74%). The largest and strongest complexes DFOM-Fe and EDTA-Ni yield corresponding to their stability constants largest recoveries. Therefore Figure 3.6 proved the direct correlation of stability constant and recovery of standard complexes.

The overlap of inorganic salts in the EDTA-Ni and Cit-Cu solutions resulted in a lower recovery of heavy metals (Figure 3.7). Nevertheless, the strong complex EDTA-Ni yield recoveries of nearly 100% in all EDTA-Ni solutions. Overlapping the inorganic Ni concentrations resulted only in higher separation of Ni in contrast to the organic ligand. This was proved in Figure 3.7 as by concentrating the EDTA-Ni 1:2 complex more than 50% Ni and in the 1:3 complex more than 33% Ni were recovered. The separation of inorganic salts was further proved as the recovery of inorganic copper (23%) in the 1:3

Cit-Cu complex was significant higher than 14%. With respect to the previously shown partly dissociation of the weak Cit-Cu complex,

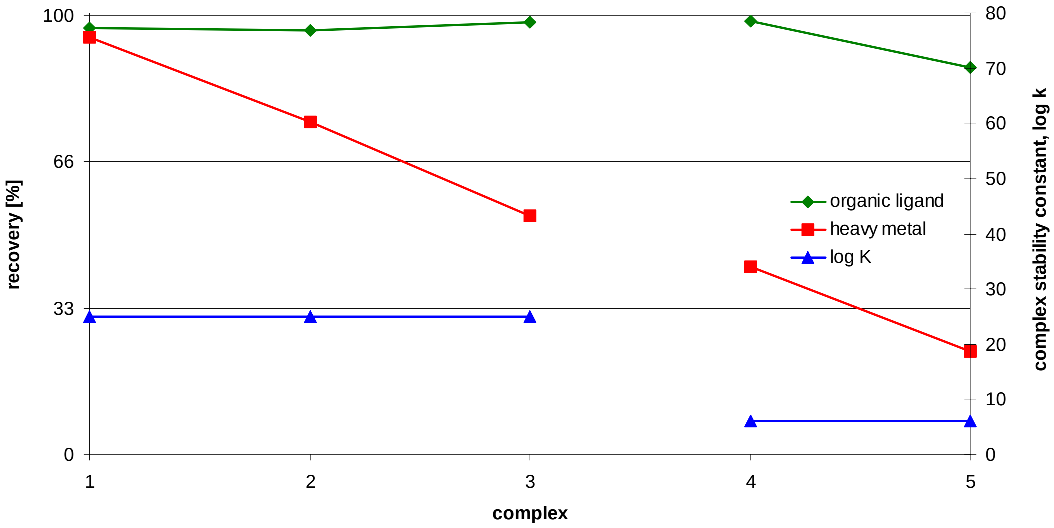


Figure 3.7: Recoveries of organic heavy metal complex solutions overlapped with corresponding inorganic heavy metal salts. Recoveries of the strong complexes EDTA-Ni 1:1 (1), EDTA-Ni 1:2 (2), EDTA-Ni 1:3 (3) and the weak complexes Citrate-Cu 1:1 (4), Citrate-Cu 1:3 (5) were plotted with respect to their complex stability constants

overlapping salt concentrations had no influence on the recovery of the organic heavy metal. If tripling the salt concentration would affect the recovery of the ligand Cu recovery in the 1:3 complex needed to be lower than 14% with respect to Cu recovery of 42% in the 1:1 complex. Consequently the second experiment confirmed the separation of inorganic salts by enrichment of organic heavy metal complexes at the same time under changing physicochemical conditions.

More details on the influence of the enrichment process to the stability of organic heavy metal complexes were achieved by HPSEC-UV investigations. Chromatograms, corresponding heavy metal concentrations and spectra of the four samples (DFOM-Fe, Cit-Fe, Cit-Cu and a mixed sample) were analyzed before and after nanofiltration (Appendix I: Figures I-1 to I-8). Concluding these chromatograms and spectra, Figure 3.8 highlights similar retention times of individual standard samples before and after the nanofiltration process. Identical retention times express the stability of the complexes during nanofiltration and also during HPSEC investigations. The stabilities of the undissociated complexes during HPSEC were independent from their stability constants. Therefore all investigated complexes overcame HPSEC undissociated.

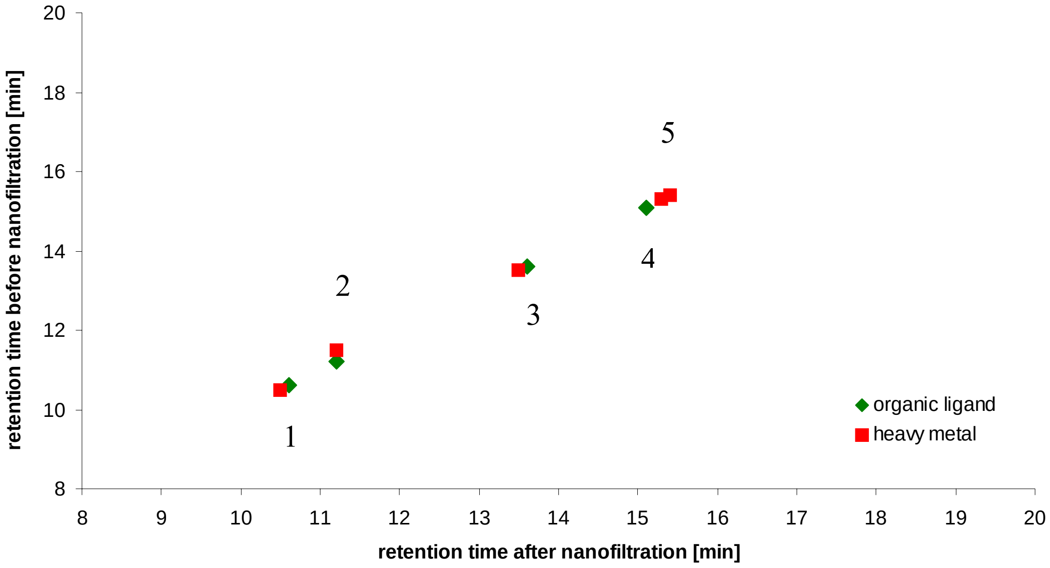


Figure 3.8: Retention times of organic heavy metal complexes before and after the nanofiltration procedure. Similar retention times represent identical complexes before and after nanofiltration. A mixed sample of DFOM-Fe, Citrate-Fe and Citrate-Cu eluted at two UV maxima representing two different complexes. The following samples were analyzed by HPSEC: DFOM-Fe (1), Citrate-Fe conc. (2), Citrate-Cu conc. (3), Mix conc. maximum 1 (4), Mix conc. maximum 2 (5)

UV traces of DFOM-Fe before and after nanofiltration had absorption maxima at 10.5 min (Appendix I: Figure: I-1). Corresponding heavy metal concentrations had also maxima at 10.5 min. Significant higher concentrations of desferrioxamine mesylate and iron after enrichment by nanofiltration were mirrored in higher UV absorption and higher heavy metal concentrations in the corresponding fractions. Identical complexes of raw water and concentrate were mirrored in spectra of the DFOM-Fe solution where the concentrated sample showed higher maximum intensities (Appendix I: Figure: I-2). Maximum UV absorption of the Cit-Fe was detected at 15.0 min (Appendix I: Figure: I-3) where the concentrated sample eluted in a significant broader maximum caused by an overload of the column. UV-spectra showed again similar traces, with higher intensities of the concentrate sample (Appendix I: Figure: I-4). Further on UV traces of Cit-Cu samples were detected at 14.9 min and corresponded with elevated copper concentrations at 14.5 min and 15.5 min (Appendix I: Figure: II-5). Similar UV spectra proved another time identical complexes in raw water and concentrate (Appendix I: Figure: I-6). The equimolar mixed samples of DFOM-Fe, Cit-Fe and Cit-Cu eluted between 9.3 min and 15.5 min (Appendix I: Figure: I-7). UV maxima were detected at 11.0 min. Both samples hold elevated heavy metal concentrations at 10.5 min and 11.5 min. Further on elevated heavy metal concentrations were detected at 13.5 min. Elevated UV absorption was correlated with elevated heavy metal concentrations at 11.0 min and 13.5 min and represented therefore two organic heavy metal species. Retention times of the mixed samples did not agree with retention times of single complex samples. This retention shift resulted either from the formation of new species immediately after mixing the standards or from interactions between sample and HPSEC column. The retention shifts resulted not from the concentration procedure because UV absorption and heavy metal retention were similar in the initial and final mixed sample. UV-spectra of the two mixed samples had a common maximum at 210 nm where the intensity at 210 nm in the concentrated sample was higher than in the initial sample (Appendix I: Figure: I-8).

Nanofiltration of inorganic and organic standard samples proved a high recovery for organic heavy metal complexes. The concentration process resulted in separation of small inorganic and organic species which were not involved in complexation. Therefore concentration of various standard solutions confirmed the enrichment of dissolved organic heavy metal complexes by nanofiltration. Additionally, chromatograms and spectra proved that organic heavy metal complexes were not modified during the concentration process.

3.2.2 Nanofiltration of field samples

The enrichment process of organic heavy metal complexes, simulated by standard solutions was finally applied to the surface water samples from the Ronneburg field site. HPSEC proved stability of complexes from the field samples during the concentration process (Figure: 3.9).

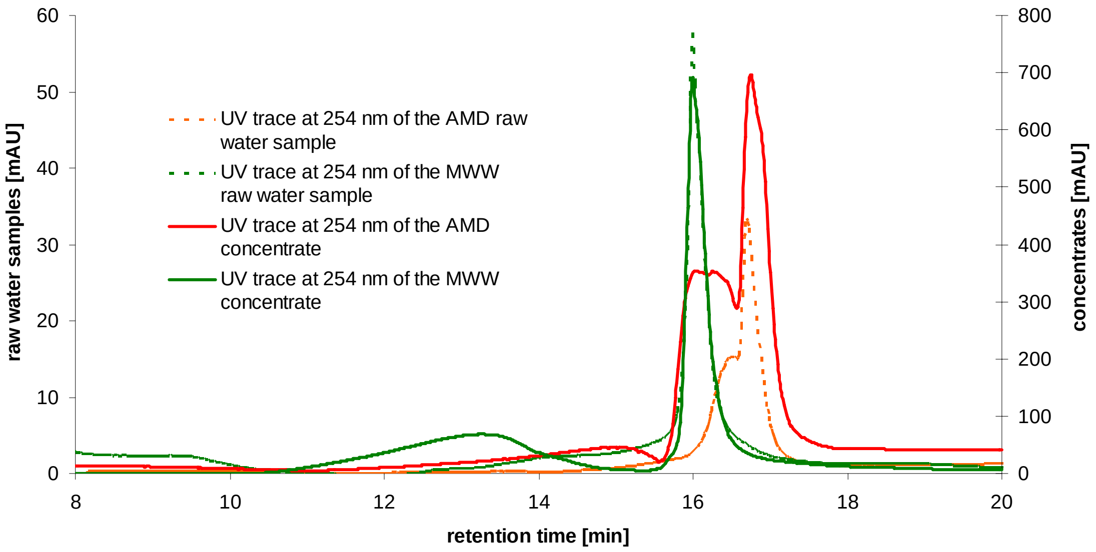


Figure 3.9: UV chromatograms of raw water samples and corresponding concentrated samples from municipal waste water (MWW) and acid mine drainage (AMD) at 254 nm

A municipal waste water sample and an acid mine drainage sample were analyzed by HPSEC-UV before and after nanofiltration. The raw sample and the concentrate of the municipal waste water had one common UV maximum at 16.0 min. The UV intensity of the concentrate was approximately 15 times higher. In addition a second maximum appeared in the concentrate at 13.5 min resulting from enrichment by nanofiltration. Raw sample and concentrate of the AMD had two corresponding UV maxima, where the maximum intensities of the concentrate were approximately 20 times higher than in the raw sample. The first maximum had a broader retention window in the concentrate, which indicated the selective enrichment of larger molecules. This was also supported by the maximum appearing at 15 min.

The initial volume (50 l) of the field samples was concentrated to final volumes between 2.7 l (MWW 2 in 2002) and 7.0 l (Mix 1 in 2003) (Table: 3.5). Slightly shifting pH values (0 to 1.2 pH units) were observed during the concentration process. Depending on the ion concentrations MWW hold lowest initial conductivity values (0.8 mS/cm – 3.2 mS/cm) where AMD samples reached values up to 16.4 mS/cm. As the mixed samples contained water from MWW and AMD their initial conductivity values were between 1.5 mS/cm and 3.6 mS/cm. Corresponding to their initial conductivity values, lowest final conductivities after concentration were achieved by MWW and highest by AMD samples. Low increase of conductivity in comparison to the corresponding enrichment factor proved the separation of inorganic ions.

Table 3.5: Initial volumes, final volumes, enrichment factors, initial pH values, final pH values, initial conductivities an final conductivities of field samples from municipal waste water, acid mine drainage and mixed samples. Given values were analyzed before and after nanofiltration in 2001, 2002 and 2003.

year	sample	initial vol [l]	final vol [l]	enrichm. factor	initial pH value	final pH value	initial cond. [mS/cm]	final cond. [mS/cm]
2001	MWW 1	50	4.7	10.6	8.0	8.2	0.8	4.3
	MWW 2	50	3.2	15.6	7.5	7.6	1.6	4.5
	AMD 1	50	7.0	7.1	3.1	3.7	10.2	14.1
	AMD 2	50	6.0	8.3	2.5	3.7	16.4	20.3
	AMD 3	50	6.2	8.1	2.7	3.5	13.2	17.5
	Mix 1	50	5.0	10.0	6.6	7.8	3.6	7.3
	Mix 2	50	5.0	10.0	7.9	7.1	1.7	5.7
2002	MWW 1	50	5.0	10.0	8.1	7.7	1.3	4.9
	MWW 2	50	2.7	18.5	7.4	7.7	1.6	1.6
	AMD 1	50	4.3	11.6	2.8	2.9	12.2	17.4
	AMD 2	50	6.0	8.3	2.9	3.1	11.3	16.9
	AMD 3	50	5.7	8.8	3.7	4.0	8.6	12.1
	Mix 1	50	4.3	11.6	7.9	7.9	1.9	1.9
	Mix 2	50	3.0	16.7	8.0	7.5	1.7	1.8
	Mix 3	50	6.5	7.7	8.0	7.8	2.1	2.4
2003	MWW 1	50	5.0	10.0	7.7	7.8	2.7	6.8
	MWW 2	50	6.5	7.7	7.6	7.5	3.2	10.8
	AMD 1	50	5.0	10.0	3.4	2.7	4.2	17.1
	AMD 3	50	8.0	6.3	3.1	4.3	14.7	18.3
	Mix 1	50	7.0	7.1	7.4	7.7	2.2	10.7
	Mix 2	50	6.0	8.3	7.5	7.7	2.3	9.5
	Mix 3	50	4.6	10.9	7.7	7.8	1.5	8.6

Separation of inorganic ions and small DOC was also mirrored by the mass balances of field samples after nanofiltration (Table: 3.6). Recoveries after nanofiltration were determined for DOC, iron, manganese, nickel, uranium, silicon, chloride and sulfate in 2001, 2002 and 2003 (Table: 3.6). Minimum recovery for DOC was 16% (AMD 2 in 2001) and maximum recovery was 83% (MWW 1 in 2001) where mean recovery was 41%. Recovery of iron was between 9% (MWW 2 in 2003) and 70% (AMD 1 in 2001) with a mean of 24%. Mn was recovered between 7% (MWW 1 in 2002) and 89% (Mix 2 in 2002) with mean recovery of 36%. Nickel recovery after nanofiltration was between 13% (AMD 2 in 2001) and 86% (MWW 1 in 2002) and had a mean of 36%. Uranium recovery was between 0.3% (MWW 1 in 2003) and 89% (MWW 2 in 2002) with mean recovery of 36%. Recovery of silicon was between 19% (MWW 1 in 2003) and 38% (AMD 1 in 2001) with a mean recovery of 29%. Anion recovery was between 43% (Mix 1 in 2002) and 60% (Mix 2 in 2003) for chloride and between 48% (Mix 1 in 2002) and 68% (MWW 1 in 2002) for sulfate with means of 49% and 55% respectively. Lower values in mass balance of sulfate in AMD samples (between 12% and 19%) proved the separation of large inorganic sulfates. This was in good accordance with elevated sulfate concentrations in raw water samples from AMD. Beside nanofiltration, separation of heavy metals was caused by precipitation mainly of iron. Finally nanofiltration of field samples mirrored the results obtained by standard solutions as inorganic species and small DOC were separated and organic heavy metal complexes were concentrated. Therefore the concentration process resulted in selective enrichment of organic heavy metal complexes from surface waters of the field site Ronneburg.

Table 3.6: Recoveries of DOC, iron, manganese, nickel, uranium, silicon, chloride and sulfate in the field samples from 2001, 2002 and 2003 after nanofiltration. Additionally to the recovery of the 17 field samples an overall mean value was calculated for each species.

				recovery [%]				
sample	DOC	Fe	Mn	Ni	U	Si	Cl^-	SO_4^{2-}
MWW 1 2001	83	n.a.	7	86	13	n.a.	53	68
MWW 2 2001	30	14	48	59	89	n.a.	54	52
MWW 1 2002	57	20	34	77	45	23	53	58
MWW 2 2002	38	18	51	46	28	27	49	62
MWW 1 2003	38	16	30	39	0.3	19	54	65
MWW 2 2003	59	9	54	50	15	n.a.	55	62
AMD 1 2001	66	70	49	52	42	38	54	19
AMD 2 2001	16	68	25	13	31	23	54	12
AMD 2 2002	42	13	42	38	27	27	46	14
AMD 2 2003	25	26	27	16	16	30	57	18
Mix 1 2001	38	31	37	39	11	28	43	16
Mix 2 2001	53	22	43	27	34	31	51	57
Mix 1 2002	52	19	12	38	69	34	43	48
Mix 2 2002	28	11	89	34	22	24	56	49
Mix 1 2003	40	26	57	59	57	32	48	52
Mix 2 2003	41	42	54	46	67	36	60	65
Mix 3 2003	54	28	15	10	60	28	51	63
Mean	45	25	40	43	37	24	52	59

n.a. = not analyzed

3.3 High Performance Size Exclusion Chromatography with UV detection (HPSEC-UV) of field samples and standard solutions

HPSEC-UV was achieved on field samples from 2001, 2002 and 2003 to isolate aqueous organic heavy metal complexes. For all samples chromatograms and spectra were recorded. Individual fractions were analyzed on UV traces corresponding with heavy metals. To guarantee reproducible results and to investigate interactions of the field samples with the stationary phase of the column, standard solutions were analyzed. Reproducibility over time of the analytical set up was proved by injecting a PEG standard continuously between HPSEC runs (Appendix I: Figure I-17)

3.3.1 HPSEC-UV investigations of standard solutions

First HPSEC-UV investigations focused on synthetic humic acid (HA)-$Ni(NO_3)_2{\cdot}6H_2O$ solutions composed of humic acid synthesized at the FZ Rossendorf and $Ni(NO_3)_2{\cdot}6H_2O$ from Fluka. This solution was analyzed to simulate the separation and retention of natural organic heavy metal complexes separated from field samples. The solutions were examined at pH 3 and pH 6. UV traces resulting from the pH 3 solution had maximum absorption at 11.6 min (Appendix I: Figure: I-9). Inorganic species had maximum concentrations additionally at the exclusion limit. HA-$Ni(NO_3)_2{\cdot}6H_2O$ solution with pH 6 hold a UV maximum at 11.5 min correlating with elevated Ni and nitrate concentrations (Appendix I: Figure: I-10). A second Ni and nitrate maximum was detected at the exclusion edge of the column. Comparing the HPSEC-UV investigations of HA-$Ni(NO_3)_2{\cdot}6H_2O$ at pH 3 and pH 6 showed that HA at pH 6 had higher affinity to $Ni(NO_3)_2{\cdot}6H_2O$. This was caused by higher solubility of HA at pH 6. Further on the separated HA-Ni complex included additionally nitrate. Nevertheless HPSEC of synthetic HA- $Ni(NO_3)_2{\cdot}6H_2O$ solution separated organic heavy metal complexes from inorganic heavy metal species. Different pH values did not influence the retention of the organic heavy metal complexes.

The two contrary field samples municipal waste water and acid mine drainage were characterized by significant different pH values, heavy metal and DOC concentrations. To simulate the impact of such physicochemical properties on the separation of organic heavy metal complexes standard solutions were analyzed by HPSEC with UV detection. The recovery in this mass balance of the 22 different standard samples was between 92% (citrate pH 8) and 108% (DFOM) for organic ligands and between 91% (Ni) and 104%

(Cu pH 6) for heavy metals (Table: 3.7). The recovery for weak complexes (citrate complexes) and strong complexes did not shift significant (between 91% and 102%) (Figure: 3.10). Therefore differing interactions of heavy metal and organic ligand with the column material causing destruction of the complexes could be excluded for complexes with stability constants between log k=5 and log k=32.

Table 3.7: Mass balance of 22 organic heavy metal complex standard solutions with corresponding complex stability constants. Recoveries were analyzed separate for ligands and corresponding donor heavy metals from the effluent of HPSEC. Recoveries of apoferritine (Apo) could not be calculated because initial DOC concentrations did not exist.

Complex	stab.const. [log k]	Ligand	recovery [%]	Heavy Metal	recovery [%]
Prot-Fe	22	Prot	94	Fe	97
Prot-Ni	n.k.	Prot	97	Ni	92
Def-Ni	n.k.	Def	95	Ni	98
Def-Cu	n.k.	Def	108	Cu	97
Apo-Ni	n.k.	Apo		Ni	98
Apo-Fe	22	Apo		Fe	94
EDTA-Ni pH 2	32	EDTA	92	Ni	92
EDTA-Ni pH 5	25	EDTA	97	Ni	92
EDTA-Ni pH 8	21	EDTA	97	Ni	95
EDTA-Ni (1:1)	25	EDTA	100	Ni	91
EDTA-Ni (1:2)	25	EDTA	100	Ni	96
EDTA-Ni (1:3)	25	EDTA	93	Ni	97
EDTA-Cu pH 3	29	EDTA	94	Cu	95
EDTA-Cu pH 6	24	EDTA	93	Cu	104
EDTA-Cu (1:1)	25	EDTA	94	Cu	97
EDTA-Cu (1:3)	25	EDTA	96	Cu	97
EDTA-Fe pH 3	36	EDTA	98	Fe	93
EDTA-Fe pH 6	30	EDTA	101	Fe	92
Cit-Cu pH 2	9	Cit	97	Cu	102
Cit-Cu pH 5	6	Cit	93	Cu	102
Cit-Cu pH 8	5	Cit	92	Cu	97
Cit-Ni	6	Cit	95	Ni	100

n.k. = not known

The influence of the pH value and the influence of inorganic salt concentrations on the separation of organic heavy metal complexes was determined by analyzing standard complexes under differing pH conditions as well as solutions holding overloads of inorganic salts (Figure: 3.10). EDTA and Citrate complexes with pH values between 2 and 6 showed similar retention times of the organic ligand and the corresponding heavy metal. Similar retention of complexes was also observed for EDTA complexes with salt overloads up to 3 times. Therefore different pH conditions and different salt concentrations caused no dissociation of the complexes during HPSEC analysis.

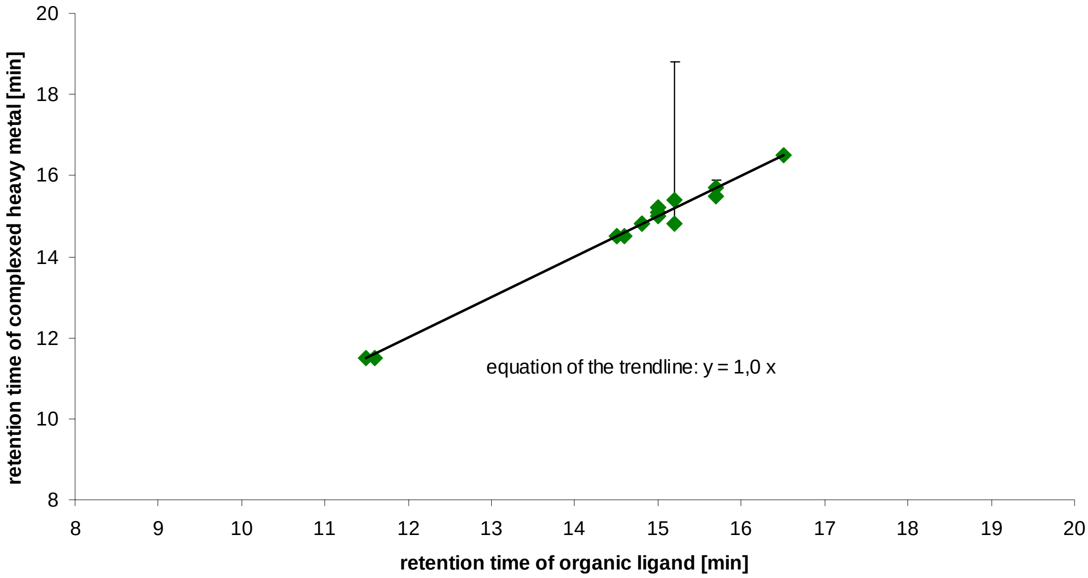


Figure 3.10: Independent retention times of organic ligands and corresponding heavy metals after HPSEC. A trend line slope of 1 proved undissociated organic heavy metal complexes. Organic heavy metal solutions were analyzed under differing pH conditions and differing heavy metal salt concentrations. Analyzed samples were (from the left to the right) humic acid-Ni pH 3, humic acid-Ni pH 6, EDTA-Ni pH 2, EDTA-Ni pH 5, EDTA-Ni pH 8, EDTA-Cu pH 3, EDTA-Cu pH 6, EDTA-Fe pH 3, EDTA-Fe pH 6, Cit-Cu pH 2, Cit-Cu pH 5, Cit-Cu pH 8, EDTA-Ni (1:1), EDTA-Ni (1:2), EDTA-Ni (1:3), Cit-Cu (1:1), Cit-Cu (1:3)

UV traces of Citrate-Cu solutions with pH values of 2, 5 and 8 showed corresponding maxima with heavy metal concentrations (Appendix I: Figure: I-11). These results were mirrored in the UV traces and corresponding heavy metal concentrations for EDTA-Cu solutions with pH values of 3 and 6 (Appendix I: Figure: I-12). EDTA-Ni solutions with pH values of 5 and 8 further on showed similar UV traces and similar heavy metal concentrations in corresponding fractions (Appendix I: Figure: I-13). At pH 2 the EDTA-Ni solution eluted with a broad maximum in contrast to the sharp maxima in the pH 5 and pH 8 solutions. However the heavy metal distribution in the fractions at pH 2 correlated with heavy metal distributions of higher pH values. EDTA-Fe solutions showed different UV maxima for pH 3 and pH 6. In both solutions the UV trace agreed with heavy metal concentrations in the fractions (Appendix I: Figure: I-14).

Elevated inorganic salt fractions in AMD were simulated with EDTA and citrate complexes. UV traces of Cit-Cu complexes with equimolar and additional 1:3 ligand donor ratio had similar adsorption maxima. Heavy metal concentrations had maxima corresponding to UV maxima and an additional maximum at the exclusion limit of the column where the not complexed inorganic salt eluted (Appendix I: Figure: I-15). EDTA-Ni solutions with ligand:donor ratios of 1:1, 1:2, and 1:3 resulted in different UV maxima at 14.9 min, 15.8 min and 16.5 minutes respectively (Appendix I: Figure: I-16). All three UV traces corresponded with maximum concentrations of its heavy metal donor ions. Overlaps of inorganic heavy metal salts were represented by heavy metal maxima at the exclusion limit of the column. Figure 3.11 shows the recovery of organic heavy metal complexes depending on their complex stability constants log k. As organic ligands and corresponding heavy metals had similar retention times it could be proved that weak and strong complexes were not dissociated during HPSEC.

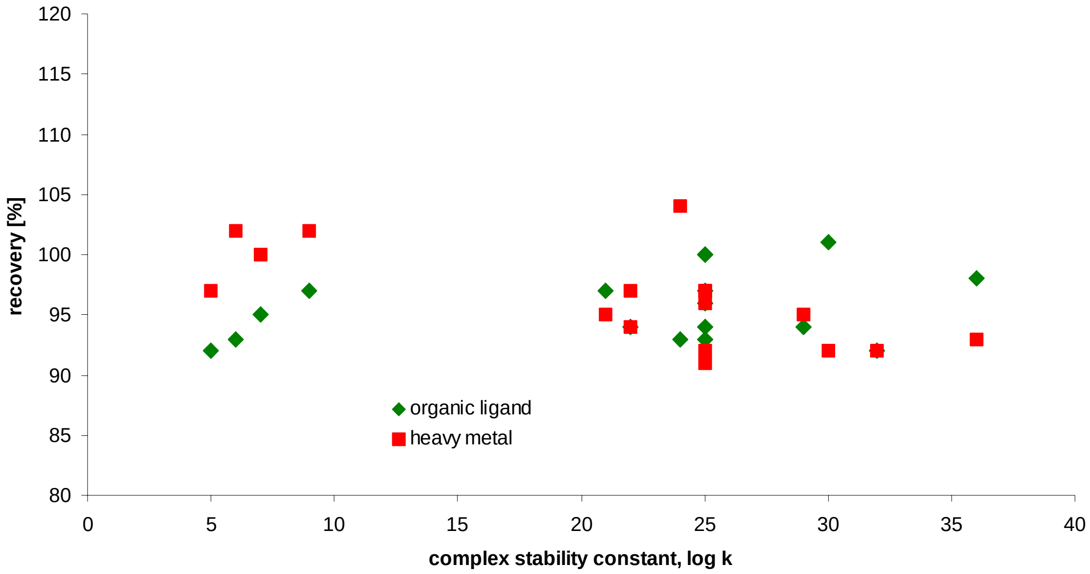


Figure 3.11: Recoveries after HPSEC investigation of organic ligands and corresponding heavy metals depending on their complex stability constants. Weak and strong complexes show similar recoveries. Recoveries near 100% prove undissociated complexes after HPSEC.

HPSEC-UV investigations of standard organic heavy metal species resulted in recoveries of weak complexes between 92% and 102% and strong complexes between 91% and 104% (Table: 3.7 and Figure 3.11). The trend that increasing recoveries correlate with increasing complex stability represented by weak complexes can not be considered as significant. As strong complexes have similar recoveries than weak complexes this trend represents scattering recoveries independent from complex stability.

Additional, separation of inorganic species from organic complexes under different pH environments simulated the separation of organic heavy metal complexes from field samples in a very good approximation. Retention shifts under modified conditions were caused by specific interactions with the stationary phase and did not reflect the dissociation of organic heavy metal complexes. The retention of organic heavy metal complexes was not influenced by their stability constants.

Beside quantitative separation of organic heavy metal complexes simulations with standard solutions showed that **size determination of organic heavy metal complexes** from field samples requires constant conditions. To achieve such constant or at least similar conditions, field samples need strong modification like pH adjustment and equal conductivities. These modifications would probably result in significant irreversible changes of the natural matrix. Consequently size investigations of field samples can only result in approximations. Further on the shape of the organic heavy metal complexes is not yet clearly defined. These complexes may have a straight-chain aliphatic or a spherical structure. Simulation of retention of spherical molecules was achieved with mono 100, mono 250 and mono 500. These molecules had a spherical shape with diameters of 100 nm, 250 nm and 500 nm. Aliphatic molecules were represented by polyethyleneglycoles (PEG) and polyethyleneoxides (PEOX) with molecular weights between 106 g/mol and 107000 g/mol (Table: 3.8).

Table 3.8: Molecular weight with corresponding retention times during HPSEC-UV of aliphatic and spherical standard molecules in aqueous solutions. Minimum and maximum lengths of the aliphatic standards polyethyleneglycole (PEG) and polyethyleneoxide (PEOX) in aqueous solutions were calculated depending on their individual ethylene units. Lengths of spherical molecules were calculated depending on their longest molecular expansion.

substance	molecular weight [g/mol]	retention time [min]	ethylene units	minimum length [nm]	maximum length [nm]
PEG 1	106	18.3	2.0	0.8	1.0
PEG 2	194	17.7	4.0	1.3	1.6
PEG 3	400	16.6	8.7	2.3	3.0
PEG 4	620	16.1	13.7	3.4	4.4
PEG 5	1080	15.4	24.0	5.6	7.5
PEG 6	1900	14.6	42.7	9.7	13.1
PEG 7	4120	13.6	93.2	20.7	28.1
PEG 8	6450	12.9	146.2	32.2	43.8
PEG 9	11840	12.0	268.7	58.9	80.2
PEG 10	22800	11.2	517.7	113.1	154.1
PEOX 1	24000	11.0	545.0	119.0	162.3
PEOX 2	50000	9.9	1135.9	247.7	337.7
PEOX 3	107000	9.2	2431.4	529.7	722.5
Mono 500	unknown	9.1			500.0
Mono 250	unknown	9.7			250.0
Mono 100	unknown	10.6			100.0
Apo	unknown	11.8			unknown
DFOM	656	12.6			5.0
Proto	561	13.1			1.5
EDTA	292	14.7			1.2
Cit	191	15.5			1.1

Size approximation with the HPSEC column was obtained with PEG and PEOX standards representing aliphatic molecules and with mono 100-500, protoporphyrine, EDTA and citrate representing spherical molecules (standard mix). For EDTA and citrate size, its longest diagonal was calculated. As the aliphatic PEG and PEOX molecules are in permanent motion size calibration was achieved with minimum and maximum lengths of these molecules. Therefore HPSEC-UV resulted in three different calibration curves (Figure: 3.12). Under stabile conditions (similar pH values and similar conductivities, similar analytical conditions) the retention time of a single molecule or organic compound corresponds to a molecular length between the curve of the standard cocktail and the curve of PEG max. This length determination could only be a rough approximation and gave only weak impression about the size of organic heavy metal complexes. Nevertheless, as the majority of molecules from the standard mix represented organic ligands it was assumed that sizes of natural organic heavy metal complexes tend more to sizes represented by the standard mix.

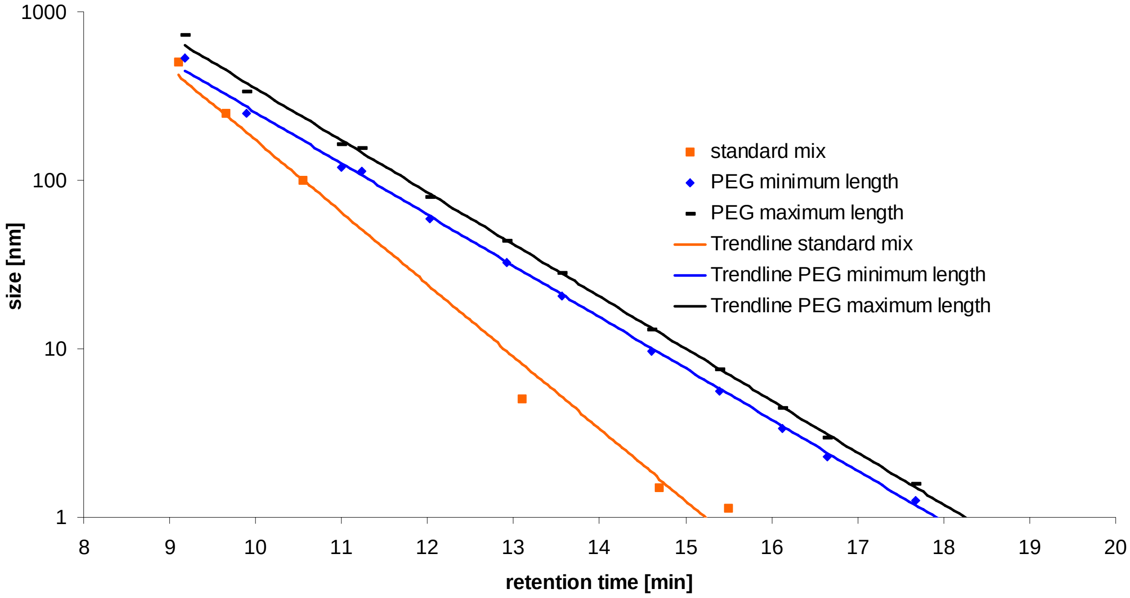


Figure 3.12: Calibration curves for size determination of aliphatic and spherical molecules in aqueous solutions investigated by HPSEC. Calculated minimum and maximum sizes of aliphatic molecules PEG and calculated sizes of spherical molecules mono 100-500, protoporphyrine, EDTA and citrate were plotted against their individual retention times.

3.3.2 Fractionation of organic heavy metal complexes from field samples by HPSEC

Preparative separation of one single fraction of organic heavy metal complexes needed approximately 100 HPSEC runs. To separate always the same fraction reproducible analytical conditions were essential. Therefore 100 runs of a municipal waste water sample and 100 runs of an acid mine drainage sample were initiated (Figure: 3.13 and 3.14). The UV traces proved a significant reproducibility of HPSEC-UV analyses of the two different field samples. Additionally a polyethylene glycol sample was injected frequently during analytical investigations to prove constant conditions of the analytical set up (Appendix: I Figure: I-17).

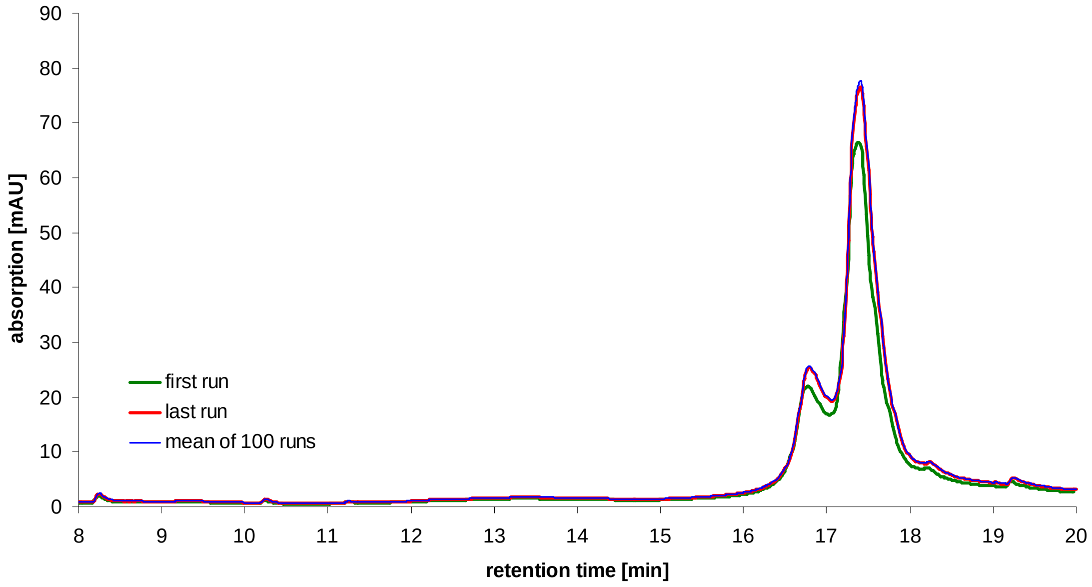


Figure 3.13: UV chromatograms of the first, the last and the mean run of 100 HPSEC runs. The reproducibility of the UV traces from municipal waste water samples confirmed the fractionation of organic heavy metal complexes

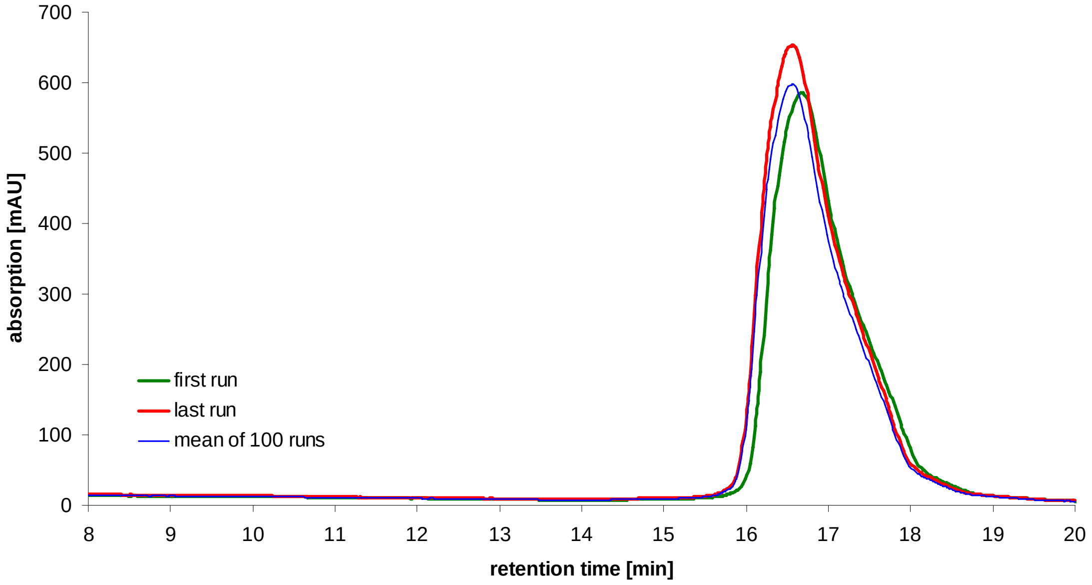


Figure 3.14: UV chromatograms of the first, the last and the mean run of 100 HPSEC runs. The reproducibility of the UV traces from acid mine drainage samples confirmed the fractionation of organic heavy metal complexes

Once a year in 2001, 2002 and 2003 samples were concentrated by nanofiltration and immediately fractionated by HPSEC with UV detection. The **mass balances of DOC, heavy metals, silicon and inorganic anions** after HPSEC-UV were satisfiable with respect to the physicochemical conditions of the field samples and reflected the results obtained by standard solutions (Table: 3.9).

Table 3.9: Recoveries of DOC, iron, manganese, nickel, uranium, silicon, chloride and sulfate in the field samples from 2001, 2002 and 2003 after HPSEC. Additionally to the recovery of the 14 field samples an overall mean recovery was calculated for each species.

				recovery [%]				
samples	DOC	Fe	Mn	Ni	U	Si	Cl^-	SO_4^{2-}
MWW 1 2001	84	n.i.	15	n.a.	2	n.a.	26	46
MWW 2 2001	35	2	30	46	83	n.a.	40	43
MWW 1 2003	35	67	13	32	0.2	17	47	54
MWW 2 2003	50	n.a.	45	48	55	n.a.	47	54
AMD 1 2001	67	n.i.	27	26	35	37	46	15
AMD 2 2001	21	0.5	18	12	28	23	50	8
AMD 2 2002	39	62	70	57	63	27	36	13
AMD 1 2003	21	24	21	14	11	30	42	11
AMD 2 2003	n.a.	53	65	65	86	98	85	10
Mix 1 2001	50	18	3	35	66	33	35	40
Mix 2 2001	29	0.17	80	66	10	22	45	45
Mix 1 2003	17	35	51	37	32	30	39	40
Mix 2 2003	23	53	4	37	57	33	35	32
Mix 3 2003	49	77	9	7	53	18	42	37
mean	40	39	29	32	34	27	37	42

n.a. = not analyzed because of too low sample volume
n.i. = not idendified because of unrealistic values over 1000% caused by analytical mistake

Smaller recoveries between 1% and 13% after HPSEC than after nanofiltration (see also table 3.6) proved minimum loss of organic heavy metal complexes during fractionation. To achieve an impression of total compound loss between sampling and separation of organic heavy metal complexes the recovery in this mass balance was calculated with initial concentrations of the raw water samples and final concentrations of the HPSEC effluent. Corresponding to recoveries after nanofiltration, recoveries after HPSEC varied strongly in individual samples. Strongest variations were found for iron recovery (0.5%-77%). These varieties of iron recovery were caused by the high precipitation potential of iron. Lower recovery varieties were found for DOC (17%-84%), manganese (3%-70%) and nickel (7%-66%). Nevertheless, high differences in recovery were analyzed for most investigated elements. These high differences indicated an elevated variety of physicochemical properties of heavy metal and organic heavy metal solutions. With view to inorganic species, potential anions chloride and sulfate recoveries changed slightly (chloride: 26%-50%; sulfate: 32%-56%) in comparison to DOC and heavy metal recoveries. Significant lower sulfate recoveries (8%-15%) referred again to elevated sulfate concentrations in raw water samples which could be separated to a large amount. This gave advice on small varieties of inorganic heavy metal compounds in contrast to elevated varieties of organic heavy metal complexes with different physicochemical parameters (e.g. complex stability or precipitation potential

Retention times of characteristic species from concentrated field samples were analyzed by using retention maxima from UV chromatograms and corresponding heavy metal concentrations in individual fractions. DOC which was able to complex heavy metals was represented by UV maxima at a wavelength of 254 nm. The occurrence of organic heavy metal complexes at UV maxima was investigated by analyzing corresponding iron, manganese, nickel, copper and uranium concentrations in isolated fractions. Further on concentration maxima of silicon, chloride and sulfate were determined. Differing DOC ligands over the three year sampling period were reflected by changing retention times of UV maxima in the municipal waste water samples (Figure: 3.15 and Appendix II: Table: II-2). Retention of elevated heavy metal concentrations along with UV maxima in MWW between 2001 and 2003 changed significant. Therefore detected organic heavy metal complexes in MWW showed very different retention times over the whole sampling period (Figure: 3.15). This indicated different sizes and structures of the complexes, predominantly of the DOC ligands. Inorganic species chloride and sulfate were detected in MWW predominantly at the exclusion edge and appeared therefore as inorganic salts. Minor detection of Silicon, chloride and sulfate was analyzed at retention times of organic heavy metal complexes.

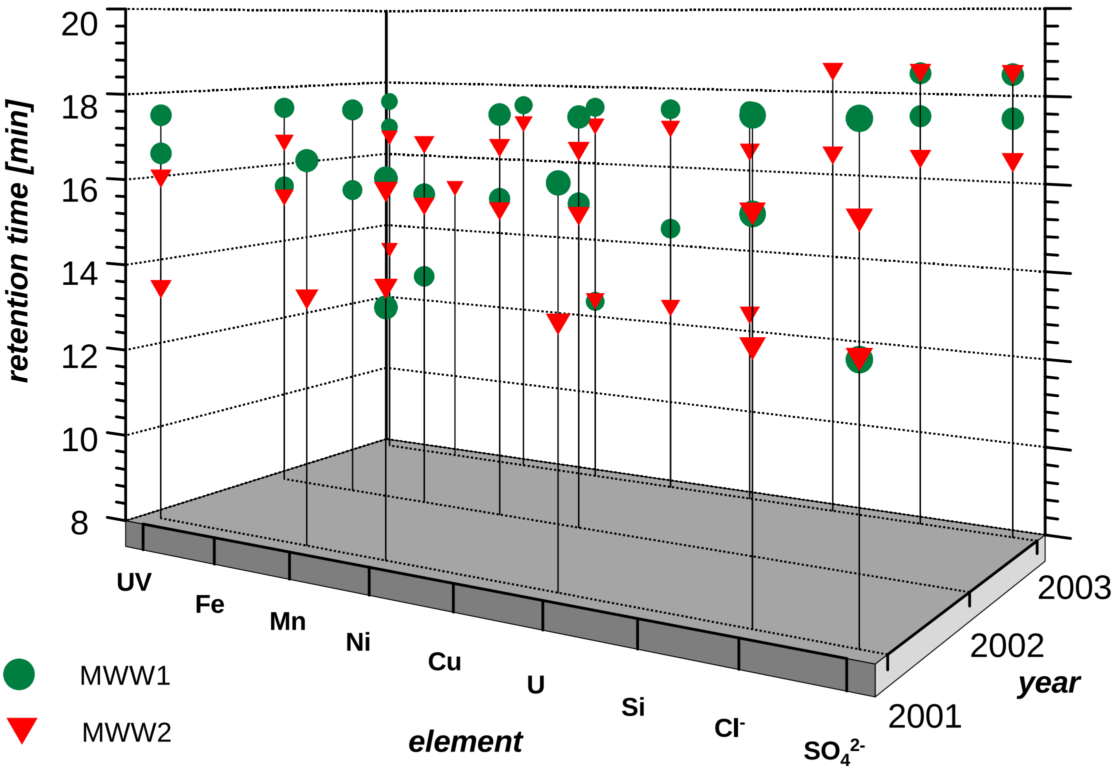


Figure 3.15: Retention times of the UV, iron, manganese, nickel, copper, uranium, silicon, chloride and sulfate maxima from municipal waste water (MWW) samples of the years 2001, 2002 and 2003. The figure clarifies high variation of retention times.

Identical retention times of DOC and corresponding heavy metals were detected over three years in the acid mine drainages (Figure: 3.16 and Appendix II: Table: II-2). Most AMD samples hold only one UV maximum corresponding with elevated heavy metal concentrations (Figure: 3.16). All AMD samples held one common heavy metal maximum over the whole sampling period. This common maximum indicated an organic heavy metal complex which was found in all samples of the years 2001, 2002 and 2003.

The retention time of the complex was between 16.2 min and 16.7 min. This recurrent retention time (labeled as green plane in Figure 3.16) indicated a stable indifferent organic heavy metal compound. Additionally, sample AMD 2 in 2003 held a second heavy metal maximum. Silicon, chloride and sulfate eluted analogical to MWW samples primary at the exclusion edge but also at retention times of organic heavy metal complexes.

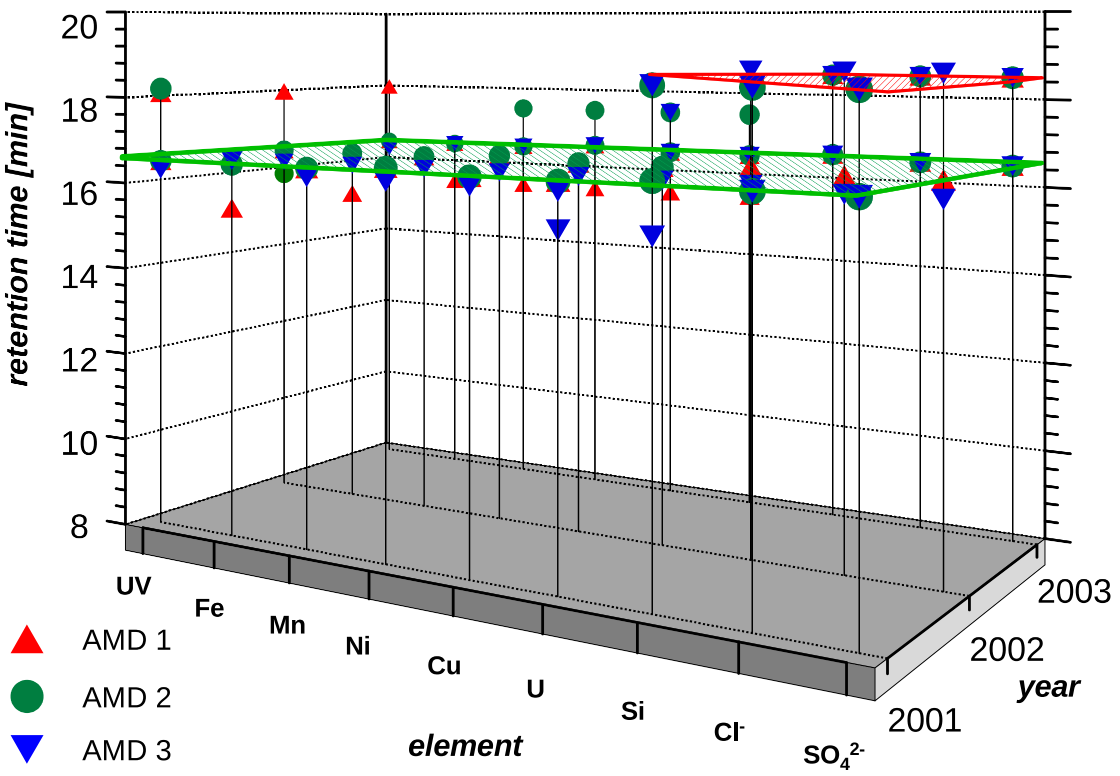


Figure 3.16: Retention times of the UV, iron, manganese, nickel, copper, uranium, silicon, chloride and sulfate maxima from acid mine drainage (AMD) samples of the years 2001, 2002 and 2003. The figure clarifies stable indifferent retention times of organic heavy metal complexes (green plane).

Downstream samples Mix 1, Mix 2 and Mix 3 hold water from municipal waste waters and acid mine drainage collectors. Mixed samples from 2001 to 2003 showed two heavy metal maxima which correlated with UV maxima (Figure: 3.17 and Appendix II: Table: II-2). These two planes of Figure 3.17 (orange and green) represent two recurrent organic heavy metal fractions of all mixed samples. The retention time of the green plane is equal to the retention time of the green plane in the AMD sample. Therefore the common plane represented similar organic heavy metal complexes, probably derived from AMD. Silicon, chloride and sulfate show once again elevated concentrations at the exclusion edge in addition to a common maximum with organic heavy metal complexes. Therefore silicon, chloride and sulfate of all detected samples eluted with organic heavy metal complexes and also as separated species.

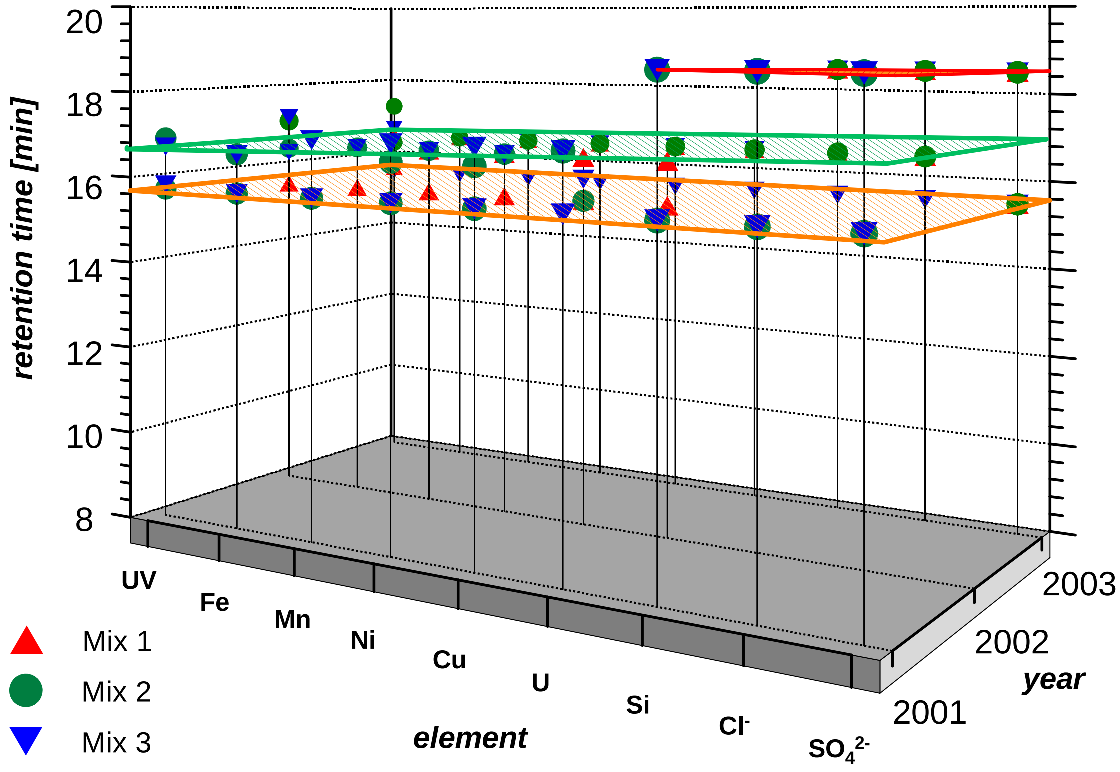


Figure 3.17: Retention times of the UV, iron, manganese, nickel, copper, uranium, silicon, chloride and sulfate maxima from mixed downstream (Mix) samples of the years 2001, 2002 and 2003. The figure clarifies stable indifferent retention times of two kinds of organic heavy metal complexes (green and orange plane).

The amount of DOC, heavy metals, silicon, chloride and sulfate detected in organic heavy metal fractions was calculated from concentrations of individual size fractions and from corresponding concentrations in raw water samples (Appendix II: Table: II-1). These calculations resulted in the percentage of complexed DOC, heavy metals, silicon, chloride and sulfate in relation to the initial concentrations in raw water samples (Figures: 3.18-3.25). Additional to the percentage of complexed material the recovery after nanofiltration was plotted. Elevated variations found in recoveries after nanofiltration were also mirrored in the content of complexed components. The interpretation of the percentage of complexed compounds with view to the relation of the recovery after nanofiltration resulted in significant smaller variations. The calculation of this relationship between complexed material and recovered material after nanofiltration resulted in mean values between 30%-45% for DOC and heavy metals and mean values between 15%-17% for silicon and anions (Figures 3.18-3.25). Therefore concentrated samples held still compounds which were not identified as organic heavy metal complexes. The portion of not complexed anions was higher than the portion of not complexed DOC and heavy metals. Small not complexed compounds, eluting at the exclusion edge of the HPSEC column were separated. Significant lower contents of inorganic anions than heavy metals and DOC proved the presence of heavy metals complexed by DOC.

Nanofiltration separated material with molecular sizes smaller 1 nm and larger than 450 nm. As proved by the mass balance after nanofiltration this amount differed significant in the investigated samples. Nanofiltration of the field samples resulted in aqueous samples, holding only dissolved material where initial inorganic salts were largely separated. With view to dissolved organic heavy metal complexes this size fraction was the base for further investigations. Therefore the relation between content of complexed material and recovery after nanofiltration will be interpreted in this paragraph. The content of DOC analyzed in organic heavy metal fractions of municipal waste waters (26%-47%), acid mine drainages (14%-50%) and mixed samples (14%-68%) differed only slightly in the three surface waters (Figure 3.18).

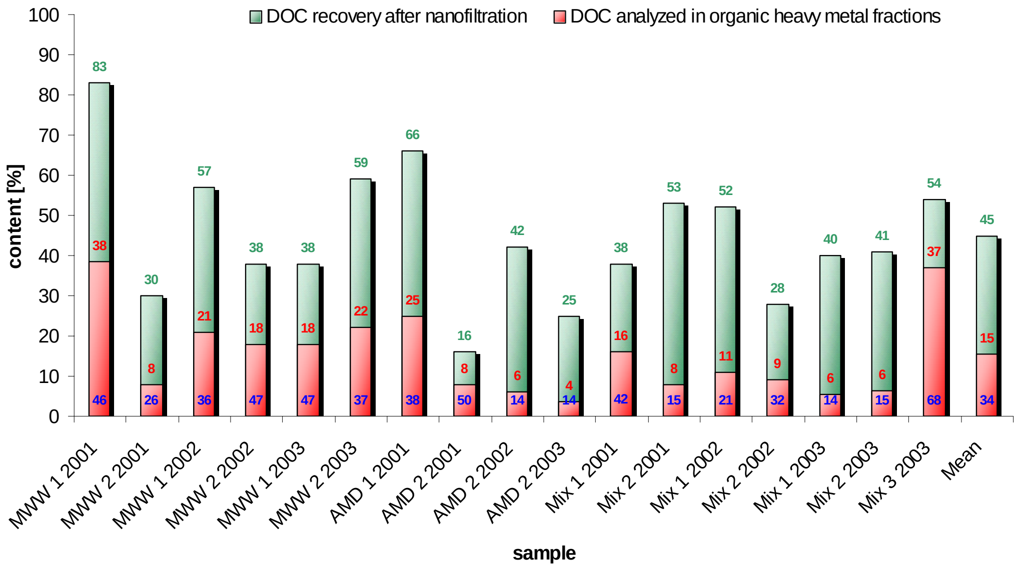


Figure 3.18: Percentage of DOC recovered after nanofiltration and percentage of DOC analyzed in HPSEC fractions of organic heavy metal complexes. Blue numbers represent the percentage of DOC found in organic heavy metal complexes in relation to recovered DOC after nanofiltration.

Changes of DOC content in municipal waste waters were slightly lower than in AMD and mixed samples. Nevertheless, between 14% and 68% of DOC from concentrated samples were recovered in organic heavy metal fractions of the surface waters.

Elevated variations of iron and manganese contents in organic heavy metal fractions were found in most samples, especially in mixed samples (Fe: 5%-81%; Mn: 3%-91%) and for manganese contents in MWW samples (4%-81%) (Figures: 3.19 and 3.20).

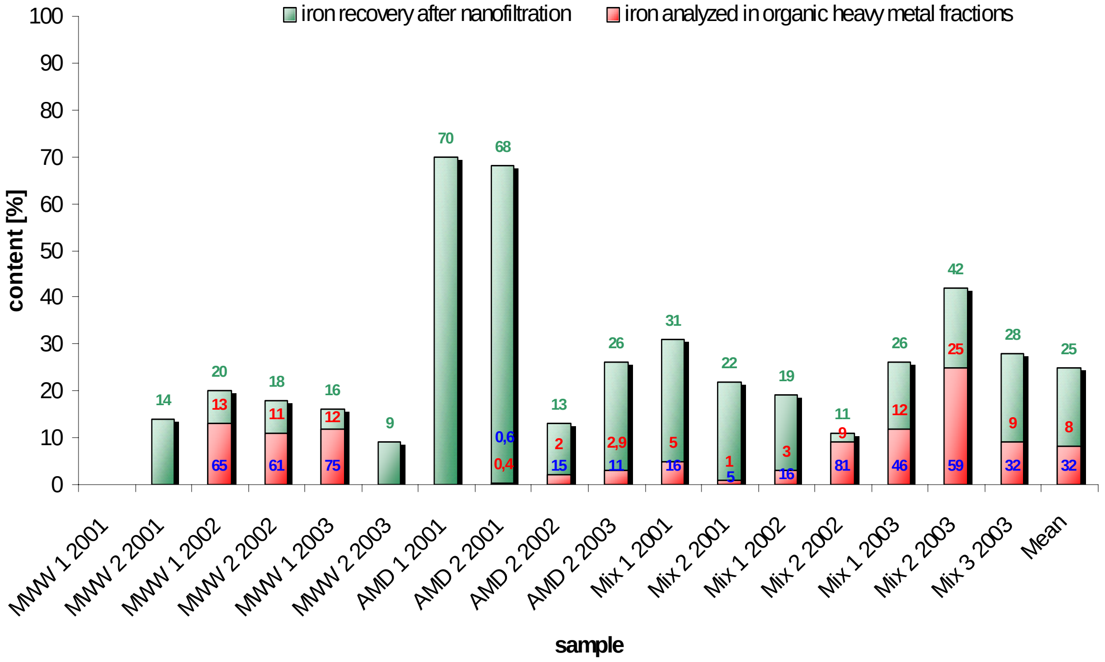


Figure 3.19: Percentage of iron recovered after nanofiltration and percentage of iron analyzed in HPSEC fractions of organic heavy metal complexes. Blue numbers represent the percentage of iron found in organic heavy metal complexes in relation to recovered iron after nanofiltration.

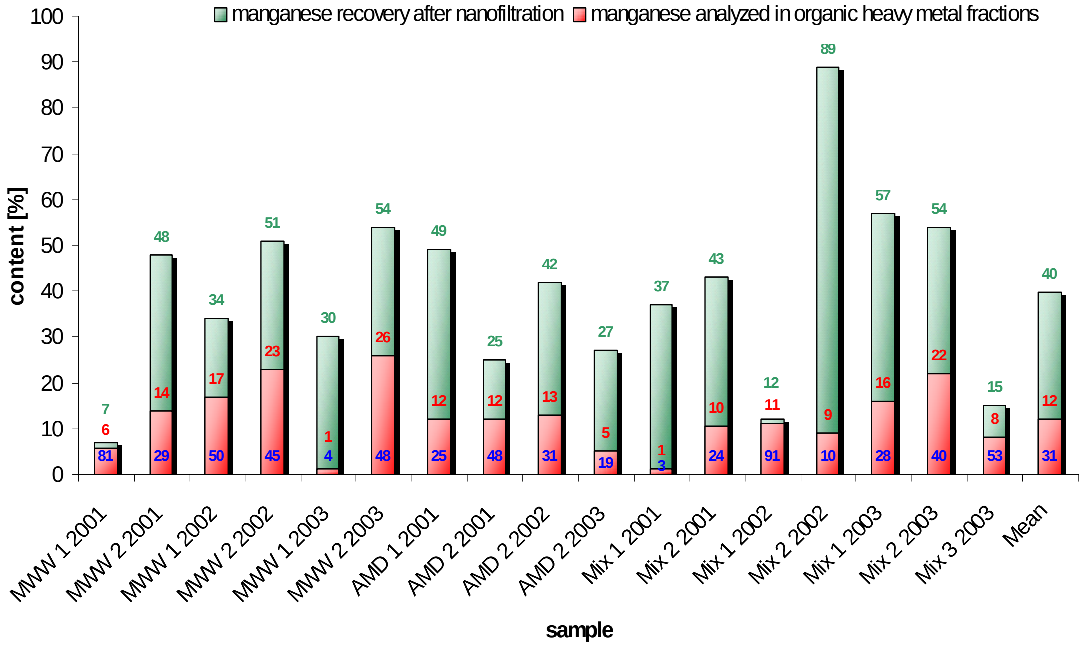


Figure 3.20: Percentage of manganese recovered after nanofiltration and percentage of manganese analyzed in HPSEC fractions of organic heavy metal complexes. Blue numbers represent the percentage of manganese found in organic heavy metal complexes in relation to recovered manganese after nanofiltration.

These variations of heavy metals resulted from elevated varieties of complexable DOC in MWW and mixed samples. Variations in iron and manganese contents indicated lower DOC-iron and DOC-manganese complexes in comparison to nickel and uranium complexes and therefore lower complexing potentials for iron and manganese. Lower contents of iron and manganese in organic heavy metal fractions from acid mine drainages were caused by higher concentrations of inorganic iron and manganese species in raw water samples.

Lower variations in contents of complexed material were found for nickel and uranium. Variations of these two metals were higher in MWW and mixed samples (Ni: 17%-37% in MWW and 28%-56% in mixed samples; U: 14%-52% in MWW and 15%-63% in mixed samples) than in AMD samples (Figures: 3.21 and 3.22).

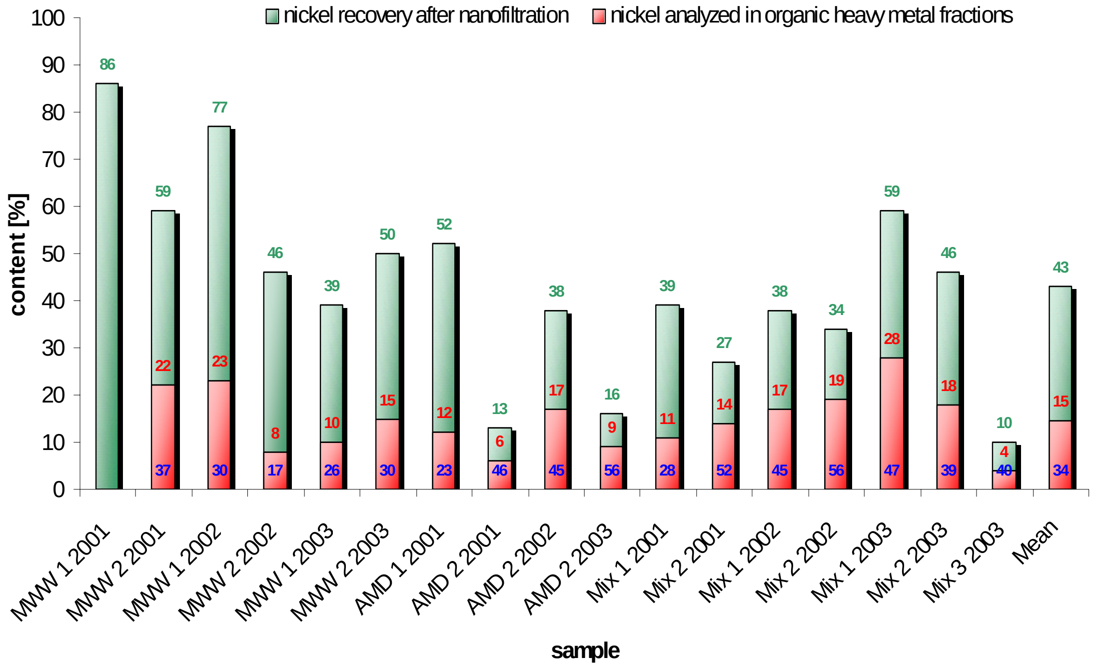


Figure 3.21: Percentage of nickel recovered after nanofiltration and percentage of nickel analyzed in HPSEC fractions of organic heavy metal complexes. Blue numbers represent the percentage of nickel found in organic heavy metal complexes in relation to recovered nickel after nanofiltration.

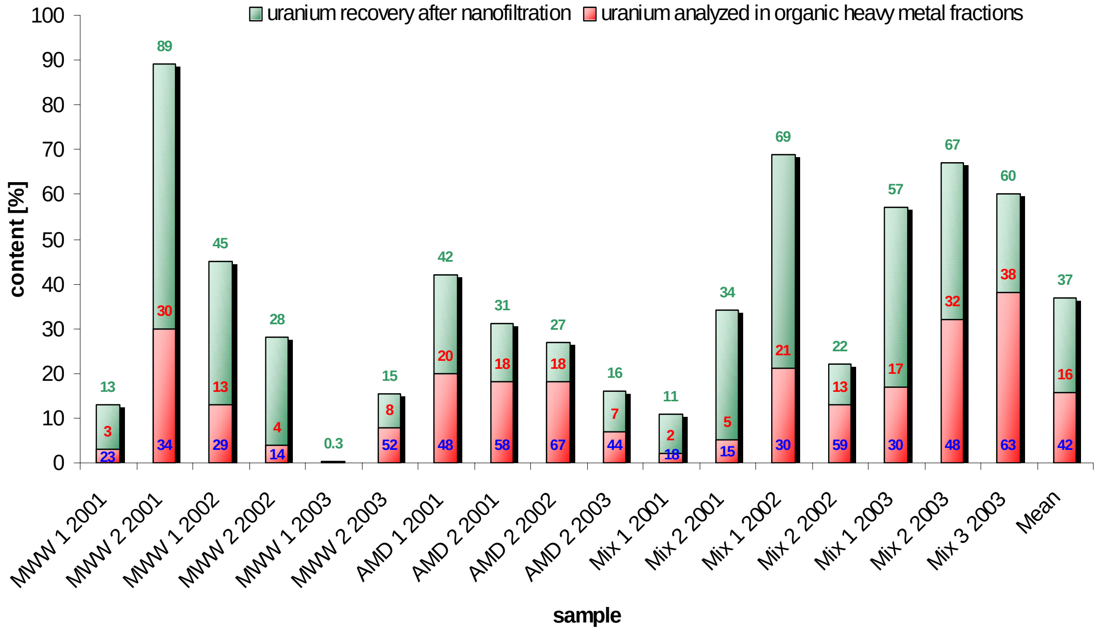


Figure 3.22: Percentage of uranium recovered after nanofiltration and percentage of uranium analyzed in HPSEC fractions of organic heavy metal complexes. Blue numbers represent the percentage of uranium found in organic heavy metal complexes in relation to recovered uranium after nanofiltration.

These higher variations in MWW and mixed samples were also caused by a higher variety of DOC resulting in a higher variety of weak and strong organic ligands. Lower variations in nickel and uranium contents in AMD samples referred to a lower variability of DOC resulting in lower amounts of potential organic ligands. These similar DOC ligands contained mostly similar amounts of nickel and uranium. Further on lower variations on nickel and uranium contents indicated a better and more constant complexation potential in contrast to iron and manganese. Lower variations of Ni and U resulted also from lower precipitation potentials which could release heavy metals out of their complexes.

Contents of silicon in organic heavy metal fractions behaved similar to inorganic species chloride and sulfate (Figures: 3.23 to 3.25).

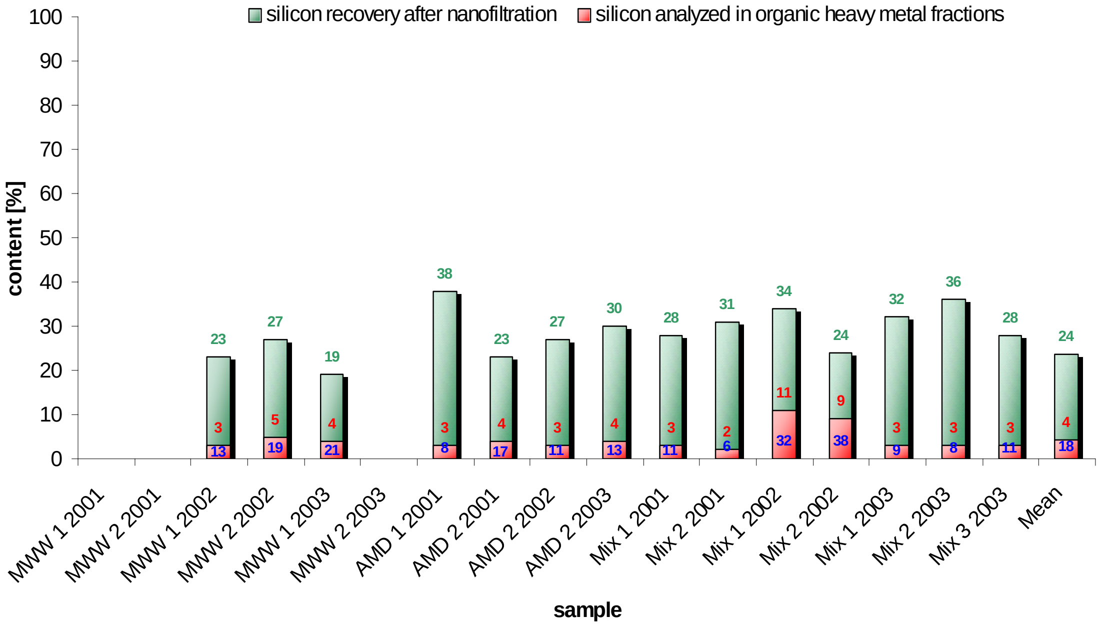


Figure 3.23: Percentage of silicon recovered after nanofiltration and percentage of silicon analyzed in HPSEC fractions of organic heavy metal complexes. Blue numbers represent the percentage of silicon found in organic heavy metal complexes in relation to recovered silicon after nanofiltration.

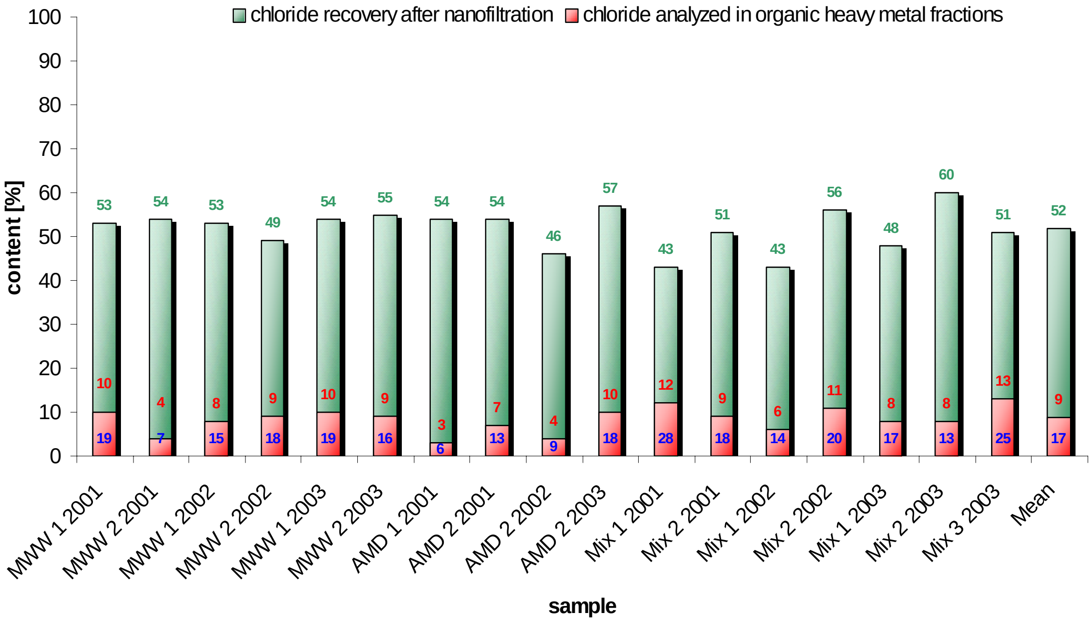


Figure 3.24: Percentage of chloride recovered after nanofiltration and percentage of chloride analyzed in HPSEC fractions of organic heavy metal complexes. Blue numbers represent the percentage of chloride found in organic heavy metal complexes in relation to recovered chloride after nanofiltration.

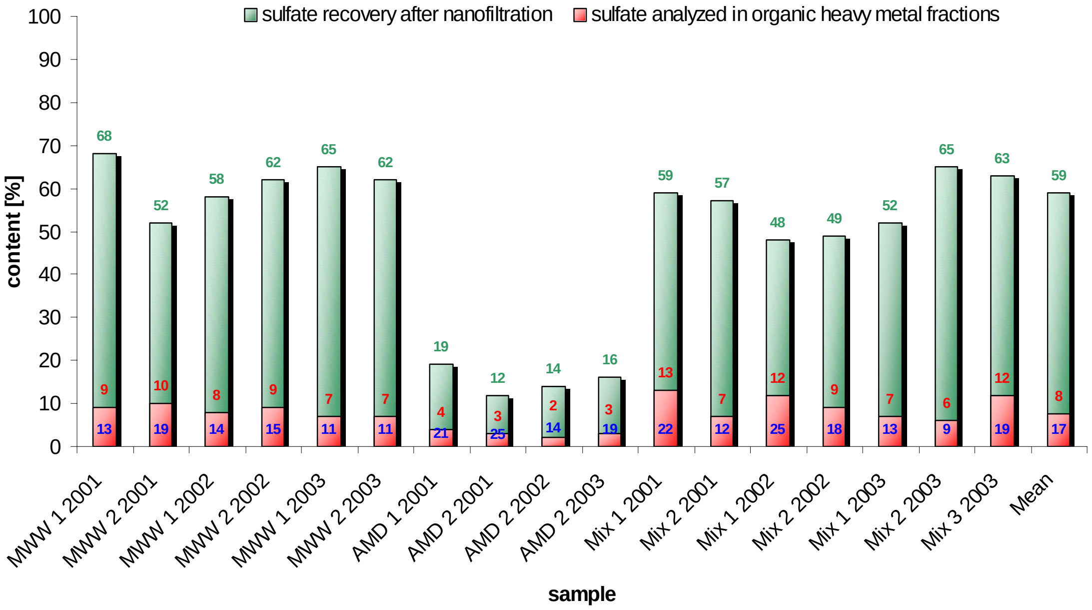


Figure 3.25: Percentage of sulfate recovered after nanofiltration and percentage of sulfate analyzed in HPSEC fractions of organic heavy metal complexes. Blue numbers represent the percentage of sulfate found in organic heavy metal complexes in relation to recovered sulfate after nanofiltration.

These compounds showed significant lower variations than DOC and heavy metals (Si: 13%-38%; chloride: 6%-28%; sulfate: 11%-25%). Variations were lowest in MWW and AMD samples. This slightly changes can also be explained by the high variety of DOC in mixed samples which resulted in the highest complexation potential of these surface waters.

The last paragraphs focused on the contents of elements which were detected in organic heavy metal fractions. As a significant proportion of heavy metals from individual fractions was complexes to more than one DOC fraction this paragraph focuses on **different affinities of heavy metals to different DOC fractions.** Heavy metal contents (µg heavy metal per 1 mg DOC) of all samples which contained two organic heavy metal fractions were plotted in figure 3.26.

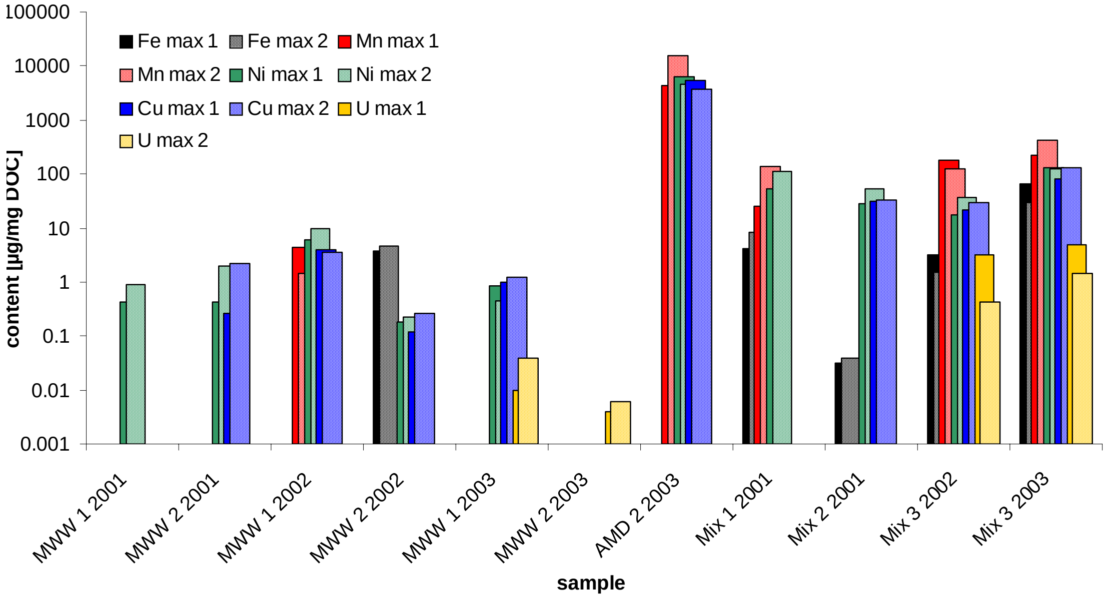


Figure 3.26: Iron, manganese, nickel, copper and uranium contents from HPSEC fractions containing organic heavy complexes. Contents are presented for water samples holding two organic heavy metal fractions to show different affinities of heavy metals to different DOC agglomerations.

It was differentiated between heavy metal contents referring to individual UV maxima 1 and heavy metals referring to UV maxima 2. It could be shown that various samples (MWW1 2001; MWW 2 2001; MWW 2 2002; MWW 2 2003; Mix 1 2001 and Mix 2 2001) had similar heavy metal affinities to different DOC fractions. Heavy metals of these samples had higher affinities to DOC detected at UV maxima 2 than to DOC detected at maxima 1. In contrast, various heavy metals from samples MWW1 2003; MWW 1 2002; AMD 2 2003; Mix 3 2002 and Mix 3 2003 had contrary affinities. In MWW 1 2003 copper and uranium had higher affinities to DOC detected at UV maximum 2 where nickel showed higher affinity to UV maximum 1. In MWW 1 2002 manganese and copper showed similar affinities in contrast to nickel which had a higher affinity to DOC detected at UV maximum 2. In sample AMD 2 2003 nickel and copper had contrary affinities to manganese where in sample Mix 3 2002 nickel and copper had contrary affinities to iron manganese and uranium. Only one AMD sample had 2 UV maxima and in this sample only manganese showed higher affinity to UV maximum 2. This proves again that most AMD samples contained of only one major DOC agglomeration to which all heavy metals had elevated affinities. In contrast MWW samples and mixed samples which had more varying DOC ligands resulted in changing affinities.

Structural correlations of organic heavy metal complexes with common retention maxima were identified by comparing their UV spectra. At the beginning of chapter 3.3.2 specific retention times of organic heavy metal complexes in natural water samples were shown. HPSEC investigations on complexes from municipal waste waters resulted in highly different retention times whereas complexes from AMD samples resulted in similar retention times. As mixed samples included MWW and AMD these samples hold 1-3 UV maxima representing organic heavy metal complexes. The last paragraphs showed for municipal waste waters high variations in heavy metal concentrations and also high variations in heavy metal affinities to corresponding organic ligands. Acid mine drainages showed in contrast to MWW and mixed samples affinities to organic ligands.

Interpretation of UV spectra resulting from UV maxima of dominant complexes shall now show if beside similar retention times also similar structural characteristics can be determined. Structural characteristics were compared using results from the years 2001, 2002 and 2003. Identification was performed by interpretation of UV traces at 254 nm, the mean heavy metal concentrations (calculated from Fe, Mn, Ni, Cu and U concentrations) in elevated fractions and corresponding UV spectra. Components of the AMD could be sub classified as persisting compounds whereas organic species of the municipal waste water were classified as permanently transforming or changing material.

UV Chromatograms of MWW 1 and MWW 2 from samples 2001, 2002 and 2003 were not identical (Appendix II: Figure: II-1). As shown before up to three UV maxima of a single MWW sample correlated with elevated heavy metal concentrations but there was no significant correlation between the retention times of these organic heavy metal complexes observed between 2001 and 2003 (Appendix II: Figure: II-2). Investigations of organic heavy metal standards showed that equal compounds in similar solutions had identical retention times. As retention times of organic heavy metals from municipal waste waters from 2001, 2002 and 2003 were not similar it can be assumed that these complexes had different characteristics.

Organic heavy metal complexes of AMD eluted with maxima between 16.2 min and 16.5 min. As shown before the fractions of organic heavy metal complexes from 3 individual samples had constant retention times and correlating heavy metal concentrations over three years (Figure: 3.16 and Appendix II: Figure: II-3 and II-4). As UV spectra resulting from organic heavy metal complexes remained also similar over the whole sampling period (maxima at 197 nm, 210 nm, 233 nm, and 262 nm) (Figure: 3.27) similar structures of the complexes could be assumed. Additionally samples AMD 1 had a common maximum at 345 nm and samples AMD 3 at 313 nm respectively. These varying intensity maxima resulted from slightly different characteristics of the organic ligands which can be explained by different locations of the sampling points.

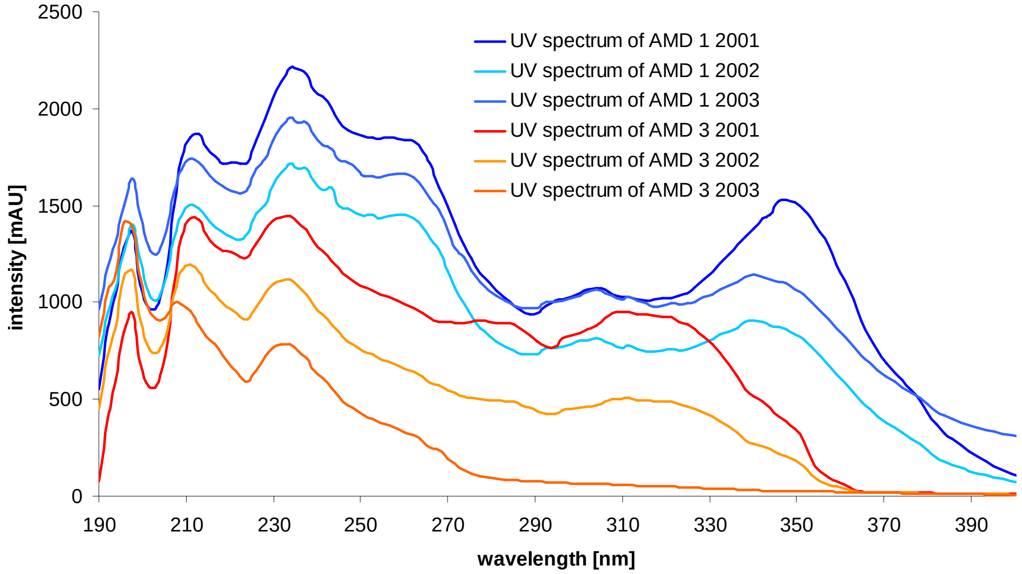


Figure 3.27: UV spectra of acid mine drainage samples from 2001, 2002 and 2003. Spectra show traces over the whole sampling period.

Separation of downstream samples resulted in two fractions representing organic compounds correlating with elevated heavy metal concentrations (Figure: 3.17 and Appendix II: Figure: II-5 and II-5). The first fraction was detected between 15.5 min and 15.7 min and the second fraction of the mixed samples was detected between 16.5 and 16.9 min (presented in an orange and a green plane in figure 3.17). These maxima of UV chromatograms represented organic heavy metal complexes. A third UV maximum detected at numerous downstream samples did not represent organic heavy metal complexes as these maxima did not correlate with elevated heavy metal concentrations. Comparison of the 8 UV spectra at maximum 1 (15.5 min to 15.7 min) resulted in significant different traces referring to different DOC species resulting from MWW (Appendix II: Figure: II-7). The interpretation of UV spectra from UV maximum 2 (16.5 min – 16.7 min) (Figure: 3.28) showed highly similar traces during the whole sampling period.

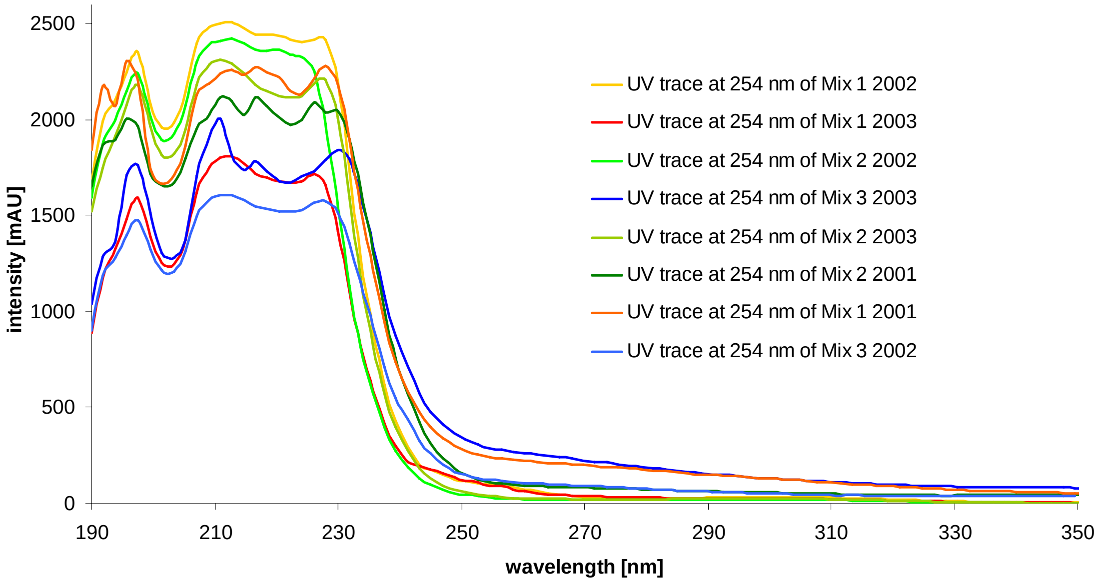


Figure 3.28: UV spectra of the common UV maximum 2 of mixed downstream samples from 2001, 2002 and 2003.

Common maximum intensities at 197 nm, 210 nm and 228 nm referred to similar DOC structures like known from AMD samples. Samples Mix 1 2001, 2 2001 and 3 2003 had a further maximum at 217 nm. The constancy of UV maximum 2 in all chromatograms and corresponding constant UV spectra gave strong impact on similar organic heavy metals in all mixed samples during the whole sampling period.

Therefore organic heavy metal complexes deriving from municipal waste water samples were dominated by changing UV maxima over the years 2001, 2002 and 2003. This reflected different DOC species resulting from continuous transformation or new generation of DOC. In contrast organic heavy metal complexes from AMD were continuously detected with similar UV spectra, reflecting dominant and constant DOC species. Downstream samples Mix 1, Mix 2 and Mix 3 included labile organic heavy metal complexes resulting from DOC similar to DOC found in municipal waste waters and also persistent organic heavy metal complexes from AMD. These persistent organic heavy metal complexes suggested a dominant influence of compounds deriving from AMD.

The focus in the last paragraph was set on the comparison of HPSEC-UV results of similar samples taken in the years 2001, 2002 and 2003. This comparison resulted in the characterization of stabile, constant and dominant organic heavy metal complexes in AMD and mixed samples. The focus in this paragraph is set on the **influence of AMD on mixed downstream samples taken in 2003**. To elaborate the suggested influence of characteristic components from AMD on the formation of organic heavy metal complexes UV traces, corresponding mean heavy metal concentrations and UV spectra were interpreted (Appendix II: Figure: II-8 and II-9). The samples MWW 1, MWW 2, AMD 2, Mix 1, Mix 2 and Mix 3 sampled in 2003 had a common UV maximum between 16.5 min and 16.7 min (Appendix II: Figure: II-8). Corresponding to that maximum, elevated mean heavy metal concentrations were detected in all samples apart from MWW 2 (Appendix II: Figure: II-10). Spectra of municipal waste water 1 and municipal waste water 2 had maxima at 197 nm and 217 nm respectively (Figure: 3.29).

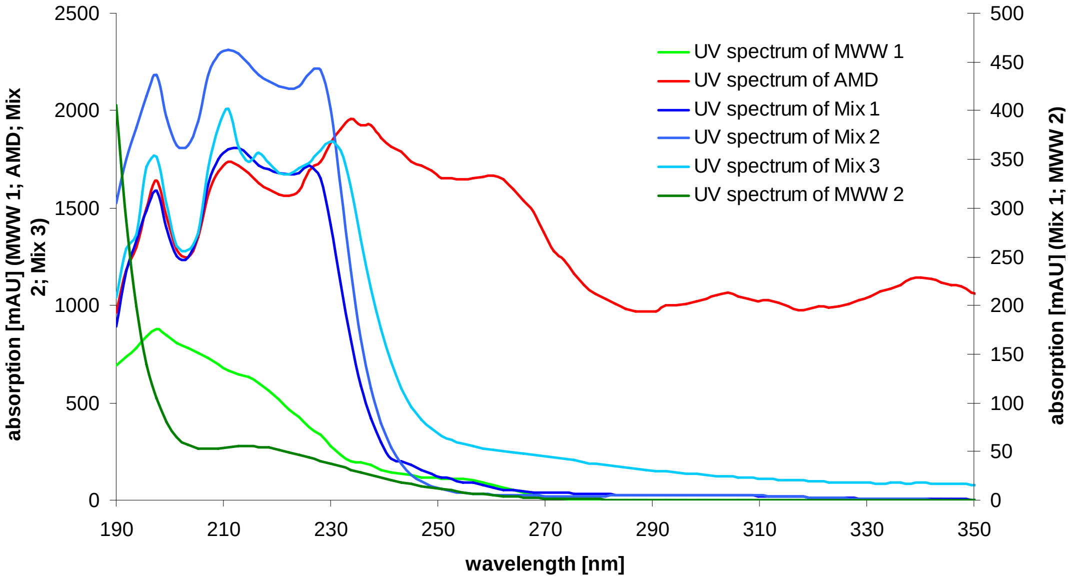


Figure 3.29: UV spectra at the common UV maximum (16.5 min-16.7 min) of municipal waste water, acid mine drainage and mixed downstream samples from 2003.

Similar traces of AMD 2, Mix 1, Mix 2 and Mix 3 showed maxima at 197 nm, 210 nm and 228 nm – 234 nm. At 230 nm the absorption of Mix 1, Mix 2 and Mix 3 decreased rapidly whereas adsorption of AMD 2 remained between 2000 mAU and 1000 mAU. Similar retention times of organic heavy metal complexes and complying UV spectra in the region between 190 nm and 230 nm from AMD 2 and Mix 1, 2 and 3 gave strong advice to common components deriving from AMD collector. These compounds seem to be major compartments of organic heavy metal complexes in AMD and mixed samples and seem to be persistent in these surface waters.

Identified fractions with continuously transforming and new generating organic heavy metal species deriving from municipal waste waters and fractions of persistent species deriving from AMD could be separated by HPSEC-UV. Fractionation of volumes up to 600 ml per separated fraction followed by freeze drying allowed a preparative isolation of organic heavy metal complexes of the investigated surface waters. Further investigations like pyrolysis could therefore be achieved on solid matter which is more stable than aqueous solutions of organic heavy metal complexes. Main focus for further characterization was set on the following organic heavy metal fractions analyzed in UV chromatograms:

- Humic acid-nickel (HA-Ni) sample at pH 6; fraction 10 min – 12 min (App. I: Figure: I-10)
- MWW 1 sampled in 2003; fraction: 15.5 min – 17.0 min (App. II: Figure: II-1)
- AMD 2 sampled in 2003; fraction: 16.0 min – 17.5 min (App. II: Figure: II-3)
- Mix 1 sampled in 2003; fraction: 16.3 min – 17.3 min (App. II: Figure: II-5)
- Mix 2 sampled in 2003; fraction: 16.0 min – 17.0 min (App. II: Figure: II-5)
- Mix 3 sampled in 2003; fraction: 16.3 min – 17.3 min (App. II: Figure: II-5)

3.4 Sources of DOC detected in surface waters

Nanofiltration and HPSEC-UV investigations resulted in the separation of organic heavy metal complexes which were individually fractionated and freeze dried. The organic ligands in individual fractions of municipal waste water were identified as less stable and metabolizing components. Additionally the amount and therefore the variety of organic ligands in MWW was higher than in AMD samples. As the majority of municipal waste waters resulted from household sewage it can be assumed that the majority of organic ligands also resulted from household sewage and biogenic metabolites of these sewages. A more detailed characterization on these compounds was done by pyrolizes-gas chromatography. The results of these analytics are described in the next chapter.

In contrast to MWW samples AMD samples hold lower concentrations of organic ligands but significant more stable and dominant species. Therefore the source of this DOC should be different to the source of DOC analyzed in MWW. As shales from the spoil pile material hold up to 6% organic carbon (Gatzweiler, Paul et al. 1997) degradation of this carbon could result in dissolved carbon compounds and could therefore result in DOC ligands for heavy metals in AMD samples. This assumption and a possible way of degradation were investigated in an additional laboratory study (Wengel, Schmidt et al. 2005). Aim of this study was to investigate the biogenic degradation of refractory organic fractions of black shales by the wood-rotting fungi *Schizophyllum commune*. Further on the associated release of heavy metals was analyzed. Incubation experiments over 1 day, 7 days, 28 days and 84 days were performed by growing the fungus in minimal medium (mainly glucose and aspartic acid) containing black shales. Additionally, blank and control samples were incubated. After harvest the samples were separated in aqueous and solid phase where DOC and heavy metal concentrations were analyzed. Analogical to the surface water samples, inoculated aqueous samples containing minimal medium (MM), grained black shales (BS) and fungi cultures (F) were additionally fractionated by HPSEC-UV to characterize organic heavy metal complexes.

Concentration of degraded organic compounds in the aqueous phase of the inoculated black shale sample (MM+BS+F) decreased from 3900 mg/l to 210 mg/l during 7 days. From day 7 to day 84 the concentration increased to 288 mg/l (Figure: 3.30). In contrast, DOC concentrations in the black shale control sample (MM+BS) decreased constant from 4210 mg/l to 3114 mg/l. This rapid decrease of DOC in all samples that included *Schizophyllum commune* during the first 7 days could be explained by metabolization of the minimal medium. After 7 days the fungus had metabolized most carbon sources and in the following period up to 84 days the DOC concentrations in the inoculated black shale sample (MM+BS+F) increased in contrast to the blank sample (MM+F) and the control samples (MM+BS).

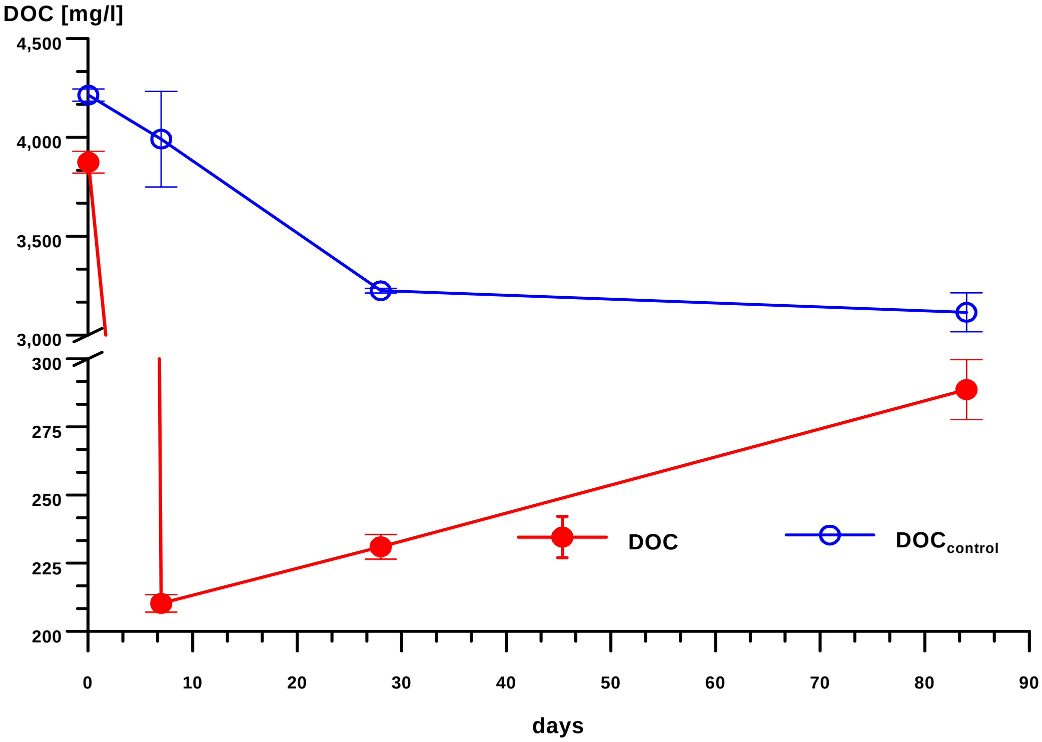


Figure 3.30: DOC concentrations in the aqueous inoculated black shale sample (MM+BS+F), DOC$_{control}$ concentrations in the aqueous control sample (MM+BS)

This significant increase was explained by degradation of high molecular weight organic molecules of the solid material by the fungus. Interpretation of UV chromatograms and

UV spectra evidenced this result. Organic heavy metal complexes in the aqueous black shale samples (MM+BS+F) were analyzed in accordance with HPSEC-UV investigations of the described natural surface water samples (Figure: 3.31). Elevated UV detection in correspondence with elevated heavy metal concentrations at retention times between 8.1 min and 10.5 min could be assigned to heavy metal complexes with organic ligands which resulted from degraded organic matter from the spoil pile material. Ligands of organic heavy metal complexes detected at higher retention times (10.5 min – 13.5 min) were identified as remains and metabolites of the minimal medium (MM).

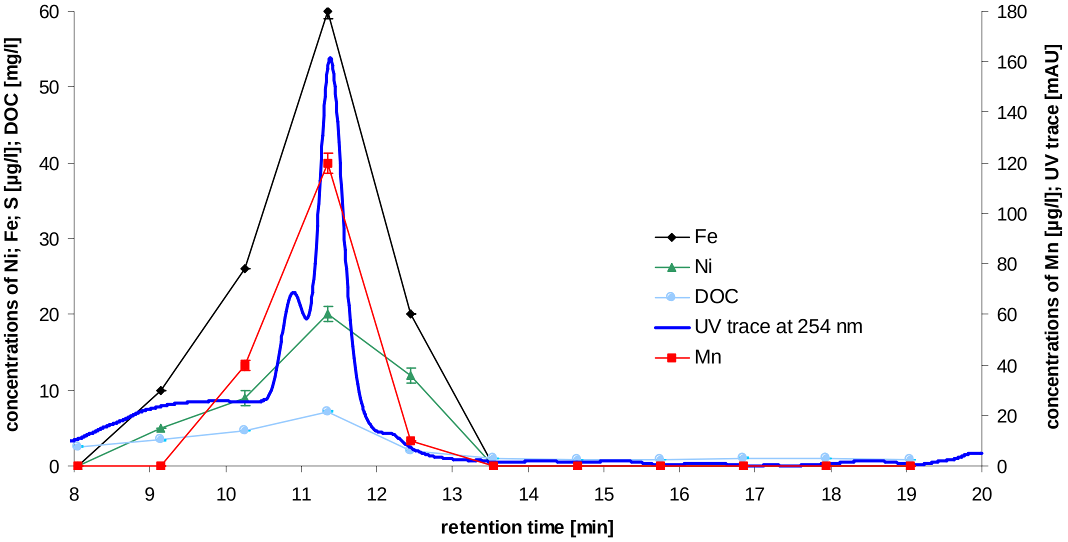


Figure 3.31: Concentrations of DOC, Fe, Ni and Mn in the individual fractions of the 84 day inoculated black shale sample (MM+BS+F) after size exclusion chromatography and UV trace of the 84 day inoculated black shale sample (MM+BS+F)

The increase of DOC in inoculated aqueous black shale samples (MM+BS+F) and the formation of new organic heavy metal complexes proved the degradation of solid organic compounds from shales by the fungus *Schizophyllum commune*. Results given in this short summary were confirmed with control and blank samples. A detailed description of the study is given in the submitted article presented in appendix III.

3.5 Further analytical investigations on freeze dried organic heavy metal fractions: pyrolyzes of freeze dried organic heavy metal fractions

As household sewages and black shales from spoil pile material were supposed to be potential sources for DOC in the investigated surface waters, pyrolytical investigations should assure these assumptions. Freeze dried fractions of organic heavy metal complexes of the synthetic HA-$Ni(NO_3)_2 \cdot 6H_2O$ sample and of the 5 samples from the sampling procedure in June 2003 (MWW 1, AMD 2 and Mix 1-3) were pyrolyzed and separated by gas chromatography. Peaks of pyrolysis products from field samples were sorted alphabetically starting with the first characterized peak of the municipal waste water 1. Peaks generated from similar chemical molecules obtained the same character and additionally an index starting with 1. Consequently, identical molecules in different samples obtained the same character (Figures: 3.32 to 3.37). The single element traces for carbon, oxygen, sulfur, nitrogen and silicon resulting from pyrolysis-GC-AED were summarized in Table 3.10.

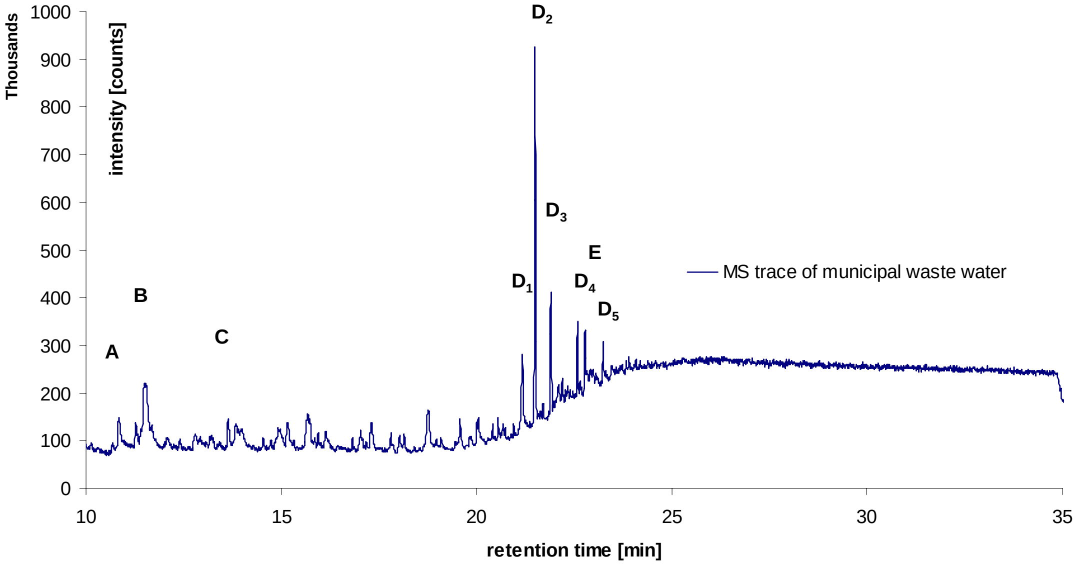


Figure 3.32: MS trace of the freeze dried organic heavy metal fraction from municipal waste water 1 with identified peaks A, B, C, $D_{1\text{-}5}$ and E

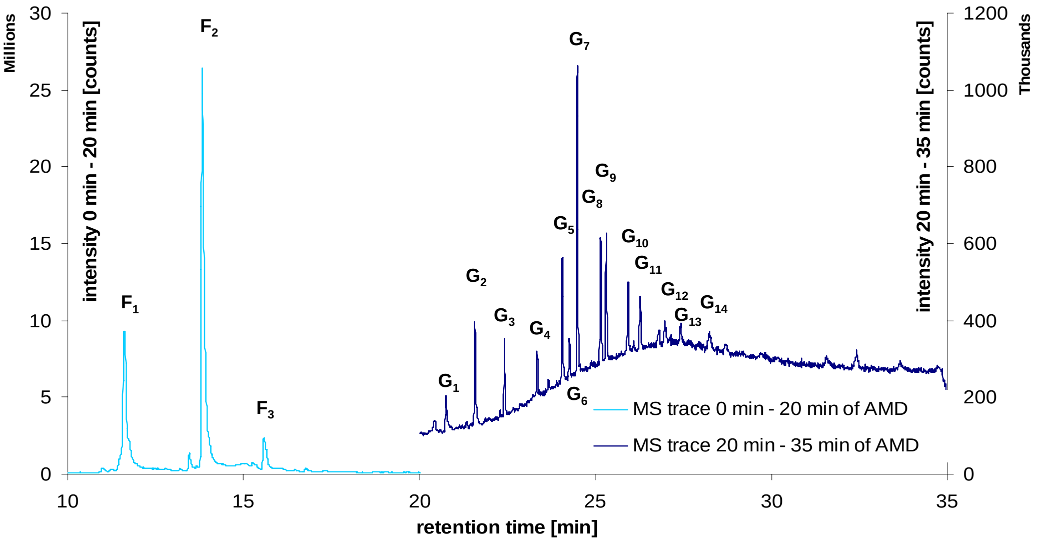


Figure 3.33: MS trace of the freeze dried organic heavy metal fraction from acid mine drainage 2 with identified peaks $F_{1\text{-}3}$ and $G_{1\text{-}14}$

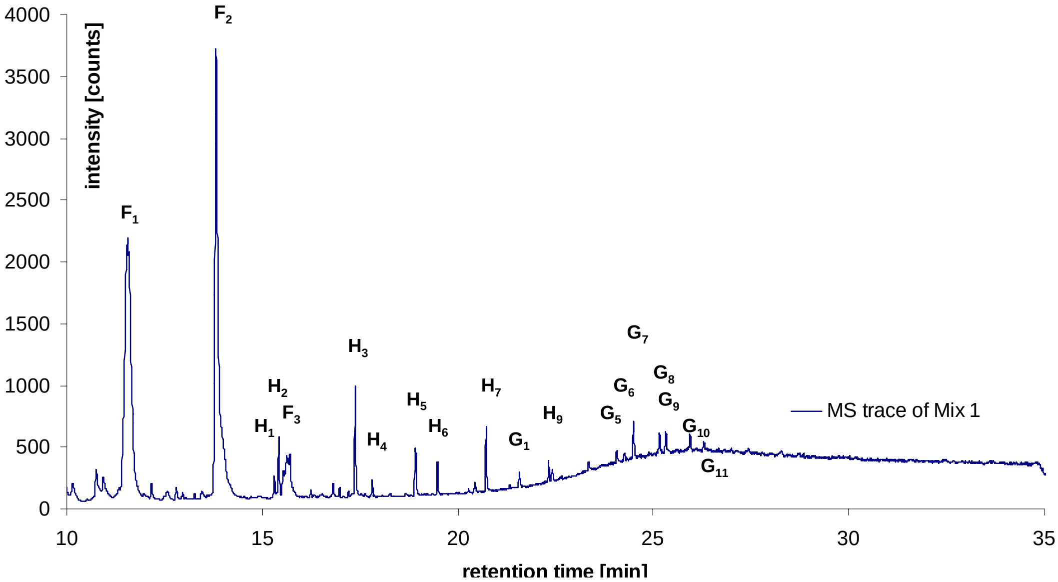


Figure 3.34: MS trace of the freeze dried organic heavy metal fraction from Mix 1 with identified peaks $F_{1\text{-}3}$, $H_{1\text{-}7}$ and $G_{1,\,5\text{-}11}$

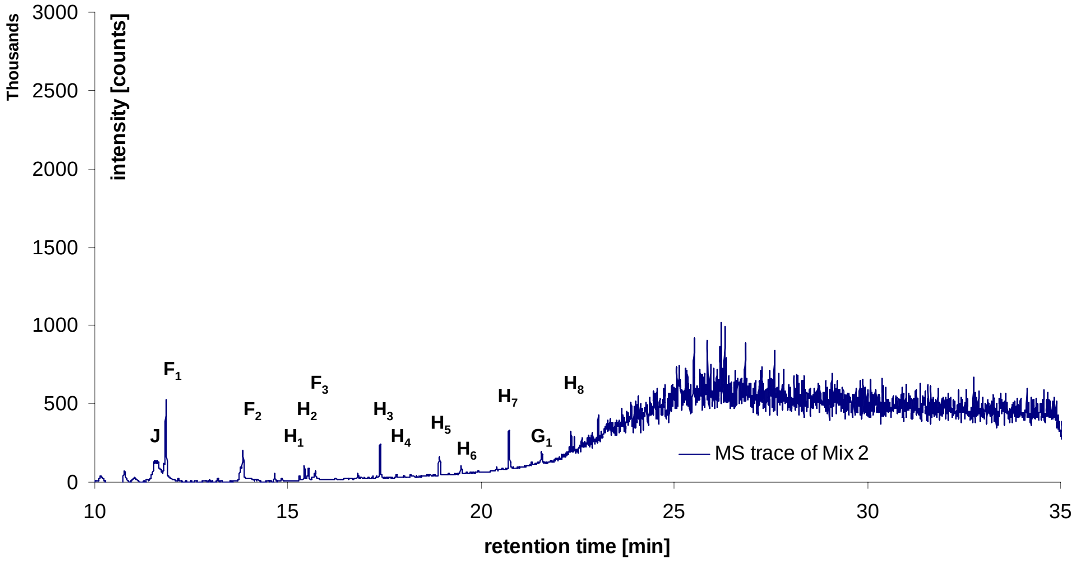


Figure 3.35: MS trace of the freeze dried organic heavy metal fraction from Mix 2 with identified peaks F_{1-3}, J, H_{1-8} and G_1

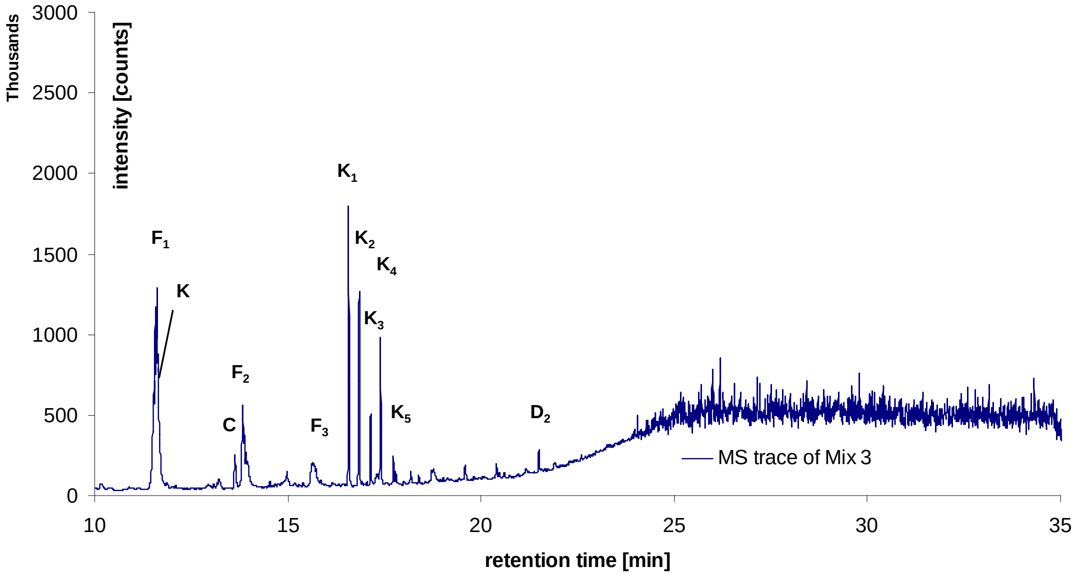


Figure 3.36: MS trace of the freeze dried organic heavy metal fraction from Mix 3 with identified peaks F_{1-3}, C, K, K_{1-5} and D_2

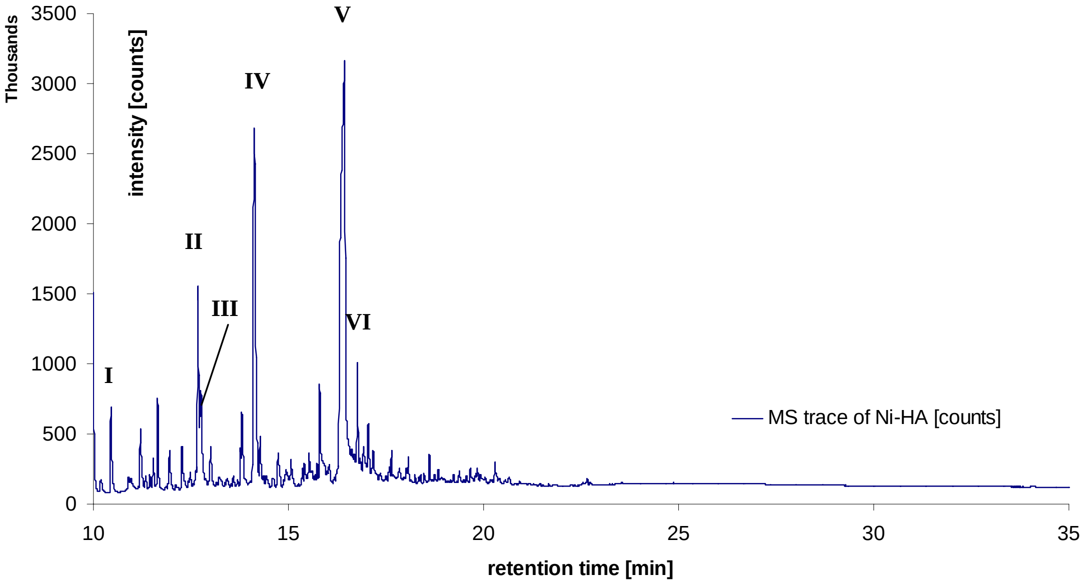


Figure 3.37: MS trace of the freeze dried organic heavy metal fraction from synthetic standard humic acid-nickel (HA-Ni) with identified peaks I, II, III, IV, V and VI.

3.5.1 Pyrolysis GC-AED

First investigations of the freeze dried samples were achieved by pyrolysis GC-AED. Attention was given to signals resulting from the elements oxygen, sulfur, nitrogen and silicon appearing simultaneous to carbon signals. Reproducibility of the analytical setup was proved by frequently injections of cellulose standards during the whole analytical investigation. Pyrograms of the different elements gave information of composition and therefore of the source of pyrolyzed organic heavy metal complexes. Table 3.10 does not show absolute intensities of oxygen, sulfur, nitrogen and silicon. It is only quoted if these elements were present or not. Pyrograms with absolute intensities are given in Appendix III: Figures III-1 to III-5.

Carbon peaks A and B at retention times 10.9 min and 11.6 min of municipal waste water 1 mirrored fragments including carbon but no oxygen, no sulfur, no nitrogen and no silicon (Table 3.10 and Appendix III: Figure: III-1). At retention times of peaks $D_{1\text{-}5}$ (21.2 min, 21.5 min, 21.9 min, 22.6 min and 23.3 min) only carbon and sulfur signals were detected. Peak E eluted at 22.8 min and represented carbon and oxygen, sulfur, nitrogen and silicon were not detected. The pyrograms of MWW 1 reflected hydrocarbon structures for fragments A, B and C whereas peaks $D_{1\text{-}5}$ and E showed beside carbon also sulfur peaks. These sulfur signals suggested hetero groups. As no oxygen was detected, inorganic SO_4^{2-} adsorptions on the detected organic material can be excluded.

Signals $F_{1\text{-}3}$ were detected in the acid mine drainage at 11.6 min, 13.8 min and 15.6 min and peaks $G_{2\text{-}4}$ were detected between 20.8 min and 28.3 min (Table 3.10 and Appendix III: Figure: III-2). Oxygen, sulfur and silicon detections beside carbon in peaks $F_{1\text{-}3}$ and $G_{2\text{-}4}$ suggested either inorganic adsorptions on the separated organic heavy metal complexes or the occurrence of heteroatoms in the DOC ligand matrix. However, the detection of carbon, oxygen, sulfur and silicon in the same fraction gave advice that these detected fragments included organic components and were indicated in the complexation of heavy metals. Detected sulfur peaks in fragments F and G referred to elevated sulfur concentrations of the AMD caused probably by pyrite oxidation of the spoil pile material. Peaks F and G were also detected in samples Mix 1, 2 and 3. It needs to be inserted on this point that peaks F and G were more detailed analyzed by mass spectrometry (MS). Interpretation of MS investigations lead to the result that peaks F and G could also result from column bleeding which could overlay carbon peaks from natural field samples. As Py-GC-AED interpretation of these peaks gave less structural information the interpretation if peaks F and G resulted from column bleeding can not finally be answered on that point and will be discussed in an extra chapter.

At the freeze dried fraction of Mix 1 GC signals F_{1-3} appeared at 11.6 min, 13.8 min and 15.6 min with signals of carbon, oxygen and silicon. Sulfur was only detected at peak F_2. Chemical group G was detected in Mix 1 between 21.6 and 26.3 min (Table 3.10 and Appendix III: Figure: III-3). As retention times and detected elements were similar to pyrolyzes fragments of the AMD it can be assumed that acid mine drainage is possibly the source of the separated organic heavy metal complexes of Mix 1. No detections of sulfur in peaks F_1 and F_3 in sample Mix 1 lead to the assumption that sulfur components of the fractionated agglomeration were separated downstream from AMD sampling point. Detected signals G represented carbon and silicon peaks corresponding to AMD. In contrast to AMD, sample Mix 1 showed more fragments holding carbon and silicon signals (G_{5-7}). The widespread chemical group H_{1-8} was detected at 15.3 min, 15.4 min, 17.7 min, 17.8 min, 18.9 min, 19.5 min, 20.7 min and 22.3 min. All detections represented only carbon signals

GC signals F_{1-3} of Mix 2 appeared in correlation with AMD and Mix 1 signals at 11.8 min, 13.8 min and 15.6 min. Like in AMD and Mix 1 oxygen and silicon was detected in all peaks F (Table 3.10 and Appendix III: Figure: III-4). These detections gave further assumptions that AMD is the potential source for organic heavy metals of the mixed samples. Additionally sulfur was detected in F_{1-2}. The detection of sulfur in the two fragments assumed another time that the organic heavy metal agglomerations consist of labile sulfur components which can be included or excluded of the matrix. Peak G_1 was detected at 21.6 min representing again the elements carbon and silicon. Signals H_{1-8} in the sample Mix 2 eluted at 15.3 min, 15.4 min, 17.4 min, 17.9 min, 18.9 min, 19.5 min, 20.7 min, and 22.4 min. These retention times and traces were identical to the programs of sample Mix 1. Therefore these peaks represented organic compounds found in both samples. According to Mix 1 no other signal beside carbon was detected. Oxygen, sulfur and nitrogen signals referring to heterogenic carbohydrates were detected at peak J which eluted at 11.6 min.

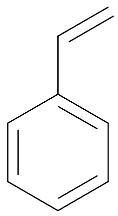
F_{1-3} eluted another time at 11.6 min, 13.9 min and 15.7 min (Table 3.10 and Appendix III: Figure: III-5). As in the previous samples oxygen and silicon signal were detected. Peak F_3 hold no oxygen trace probably because of too low concentrations. Signals K_{1-5} eluted between 16.6 and 17.7 minutes. No oxygen, sulfur, nitrogen and silicon were detected at these retention times. Therefore these peaks suggested carbohydrates. Peak D_2 was detected at 21.5 min as carbon and sulfur trace representing sulfur compounds in the carbon matrix.

Signals F_{1-3} appearing in samples AMD, Mix 1, Mix 2 and Mix 3 showed decreasing intensities from AMD downstream to Mix 3. With respect to the possibility that silicon and carbon peaks of signals F and G resulted from column bleeding decreasing intensities mirrored nevertheless dilution effects of these compounds in the downstream samples. Further interpretation of dominant species which resulted in peaks F and G is given in an extra chapter. Less dominant and less mobile organic compounds were mirrored in fragments A, B, D, J, H, and K as these peaks were detected in only one or maximal two following downstream samples.

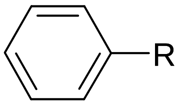
3.5.2 Py GC-MS

First the freeze dried organic-nickel fraction of the synthetic humic acid-nickel solution was analyzed (Figure: 3.37). The pyrograms hold MS peaks in the retention area between 10 min and 24 min. Corresponding to its precursor substances 6 peaks were identified as methylfuran (I), dimethylfuran (II), propylfuran (III), C3 alkenes (IV), cyclic or aromatic amine (V) and furan (VI). Major pyroysis fragments of the organic ligand were therefore furans. This was in good accordance with fragments of the initial humic acid. Pyrolysis of the humic acid raw material attempted in the Forschungszentrum Rossendorf (FZR) resulted in the identification of methylfuranes and dimethylfuranes as major fragments beside CO_2 and acetone (personal report of S. Sachse from FZR). Therefore the humic acid-nickel complex hold major compounds of the initial humic acid which proved the elevated complexing potential of humic acid to nickel ions.

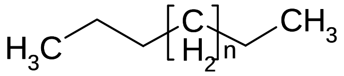
Signals of pyrograms of the organic heavy metal fractions from the 5 samples from June 2003 were characterized like described before. Figures 3.32 to 3.36 show representative pyrograms with identified signals. Characterization was achieved by means of data bases and literature search. Signals could be identified or at least a good approximation was possible (Table: 3.10 and Appendix III; Figures III-7 to III-11). Main signals which needed to be identified were signals F_{1-3} which appeared in the acid mine drainage and in all mixed samples and signals H_{1-8} which appeared in the samples of Mix 1 and Mix 2 (Table: 3.10 and Appendix III: Figures III-7 to III-11). Identification of few signals needed to be proceeded only manual as the digital library search of the MS system could not exclude background and overlapping signals. Figure 3.38 shows a variety of identified structures.



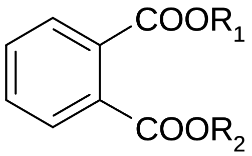
B
(phenyl derivates)



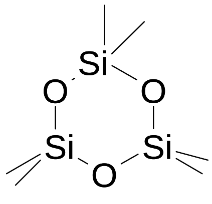
C
(styrene)



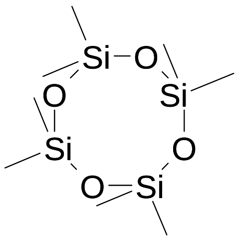
D_{1-5}
(alkanes)



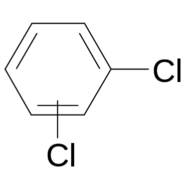
E
(phthalates)



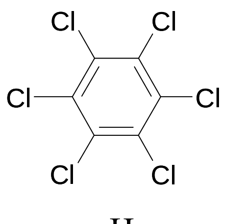
F_2
(cyclotrisiloxane hexamethyl)



F_1
(cyclotetrasiloxane octamethyl)



$H_{1/2}$
(dichlorobenzene)



H_8
(hexachlorobenzene)

Figure 3.38: Structures of pyrolyzes products detected by mass spectrometry. Analyzed freeze dried fractions hold organic heavy metal complexes from municipal waste water, acid mine drainage and mixed samples in 2003

Fragments identified in the municipal waste water were characteristic for DOC of household sewages and untreated sewage effluents (Kracht 2001). Signal A of the municipal waste water 1 was identified as alkene with a chain length between C5 to C7. Signal B represented a phenyl derivate whereas signal C resulted of a styrene. Peaks D_{1-5} were identified as aliphatic alkanes and aromates with aliphatic alkene side chains. Fragment E resulted of a phthalate unit (Figure 3.32, Table 3.10 and Appendix III: Figure III-6). All these fragments are possible organic ligands for heavy metals.

The major fragments of the AMD were signals F_{1-3}. These cyclosiloxanes were analyzed more detailed in an extra paragraph. Signals G_{1-14} of the AMD mirrored silyl organic compounds. The organic parts of the molecules were identified as alkyl units (Figure 3.33, Table 3.10 and Appendix III: Figure: III-7). Identified structures are given in figure 3.38. Reproducibility of the detected fragments was proved by pyrolyzing the corresponding, separated organic heavy metal fraction of sample AMD 2 2001 (Appendix III: Figure: III-11). Peaks F_{1-3} were also found in this sample. Minor detection was found for peaks G_{1-14}. Therefore reproducible structure analyses of the organic heavy metal fractions from AMD resulted in the identification of organic silicon compounds. The organic parts of the fragments were predominantly methyl groups. Typical fragments for DOC (Kracht 2001) without silicon units could not be identified in AMD samples. As no organic fragments without silicon units were identified and as methylsiloxanes were major part of the GC column material it needed to be considered that the analyzed peaks resulted from column bleeding. If column bleeding would cause the analyzed peaks, components from separated organic heavy metals of the AMD would be responsible for strong interactions with the column material, resulting in column peaks which overlapped the DOC peaks from the natural complexes.

According to the AMD sample signals F_{1-3} of the samples Mix 1 and Mix 2 were also identified as cyclosiloxanes. Further on both samples included fragments H_{1-8}. Fragments H_{1-2} were identified as different stereo isomers of dichlorobenzene. H_{3-4} reflected isomers of trichlorobenzene, H_{5-6} represent tetrachlorobenzene whereas H_7 represented pentachlorobenzene and H_8 was identified as hexachlorobenzene (Figures: 3.34 and 3.35, Table 3.10 and Appendix III: Figures: III-8 and III-9). Signal J which appeared only in Mix 2 was identified as a phenyl derivate which is typical for DOC. Reproducibility of silicon organic compounds in Mix 2 was proved as similar peaks were also detected in the corresponding organic heavy metal fraction of sample Mix 2 2001. Beside further organic silicon compounds no chlorobenzenes were detected in 2001. In separated and pyrolyzed organic heavy metal fractions from samples Mix 1 and Mix 2 were in contrast to the AMD sample beside silicon organic compounds further organic compounds identified. These were mainly chlorobenzenes (Table: 3.11).

Table 3.11: Relative intensities of specific m/z values resulting from Py-GC-MS of freeze dried organic heavy metal fractions. Relative intensities of analyzed chlorobenzenes from Mix 2 and Mix 3 were compared with literature values.

		relative intensity [%]		
$H_{1/2}$	m/z	Mix 1	Mix 2	databa/lit
Dichlorobenzene	38	8	21	5-19
MW= 146 g/mol	50	40	88	12-45
	75	81	110	18-60
	111	52	47	27-62
	146	100	100	100
$H_{3/4}$	m/z	Mix 1	Mix 2	databa/lit
Trichlorobenzene	74	72	110	12-40
MW=180 g/mol	109	37	63	13-40
	145	31	47	26-42
	180	100	100	100
$H_{5/6}$	m/z	Mix 1	Mix 2	databa/lit
Tetrachlorobenzene	37	8	12	2-15
MW= 214 g/mol	50	5	18	2-8
	74	51	80	6-40
	108	24	32	12-35
	216	100	100	100
H_7	m/z	Mix 1	Mix 2	databa/lit
Pentachlorobenzene	37	4	8	0-24
MW= 248 g/mol	73	30	50	1-40
	108	27	34	10-62
	125	2	3	2-13
	143	14	16	7-24
	178	9	13	7-17
	215	24	24	14-35
	250	100	100	100
H_8	m/z	Mix 1	Mix 2	databa/lit
Hexachlorobenzene	36	17	10	15-20
MW= 282 g/mol	71	20	39	7-27
	107	27	50	18-42
	142	34	64	30-48
	177	20	28	8-15
	214	30	32	15-30
	249	49	50	25-35
	284	100	100	100

Relative intensities resulting from MS investigations were in good accordance with intensities of database spectra (between 93% and 99%). As these chlorobenzenes were identified in organic heavy metal fractions it can be assumed that they act as organic ligands in samples Mix 1 and Mix 2. Such a compound could possibly be sandwich complexes (Hollemann and Wiberg 1995) or adsorptions of heavy metal cat ions on electronegative chloride units. In contrast to silicon organic compound these chlorobenzenes were detected only in samples from 2003. This referred to organic ligands which were found only once and indicated a temporary source resulting e.g. from agricultural or anthropogenic pollution near sampling points Mix 1 and Mix 2. Fragments of the furthest downstream sample Mix 3 mirrored signals of municipal waste water and AMD. Signal C identified as styrene and signal D_2 a substituted aromat were also detected in the municipal waste water. Signals F_{1-3} resulting of cyclosiloxanes from AMD were also detected in sample Mix 3. Further on signals K_{1-5} were identified as substituted aromates (Figure 3.36, Table 3.10 and Appendix III: Figure III-10). Signals C and D_2 were also detected in the organic heavy metal fraction of the municipal waste water. As these fragments and also fragments K_{1-5} represented typical DOC units it can be resumed that they are molecular units of the DOC ligands.

Special interest was set on the identification and characterization of cyclosiloxanes in the fractions of AMD, Mix 1, Mix 2 and Mix 3. Signal F_1 represents cyclotrisiloxan hexamethyl, F_2 cyclotetrasiloxan octamethyl and F_3 cyclopentasiloxan decamethyl. These three signals were detected in all four samples at similar retention times with decreasing intensities from AMD downstream to Mix 3. Data base and literature search on these compounds leaded to specific mass spectra of the three cyclosiloxanes. Specific m/z values from literature and the corresponding analyzed relative intensities are given in Table 3.12. Analyzed MS spectra are given in Appendix III: Figures III-7 to III-10. Most specific m/z values of cyclotrisiloxan hexamethyl (molecular weight 222 g/mol), cyclotetrasiloxan octamethyl (molecular weight 296 g/mol) and cyclopentasiloxan decamethyl with a molecular weight of 370 g/mol were in good accordance with literature values (Table: 3.12).

Cyclosiloxane fragments in organic heavy metal fractions were identified in AMD and mixed samples from 2003. Identification of these species in samples from 2001 (Appendix III: Figure III-11) proved the reproducibility of the fragments. Nevertheless, as in AMD samples no further DOC fragments were detected it must be taken into account that all silicon organic peaks refer to column bleeding caused by components of the organic heavy metal fraction. Such components could e.g. be traces of inorganic salts which were accelerated during pyrolysis and caused local damages of the GC column by striking out organic silica. Therefore this organic silica could result in peaks which overlap DOC peaks from natural organic heavy metal complexes.

Table 3.12: Relative intensities of specific m/z values resulting from Py-GC-MS of freeze dried organic heavy metal fractions. Relative intensities of analyzed cyclosiloxanes from AMD, Mix 1, Mix 2 and Mix 3 were compared with literature values.

fragment		**relative intensity [%]**				
F_1	m/z	AMD	Mix 1	Mix 2	Mix 3	databa/lit
Cyclotrisiloxan	73/75	6	5	9	4	4
hexamethyl	95/97/98	5	6	13	6	14-35
MW=222g/mol	119/120	4	3	6	2	5
	133/134	11	13	33	10	10-30
	191/192/193	25	25	32	25	10-30
	207/208/209	100	100	100	100	100
	222-227	60	65	70	64	1
F_2	m/z	AMD	Mix 1	Mix 2	Mix 3	databa/lit
Cyclotetrasiloxan	73/75	13	13	28	13	5-11
octamethyl	103	1	1	4	1	3
MW=296 g/mol	133/134	3	3	7	5	5-13
	191-195	16	20	30	30	12-14
	207-209	12	14	34	22	5-13
	249-251	18	18	22	21	4-10
	265-267	58	62	76	80	6-15
	281	100	100	100	100	100
	299	44	43	55	48	1
F_3	m/z	AMD	Mix 1	Mix 2	Mix 3	databa/lit
Cyclopentasiloxan	43	13	25	60	39	6-13
decamethyl	73-75	100	100	100	100	85-100
MW=370 g/mol	133	1	3	4	4	0-3
	179	3	4	2	3	0-5
	207-211	15	14	13	11	3
	248-253	12	9	6	7	4-10
	267	65	57	45	42	40-70
	285	50	50	50	37	0
	323-325	3	5	2	2	1-6
	355	10	5	1	3	25-100

3.5.2 Py GC-IRMS

The GC effluent of the organic heavy metal fractions from 5 samples of June 2003 was splited. The minor part was detected as CO_2 in the ion trap after oxidation. The CO_2

traces of the fractions from municipal waste water 1, AMD, Mix 1, Mix 2 and Mix 3 from 2003 were analyzed depending on its carbon isotope ratios.

As assumed the $\delta^{13}C$ values resulting from signals of the municipal waste water 1 were between -24‰ and -32‰ (Table: 3.10). The $\delta^{13}C$ values of the peak A was -28‰, where peaks B and C reached -32‰ and -29‰ respectively. Peaks D_{1-5} resulted in values of -29‰, -24‰, -30‰, -30‰ and -30‰. Finally peak E, the phthalate resulted in a $\delta^{13}C$ value of -29‰. These values reflected good accordance with $\delta^{13}C$ investigations of sewage effluents and waste water treatment plants.

Analyzed CO_2 peaks of the AMD represented mainly silicon organic compounds. The cyclosiloxanes F_{1-3} resulted in relatively low $\delta^{13}C$ values (-48‰ - -47‰). Silylorganic fragments G_{1-15} showed higher values (-45‰ - -32‰). According to AMD peaks separated and freeze dried organic heavy metal fraction from sample Mix 1 showed similar values for the peaks F_{1-3} (-49‰ - -47‰). The values for chlorobenzenes of these sample were between -29‰ and -27‰. Cyclosiloxanes in the sample Mix 2 had $\delta^{13}C$ values between -48‰ and -45‰. Chlorobenzenes of Mix 2 appeared in the CO_2 trace between -31‰ and -28‰. The $\delta^{13}C$ value of the phenyl derivate was -33‰. Styrene found in sample Mix 3 had a $\delta^{13}C$ value of -28‰ and the identified peak D_2 -24 ‰ respectively. Cyclosiloxanes had isotopic carbon ratios between -52‰ and -44‰. Substituted aromates K_{1-5} had a $\delta^{13}C$ values between -24‰ and -27‰.

Isotopic carbon ratios of cyclosiloxanes detected in organic heavy metal fractions of AMD, Mix 1, 2 and 3 had low $\delta^{13}C$ value for surface waters. The low $\delta^{13}C$ values of these compounds need to be discussed in detail. Further isotopic carbon ratios were typical for DOC resulting from biogenic compounds or untreated sewages.

4 Discussion

4.1 Investigations on heavy metals and DOC from raw water samples

Investigations of heavy metal concentrations in raw water samples resulted in a not linear decrease of heavy metal concentrations from the AMD 2 downstream to sample Mix 3 (Figure 3.5). As there were two dilution points (when the AMD entered the creek and when the creek Gessenbach run into the creek Badergraben) a higher decrease of heavy metals was expected. Relatively high heavy metal concentrations in the farer downstream sample may resulted either of diffuse entries of heavy metals in the creek or of increasing mobility of inorganic heavy metals caused by DOC complexation. Diffuse runnels held elevated heavy metal concentrations during remediation operations (Geletneky 2002) which decreased significant to concentrations under the critical value by finishing remediation between 2001 and 2003. Therefore it could be assumed that in samples from 2001, 2002 and 2003 the non linear decrease of heavy metal concentrations in downstream samples was caused by DOC complexation resulting in an increase of heavy metal mobility.

Between the sampling point of AMD and Mix 1 strong oxidizing conditions (Eh between 400 mV and 600 mV), low DOC and elevated heavy metal concentrations resulted in precipitation of manganese IV and iron III species. The elements nickel and copper, occurring mainly as water soluble Cu II and Ni II species had more mobile inorganic compounds than iron and manganese and did therefore not precipitate (Falbe J. 1996-1999; Lin, Burns et al. 1998; Green, Heil et al. 2003). Hence, nickel and copper solubility was less influenced from surrounding redox conditions and both elements showed higher or similar fluxes in downstream samples. Largest physicochemical changes of the surface waters were analyzed between sampling point AMD 2 and Mix 1. These changes in conductivity, pH value and redox potential were the most influencing factors on heavy metal mobility between these two sampling points. Under the analyzed conditions iron and manganese were less mobile than copper, nickel and uranium (Figure 3.5). Like shown before, iron mobility was strongly influenced by the redox potential. Strong oxidizing conditions resulted in precipitates which were blocked by the anthropogenic barrier. This barrier was installed after the first sampling procedure in 2001. Therefore investigations at Mix 1 in 2001 resulted in elevated relative iron fluxes (22%) in contrast to investigations at Mix 1 in 2002 (0.1%) and 2003 (0.4%). These different concentrations caused by blocking of iron precipitates resulted in high error bars for iron in figure 3.5. Elevated error bars were also calculated for uranium fluxes. In contrast to iron, uranium showed elevated flux changes during the whole sampling procedure at all sampling points. This could be explained as iron fluxes were mainly influenced by the redox potential of the surface water and uranium fluxes were additionally influenced by changing pH values. Uranium forms a higher variety of soluble species than iron (e.g. uranyl complexes). These species are more sensitive to changing physicochemical conditions than species of the other investigated heavy metals.

Downstream of sampling point Mix 1 the redox potential decreased up to 150 mV which could result in an increase of the solubility potential of manganese and iron species between 100 and 1000 times (Porter, Bajita-Locke et al. 2004). Significant changes of redox potential, conductivity and pH value between sampling points AMD 2 and Mix 1 were not continued in the downstream samples Mix 2 and Mix 3 therefore further precipitations of heavy metals between Mix 1 and Mix 3 could be neglected. As DOC concentrations increased significant in the downstream samples Mix 1-3 in contrast to AMD samples, heavy metal mobility was downstream from Mix 1 on stronger influenced by DOC complexation. Organic heavy metal complexes generated upstream of sampling point Mix 1 were probably still persistent in the downstream samples despite of slightly changing redox potential and pH values (Garcia-Casal and Layrisse 2001). The presence of DOC as potential ligand for heavy metals increases their mobility significant (Andrade, Miyazawa et al. 2002). As more DOC reflected a broader range of available ligands, a higher variety of mobile organic heavy metal complexes could be formed (Yin, Impellitteri et al. 2002; Chen, Wang et al. 2004). Nevertheless precipitations of weak bound heavy metals would also occur under slightly changing conditions. Such immobile precipitates could be remobilized by complexation caused by changing physicochemical or biogenic conditions (Kuma, Katsumoto et al. 2000; Porter, Bajita-Locke et al. 2004). Therefore heavy metal precipitates were not permanent immobile and could change their mobility depending on the physicochemical and biogenic environment. As biogenic conditions in surface waters of the creeks Gessenbach and Badergraben changed frequently because of seasonal incidents the main influence on heavy metal mobility beside precipitation were changing DOC concentrations (Bourg and Bertin 1994).

The mobility of iron and manganese species was dominated by the redox potential and also by DOC concentrations of the surrounding environment. In contrast, nickel and manganese mobility was mainly dominated by DOC concentrations and was therefore higher in the investigated field site. Caused by different oxidation states and multiple soluble and insoluble oxide species estimation on uranium mobility was more speculative. Nevertheless investigated results acted on the assumption of higher uranium mobility than iron and manganese species but lower or similar mobility than nickel and copper species.

4.2 Validation of the analytical methods nanofiltration and HPSEC-UV by standard solutions

4.2.1 Validation of the nanofiltration set up

Standard samples of individual weak and strong organic heavy metal complexes retained unmodified after nanofiltration. Chromatograms and spectra of a mixed standard sample obtained before and after the concentration process were also similar whereas its retention maxima did not correlated with retention maxima of its educts (Appendix I: Figure I-7). With respect to the validity of UV spectra on molecular structures, spectra of the mixed samples correlate with spectra of its educts (Appendix I: Figure I-8). Consequently the retention shift of the mixed sample was not caused by the concentration process. This retention shift resulted either from interactions with the stationary phase of the HPSEC column or from the formation of new species immediately after mixing the standard solutions. Both reasons causing retention shifts were minor important for the nanofiltration of field samples. The modification of strong organic heavy metal complexes in natural raw waters under identical conditions will not occur because these samples are in a chemical equilibrium. Modifications of complexes with stability constants between log k = 6 and log k = 36 during the concentration procedure were also excluded by concentrating relevant standard samples. Further on the reproducibility of chromatograms from field samples obtained before and after nanofiltration confirmed that no modification occurred during concentration by nanofiltration.

4.2.2 Validation of the HPSEC-UV system

HPSEC investigations of standard complexes showed influences on retention time caused by pH value and inorganic salt concentration of the aqueous samples. These influences resulted from interactions of the analyte with the stationary phase. A decrease of such interactions is generally obtained by using phosphate buffers and/or EDTA additives as mobile phase (Sigg and Stumm 1996; Piccolo, Conte et al. 2002; Diallo, Simpson et al. 2003). Using such a mobile phase heavy metals of the AMD would precipitate as heavy metal phosphates in the HPSEC system. EDTA would complex heavy metals and destroy weaker naturally occurring organic heavy metal complexes. As these side reactions would strongly modify the separation of organic heavy metal complexes deionized water was used as mobile phase. Deionized water increases hydrophobic interactions of the analyte and the stationary phase. Further on changes of the ionic strength in the HPSEC system influence the retention of organic compounds. Nevertheless, like shown on standard solutions this retention effects did not change the composition of the organic heavy metal complexes. With view to the intension of preserving natural conditions it was important to obtain unmodified organic heavy metal complexes after HPSEC. Retention shifts, influencing size determinations were less important than the exclusion of heavy metal complex modification by mobile phase additives. The reproducibility of the method was proved by reproducible chromatograms of PEG solutions which were injected frequently (Appendix I: Figure I-17).

Main retention shifts caused on different ionic strengths were detected at EDTA-Ni standard solutions holding $Ni(NO_3)_2$ overloads. The separation of the overloaded inorganic fraction and the EDTA-Ni complex was proved in chromatograms (Appendix I: Figure I-16). Beside this separation the organic EDTA-Ni fraction in the 1:1 sample eluted earlier than in the 1:2 and 1:3 samples. This effect could only be explained by specific interactions between the complex and the column material. As adsorption of inorganic ions on the organic complex increased with increasing $Ni(NO_3)_2$ concentrations the size of the complex increased too. This inorganic ions bound on EDTA-Ni increased its ionic strength. Different ionic strengths resulted in different retention of the EDTA-Ni solutions with overloads 1:1, 1:2 and 1:3. Further retention shifts were detected in EDTA-Fe solutions at pH values 3 and 6. As the adjustment of EDTA-Fe to pH 3 was done with HCl the ionic strength in the solution was also changed. Cl^- ions adsorbing to EDTA changed the ionic strength of the complex and therefore caused specific interactions with the column material, resulting in retention shift. Further pH values were adjusted with different acids (HNO_3, H_2SO_4) causing no retention shifts. These few critical results, obtained with standard solutions did not correlate with results observed in HPSEC investigations of the contrary field samples MWW and AMD. Chromatograms detected before and after nanofiltration of these two samples showed identical maxima. Additional to maxima detected in the raw water samples two more maxima were detected in the concentrate samples (Figure: 3.9). These maxima resulted from enrichment of natural components by nanofiltration. Therefore changing pH values and inorganic salt concentrations did not cause modification of the detected species. This can be explained as retention shifts caused by changing inorganic strengths occur only at ionic species which were able to interact with organic heavy metal species. However retention shifts caused by changing pH values and inorganic salt concentrations could not be excluded. According to the results obtained by HPSEC of standard complexes retention shifts were caused by changing inorganic strengths and not by modification of organic heavy metal complexes. These simplified standard materials could not completely meet the conditions of the field samples and the reproducibility of two field samples could not represent all field samples. Nevertheless the investigations of standard materials proved a sufficient efficiency of nanofiltration and HPSEC for the enrichment and separation of organic heavy metal complexes from surface waters. Consequently the method allowed the separation of freeze dried organic heavy metal complexes from surface waters.

4.2.3 Size validation of the HPSEC column by standard solutions

Retention shifts caused by changing pH values and inorganic salt concentrations interfered strongly the size determination of organic heavy metal complexes. Approximations of size determination were only possible for retention maxima of the same or of similar samples. Interpretations of standard samples showed that a correlation between salt concentration and degree of retention shift could not be calculated. Therefore size determinations or size comparison of organic heavy metal complexes from the contrary samples MWW and AMD would probably result only in roughly approximations. As all organic heavy metal complexes from surface waters were separated and the HPSEC column separates between 9.5 min and 18.5 min an approximation of sizes could be a length between 1 nm and 400 nm which correlated approximately with 1 kDa to 400 kDa. As AMD helds elevated to high salt concentrations these approximations probably mirrored too small sizes. Nevertheless using spherical and aliphatic molecules for size calibration is a powerful tool. Size determination of aqueous samples with similar physicochemical parameters should result in good approximation. Most size determination studies investigated only straight-chain aliphatic standards. Using straight-chain aliphatic and spherical standards resulted in two calibration curves representing upper and lower size limits at a specific retention edge (Figure: 3.12). Further on size standards should be used as internal standards.

4.3 Analytical investigations on natural field samples

4.3.1 Recoveries of DOC, heavy metals, silicon and anions after nanofiltration and HPSEC

Comparing overall mass balances after nanofiltration and HPSEC resulted for most elements in lower recoveries after HPSEC than after nanofiltration. This was predominantly found for precipitating heavy metals iron and manganese in the years 2001 and 2002. Lowered recoveries of DOC, Ni, Cu or U resulted probably on co precipitation. These low precipitations occurred continuously during the analytical process and could only be stopped by freezing or freeze drying of the samples. Therefore lower recoveries after HPSEC of few samples resulted from precipitation after nanofiltration. As in handling of samples from 2002 and 2003 extreme attention was given on fast proceeding and generation of freeze dried material recovery was improved. Nevertheless precipitation could not completely be stopped. However as mainly inorganic compounds precipitated the separation of organic heavy metal complexes was only slightly influenced. With view to varying recoveries, the content of elements and ions in the fractionated organic heavy metal agglomerations also varied. Nevertheless, calculation of the content of DOC, heavy metals, silicon, chloride and sulfate proved that a higher percentage of DOC and heavy metals was included in organic heavy metal fractions than occurred as inorganic salts. With respect to the calculated recoveries after nanofiltration detected compounds in organic heavy metal fractions showed mostly contents smaller than 50% of the recovered element (Figures: 3.18-3.25). This high variability of values resulting from total mass balance after nanofiltration can be explained by strongly varying sizes of dissolved compounds resulting in strongly varying amounts of separation. As after nanofiltration all samples include dissolved components of the same size window (1 nm-450 nm) the amounts of inorganic salts and further on of dissolved compounds separated by HPSEC differed slightly. As similar amounts of dissolves compounds were separated by HPSEC, the portions of complexed material with view to recovered material after nanofiltration were also similar.

4.3.2 HPSEC investigations on field samples

HPSEC of field samples was performed on concentrates from 2001, 2002 and 2003. DOC from municipal waste water samples of the villages Ronneburg and Kauern eluted at varies retention times. 1-3 elution maxima of the MWW samples represented organic heavy metal complexes. Physicochemical and biogenic parameters of these MWW samples were similar. Therefore identical retention times represented similar organic heavy metal complexes. However, as retention times of these similar waters were not constant over the sampling period size and composition of DOC and organic heavy metal compounds varied significant. Varying DOC composition leaded to the assumption that characteristics of the complexing DOC from MWW changed frequently.

Organic heavy metal compounds from 3 samples of acid mine drainages eluted at similar retention times and had similar UV spectra over the whole sampling period. As there was no shift in the retention of organic heavy metal complexes (Appendix II: Figure: II-3 and Figure 3.27) remediation actions and seasonal weather effects probably changed the concentrations of DOC and heavy metals but not the characteristics of the organic heavy metal complexes. Consequently compounds from AMD were constant in size and composition. The degree of heavy metal release from AMD into the creeks Gessenbach und Badergraben was closely connected to the redox stage of the heavy metals. Less release of iron and manganese into the creeks was caused by precipitation under oxidizing conditions. In contrast to less mobile precipitates of iron and manganese mobility of soluble nickel, copper and uranium species was minor affected by the displacement of the creek Badergraben.

Sources of organic ligands analyzed in the mixed water samples were municipal waste water and AMD. The dominance of organic ligands from AMD could be described with UV traces and spectra of the aqueous samples. Chromatograms of the mixed water samples showed two fractions representing dissolved organic heavy metal complexes. DOC of the first fraction (retention time 15.5 min to 15.7 min) could be allocated to municipal waste waters. UV spectra of this fraction differed strongly which referred to different organic ligands and therefore to a variety of DOC species (Appendix II: Figures II-5 and II-7). As AMD samples hold low DOC concentrations in contrast to MWW samples elevated variety of DOC species in mixed samples referred also to MWW as DOC source. The pyrograms und mass spectra of MWW samples were only slightly mirrored in the mixed downstream samples. Finally it can be said, that DOC from municipal waste waters in the Ronneburg field site was ligand for heavy metals but its composition and size changed continuously.

DOC reflected by the second fraction (16.5 min to 16.7 min) could be allocated to AMD (Appendix II: Figure II-5). UV spectra of these samples were very similar and agreed between 190 nm and 230 nm with the UV spectra of the acid mine drainage (Figures 3.28

and 3.29). The UV spectra differed only slightly in the individual mixed samples over the whole sampling period. Further on UV spectra of the downstream mixed samples of the same year were very similar. Pyrograms of the AMD sample and the 3 mixed water samples had several common peaks which were characterized in mass spectrometry as very similar silicon organic molecules. If these common peaks resulted from fragments of the field samples or from overlapping column bleeding will be discussed in the next chapter. However, UV chromatograms and corresponding UV spectra identified common organic heavy metal complexes in AMD, Mix1, Mix 2 and Mix 3. These separated fractions represented stable compounds occurring in the whole investigated surface waters downstream from AMD. These complexes hold more constant structures than complexes from MWW samples. Therefore it can be assumed that organic compounds from AMD dominated the organic transport of heavy metals. MWW samples included a variety of organic ligands with different physicochemical and biogenic properties. Organic heavy metal complexes resulting from this DOC were less stable compared to organic heavy metal complexes resulting from AMD. As DOC in AMD had significant lower concentrations, the range of organic ligands was also lower. Therefore only very similar organic heavy metal complexes were detected in AMD samples.

Inorganic species Si, Cl^- and SO_4^{2-} had exclusion maxima predominantly at the exclusion limit of the HPSEC column. This indicated the occurrence of inorganic salts of the three species. Nevertheless minor concentrations of Si, Cl^- and SO_4^{2-} correlated with UV maxima and elevated heavy metal concentrations. This second elution maxima supported the fact that separated organic heavy metal complexes did still include inorganic species.

UV detection of DOC which is able to complex heavy metals was achieved at a wavelength of 254 nm. This wavelength was widely used in DOC research (Vogl and Heumann 1997; Specht, Kumke et al. 2000; Perminova, Frimmel et al. 2003). Investigated UV spectra resulted additionally in maxima at other wavelengths. Such maxima were for example detected in the wavelength region 210 nm (AMD) or 230 nm (AMD and mixed samples). Low to moderate UV absorption at 254 nm was detected from most samples whereas maximum absorption at 254 nm was less detected. This can be demonstrated by comparing spectra from mixed samples and AMD (Fig.: 3.29). Maximum absorption was obtained at wavelengths smaller 250 nm where a strong increase of absorption was detected in the region 254 nm. Therefore further investigations should focus on several specific wavelengths for different water samples.

Especially for AMD samples the 210 nm trace seemed to be more specific than the 254 nm trace.

4.4 Investigations on the DOC sources of AMD

Investigations on potential sources for DOC ligands of AMD samples focused on the degradation of refractory organic matter from spoil pile material. These organic compounds from black shale were found to be degradable by fungus. The degradation of solid organic compounds by the wood-rotting fungus Schizophyllum commune could be proved in a laboratory study. It was proved that the solid material was metabolized or transformed into soluble material. It could also be shown that this degradation was accompanied by the release of heavy metals. It could not ascertain if heavy metals from black shale were inert to metabolization processes of the fungus. Possibly, heavy metals were absorbed by fungus, metabolized and released as organic heavy metal complexes. Another opportunity is that heavy metals were not involved in metabolization processes and exclusively acted as donor elements in the aqueous phase to complex all possible kind of DOC. Nevertheless organic heavy metal complexes resulted from biogenic degradation of organic spoil pile material.

4.5 Characterization of fragments from selected fractions of freeze dried organic heavy metal complexes by pyrolysis-gas chromatography (Py-GC)

4.5.1 Analytical investigations resulting from Py-GC coupled with atomic emission detection (AED) and mass spectrometry (MS)

Results of analytical investigation from Py-GC-AED and Py-GC-MS were conform in most pyrograms. Fragments detected in AED were mostly confirmed in MS. Almost all fragments of the municipal waste water sample were found with both detection methods and could be characterized as typical DOC components. Peak C of the municipal waste water was only found in mass spectrometry. As this styrene fragment showed low detection in MS and MS was more sensitive as AED for carbon detection it could be assumed that peak C was overlapped from background detection in AED. Fragments D (substituted aromates) included sulfur signals in AED which could not be conformed in MS. Most likely the sulfur peaks in AED resulted from carbon emission on the sulfur wavelength as carbon emits beside 193 nm also at 179 nm and sulfur at 181 nm.

Low sensitivity for oxygen in AED caused minor detection of fragments G (aliphatic siloxanes) in AMD and mixed samples. Therefore fragments G with low intensities in chromatograms from Py-GC-MS could not be found in AED pyrograms. Most fragments F and G (silicon organic compounds) were found to have additional sulfur peaks. As these sulfur atoms were not found in MS investigations carbon emissions on the sulfur wavelength were most likely the cause of these detections. AED and MS analytics of AMD found exclusively silicon organic species. These fragments were identified as cyclic methylsiloxanes, aliphatic methylsiloxanes and further organic silyl compounds. DOC species comparable with species found in municipal waste waters could not be identified in AMD fragments.

A major question which needed to be discussed is about the source and generation of these organic silicon compounds detected in mass spectrometry. Interpretation of the pyrograms and mass spectra leaded to the assumption that these silicon organic compounds, predominantly methyl siloxanes resulted from column bleeding. This assumption seemed to be correct as the stationary phase of the used GC column mainly consists of dimetyl polysiloxanes. Strong column bleeding is known to result in homogeneous signals with significant higher intensities than the corresponding background (http://www.sge.com/htm/support/troubleshooting/gc_columns/column_bleed_3.asp). If column bleeding was responsible for these peaks, AMD must be the source of the compounds causing the bleeding.

Source of such strong column bleeding could possibly be inorganic salts which were fractionated with organic heavy metal complexes. AMD hold elevated concentrations of inorganic salts in its raw water samples. Lower concentrations of inorganic salts were found in downstream samples Mix 1-3. It was also found that traces of inorganic salts overcame nanofiltration and could not be separated by HPSEC. These inorganic compounds were closely connected with separated organic heavy metal complexes and were therefore also part of the freeze dried fraction. Such salts could cause small damages of the GC column during pyrolysis. These damages could result in loss of column material which will be detected as methylsiloxanes and cyclosiloxanes. As AMD hold higher salt concentrations than Mix 1, 2 and 3 this column bleeding was also highest in fractions from AMD. Therefore inorganic salt components of the organic heavy metal fractions from AMD most likely caused column bleeding which overlapped DOC fragments from natural complexes. Lower inorganic salt concentrations in mixed samples resulted in less intensive column bleeding. Therefore fragments of DOC could be detected parallel to column bleeding.

As silicon was also analyzed as compound of the organic heavy metal agglomeration in AMD and mixed samples it was fractionated and freeze dried within the organic heavy metal fraction. Therefore the stationary phase was may be not the only source for silicon. Additionally all detected signals were representative in its retention times but the $\delta^{13}C$ values of the signals differed significant. As one can assume that the stationary phase of the column has only one carbon source the $\delta^{13}C$ values should be constant for all signals resulting from column bleeding. These $\delta^{13}C$ values further suggest that the signals from silicon organic carbon did not exclusively derive from the stationary phase of the GC column.

The possibility of the presence of organic silicon compounds in the natural fraction could not completely be excluded as water soluble synthetic silicon organic materials are well known (Kolesnik, Goloshchapov et al. 1990). Such compounds especially siloxanes are also known to absorb DOC and act as chelating ligand for heavy metals under norm conditions (Matheisen, Nazarov et al. 1993; Cazacu, Marcu et al. 2004; Czerwinski, Ostrowska-Gumkowska et al. 2004). Anthropogenic silicon organic compounds were detected in the environment and are chemical and biological reactive. Investigations showed for example the condensation of siloxanes with hydroxide groups of cellulose and lignin (Castellano, Gandini et al. 2004). Further investigations asserted the biological degradation of such compounds (Graiver, Farminer et al. 2003). Analytical investigations of aqueous field samples showed that the organo silicon material including siloxanes remained unchanged after HPSEC (Varaprath and Cao 2000) and that these compounds withstand heating up to 250 degrees centigrade (Marciniec, Malecka et al. 2001). At higher temperatures silicon organic material can forms cyclic siloxanes which elute in GC-MS with characteristic mass spectra (Table: 3.11) (Varaprath and Larson 2002; Chojnowski, Cypryk et al. 2003). Such anthropogenic silicon organic materials may possibly find their way into the environment and in the AMD samples by mining activity and environmental pollution in the past.

Beside this anthropogenic source of silicon organic material in aqueous environments only one other aqueous silicon source is yet known in literature. These are biogenic silica which characterization is a main topic in actual research projects. Generation of biogenic silica results on the formation of external skeletons made of amorphous and highly porous silica by aquatic organisms like diatoms or unicellular algae (Gendron-Badou, Coradin et al. 2003). Adsorption of heavy metals and ooze by biogenic silica occurs in pH regions between 3 and 7 (Dixit and Van Cappellen 2002; Michalopoulos and Aller 2004). Beside its function as carrier for heavy metals and pyrite this inorganic silica covered with a biofilm form on its surfaces covalent and electrostatic silica-organic bonds. These molecular units are predominantly silanoles and after condensation siloxanes (Dixit and Van Cappellen 2002; Gendron-Badou, Coradin et al. 2003). However, only small units of biofilms and biogenic silica could be fractionated as only dissolved compounds were sampled. Biofilms are a heterogeneous system of clusters and microbial cells interspersed with channels. Clusters show typical dimensions of 150 µm – 200 µm. These clusters are separated by voids of 50 µm – 100 µm (Beyenal, Donovan et al. 2004; Zacarias, Ferreira et al. 2005). Therefore separated organic heavy metals with traces of silicon were significant smaller than biofilms. These compounds could possibly only be the stable part of an accumulation on a biofilm occurring in biofilm voids. The accumulation needed to overcame the prefiltration process in contrast to the whole biofilm which was separated on the 450 nm prefilter.

Nevertheless, mass spectra of alkane cyclosiloxanes and other silyl organic materials in the AMD and mixed downstream samples did most likely result from column bleeding. The column bleeding was probably caused by inorganic salts which were included in the organic heavy metal fraction. Traces of naturally occurring organic silicon compounds could be a minor source for silicon organic fragments and gave first advice of accumulations of heavy metals on biofilms and biogenic silica. If DOC in samples AMD, Mix 1, 2 and 3 resulted from biofilms or biogenic silica could not definitely clarified. As biofilm formation begins with the adsorption of macromolecules (proteins, polysaccharides and humic acids) and also smaller molecules (fatty acids and lipids) at liquid/solid (e.g. silica minerals or amorphous diatoms) interfaces they held excellent potential for heavy metal ligands (Hamers 1997). As applied analytical investigations were not meaningful enough for the complete characterization of the complex organic heavy metal matrix further investigations need to be attempted. Such investigations should probably start with microscopic analyses of the raw samples to prove the presence of biogenic silica and biofilms.

4.5.2 Interpretation of carbon isotop rations from Py-GC-IRMS of separated fractions from organic heavy metal complexes

Municipal waste waters like effluents from sewage treatment plants generally show values between -21‰ and -34‰. These values agree with the values in the analyzed municipal waste water samples and the values C, D_2 and K_{1-5} of Mix 3. These values were in good accordance with literature (Kracht 2001). Therefore the sources of complexing DOC were household sewages or biogenic metabolites of household sewages. Similar $\delta^{13}C$ values of silicon organic compounds found in the AMD and mixed water samples could be separated in two groups. The first group represented values of methyl cyclosiloxanes (-44‰ to -52‰). The second group, represented values of aliphatic silyl organic compounds (-32‰ to -45‰).

Consequently these low $\delta^{13}C$ values of methyl siloxanes did not originate from municipal waste. Most likely these $\delta^{13}C$ values were in correspondence with the column bleeding caused by inorganic salts. As the $\delta^{13}C$ values scattered between -52‰ and -32‰ further explanations needed to be taken into account. One further explanation beside column bleeding for this low values is the integration of methane into DOC. Methane from soil or surface water could be fixed in the aqueous phase by oxidation processes (van Breukelen and Griffioen 2004). These oxidized molecules could interact with biofilms or other dissolved organic compounds (Grossman, Cifuentes et al. 2002). As methane found in soils had $\delta^{13}C$ values between 50‰ and 57‰ and biogenic matter has $\delta^{13}C$ values between -9‰ and -32‰ a molecule resulting of the interaction of methane and biogenic matter probably has $\delta^{13}C$ values between -28‰ and -52‰.

As degradation of kerogenes found in spoil pile material by biogenic species is known (Wengel, Schmidt et al. 2005) this dissolved oxidized species could e.g. appear as carboxylic acids. If these degraded kerogenes were involved in the formation of silica organic heavy metal complexes its isotopic signal shifted extremely to lower values by metabolization because of biological degradation. This would also be an explanation for the metabolization of kerogenes from water insoluble into water soluble molecules.

However, Py-GC-IRMS is a very powerful analytical method to determine the sources of DOC found in the surface waters.

It is known that solid organic compounds from black shales are degradable by biogenic processes. It is also known that this degradation is accompanied by the release of heavy metals and the formation of dissolved organic heavy metal complexes (Schmidt 2005; Wengel, Schmidt et al. 2005). For comparison of these degraded organic compounds with organic ligands found in AMD and mixed samples Py-GC-IRMS analyses of both species are needed. The interpretation of these analyses of samples AMD, Mix 1, 2 and 3 strongly depends on inorganic salt concentrations in organic heavy metal fractions. Therefore salt concentrations need to be decreased in separated fractions. This would probably result in more specific detection of DOC fragments. Comparing the $\delta^{13}C$ values from degraded kerogenes with signals resulting from AMD, Mix1, 2 and 3 could finally answer the question if the DOC ligands of surface waters resulted from spoil pile material or from different biogenic material. Nevertheless it is questionable how strong the separation of inorganic salts would influence the whole organic heavy metal agglomeration. The analytical methods nanofiltration and HPSEC did not completely separate inorganic salts from organic heavy metal fractions. This result confirmed that agglomerations of organic heavy metal complexes include inorganic salts. The complete separation e.g. by ion exchange would probably change natural characteristics significantly. Such chemical methods would not be possible without changing DOC concentrations.

5 Conclusions

Interpretation of DOC and heavy metal concentrations in raw water samples allowed the calculation of heavy metal fluxes and therefore heavy metal transport in the investigated surface waters. These results led to the assumption that elevated mobility of heavy metals in downstream samples was caused by complexation with DOC. Investigations on mobility and complexation behavior for iron, manganese, nickel, copper and uranium obtained similar properties for iron and manganese contrary to similar properties for the more mobile species nickel, copper and uranium. As the mobility of iron and manganese was strongly influenced by their redox potential both metals were less mobile under oxidizing conditions. Nevertheless mobility of both metals could be improved by complexation with organic ligands.

For environmental protection precipitated iron and manganese species could not be regarded as constant immobile. Slightly changing biogenic conditions causing generation of organic ligands could remobilize iron and manganese precipitates as organic heavy metal complexes. Toxification of the environment by these heavy metals could only be prohibited by separation. Nickel and copper were mobile in most inorganic and organic species and existed therefore as both species in surface waters. Methods for separation of inorganic species are well known whereas the separation of strong organic heavy metal complexes is more difficult and less investigated. Finally it can be said that spreading of heavy metals in the environment as organic heavy metal complexes occurred for all investigated heavy metals. The spreading of iron and manganese needed more time than the spreading of nickel, copper and uranium.

Inorganic and organic standard solutions proved that nanofiltration was the most suitable method to enrich organic heavy metal complexes. This enrichment for further preparative investigations was achieved without destroying the complexes. Fractionation and separation of organic heavy metal complexes by HPSEC was achieved without modification of the complexes. Consequently quick sampling followed by immediately nanofiltration, HPSEC and freeze drying insured the transfer of non to slightly modified organic heavy metal complexes from aqueous field samples to stable freeze dried material.

Retention times of aliphatic and spherical standard molecules with known molecular weight and size resulted in two calibration curves. Size determination of field samples using this calibration curves resulted in insufficient approximations caused by interactions of the analyte with the stationary phase. With respect to the transfer of unmodified organic heavy metal complexes these interactions were not reduced by modification of the mobile phase. Nevertheless using two calibration curves obtained by spherical molecules and additionally by aliphatic molecules was a first step for better size determination of aqueous molecules. The use of aliphatic and spherical standard molecules in future investigations would have the advantage to get upper and lower boarder for molecular sizes determination of dissolved compounds. To avoid problems in size determination caused by differing chemical conditions like ionic strength the method needs to be modified. A promising modification would be the use of the described standards as internal standards. Therefore the direct comparison of standard size and analyte size would be possible.

DOC and organic heavy metal complexes detected in municipal waste water samples were not constant in size and stability. These samples were characterized by constant changes of organic species resulting from household sewage. This could also be shown on changing affinities of heavy metals to DOC in MWW samples. Due to HPSEC investigation, DOC and heavy metals leaving the spoil pile dominated the creek Badergraben and were found as organic heavy metal compounds in all downstream samples. Beside these organic heavy metal complexes from AMD which had strong and focusing affinities to only one common DOC fraction, less stable complexes were detected in mixed samples. Such compounds were detected over shorter distances and resulted most likely from household sewages and diffuse runnels of the surrounding agriculture.

Finally it can be concluded that all investigated heavy metals were mobile. One needs to differentiate between inorganic and organic heavy metal species. The mobility of inorganic heavy metal species is easier to predict than the mobility of organic heavy metal complexes. All investigated heavy metals formed beside their inorganic species organic heavy metal complexes where its mobility strongly depended on the stability of the complex and therefore on the composition of DOC. However, heavy metals in aqueous systems can never be regarded as immobile and can therefore be transported on all aqueous pathways in the environment. The degree of mobility is only responsible for the time heavy metals need to spread in the environment.

An incubation experiment proved the degradation of organic matter and the release of heavy metals from black shale by fungi. Therefore black shale from spoil pile material is a potential source for dissolved organic heavy metal ligands. UV detection of the aqueous phase of the degraded material proved the formation of new and larger heterogenic organic compounds by biological degradation of black shale. Interpretation of UV spectra, unfortunately yield less information on the molecular structure. However, UV absorptions indicate aromatic compounds such as benzenes and phenols, carboxylic groups or long chain alkenes which could be degradation products of black shale. Therefore, organic heavy metal complexes from refractory organic matter were released biogenical and entered subsequently the environment. Unfortunately degraded dissolved organic compounds could not be detected in freeze dried organic heavy metal fractions of AMD and mixed samples. This was probably caused by elevated detections of organic siloxanes overlapping natural DOC signals.

MS detections of freeze dried fractions from AMD were correlated with elevated silicon concentrations. At the actual state of scientific knowledge the generation of organic silicon compounds can not be clarified. However the detection of silicon beside DOC in equal HPSEC fractions must be considered. This indicates the existence of silicon species in the organic heavy metal fractions.

It was further found that organic heavy metal agglomerations hold beside silicon other inorganic salts. This was mainly found in AMD and mixed samples. The mild separation methods nanofiltration and HPSEC could not completely separate these salts. Beside such salts it was proved that separated and freeze dried fractions hold organic heavy metal complexes from field samples. Such matrices could exist of small parts of biofilms complexing heavy metals which were composed of biogenic, geogenic or anthropogenic organic matter. Most likely biofilms were composed of symbioses of different organic matter. Precursor substance could be geogenic kerogenes from the spoil pile, biogenic aquatic matter and organic matter resulting from anthropogenic pollution. Pyrolytical investigations of such diverse mixtures of organic and inorganic material were complicated or partly impossible as inorganic salt components caused strong column bleeding.

In conclusion the separation method was able to produce freeze dried samples of organic heavy metal complexes but this sample material was not boundless suitable for further characterizations. As the organic heavy metal complexes consist of complicated individual matrices the freeze dried samples need special treatment depending on the characterization method. For such characterization it must be decided how strong the sample matrix needs to be changed and how strong this treatment will affect the organic ligands. Further investigations for structure characterization like infrared spectroscopy or

NMR could possibly result in more detailed structure characteristics. Such further investigations on up to now less studied organic heavy metal complexes are important for the knowledge on mobility and transport of heavy metals as well as on complexation potential of DOC.

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Appendix I

Appendix I presents UV chromatograms and UV spectra from standard solutions. These chromatograms and spectra of inorganic salts and organic heavy metal complexes were detected to validate the enrichment by nanofiltration and the separation and fractionation of organic heavy metal complexes by high performance size exclusion chromatography (HPSEC). Different physicochemical conditions were adjusted on standard solutions to simulate interactions between the analyte and the stationary phase of the HPSEC column. Additionally, appendix I presents UV chromatograms and pyrograms from the standards proving the stability and reproducibility of the analytical methods.

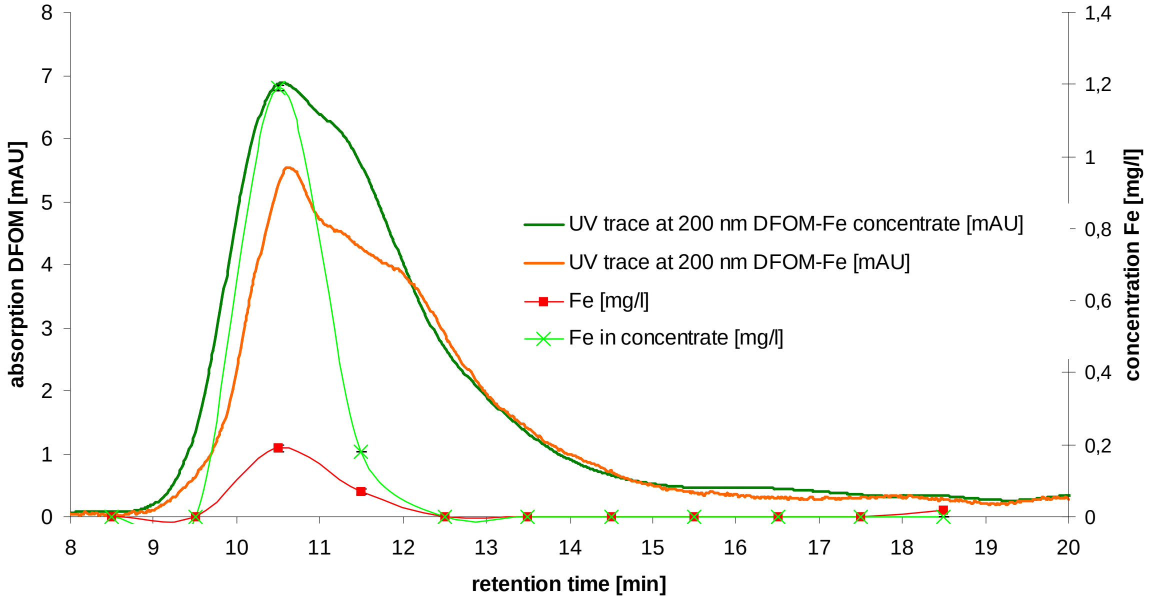

Figure I-1: UV chromatograms and corresponding heavy metal concentrations of the organic heavy metal complex DFOM-Fe before nanofiltration and in the concentrated sample after nanofiltration

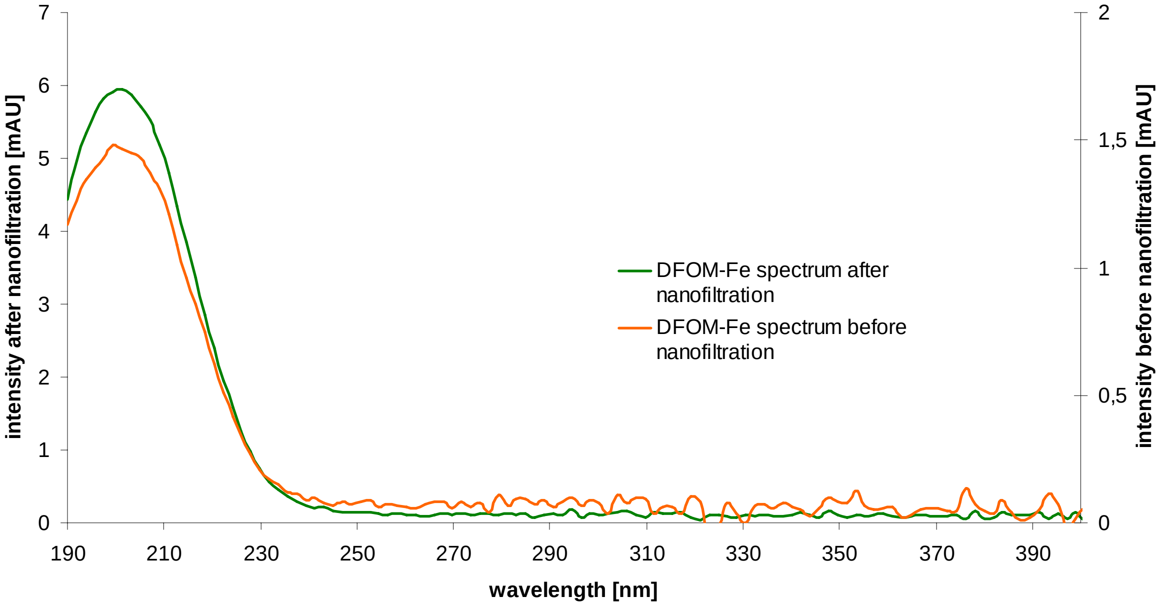

Figure I-2: UV spectra of the organic heavy metal complex DFOM-Fe before nanofiltration and in the concentrated sample after nanofiltration

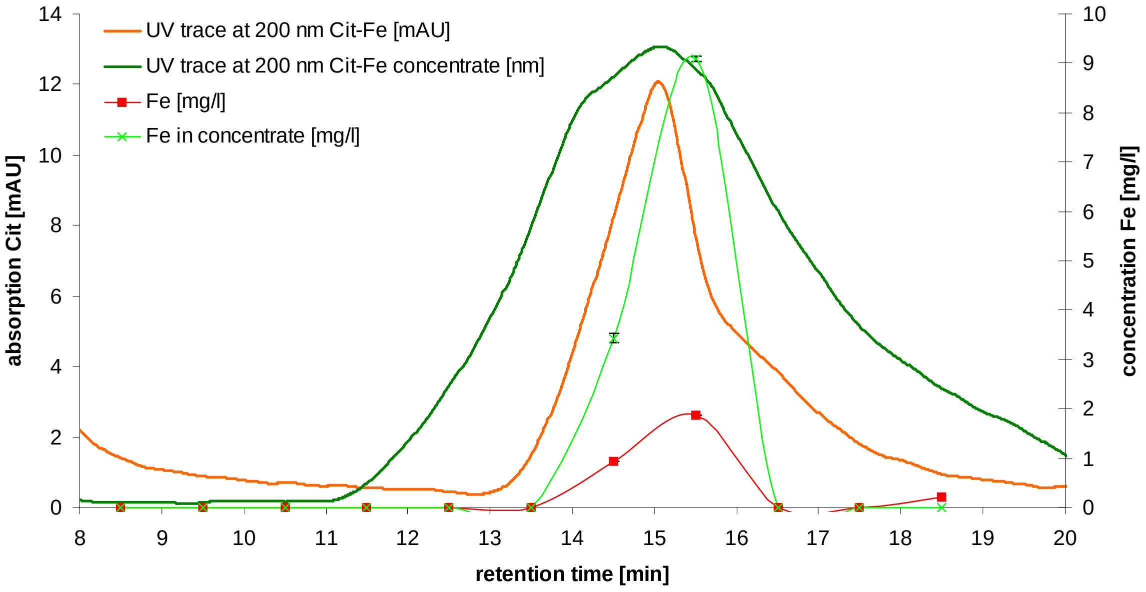

Figure I-3: UV chromatograms and corresponding heavy metal concentrations of the organic heavy metal complex Cit-Fe before nanofiltration and in the concentrated sample after nanofiltration

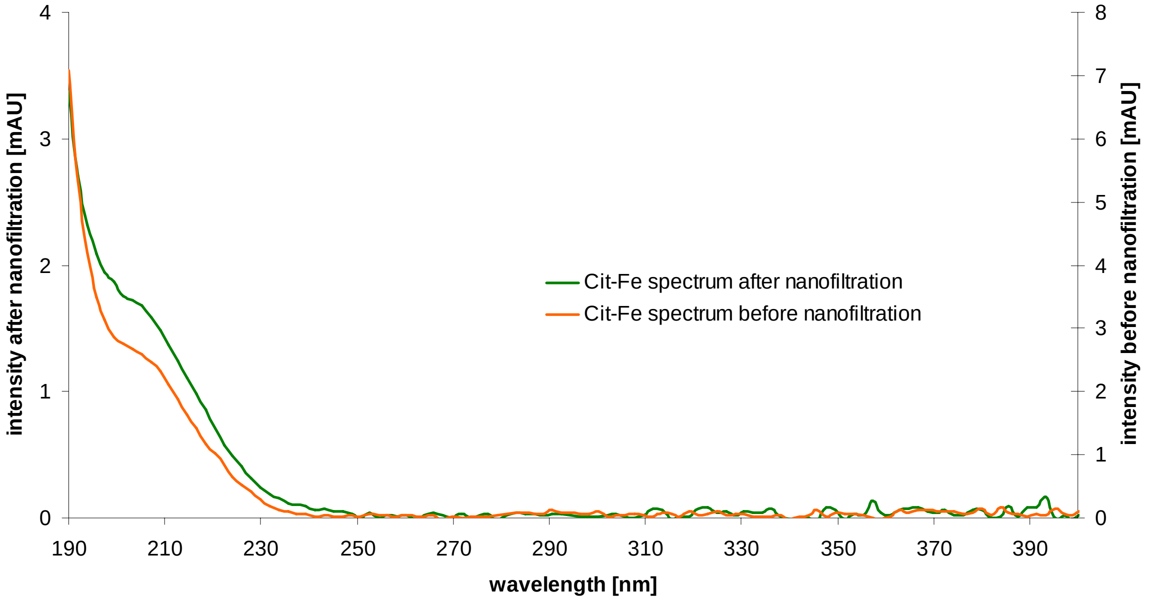

Figure I-4: UV spectra of the organic heavy metal complex Cit-Fe before nanofiltration and in the concentrated sample after nanofiltration

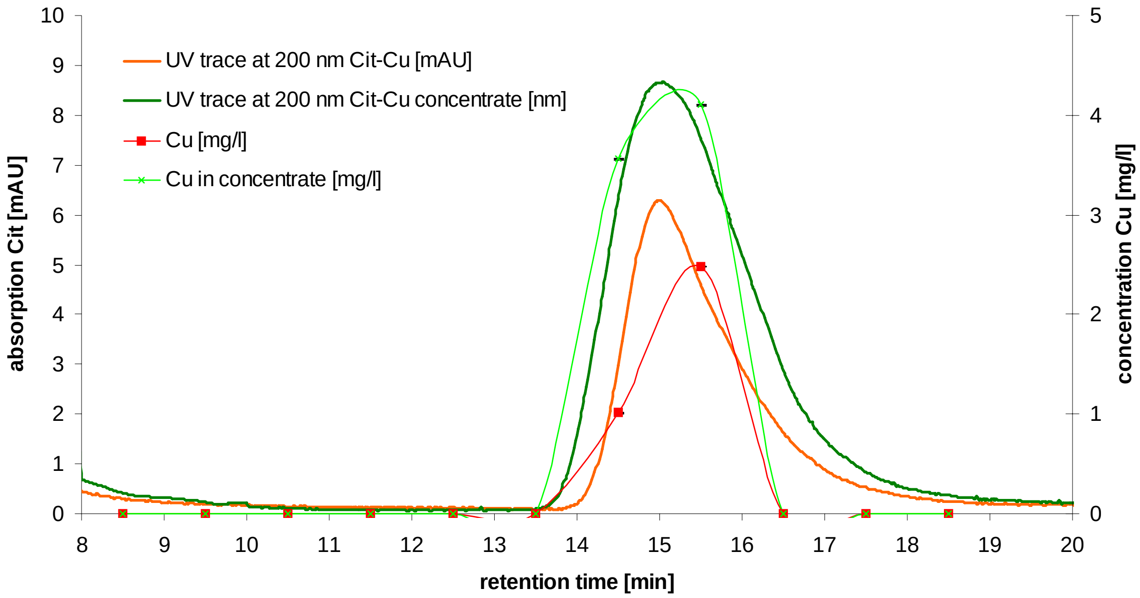

Figure I-5: UV chromatograms and corresponding heavy metal concentrations of the organic heavy metal complex Cit-Cu before nanofiltration and in the concentrated sample after nanofiltration

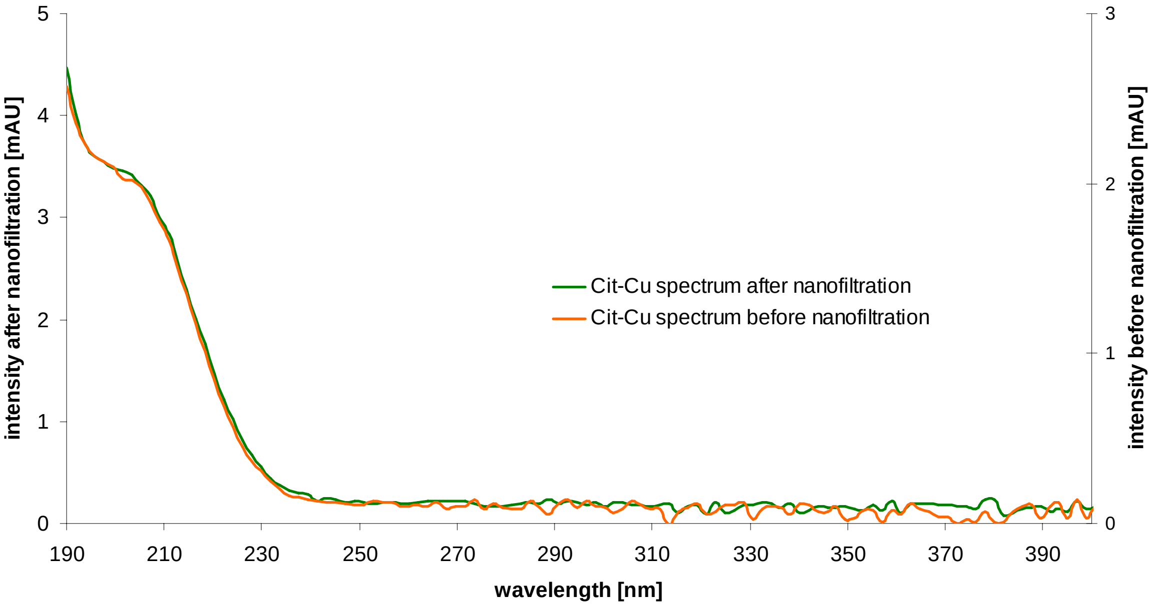

Figure I-6: UV spectra of the organic heavy metal complex Cit-Cu before nanofiltration and in the concentrated sample after nanofiltration

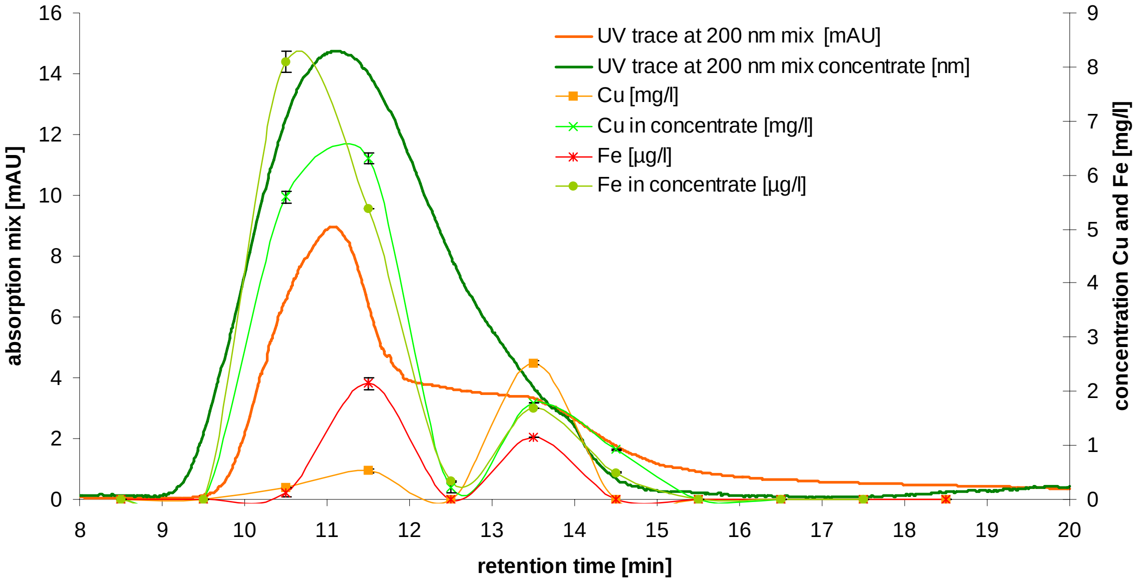

Figure I-7: UV chromatograms and corresponding heavy metal concentrations of a mixture of DFOM-Fe and Cit-Cu before nanofiltration and in the concentrated sample after nanofiltration

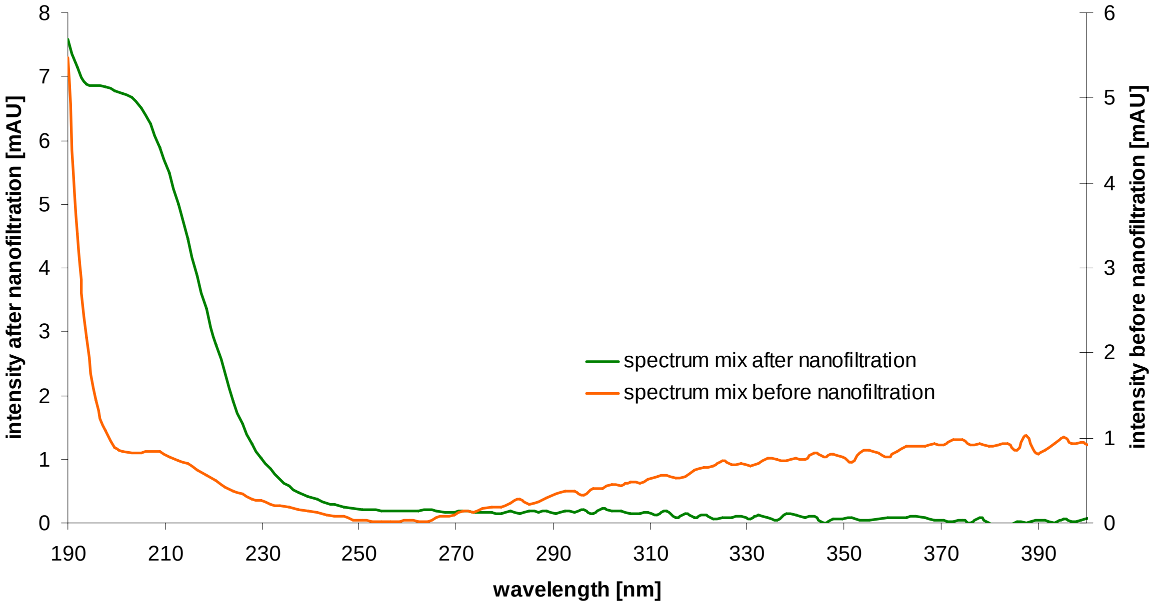

Figure I-8: UV spectra of a mixture of DFOM-Fe and Cit-Cu before nanofiltration and in the concentrated sample after nanofiltration

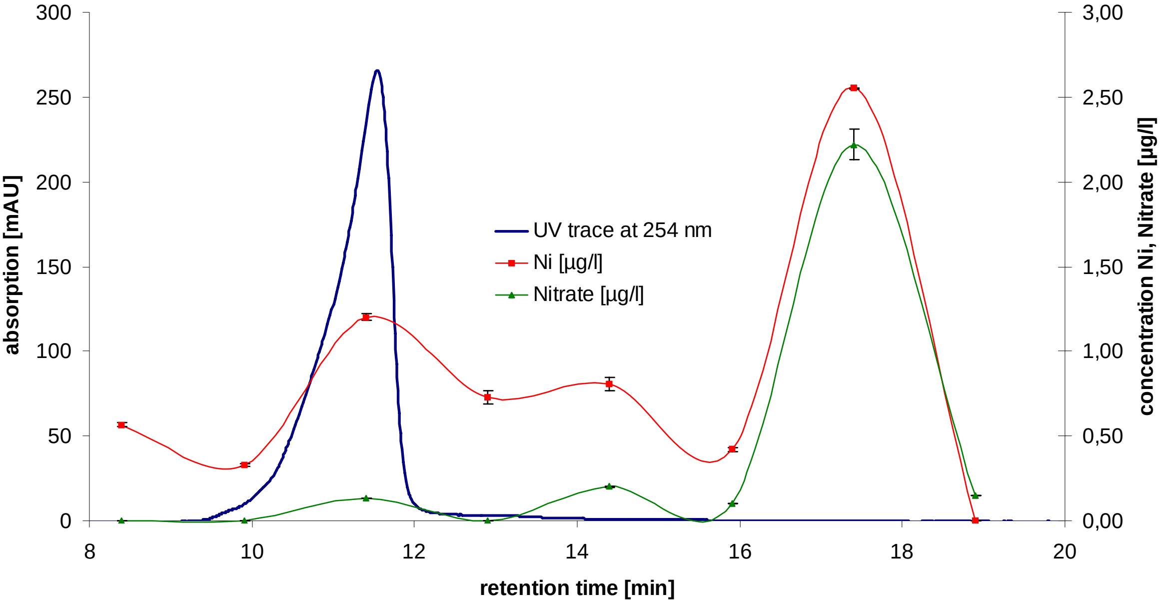

Figure I-9: UV chromatogram and corresponding heavy metal concentrations of humic acid (HA)-Ni(NO$_3$)·6H$_2$O. The organic heavy metal solution was set to pH 3. The chromatogram shows the separation of organic fraction from inorganic salt fraction.

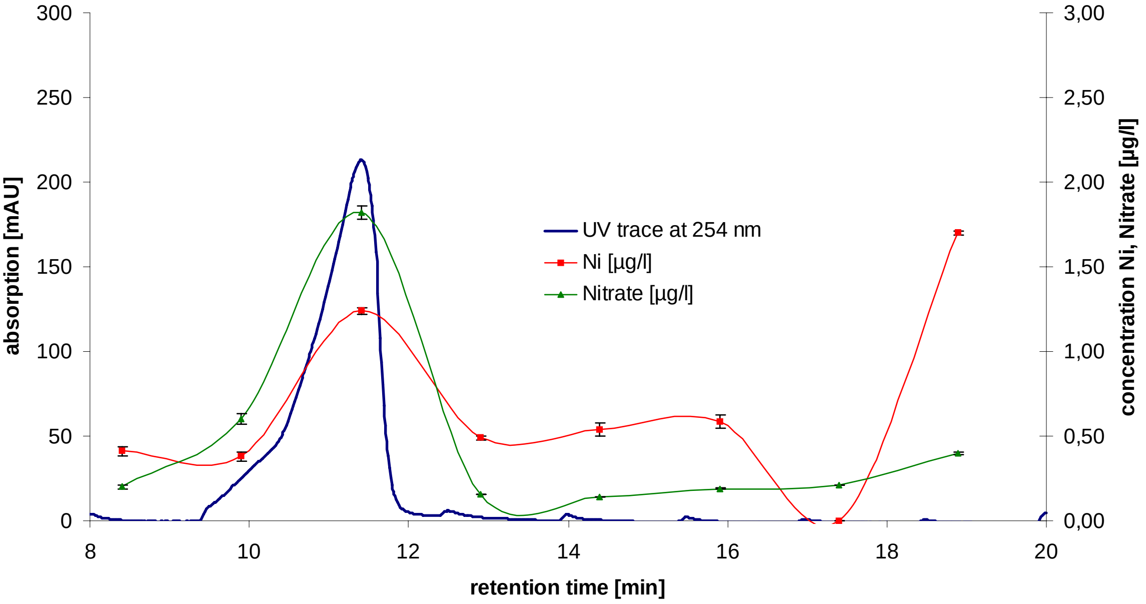

Figure I-10: UV chromatogram and corresponding heavy metal concentrations of humic acid (HA)-Ni(NO$_3$)·6H$_2$O. The organic heavy metal solution was set to pH 6. The chromatogram shows the separation of organic fraction from inorganic salt fraction.

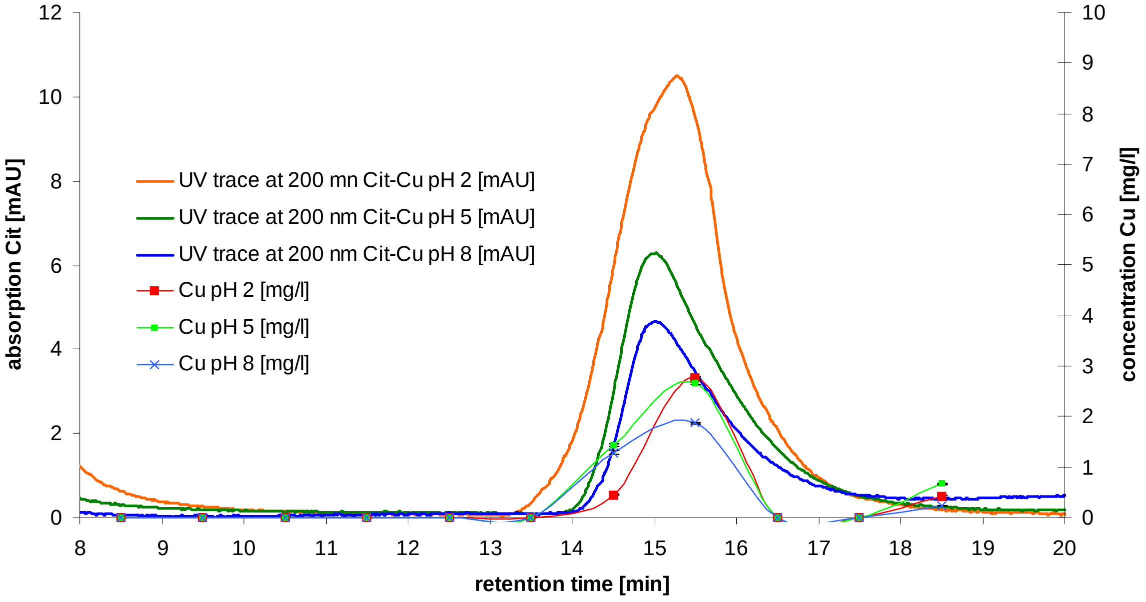

Figure I-11: UV chromatograms and corresponding heavy metal concentrations from organic heavy metal standard solutions. The organic heavy metal complex citrate (cit)-Cu was adjusted to pH values 2, 5 and 8 before analyzing by HPSEC-UV

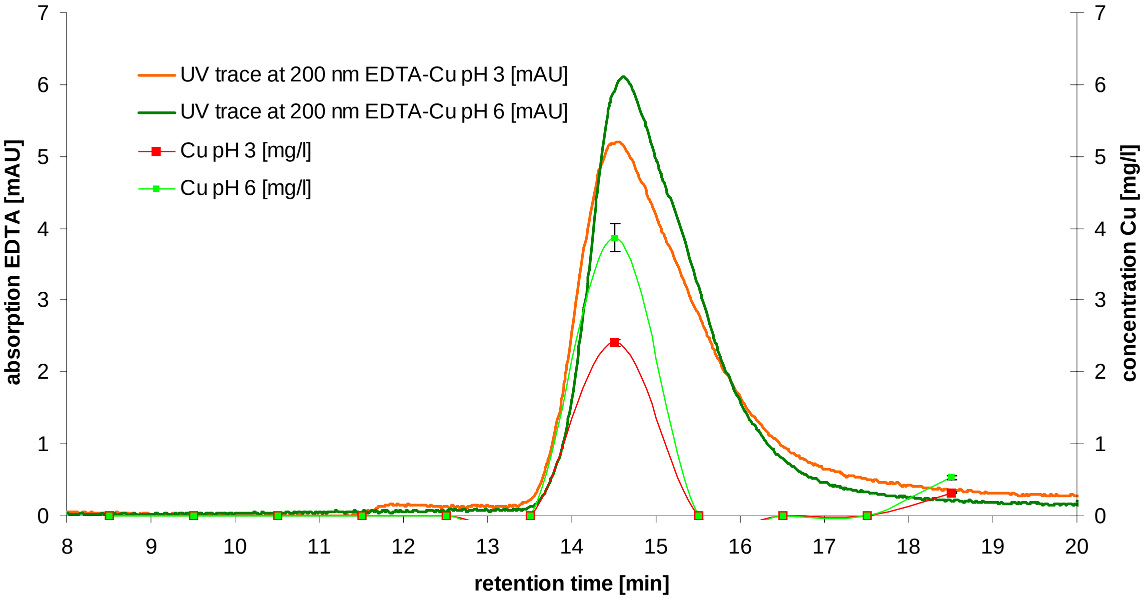

Figure I-12: UV chromatograms and corresponding heavy metal concentrations from organic heavy metal standard solutions. The organic heavy metal complex EDTA-Cu was adjusted to pH values 2 and 6 before analyzing by HPSEC-UV

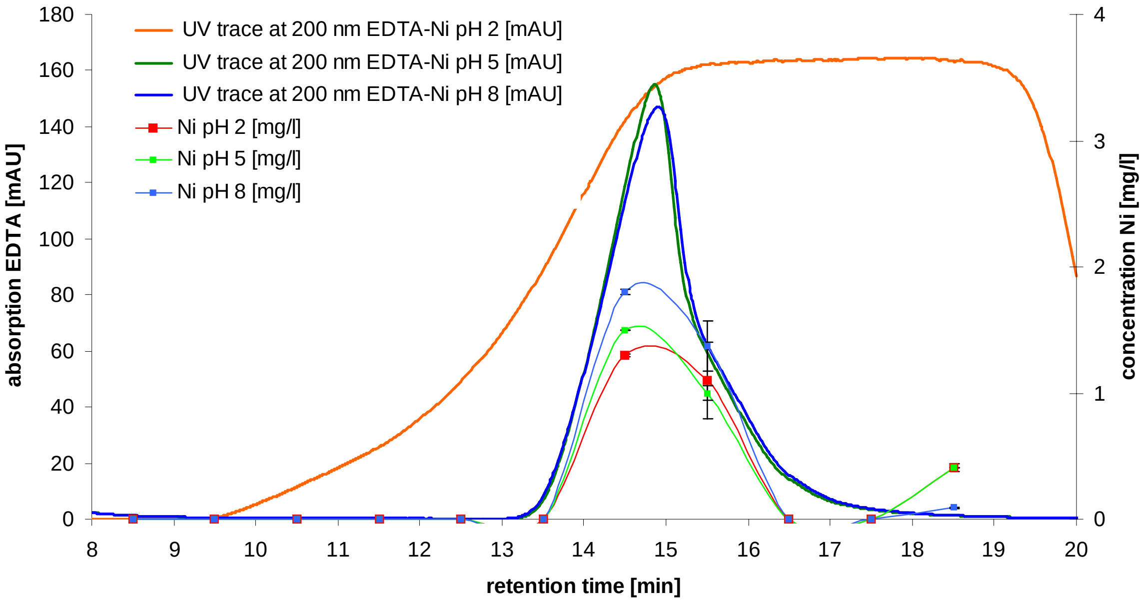

Figure I-13: UV chromatograms and corresponding heavy metal concentrations from organic heavy metal standard solutions. The organic heavy metal complex EDTA-Ni was adjusted to pH values 2, 5 and 8 before analyzing by HPSEC-UV

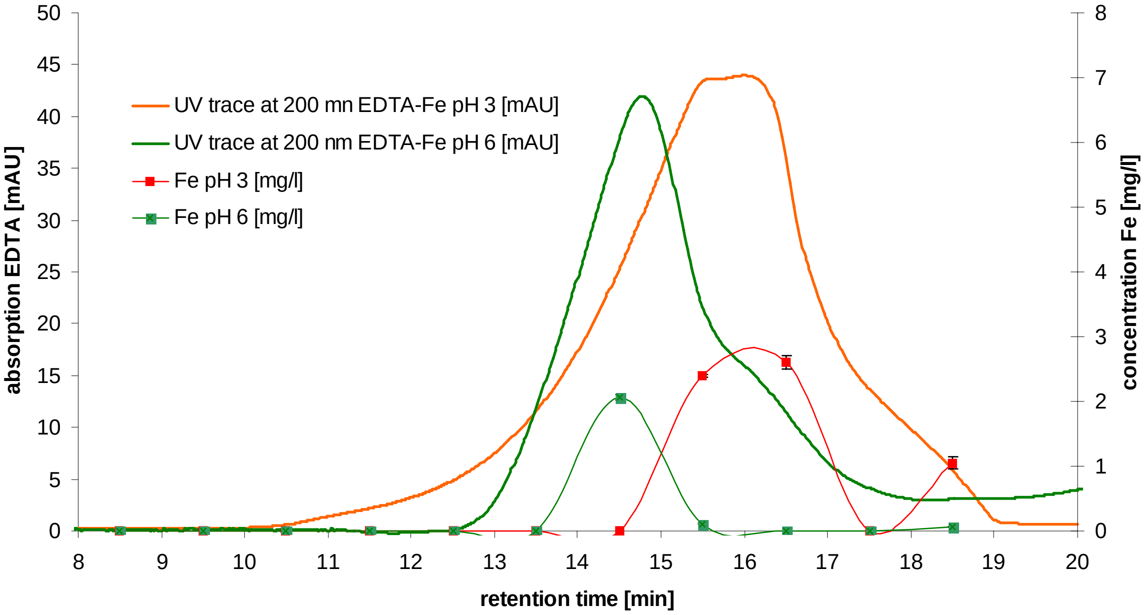

Figure I-14: UV chromatograms and corresponding heavy metal concentrations from organic heavy metal standard solutions. The organic heavy metal complex EDTA-Fe was adjusted to pH values 3 and 6 before analyzing by HPSEC-UV

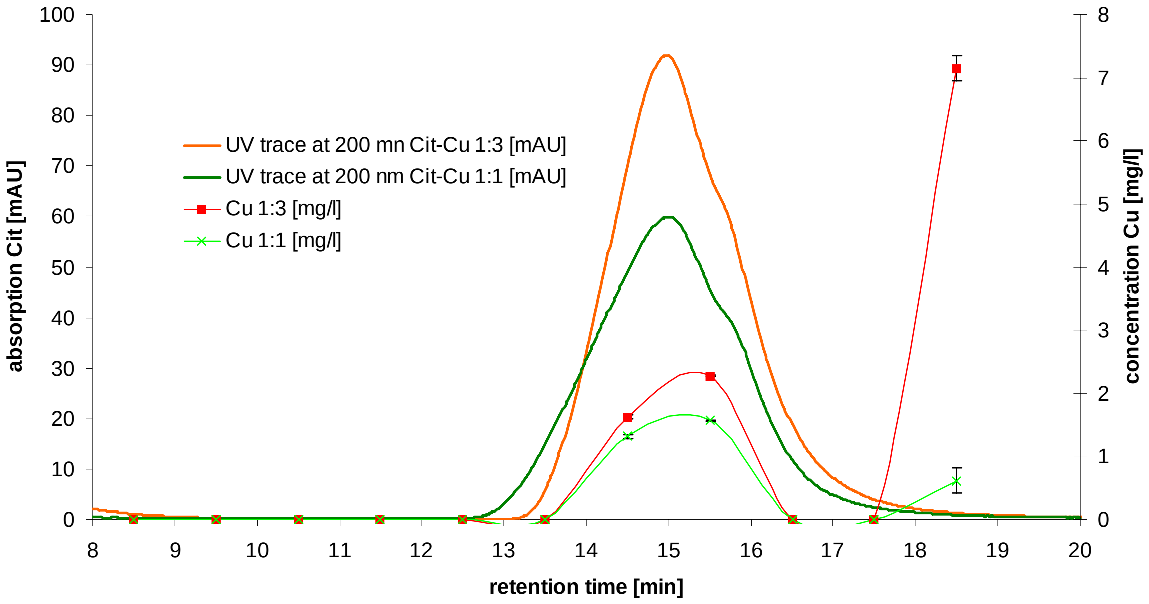

Figure I-15: UV chromatograms and corresponding heavy metal concentrations from organic heavy metal standard solutions. The organic heavy metal complexe citrate (cit)-Cu with ligand:donor ratios 1:1 and 1:3 were analyzed by HPSEC-UV

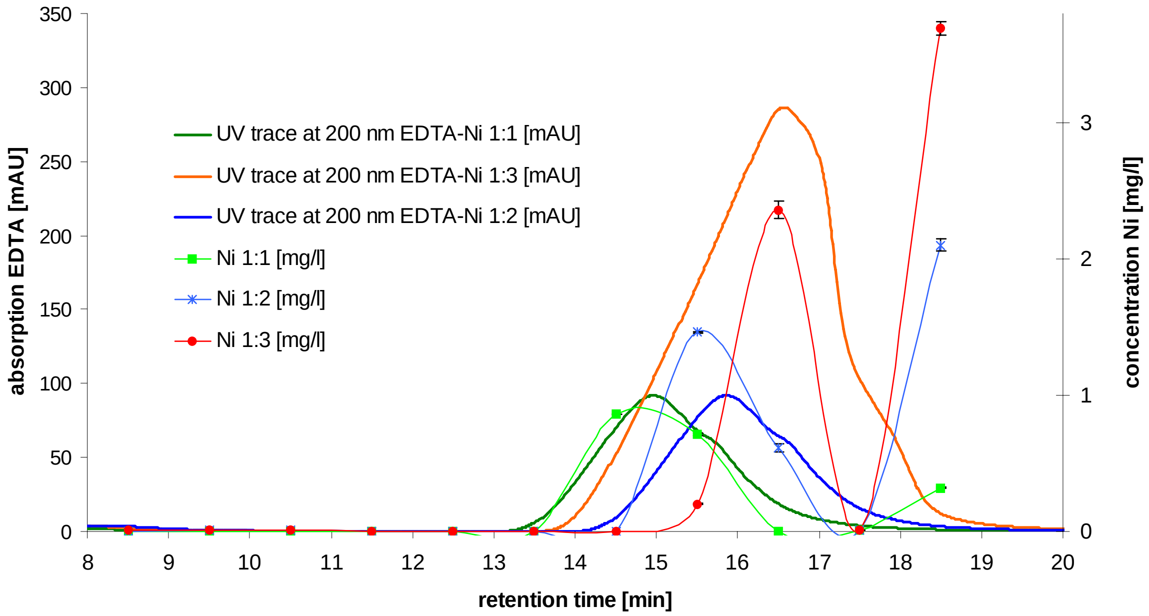

Figure I-16: UV chromatograms and corresponding heavy metal concentrations from organic heavy metal standard solutions. The organic heavy metal complexe EDTA-Ni with ligand:donor ratios 1:1, 1:2 and 1:3 were analyzed by HPSEC-UV

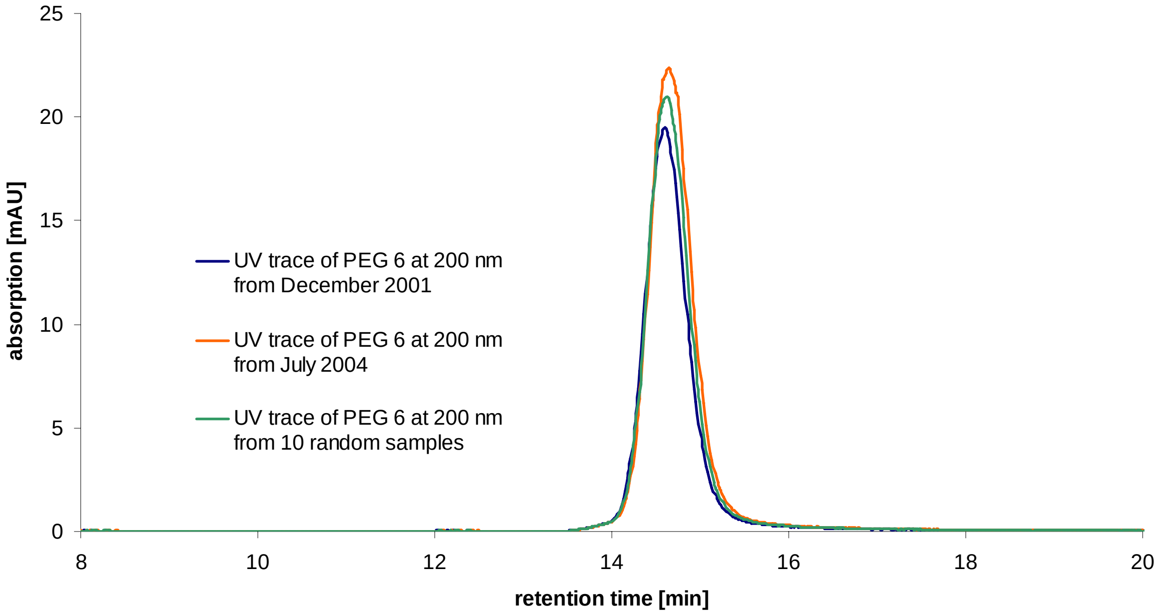

Figure I-17: UV chromatograms of polyethyleneglycole 6 (PEG 6)) detected over the whole fractionation procedures beginning in December 2001 and ending in July 2004. Additional a UV chromatogram with the mean trace of 10 random PEG 6 runs detected between 2001 and 2004 is shown.

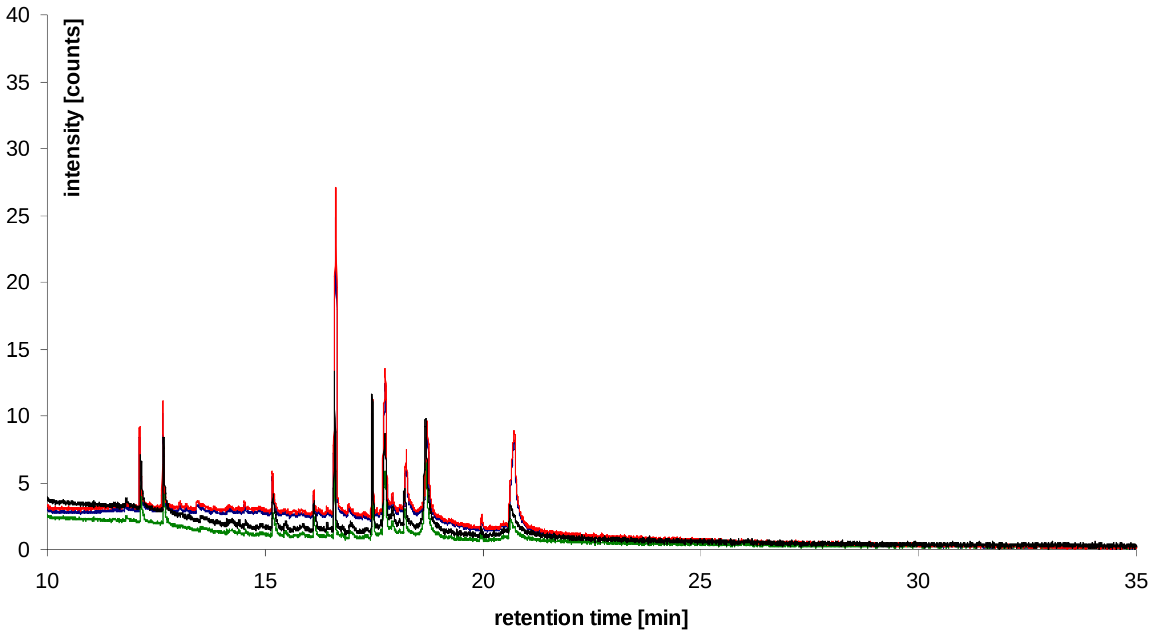

Figure I-18: Carbon pyrograms of 4 cellulose samples detected by AED. Similar traces prove reproducible analysis by Py-GC-AED.

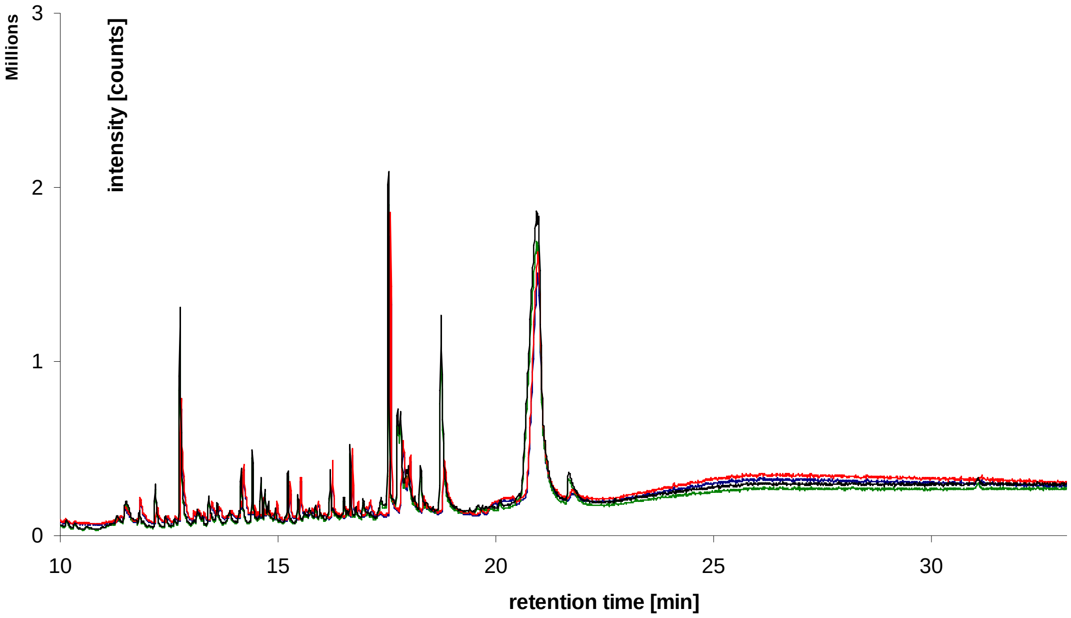

Figure I-19: Carbon pyrograms of 4 cellulose samples detected by MS. Similar traces prove reproducible analysis by Py-GC-MS.

Appendix II

Appendix II presents tables with retention times and corresponding intensities from natural field samples. Additionally, UV chromatograms, UV spectra and corresponding heavy metal concentrations from field samples are shown. Characteristics of municipal waste water samples, acid mine drainage and mixed downstream samples are shown on specific UV traces and heavy metal concentrations detected from samples of 2001, 2002 and 2003.

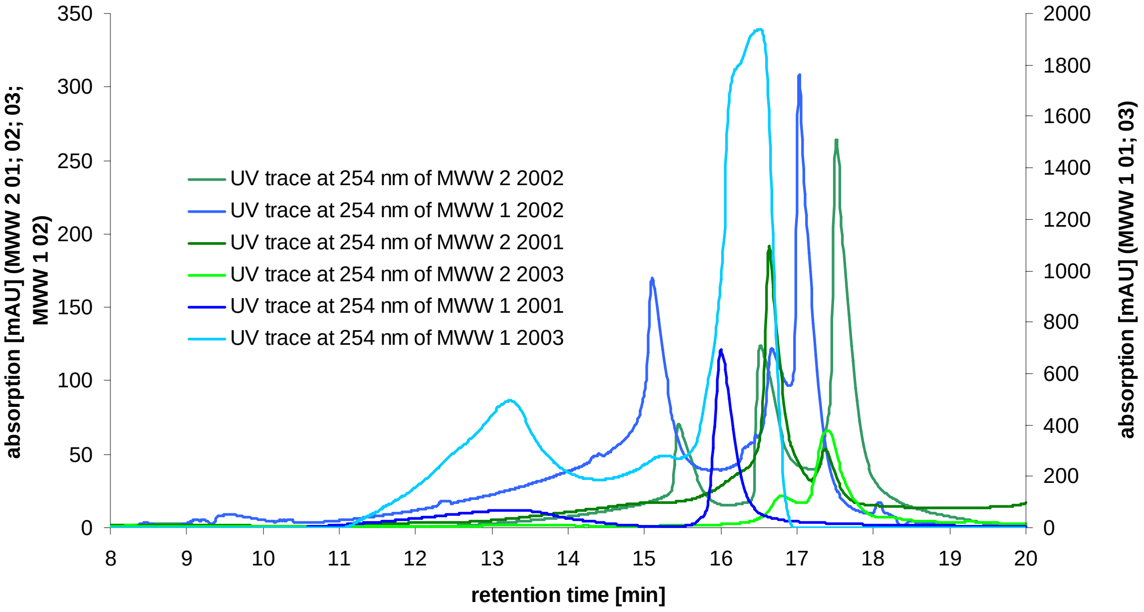

Figure II-1: UV chromatograms of municipal waste water samples from 2001, 2002 and 2003. UV traces show no similar maxima over the whole sampling procedure.

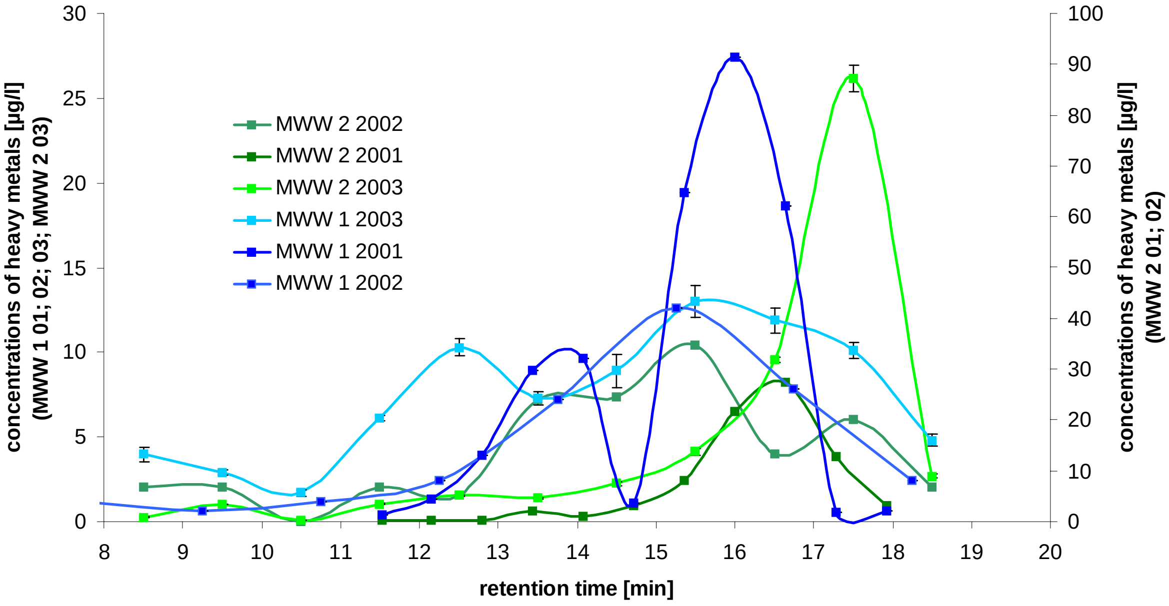

Figure II-2: Mean heavy metal concentrations (calculated from Fe, Mn, Ni, Cu and U concentrations) in the HPSEC effluent of municipal waste water samples from 2001, 2002 and 2003. Heavy metal concentrations show no similar maxima over the whole sampling period.

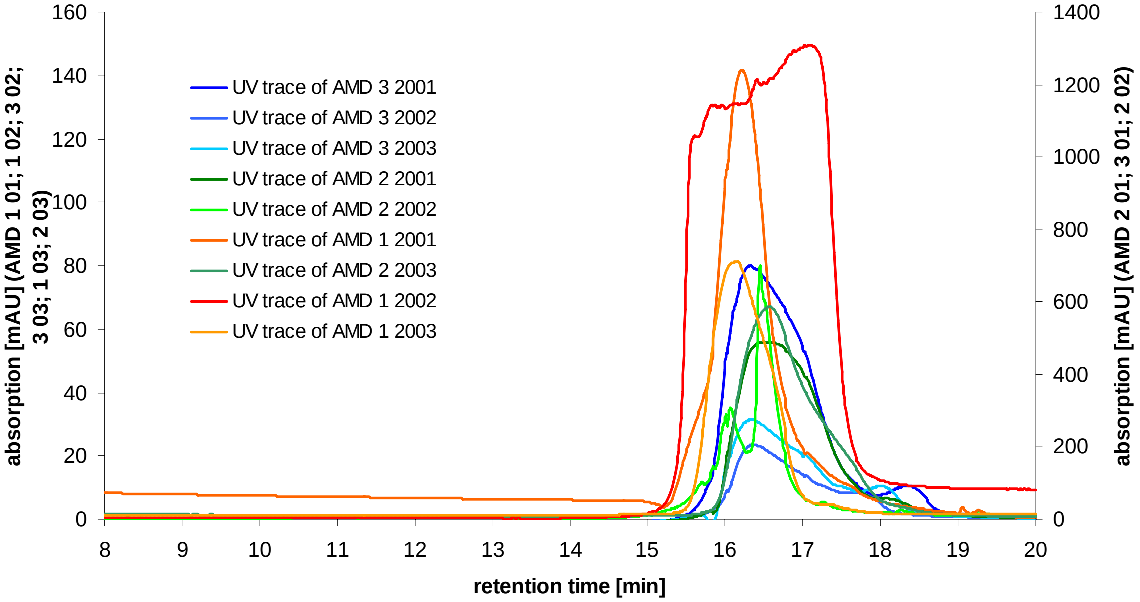

Figure II-3: UV chromatograms of acid mine drainage samples from 2001, 2002 and 2003. UV traces show similar maxima over the whole sampling procedure.

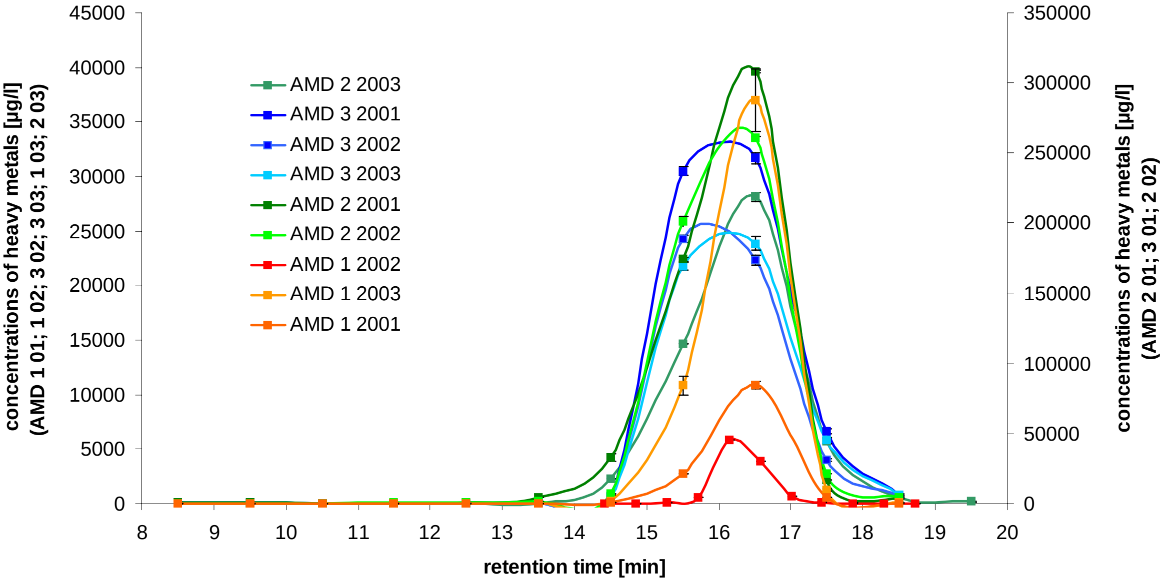

Figure II-4: Mean heavy metal concentrations (calculated from Fe, Mn, Ni, Cu and U concentrations) in the HPSEC effluent of acid mine drainage samples from 2001, 2002 and 2003. Heavy metal concentrations show similar maxima over the whole sampling period.

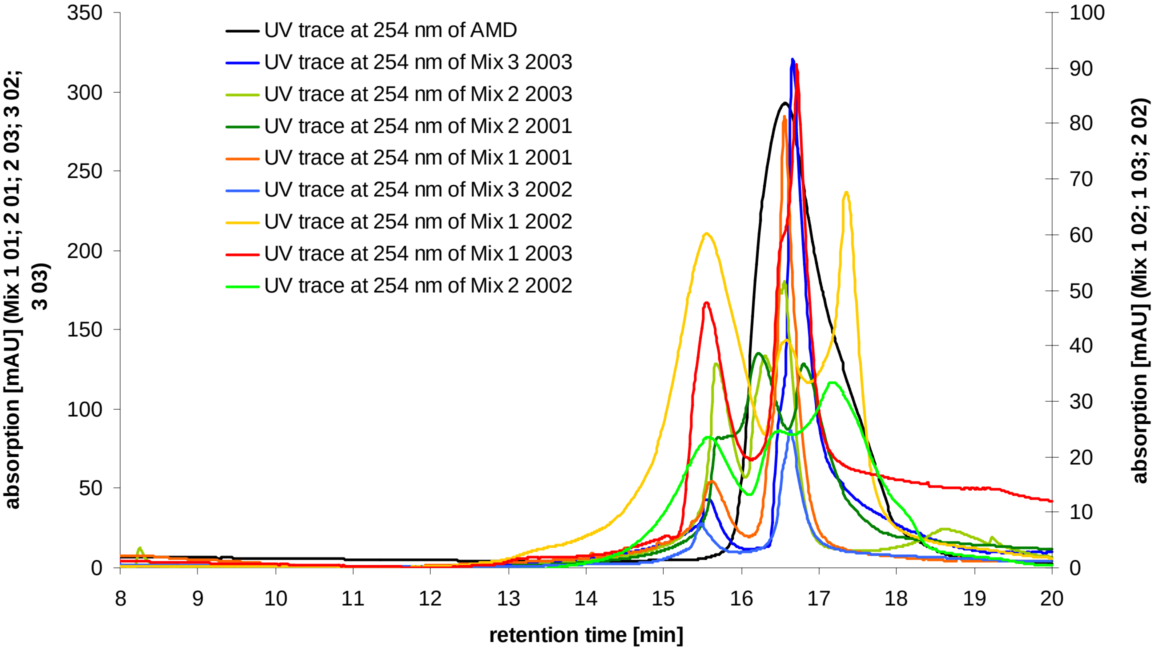

Figure II-5: UV chromatograms of mixed downstream samples from 2001, 2002 and 2003. UV traces show two similar maxima over the whole sampling procedure. (AMD values are reduced to a factor of 2)

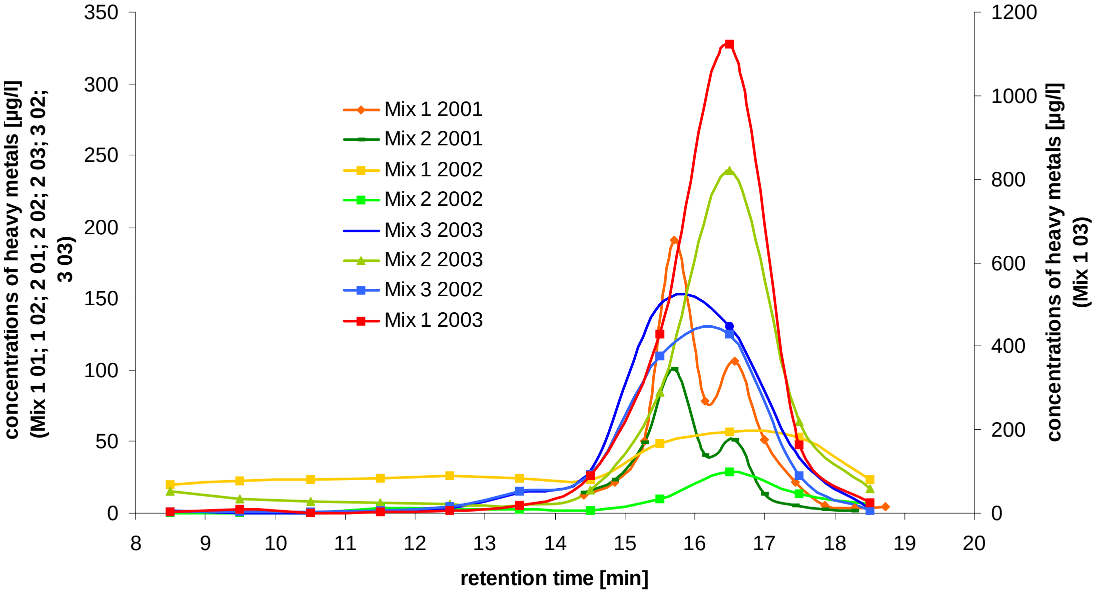

Figure II-6: Mean heavy metal concentrations (calculated from Fe, Mn, Ni, Cu and U concentrations) in the HPSEC effluent of mixed downstream samples from 2001, 2002 and 2003. Heavy metal concentrations show two similar maxima over the whole sampling period.

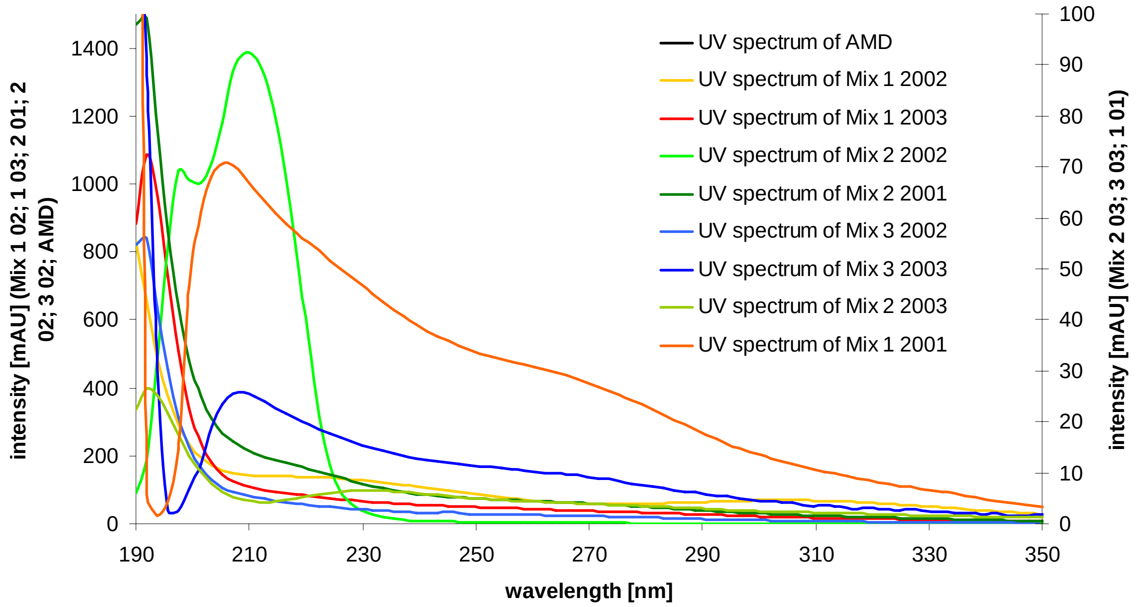

Figure II-7: UV spectra of the common maximum 1 of mixed downstream samples from 2001, 2002 and 2003. Spectra show no identical traces over the whole sampling procedure.

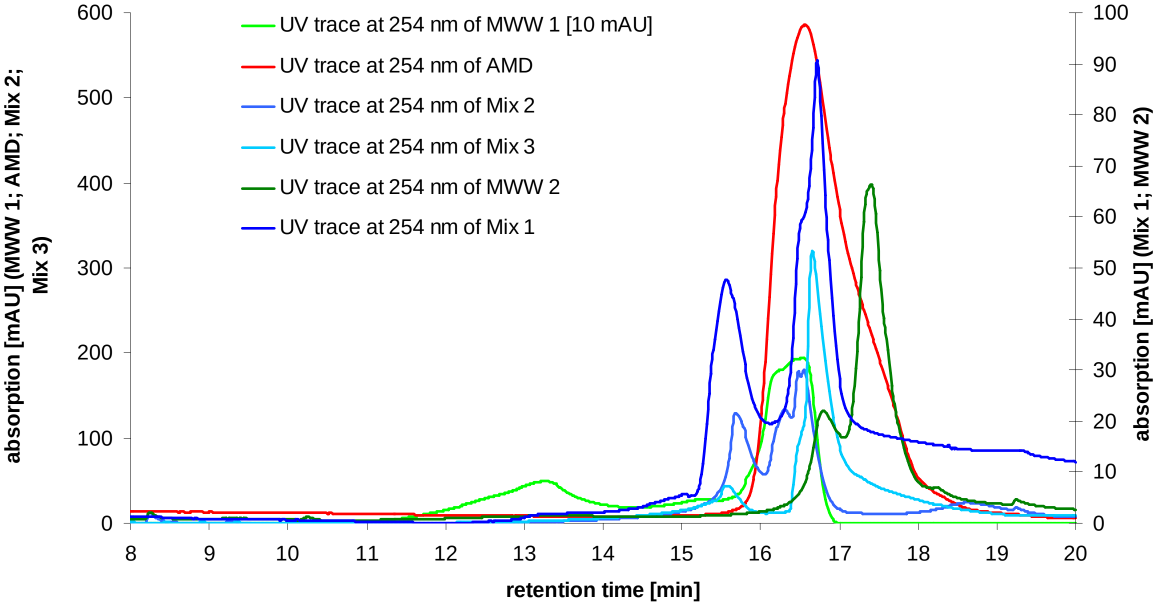

Figure II-8: UV chromatograms of municipal waste water, acid mine drainage and mixed downstream samples from 2003. UV traces show one similar maximum between 16.5 min and 16.7 min.

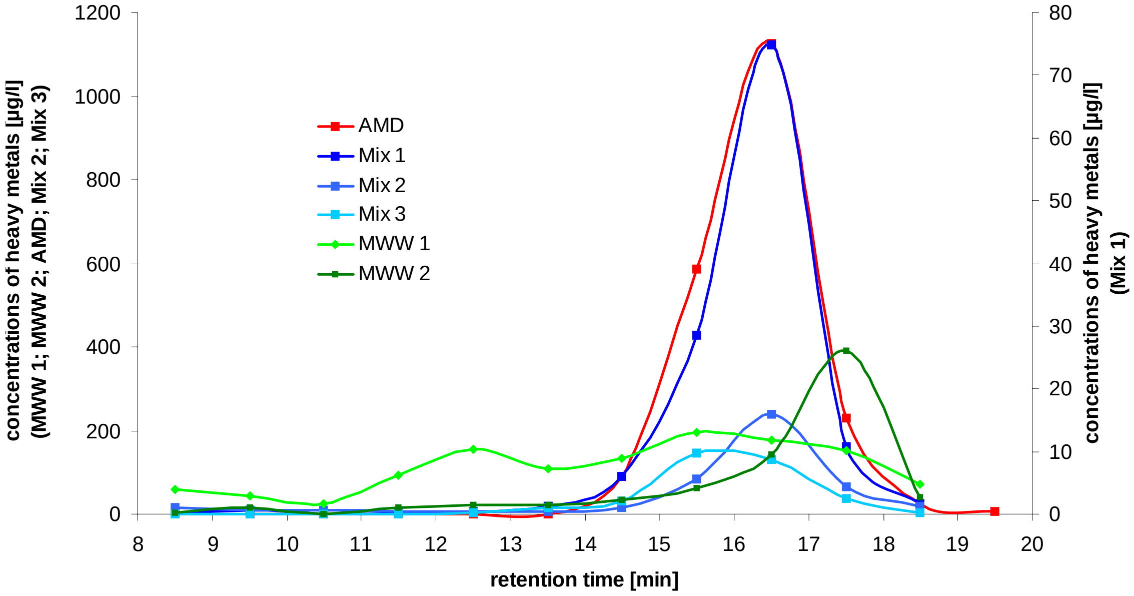

Figure II-9: Mean heavy metal concentrations (calculated from Fe, Mn, Ni, Cu and U concentrations) in the HPSEC effluent of municipal waste water, acid mine drainage and mixed downstream samples from 2003. Beside MWW 2 all samples show elevated heavy metal concentrations at 16.5 min. (AMD values were reduced by a factor of 25)

Appendix III

Appendix III shows traces of carbon, oxygen, sulfur, nitrogen and silicon of five freeze dried organic heavy metal fractions from municipal waste water samples, acid mine drainage and mixed samples from 2003. Detected was done by an atomic emission detector (AED) after Py-GC. Additional appendix III lists all characterized mass spectra of pyrolysis products obtained by GC-MS. The indexes refer to the samples and the corresponding peaks used in the text. Identification of the mass spectra was preceded by using data bases and libraries (Wiley 6.0 and NIST extension). Further on pyrograms of AMD and Mix 2 from 2001 prove reproducibility of silicon organic fragments in Py-GC-MS.

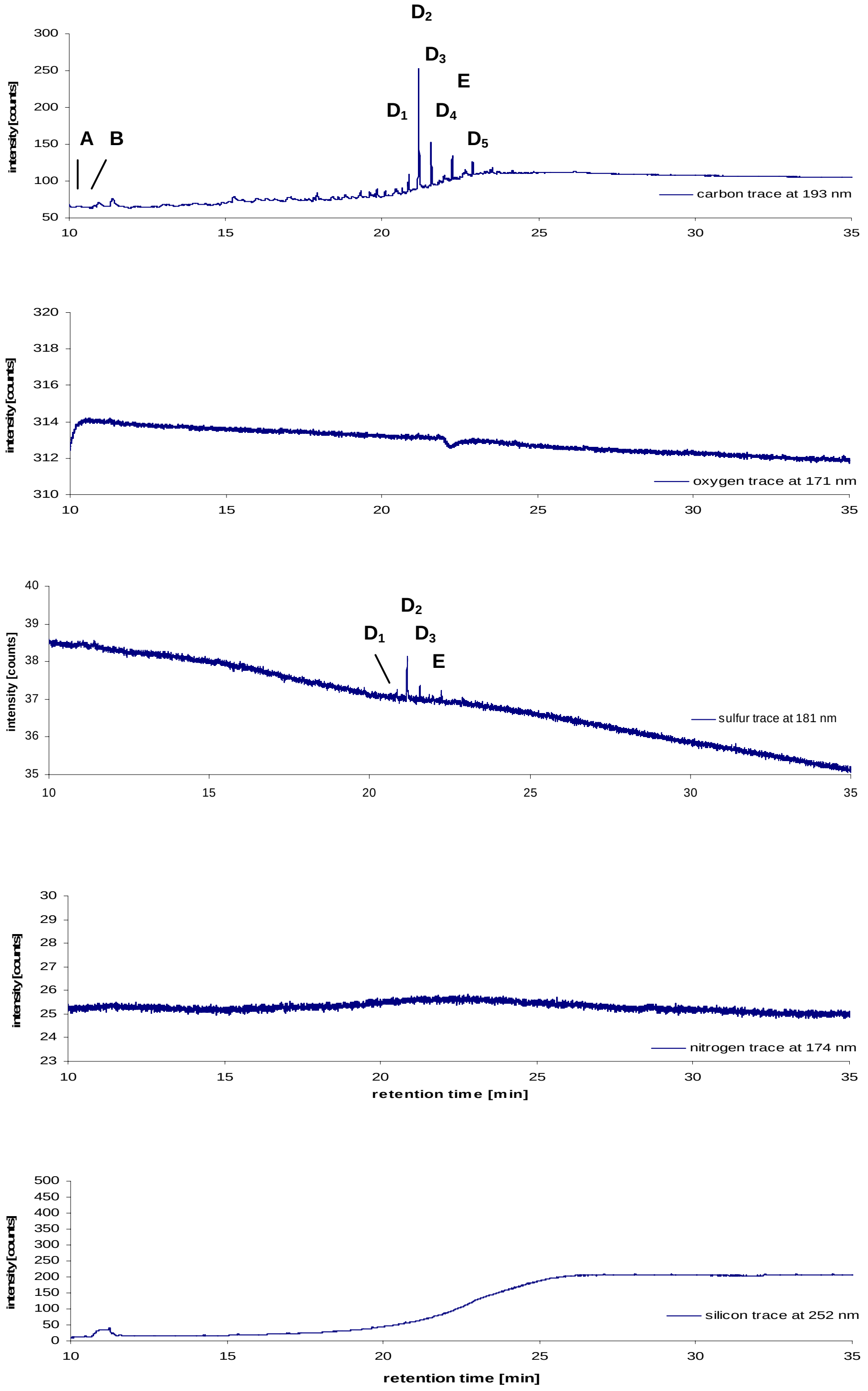

Figure III-1: Carbon, oxygen, sulfur, nitrogen and silicon traces of municipal waste water 2 from 2003. Given traces resulted from freeze dried organic heavy metal fraction and were analyzed by Py-GC-AED.

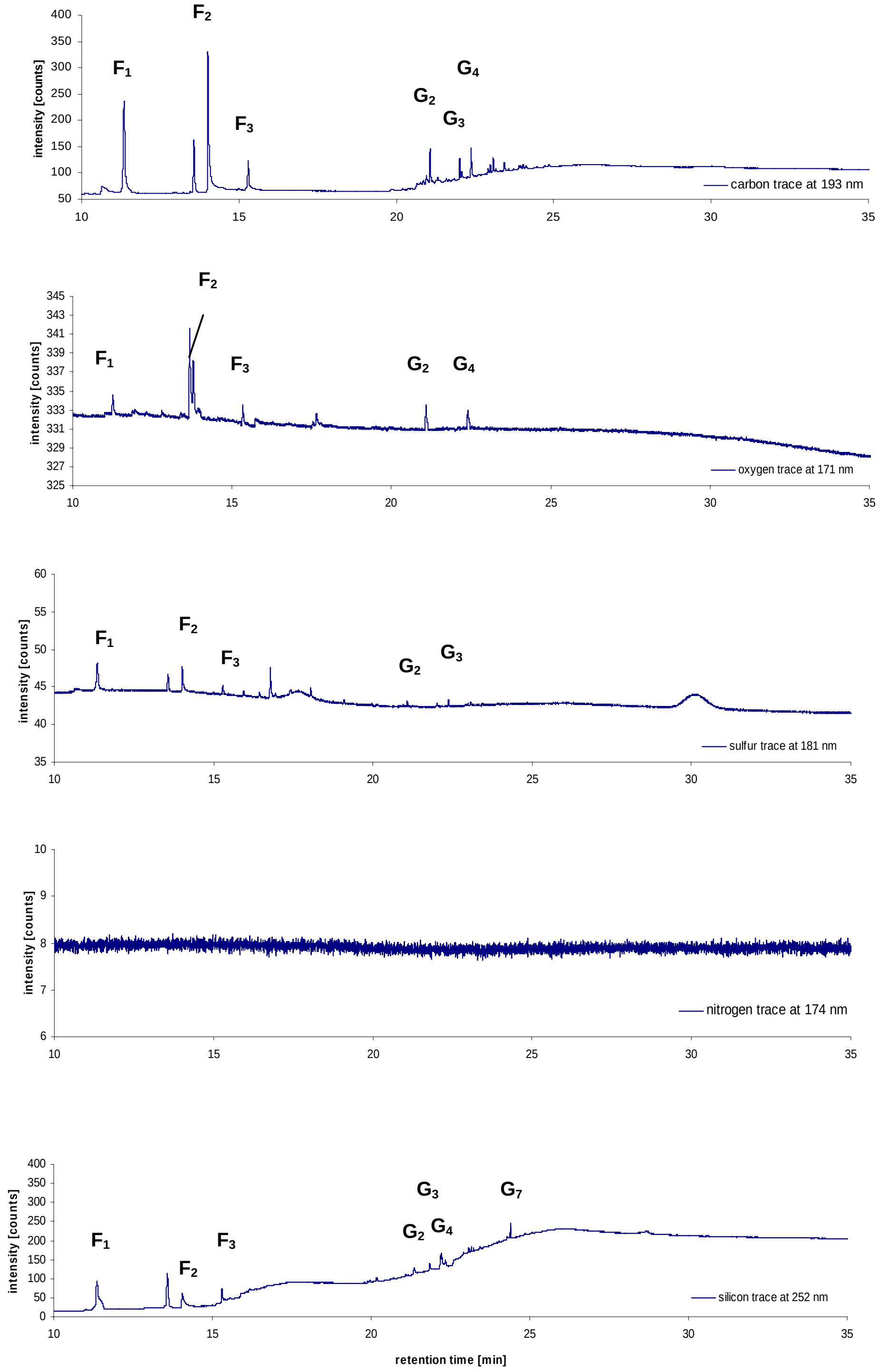

Figure III-2: Carbon, oxygen, sulfur, nitrogen and silicon traces of acid mine drainage from 2003. Given traces resulted from freeze dried organic heavy metal fraction and were analyzed by Py-GC-AED.

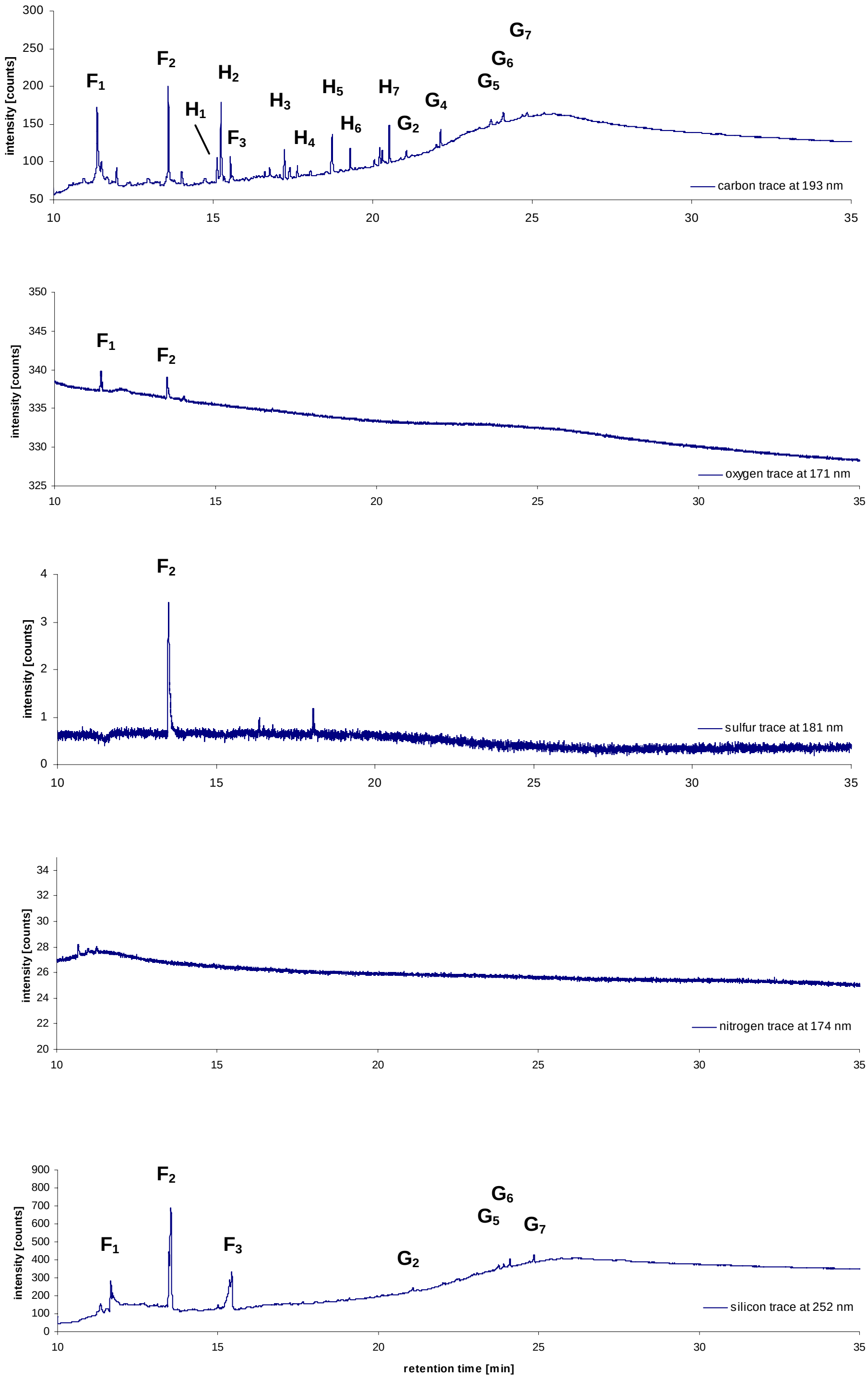

Figure III-3: Carbon, oxygen, sulfur, nitrogen and silicon traces of Mix 1 from 2003. Given traces resulted from freeze dried organic heavy metal fraction and were analyzed by Py-GC-AED.

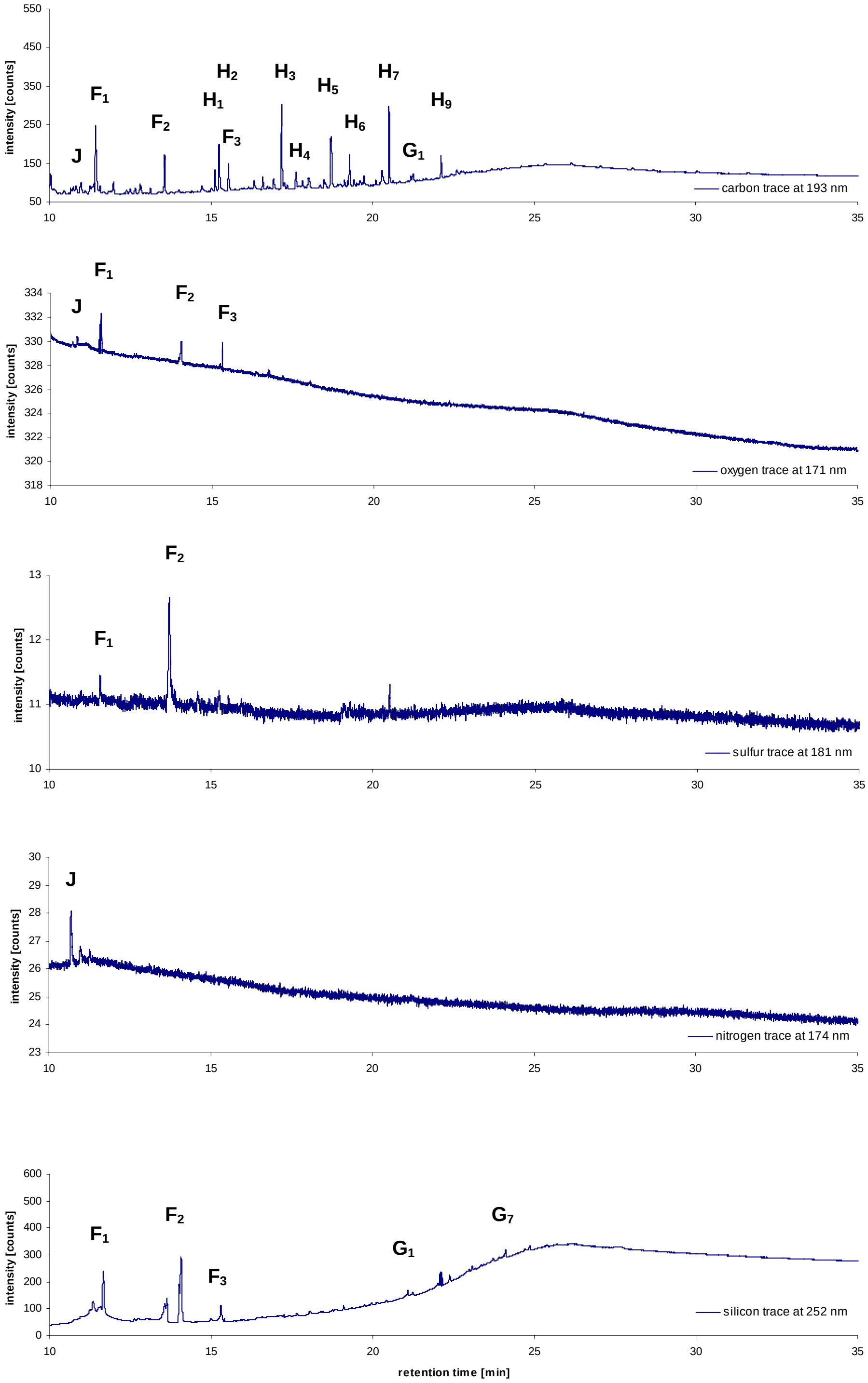

Figure III-4: Carbon, oxygen, sulfur, nitrogen and silicon traces of Mix 2 from 2003. Given traces resulted from freeze dried organic heavy metal fraction and were analyzed by Py-GC-AED.

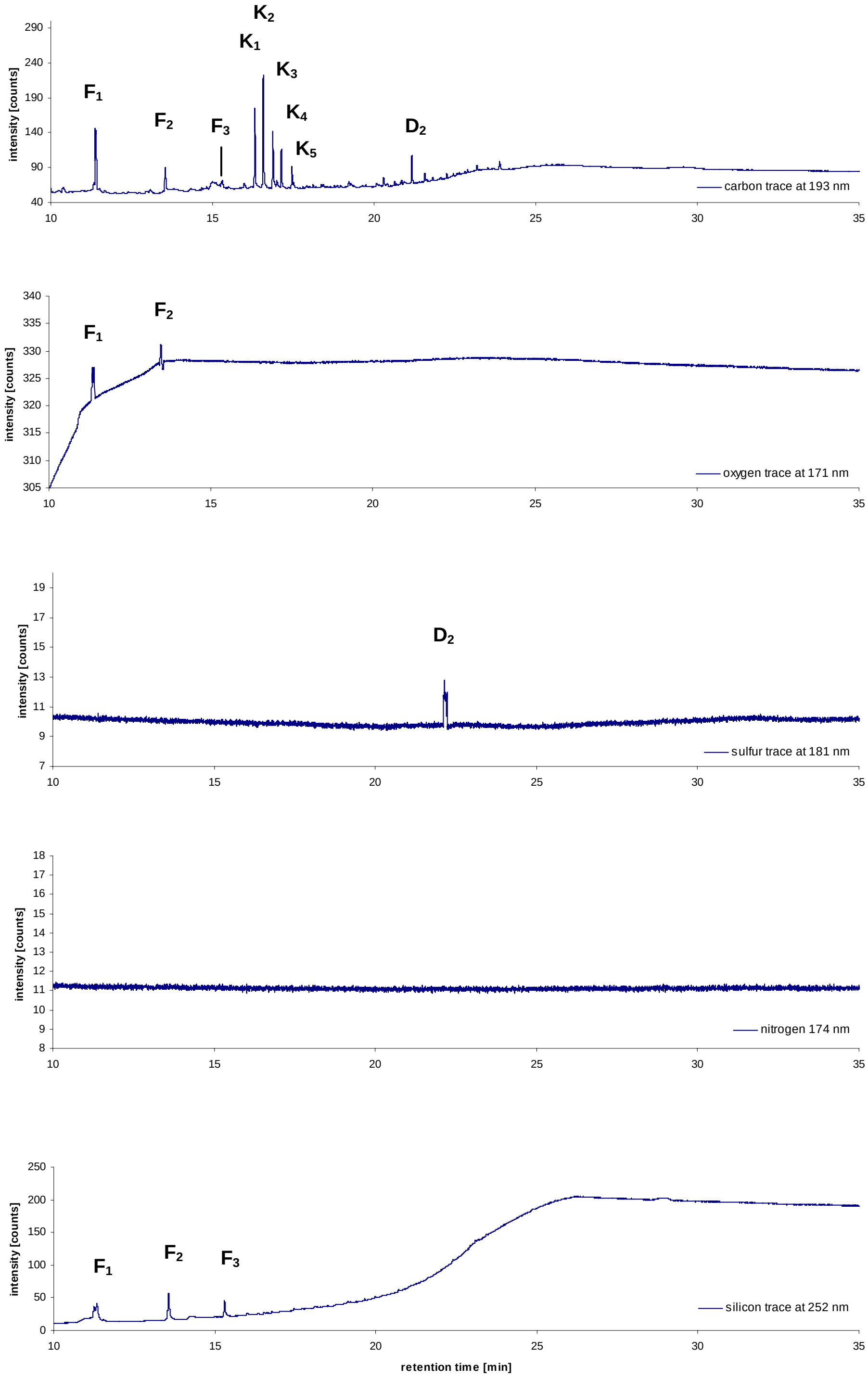

Figure III-5: Carbon, oxygen, sulfur, nitrogen and silicon traces of Mix 3 from 2003. Given traces resulted from freeze dried organic heavy metal fraction and were analyzed by Py-GC-AED.

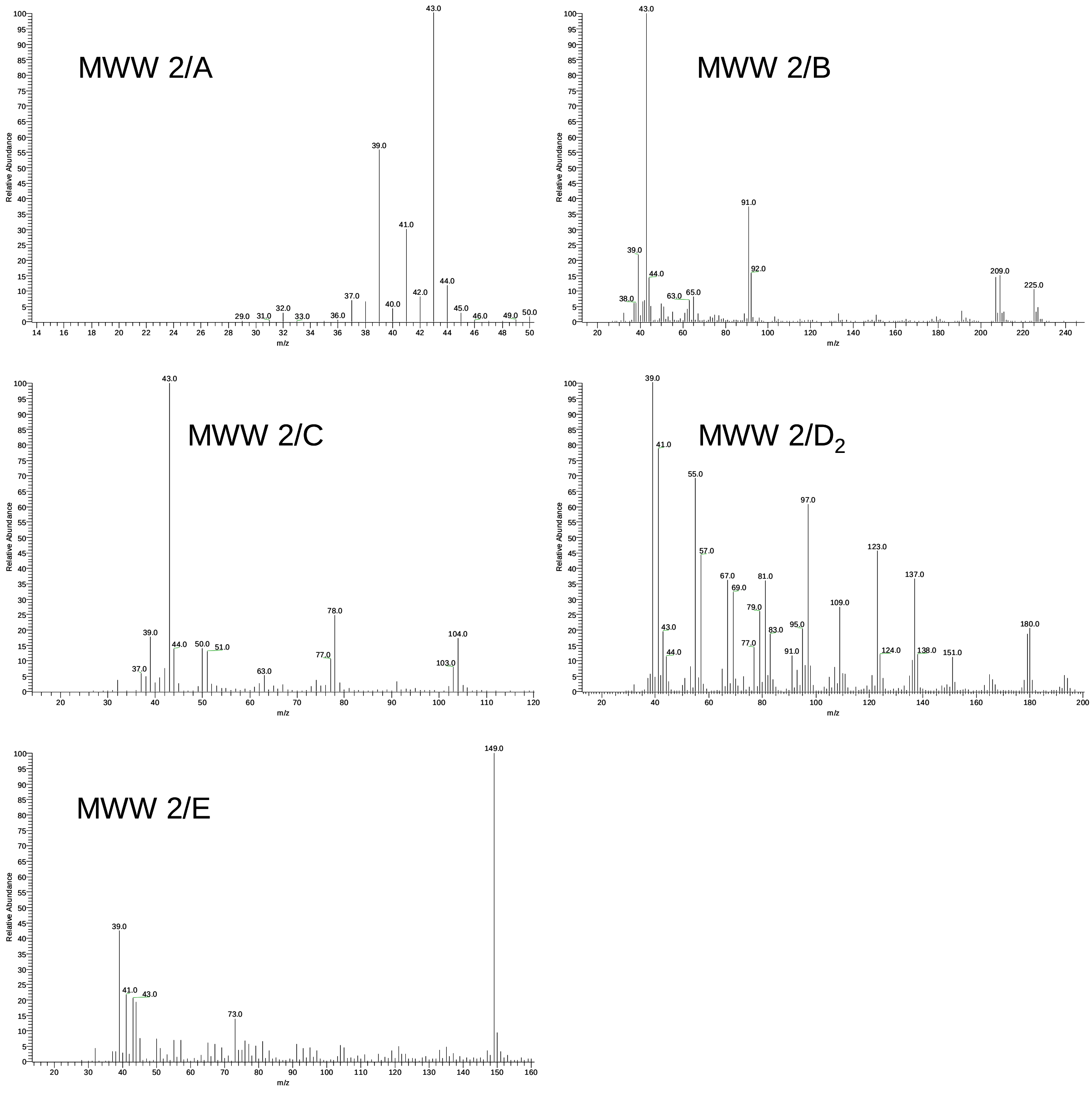

Figure III-6: Mass spectra of maxima A, B, C, D_2 and E from the pyrogram resulting from the organic heavy metal fraction of the municipal waste water 1 2003

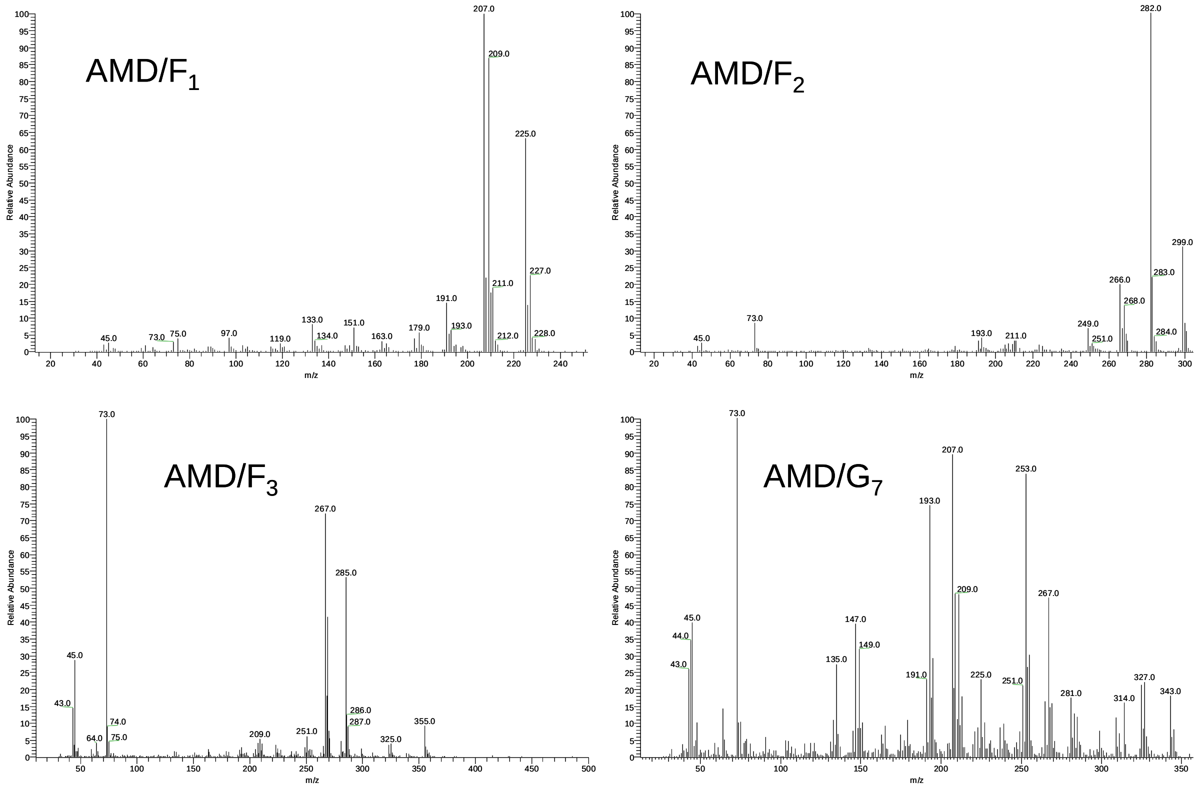

Figure IV-7: Mass spectra of maxima F_1, F_2, F_3, and G_7 from the pyrogram resulting from the organic heavy metal fraction of acid mine drainage 2 2003

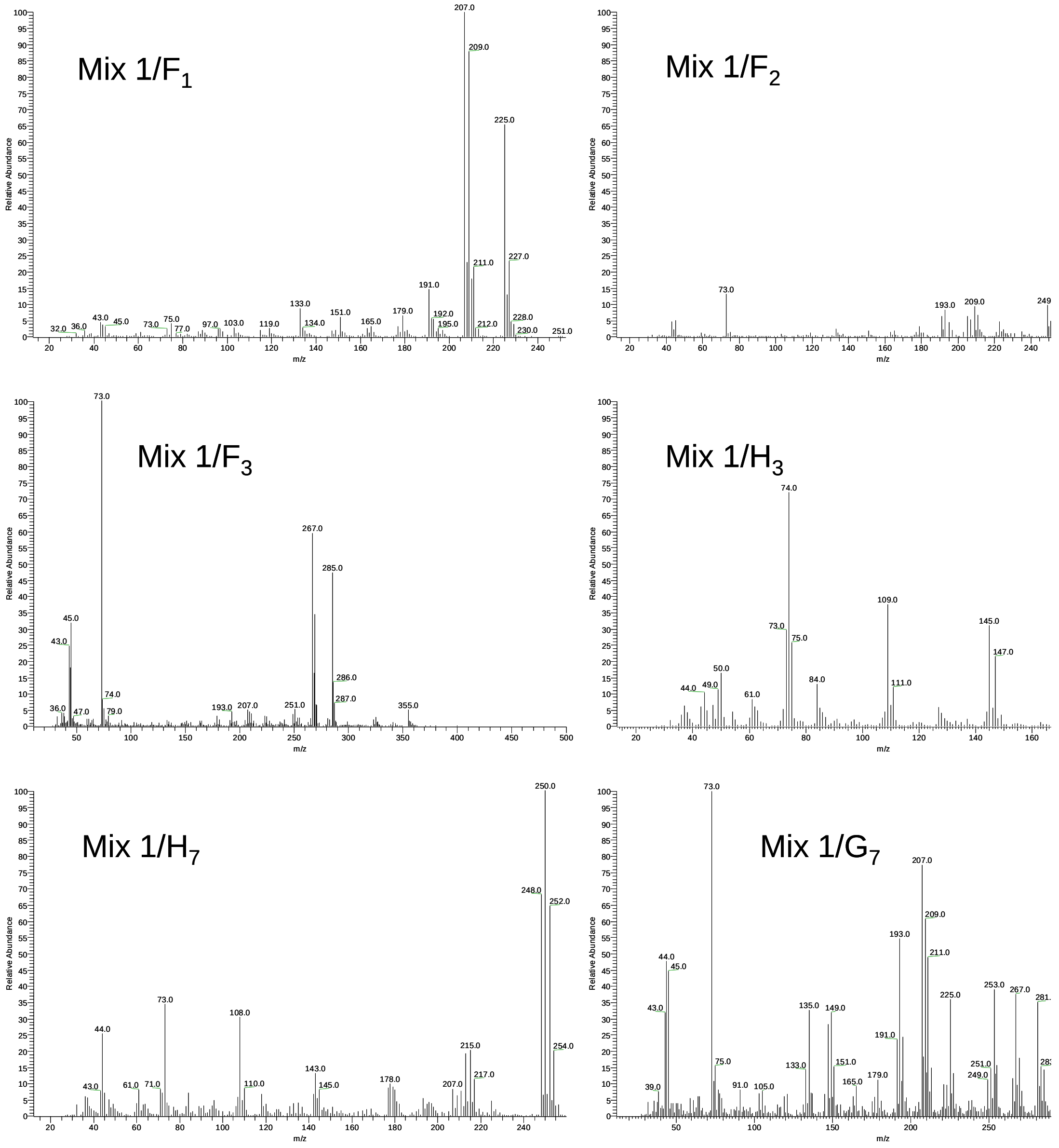

Figure III-8: Mass spectra of maxima F_1, F_2, F_3, H_3, H_7 and G_7 from the pyrogram resulting from the organic heavy metal fraction of Mix 1 2003

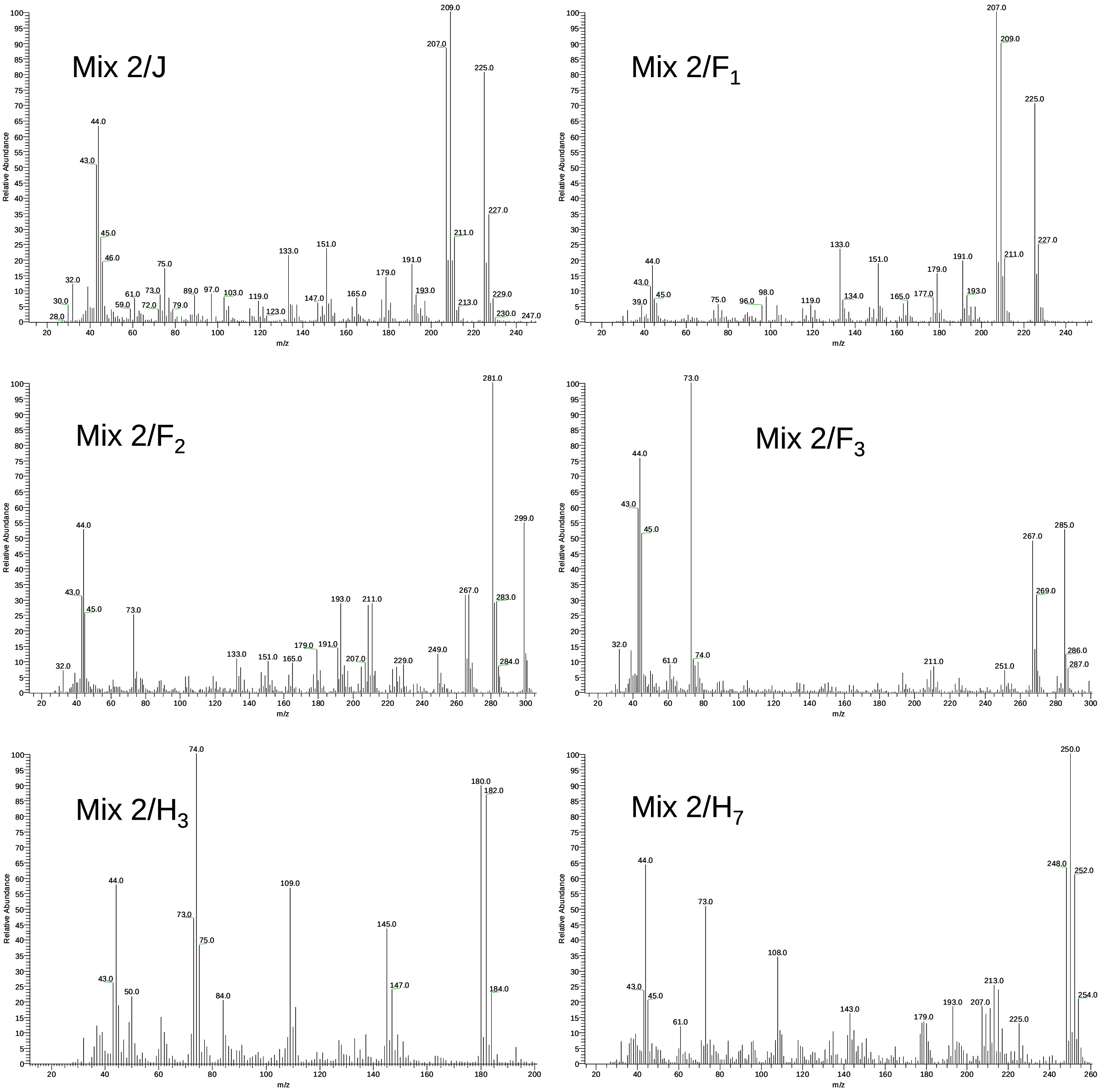

Figure III-9: Mass spectra of maxima J, F_1, F_2, F_3, H_3, and H_7 from the pyrogram resulting from the organic heavy metal fraction of Mix 2 2003

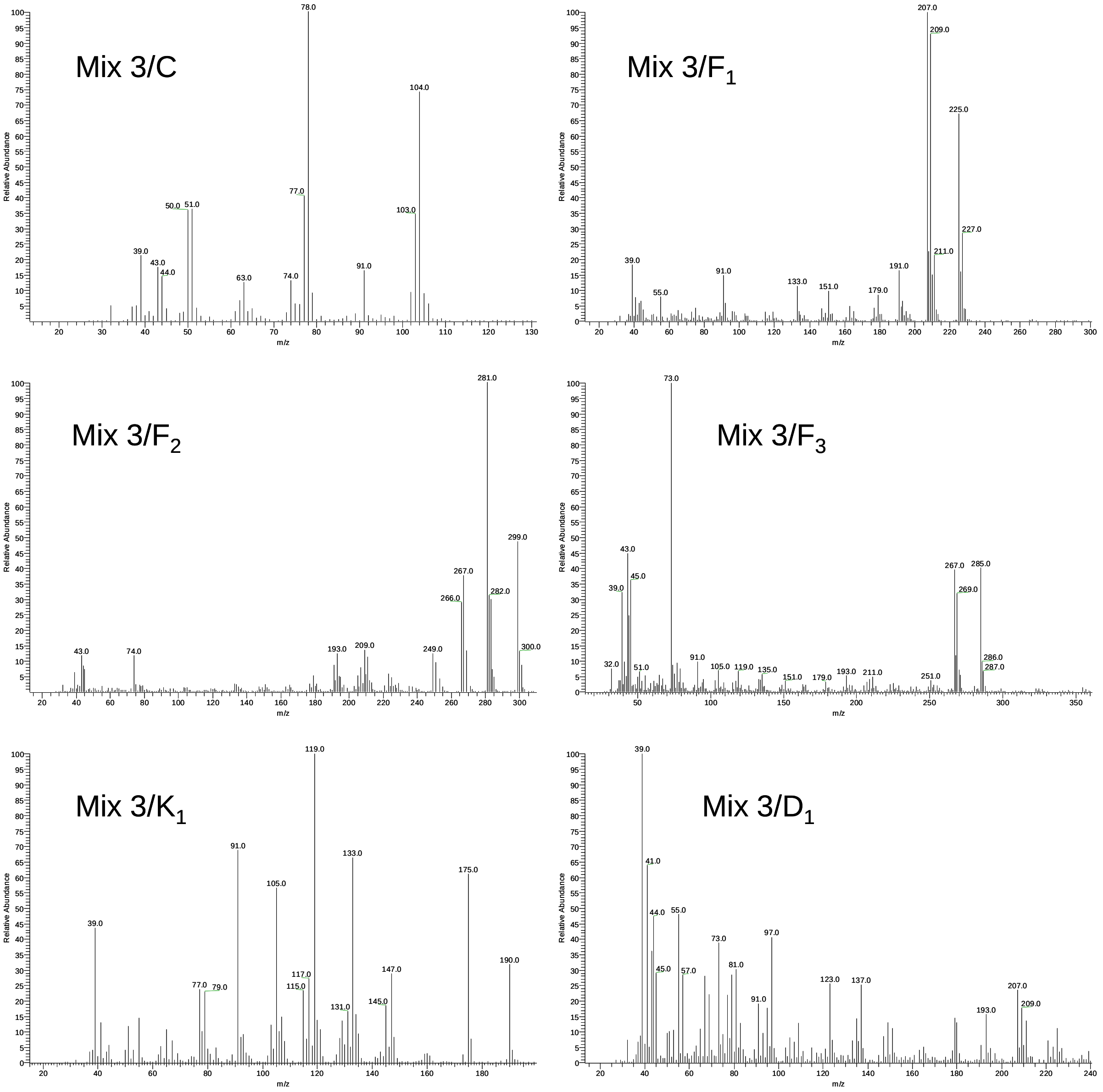

Figure III-10: Mass spectra of maxima C, F_1, F_2, F_3, K_1, and D_1 from the pyrogram resulting from the organic heavy metal fraction of Mix 3 2003

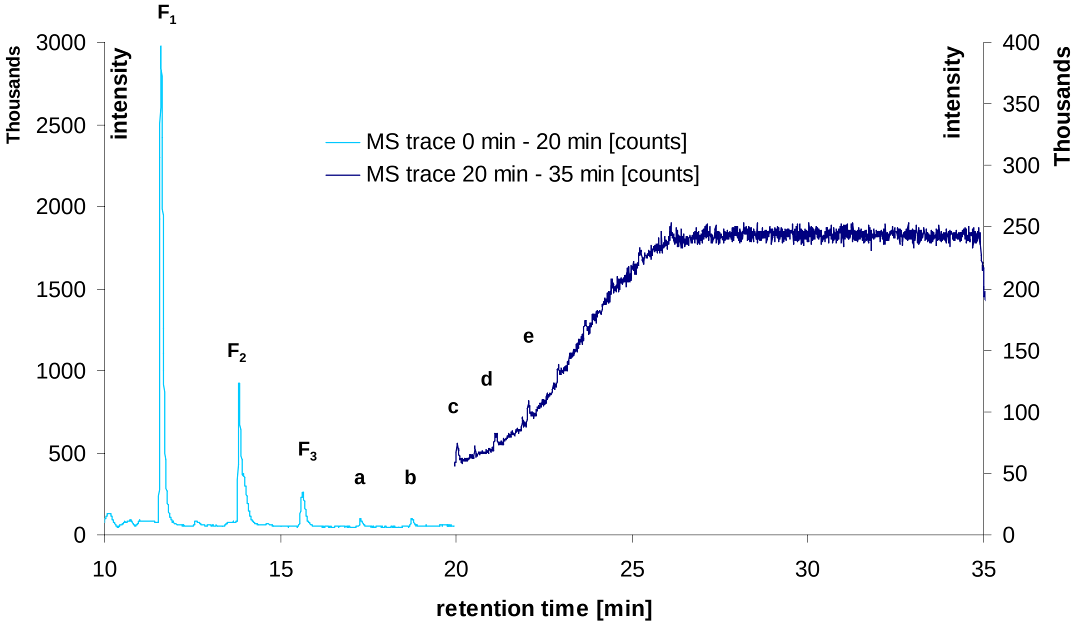

Figure III-11: MS trace of the freeze dried organic heavy metal fraction from acid mine drainage from 2001 with correlating peaks F_{1-3} and further organic silicon peaks a-e.

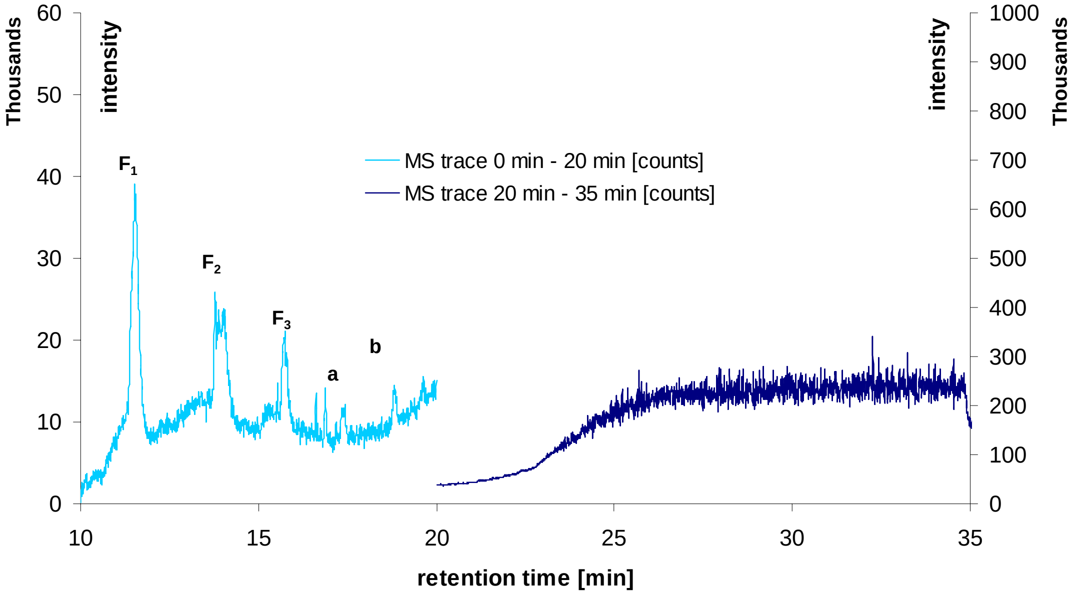

Figure III-12: MS trace of the freeze dried organic heavy metal fraction from Mix 2 from 2001 with correlating peaks F_{1-3} and further organic silicon peaks a and b.

Appendix IV

Appendix IV presents the submitted article:

Degradation of organic matter from black shales and charcoal by the wood-rotting fungus *Schizophyllum commune* and release of DOC and heavy metals in the aqueous phase

Marcus Wengel[1], Christian M. Schmidt[2], Gerd Gleixner[1], Klaus Heide[2], Erika Kothe[3]*

Degradation of organic matter from black shales and charcoal by the wood-rotting fungus ***Schizophyllum commune*** and release of DOC and heavy metals in the aqueous phase

Marcus Wengel[1], Christian M. Schmidt[2], Gerd Gleixner[1], Klaus Heide[2], Erika Kothe[3]*

1 Max Planck Institute for Biogeochemistry, Jena, Germany.

2 Friedrich Schiller University Jena Institute for Geosciences

3 Friedrich-Schiller-University Jena Institute for Microbiology, Germany

mwengel@bgc-jena.mpg.de

christian.m.schmidt@uni-jena.de

ckh@rz.uni-jena.de

erika.kothe@rz.uni-jena.de

*Corresponding author: PD Dr. Gerd Gleixner

Postbox 10 01 64

07701 Jena / Germany

Phone +49(0) 3641 576172

Fax +49(0) 3641 577100

E-mail: gerd.gleixner@bgc-jena.mpg.de

Abstract

We investigated the degradation of refractory organic matter (OM) by fungi and the role of dissolved organic compounds in heavy metal transport. In this study we focused on two organic compounds. First, charcoal (CC), as short time end product of high tempered wood in the absence of oxygen, composed mainly of pure organic matter. Second, black shales (BS) which were formed in an abiotic, geogenic long time process, composed of clay minerals, quartz, sulfides and organic matter. Charcoal consists mainly of polyaromatic hydrocarbons and the organic matter fraction of black shales consists mainly of saturated aliphatic macromolecules. We investigated their degradability by laccase, an oxidative exoenzyme produced by the fungus (F) *Schizophyllum commune*.

Black shales hold iron up to 13.7 g/kg and sulfide up to 9.3 g/kg. Charcoal hold manganese up to 1.1 g/kg. Therefore, in addition to biological degradation of the organic backbone by fungal laccase, competitive abiotic degradation by oxidation processes and removal of heavy metals could occur. This processes increase bioavailability of heavy metals and their potential toxicity.

Ground black shale and charcoal samples were inoculated with minimal medium consisting mainly of aspartic acid, glucose (1:10), and *Schizophyllum commune*. The aqueous and solid phases were sampled after 1 day, 7 days, 28 days and 84 days. Dissolved organic carbon (DOC) was measured as non purgeable carbon (NPOC) and characterized by size exclusion chromatography (SEC) and UV detection. Carbon concentrations of the solid phase were determined by elementary analyses. Ni, Mn, Fe and S were analyzed by ICP-OES.

After initial decrease of the DOC concentrations due to the degradation of carbon from the minimal medium, the DOC increased up to 80 mg/l after 84 days. Carbon decrease in the solid fraction confirmed that this C was released as DOC by the fungus. Biological oxidation processes also increased the Fe and Mn concentrations significant after 84 days in the aqueous phase. Accordingly, Fe and Mn concentrations decreased in the solid fraction, apart from one sample where the Fe concentration increased after 7 days.

DOC from the growth medium and DOC resulting from biological degradation of charcoal and shale were distinguished by SEC with UV detection. The newly generated DOC formed larger molecules than the DOC of the growth medium and interacted quantitatively stronger with heavy metals. The investigation proved that the degradation of persistent carbon sources, such as charcoal and black shale, is accelerated by fungal activity. Consequently the associated release of heavy metals is also accelerated by the fungus. Main products of the biological degradation processes were organic heavy metal complexes which can enter the environment.

Keywords

Size Exclusion Chromatography (SEC), organic matter (OM), weathering, refractory

Introduction

Weathering of ancient organic matter (OM) is one of the key processes in the geochemical cycles of carbon and oxygen (Berner 1989). Together with the burial of recent OM in young sediments these two effects control the concentration of oxygen and carbon dioxide in the atmosphere on a geological timescale (Holland 1978; Lasaga et al. 2002). In general it is assumed that during the weathering process OM is completely oxidized to CO_2. Outcrops of OM weathering profiles developed on shales show a loss of the initial OM content between 60 % and nearly 100 % (Petsch et al. 2000).

However, several studies have shown that some part of the initial OM escapes mineralization (Rowland et al. 1984; Eglinton et al. 1997; Eglinton et al. 1998). Laboratory studies resulted in the formation of dissolved CO_2 and dissolved organic carbon (DOC) as major products of degradation processes of coal under abiotic conditions (Chang et al. 1998; Chang et al. 1999). In addition to abiotic degradation, microbiological activity plays a major role in degradation processes (Robinson et al. 1999; Gadd 2000; Jenisch-Anton et al. 2000; Petsch et al. 2001; Fauser et al. 2002; Petsch et al. 2003). Biological degradation of aromatic macromolecules like lignin is widespread in nature (Gleixner et al. 2001). Exoenzymes produced by wood-rotting fungi attack and metabolize such macromolecules (Leonowicz et al. 1999). Further investigations have shown the ability of fungi to transform and depolymerise coal (Catcheside et al. 1991; Ralph et al. 1996; Hofrichter et al. 1997; Hofrichter et al. 1997; Burford et al. 2003). In natural systems degradation of organic matter from coal and shales is usually accompanied by the release of inorganic compounds (Littke et al. 1991). Inorganic species like heavy metals enter the environment either as inorganic salts or organic heavy metal compounds and consequently increase its bioavailability and its potential toxicity (Stone 1997; Burba et al. 2000; Wu et al. 2001). Therefore microorganism like fungi affect heavy metal mobilization and transfer between organic and inorganic locations (Lin 1997; Gadd 2000). The extend to which this mobilization and transfer occur depends on the chemical form of the heavy metal and on the physical and chemical nature of the site (Alastuey et al. 1999; Gadd 2001). As DOC has a high potential to complex heavy metals it is an important carrier for heavy metals from refractory organic matter in surface water and into the sediment (Ran et al. 2000; Croue et al. 2003).

Aim of this study was to investigate the degradation of refractory OM from charcoal and black shales by the ubiquitous wood-rotting fungi *Schizophyllum commune*. Due to their environmental significance another focus was set on the release of heavy metals. Special emphasis was laid on the interaction of abiotic and biological degradation and on the interaction between degradation of OM and release of heavy metals. We performed incubation experiments over 1 day, 7 days, 28 days and 84 days growing the fungus in minimal medium containing either charcoal or black shales. Additionally, blank and control samples were incubated. Samples were separated in aqueous and solid phase and analyzed separately for DOC and heavy metal concentrations. Aqueous samples were fractionated depending on their molecular size by size exclusion chromatography (SEC) to investigate the formation of organic heavy metal complexes. UV absorption was detected at 254 nm because this wavelength is an indicator for organic heavy metal complexes(Hesse et al. 1997; Schmitt et al. 2001)

Material and Methods

Schizophyllum commune strains 4-40 and 12-43 (strain collection of the institute of microbiology, university of Jena) were grown in minimal medium (MM) holding 2 g/l trypticase peptone, 2 g/l yeast extract, 20 g/l glucose, 0.5 g/l $MgSO_4 * 7 H_2O$, 0.5 g/l KH_2PO_4 and 1 g/l K_2HPO_4) (Schwalb, M. N. et al. 1967; Schwalb, M. V. et al. 1967). 2 flasks with 400 ml deionized water were inoculated with 2 cm^2 of a *Schizophyllum commune* colony and shaken for 5 days at 30 °C. Pregrown cultures were centrifuged, washed twice in distilled water and inoculated in half-concentrated minimal medium, containing charcoal and black shales. 100 ml cultures hold 10 g/l glucose, 1g/l aspartic acid, 0.25 g/l $MgSO_4 * 7 H_2O$, 0.25 g/l KH_2PO_4, 0.5 g/l K_2HPO_4, 60 µg/l thiaminium chloride, 3 g black shales (63 µm fractionized) or 3 g charcoal (63 µm fractionized) and 7 ml pre-grown culture. All samples were adjusted to pH 6.3 with 5 N NaOH and incubated at room temperature. Control samples without the fungus (MM+BS and MM+CC) and blank samples without black shales and charcoal (MM+F) were incubated under identical conditions. Two replicates of 4 charges were cultivated in parallel. Charges were analyzed after 1 day, 7 days, 28 days and 84 days.

For harvest, the samples were centrifuged at 10000 rpm (Megafuge, Heraeus, Goettingen, Germany). The solid phase was freeze dried and digested. 100 mg of each solid sample were digested with 3 ml suprapure HNO_3 (Merck, Darmstadt, Germany), using high pressure microwave digestion (Multiwave, Anton Paar, Graz, Austria) at 1000 W for 20 minutes. The aqueous phase was filtered to 0.45 µm. Size exclusion Chromatography (SEC) was applied for the fractionation of the aqueous samples. The SEC system consisted of a pump (P580, Dionex, Idstein, Germany), an auto sampler (ASI 100, Dionex, Idstein, Germany), a column oven (STH 585, Dionex, Idstein, Germany), a guard column (TSK-GEL PWKL, TOSO HAAS, Stuttgart, Germany), a column (TSK-GEL PW 3000 XL, TOSO HAAS, Stuttgart, Germany), an UV-VIS detector (PDA-100, Dionex, Idstein, Germany) and a multi valve (VALCO VICI CSD 12 UW, Schenkos, Switzerland) to collect fractions at given elution times. Deionized water (MembraPur, Bodenheim, Germany) was pumped with a constant flow of 0.6 ml/min. The column oven was held at 20 °C.

The exclusion limit and total permeation limit of the column were determined by injecting 250 µl of polyethylene oxide solution ($7.7 * 10^{-7}$ g/mol; $M=9.2 * 10^5$ g/mol) (PolymereLabs, Darmstadt, Germany) and 250 µl of 0.01 molar NaCl and $Ni(NO_3)_2$ solutions (Fluka, Munich, Germany). Chromatograms at the wavelength 254 nm were recorded to detect organic compounds which are able to complex heavy metals. Additionally, UV-spectra were recorded continuously from the column effluent at wavelengths between 190 nm and 350 nm. Identification of the MM components was performed using the standards glucose (Fluka, Munich, Germany), aspartic acid (Fluka, Munich, Germany), and L-tryptophan (Fluka, Munich, Germany).

A multi port valve separated the effluent into 11 fractions of 50 ml each and collected the fractions in separate glass tubes. To collect 11 fractions of 50 ml, approximately 100 runs of 20 minutes were needed. Aqueous fractions and the digested solid samples were analyzed for DOC, Fe, Mn, Ni and S. DOC (High TOC, Elementar, Hanau, Germany) was measured by swamping inorganic carbon out and combusting the nonpurgeable organic carbon. The samples were analyzed in the calibration area between 0 and 500 mg/l. For calibration, we used 4412.2 mg/l sodium carbonate and 1260.7 mg/l trishydroxymethylaminomethane. The external standard was Super-2 (National Water Research, Canada). Heavy metal concentrations were analyzed using ICP-OES (Optima 3000 DV, Perkin Elmer, Norwalk, USA,), calibrated with multi element standards (Super Certi Prep, Metuchen, USA) in the area between 0 mg/l and 2.5 mg/l. External standards were SPS-WW 1, SPS-WW 2 and SPS-SW 2 (Spectrapure Standards, Danmark).

Results

DOC concentration in the aqueous phase of the inoculated black shale sample (MM+BS+F) decreased from 3900 mg/l to 210 mg/l during 7 days. From day 7 to day 84 the concentration increased to 288 mg/l (Fig. 1). DOC concentrations in the control sample MM+BS decreased from 4210 mg/l to 3114 mg/l. In accordance with the DOC increase at the end of the incubation, carbon concentrations in the solid MM+BS+F sample decreased from 116 g/kg to 74 g/kg. C concentrations in the control sample increased from 71 mg/kg to 95 mg/kg. Similar concentration changes were analyzed in the MM+CC+F sample (Fig. 2). DOC concentrations decreased from 3876 mg/l to 261 mg/l during the first 7 days and up to 84 days the DOC concentrations increased to 290 mg/l. Carbon concentrations of the solid MM+CC+F sample decreased from 823 mg/kg to 762 mg/kg during 84 days. Control samples showed similar concentration changes as the controls of the MM+BS+F samples. DOC concentrations in the blank MM+F sample decreased during 7 days from 3900 µg/l to 270 µg/l and decreased further during the remaining experiment to 240 µg/l.

Iron concentrations in the aqueous phase increased in 84 days from 220 µg/l to 330 µg/l in the MM+BS+F sample, while Mn concentrations increased from 1300 µg/l to 2430 µg/l. In contrast to Fe and Mn, Ni concentrations were nearly identical in the 1 day, 7 days, and 28 days samples of the MM+BS+F sample and decreased after 84 days from 1090 µg/l to 410 µg/l (Fig. 3). Fe concentrations in the aqueous MM+CC+F sample increased during 84 days from 30 µg/l to 90 µg/l and Mn concentrations increased from 1000 µg/l to 2340 µg/l, respectively. Ni concentrations increased up to 10 µg/l during 7 days and remained nearly constant until 84 days (Fig. 4). Sulfur concentrations slightly increased after 7 days in all charcoal and black shale samples in comparable amounts. In the 7 days, 28 days and 84 days samples sulfur concentrations varied insignificantly. There were no significant concentration changes in the control samples (MM+CC, MM+BS) and in the blank sample (MM+F).

The increased Fe and Mn concentrations in the aqueous phase were mirrored by decreasing concentrations in the solid phase. Fe and Mn concentrations decreased from 1 day to 84 days in the MM+BS+F sample (Fe from 11978 mg/kg to 10078 mg/kg; Mn from 116 mg/kg to 110 mg/kg) and in the MM+CC+F sample (Fe from 40 mg/kg to 34 mg/kg; Mn from 500 mg/kg to 470 mg/kg) with exception of Fe in the 7 days MS+CC+F sample which increased from 29 mg/kg to 40 mg/kg. Nickel concentrations also decreased significant in these samples from 150 mg/kg to 131 mg/kg (MM+BS+F) and from 10 mg/kg to 7 mg/kg (MM+CC+F). The heavy metal concentrations of the control samples did not change significant. Sulfur changes were also not significant.

Fractionation and analytical investigations of the aqueous 1 day MM+BS+F and 1 day MM+CC+F samples by SEC, UV detection at 254 nm as characteristic wavelength for organic ligands, ICP-OES- and DOC measurements resulted in a differentiation of the organic substances. The exclusion limit of the SEC column was at 8.1 min. The total permeation limit was at 18.3 min. DOC concentrations in the fractions of the 1 day MM+BS+F sample appeared in two maxima (Fig. 5). The DOC maximum at lower retention times correlated with the maximum of the UV trace at 254 nm and with maximum heavy metal concentrations, where maximum iron concentrations eluted slightly earlier. The second DOC maximum correlated with elevated Fe concentrations but did not correlate with the second UV maximum at 254 nm.

Similar correlation was detected in the 1 day MM+CC+F sample where DOC, heavy metals and UV trace had a maximum at 13.5 minutes (Fig. 6). At 16.5 minutes only DOC and Fe had a maximum. An other maximum of the UV trace at 254 nm was detected at 15.2 min.

UV spectra of the first maxima in both 1 day samples (13.5 min and 14.5 min) were similar to amino acid standards, especially to aspartic acid. The spectra of the second maxima (16.5 min) were similar to glucose (Fig. 7).

After 84 days, fractions holding elevated concentrations of DOC and heavy metals shifted to shorter retention times. This shift of the MM+BS+F and MM+CC+F samples was also realized at he UV trace at 254 nm. Both samples had one common maximum of DOC, heavy metals and UV trace at 254 nm and two more UV maxima. In the MM+BS+F sample (Fig. 8) the first UV maximum between 8.1 min and 10.5 min correlated moderately with DOC and heavy metal concentrations. The second and the third maximum (10.5 min to 12 min) correlated with elevated DOC and heavy metal concentrations.

The first UV maximum of the 84 days MM+CC+F sample (Fig. 9) (8.1 min to 11.0 min) correlated with DOC and heavy metal concentrations. The other two maxima (11.5 min and 12 min) correlated only moderately with DOC and Fe concentrations.

UV spectra of the 84 days samples had elevated intensities at the third maximum from 210 nm to 230 nm and from 250 nm to 270 nm (Figs. 10 and 11). At the second maximum both samples showed decreasing intensities from 190 nm to 350 nm. At the first maximum of the MM+BS+F sample elevated intensities were detected from 210 nm to 240 nm and from 250 nm to 300 nm. The MM+CC+F sample had intensity maxima at 197 nm, 235 nm and 275 nm.

Discussion

The investigation demonstrated the impact of the wood-rotting fungus *Schizophyllum commune* on the degradation of DOC and the release of heavy metals into solution. The degradation of black shales and charcoal by the fungus resulted in a significant increase of DOC and heavy metal concentrations in the aqueous samples and decrease of DOC and heavy metals in the solid samples. Insignificant concentration changes of Ni and relatively low concentrations of Fe in the aqueous phase are probably results of assimilation by fungus in the aqueous phase and iron precipitation initiated by abiotic oxidation processes (Lin 1997).

In contrast to the release of heavy metals by the fungus there was no detectable release of sulfur by the fungus. This could be shown, as sulfur concentrations in the inoculated samples and in the control samples showed only slightly different changes. Thus, pyrite oxidation was in this case not the reason for heavy metal release.

DOC concentrations decreased rapidly in all samples that included *Schizophyllum commune* during the first 7 days. After 7 days the fungus had metabolized most carbon sources of the growth medium (MM). In the following period up to 84 days the DOC concentrations in the MM+BS+F and MM+CC+F samples increased in contrast to the blank (MM+F) sample and the control (MM+BS and MM+CC) samples. This significant increase is explained by the degradation of high molecular weight organic molecules in the solid material by the fungus as evidenced by interpretation of UV chromatograms and UV spectra.

Nearly constant Ni concentrations and relatively low Fe concentrations in the aqueous phase were results of precipitation processes and assimilation of heavy metals by the fungus. It could not ascertained if heavy metals from black shale and charcoal are inert to metabolisation processes of the fungus. Possibly, heavy metals are absorbed by fungus, metabolized and released as organic heavy metal complexes. An other opportunity is that heavy metals are not involved in metabolisation processes and exclusively act as donor elements in the aqueous phase to complexe all possible kind of DOC.

Organic matter of the fractions with maximum DOC concentrations in the MM+BS+F and MM+CC+F sample after 1 day were identified to be the main carbon components of the minimal medium. This could be shown by identical UV spectra of pure glucose and maximum 2 and identical spectra of a cocktail of pure aspartic acid with traces of L-tryptophan and maximum 1. Although there were higher concentrations of glucose in the minimal medium samples, the UV active amino acids resulted in higher UV absorption at 210 nm and between 260 nm and 280 nm in contrast to the less UV active glucose (Feinstein 1995; Field et al. 2000; Pretsch 2000). UV absorption between 260 nm and 280 nm in the aqueous samples resulted from traces of aromatic amino acids from the preculture. Identical retention times of heavy metals and the identified aspartic acid of the 1 day samples suggests the complexation of heavy metals by aspartic acid which was not observed with glucose. No UV absorption at 254 nm and elevated Fe concentrations at DOC maxima 2 of the 1 day MM+BS+F sample and the MM+CC+F sample refer to organic matter not forming iron complexes and to inorganic Fe species.

UV absorption of the 1 day MM+BS+F sample at 16.5-18.5 min and of the 1 day MM+CC+F sample at 15 min are not coincident with heavy metal concentration. These absorption maxima represent smaller DOC constituents, which are probably excreted by fungus. The shift to smaller retention times of the organic compounds after 84 days gave evidence of the metabolization or modification of the minimal medium and furthermore of the formation of new DOC compounds. These newly formed organic compounds and organic heavy metal complexes were results of degradation and release processes of black shales and charcoal.

The second UV maximum of the 84 days MM+BS+F sample (10.5 min) and the second maximum of the 84 days MM+CC+F sample (11.5 min) had similar UV spectra. The third maxima of both samples at 12 minutes had also similar UV spectra. As similar UV spectra refer to similar organic compounds it can be assumed that these spectra refer to metabolized compounds of the minimal medium. The broad, first UV maximum in the 84 days MM+BS+F and MM+CC+F samples appeared at lower retention times compared to the first maxima in the 1 day samples. In addition, the UV spectra differed significantly from each other and from these of the 1 day samples. These facts demonstrate of the formation of new and larger heterogenic organic compounds by biological degradation of black shales and charcoal. Interpretation of UV spectra, unfortunately yield less information on the molecular structure. However, elevated UV absorption between 210 nm and 240 nm and between 250 nm and 300 nm could indicate aromatic compounds such as benzenes and phenols or carboxylic groups or long chain alkenes which could be degradation products of black shales and charcoal (Feinstein 1995; Pretsch 2000). Organic compounds absorbing at the first UV maximum of the 84 days MM+CC+F sample were more specific for complexing heavy metals than compounds absorbing at the second and the third maximum. In the 84 days MM+BS+F sample organic molecules absorbing at the second and the third UV maxima are more specific ligands for heavy metals. These different heavy metal specificities of the newly formed DOC indicated different molecular structures of the degradation products resulting in different complexing potentials. Therefore, organic heavy metal complexes from refractory organic matter can be released biogenically and subsequently enter the environment

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FIGURE CAPTIONS

Figure 1: DOC concentrations in the aqueous inoculated black shale sample (MM+BS+F), DOC_c concentrations in the aqueous control sample (MM+BS), DOC_b concentrations in the aqueous blank sample (MM+F), Carbon (C) concentrations in the solid black shale sample (MM+BS+F) and C_c concentrations in the solid control sample (MM+BS)

Figure 2: DOC concentrations in the aqueous inoculated charcoal sample (MM+CC+F), DOC_c concentrations in the aqueous control sample (MM+CC), DOC_b concentrations in the aqueous blank sample (MM+F), Carbon (C) concentrations in the solid charcoal (MM+CC+F) sample and C_c concentrations in the solid control sample (MM+CC)

Figure 3: Concentrations of Fe, Ni, Mn and S in the aqueous inoculated black shale sample (MM+BS+F) after 1 day, 7 days, 28 days and 84 days and Fe_c, Ni_c, Mn_c, and S_c concentrations of the control sample (MM+BS)

Figure 4: Concentrations of Fe, Ni, Mn and S in the aqueous inoculated charcoal sample (MM+CC+F) after 1 day, 7 days, 28 days and 84 days and Fe_c, Ni_c, Mn_c, and S_c concentrations of the control sample (MM+CC)

Figure 5: Concentrations of DOC, Fe, Ni and Mn in the individual fractions of the 1 day inoculated black shale sample (MM+BS+F) after Size Exclusion Chromatography. UV trace of the 1 day inoculated black shale sample (MM+BS+F) with the absorption maxima 1 and 2

Figure 6: Concentrations of DOC, Fe, Ni and Mn in the individual fractions of the 1 day inoculated charcoal sample (MM+CC+F) after Size Exclusion Chromatography. UV trace of the 1 day inoculated charcoal sample (MM+CC+F) with the absorption maxima 1 and 2

Figure 7: UV spectra of the aqueous 1 day inoculated samples (MM+BS+F and MM+CC+F) at the absorption maxima 1 and 2. UV spectra of the ingredients aspartic acid/tryptophan and glucose of the minimum medium

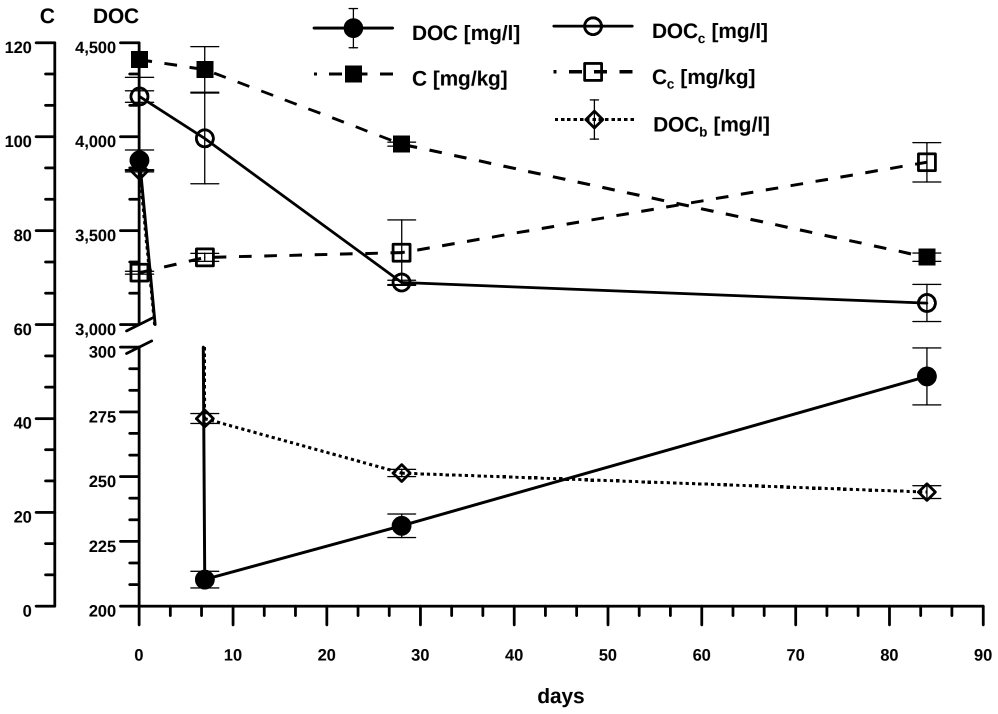
Figure 8: Concentrations of DOC, Fe, Ni and Mn in the individual fractions of the 84 day inoculated black shale sample (MM+BS+F) after Size Exclusion Chromatography. UV trace of the 84 day inoculated black shale sample (MM+BS+F) with the absorption maxima 1 and 2

Figure 9: Concentrations of DOC, Fe, Ni and Mn in the individual fractions of the 84 day inoculated charcoal sample (MM+CC+F) after Size Exclusion Chromatography. UV trace of the 84 day inoculated charcoal sample (MM+CC+F) with the absorption maxima 1 and 2

Figure 10: UV spectra of the aqueous 84 day inoculated black shale sample (MM+BS+F) at the absorption maxima 1, 2 and 3.

Figure 11: UV spectra of the aqueous 84 day inoculated charcoal sample (MM+CC+F) at the absorption maxima 1, 2 and 3.

Figure 1



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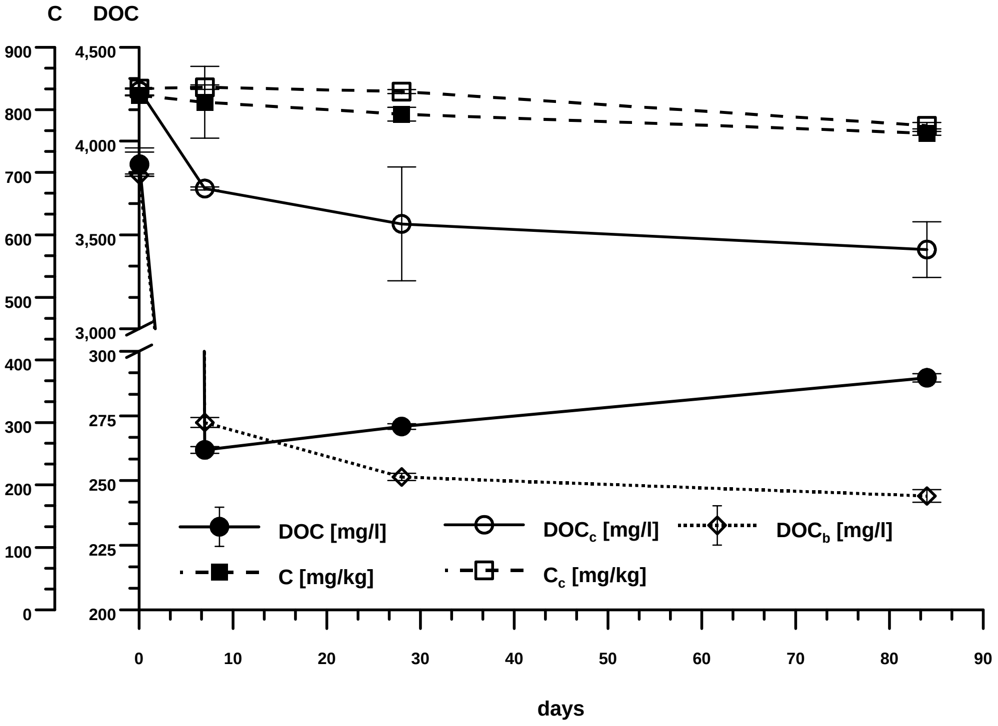

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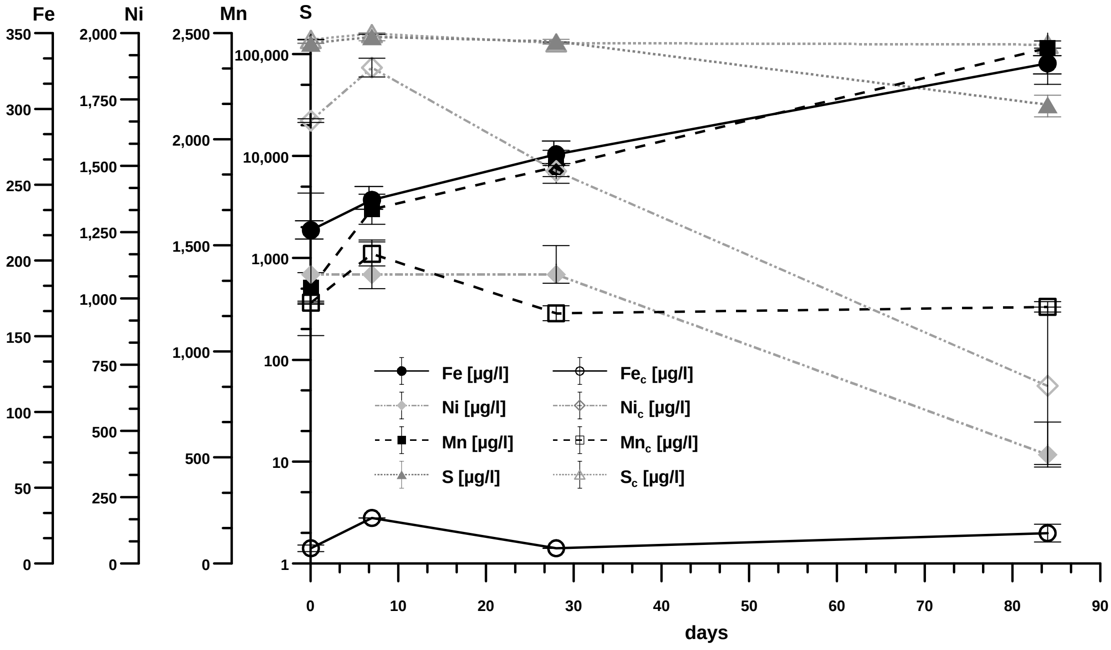

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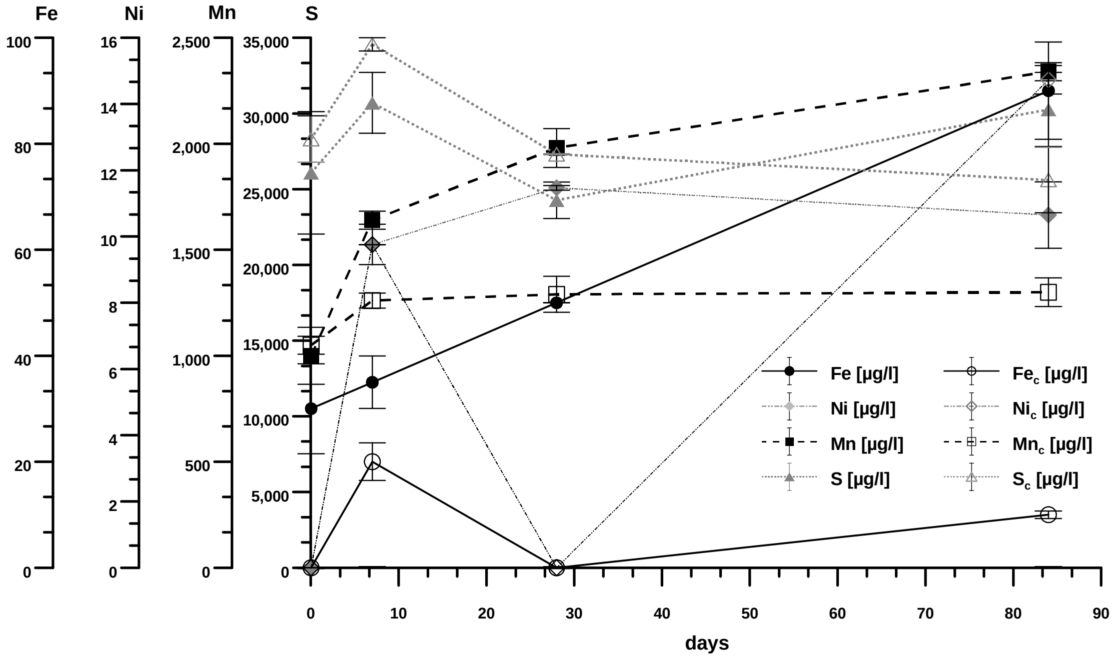

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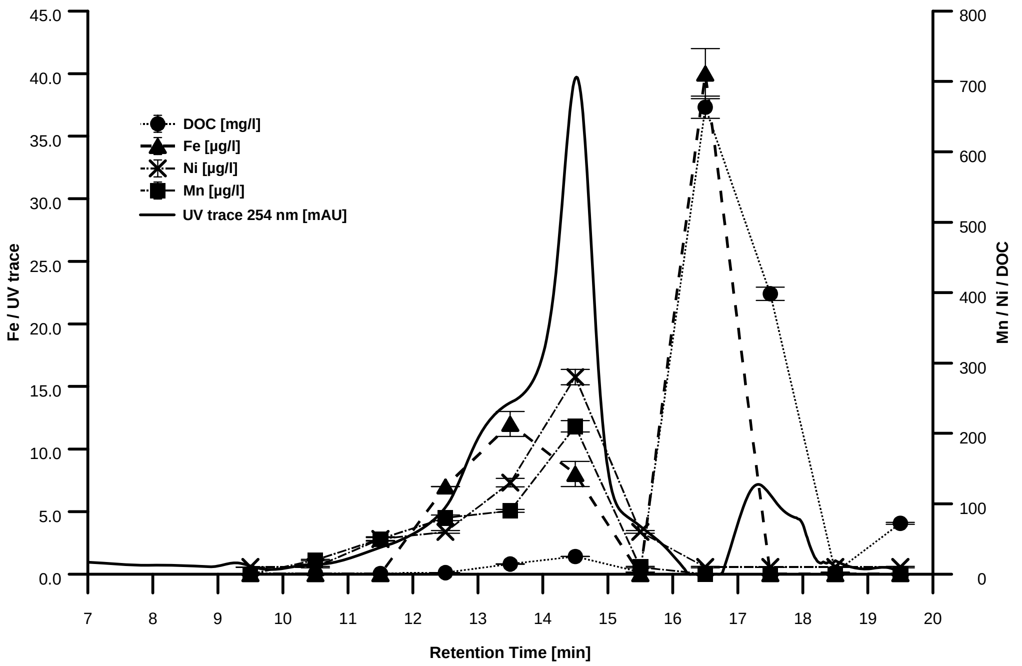

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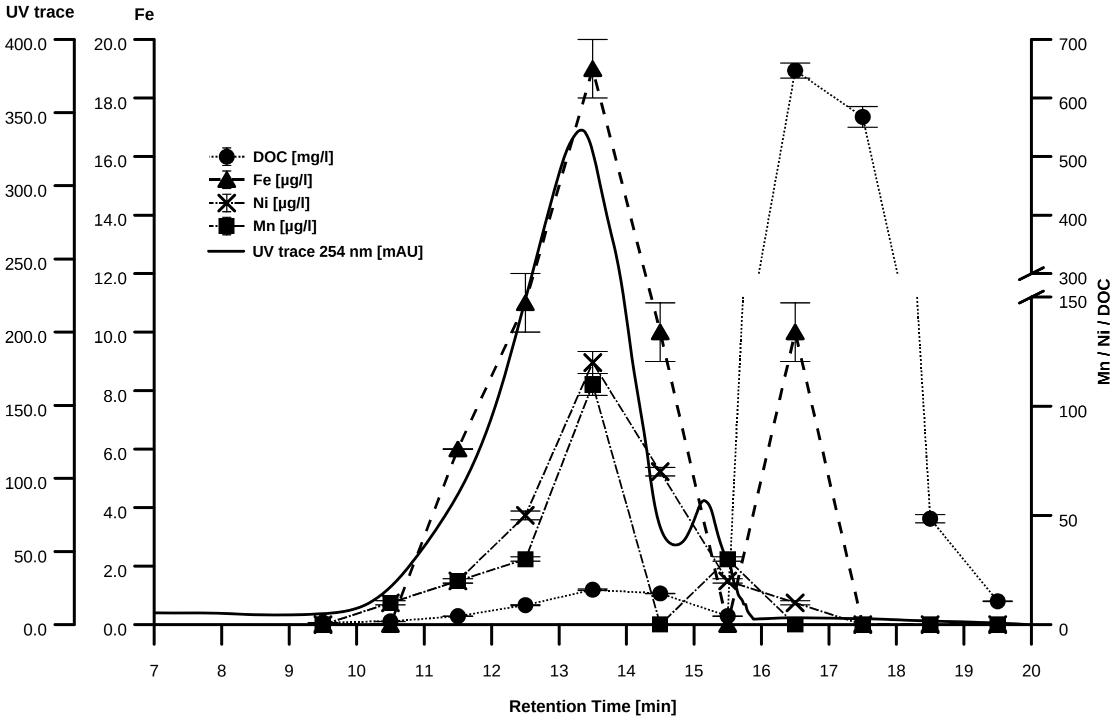

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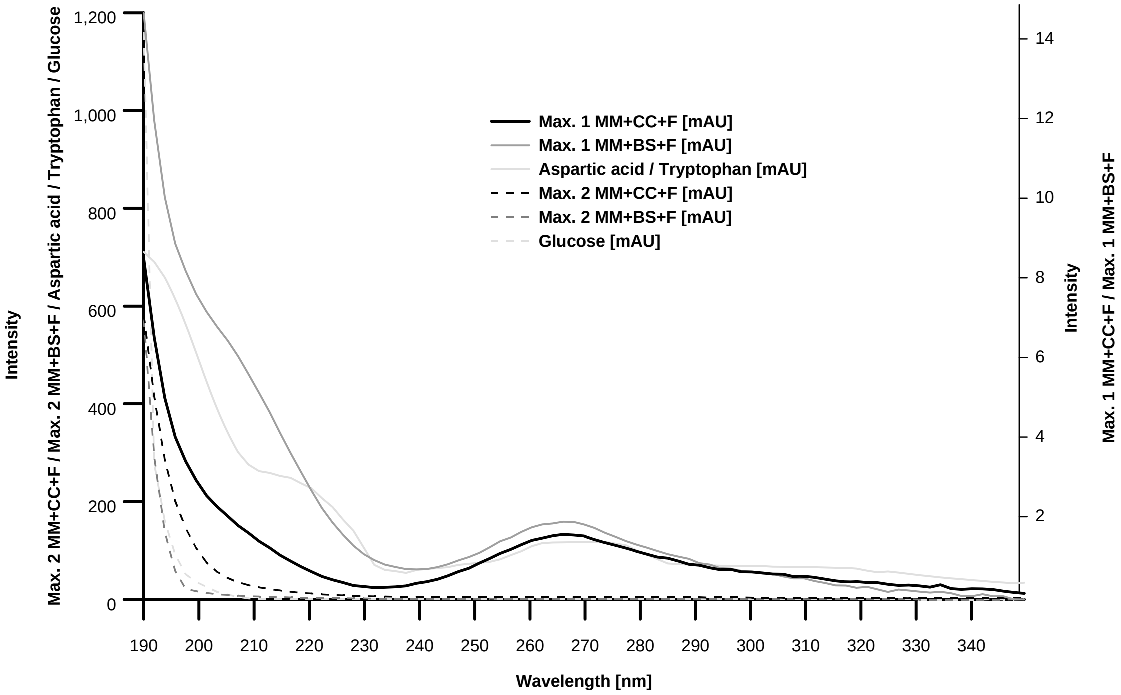

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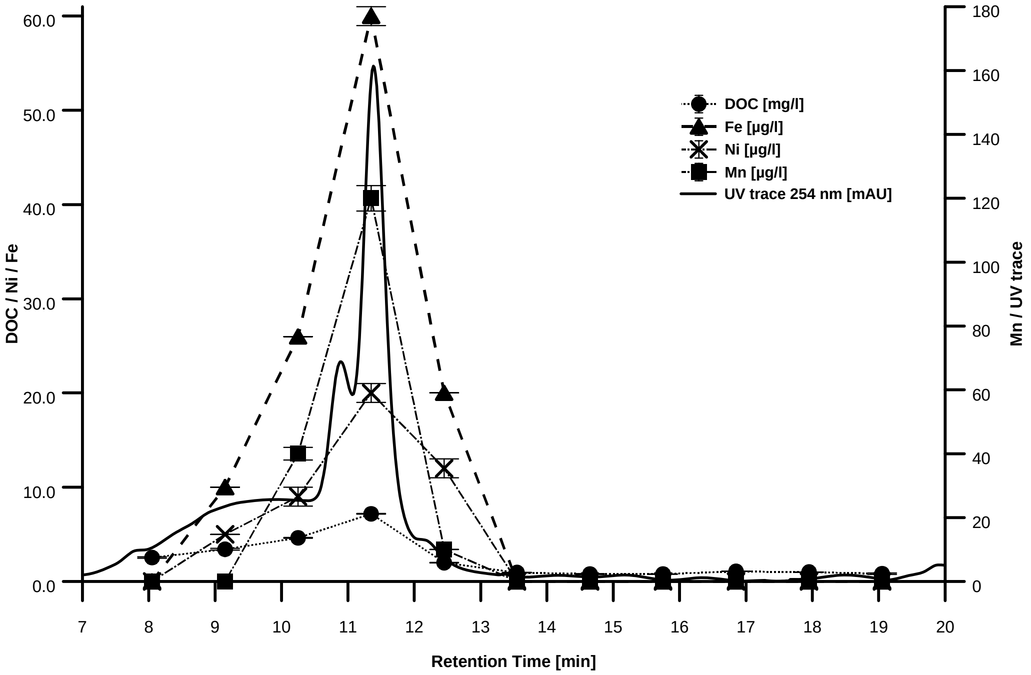

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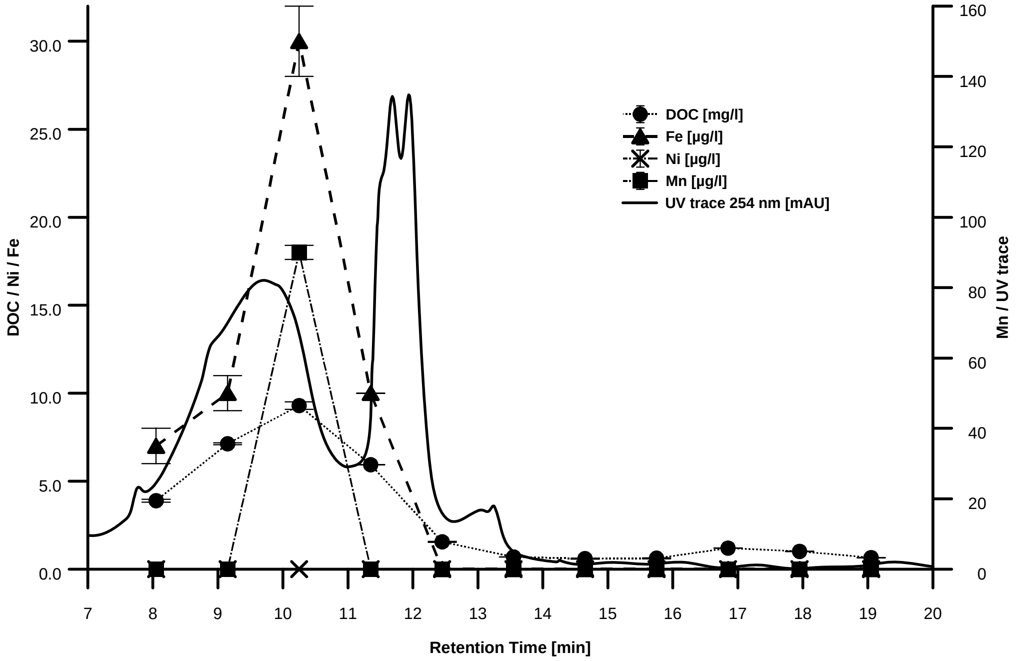

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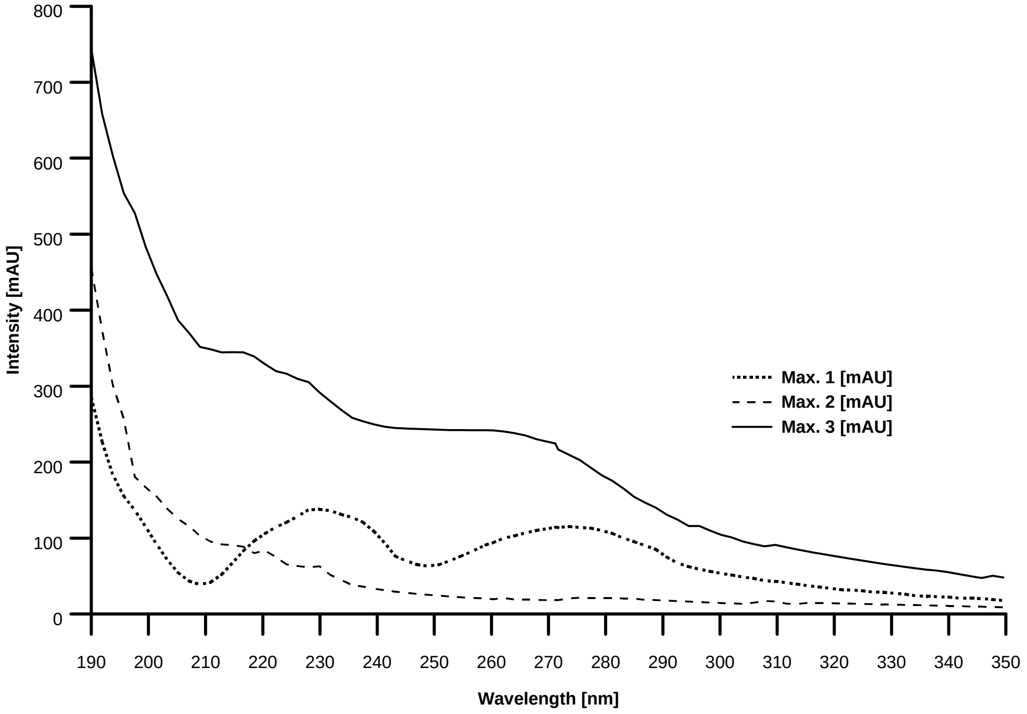

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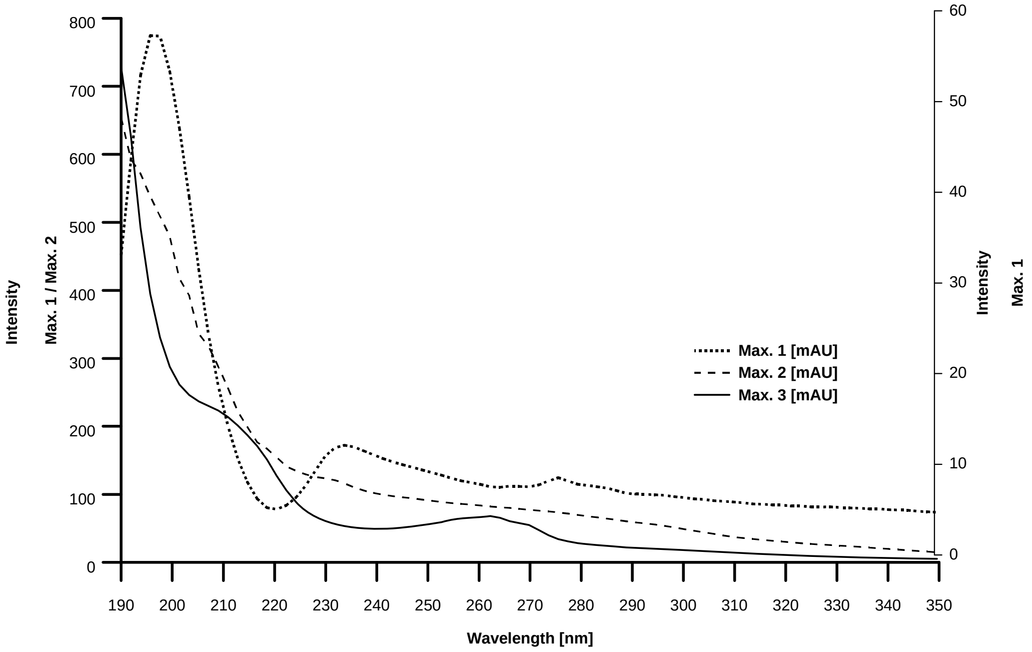
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260
270
280
290
300
310
320
330
340
350
Wavelength [nm]
60
50
40
30
20
10
0
Intensity
Max. 1
Max. 1 [mAU]
Max. 2 [mAU]
Max. 3 [mAU]

Curriculum Vitae

Name: Marcus Wengel
Geburtsdatum/-ort: 25.09.1973 in Zwickau, Sachsen, Deutschland
Familienstand: ledig

Werdegang:

1980-1986	Politechnische Oberschule in Mosel, Sachsen
1986-1994	Rechberggymnasium in Donzdorf, Baden-Württemberg
06/1994	Abitur mit Leistungskursen Chemie und Deutsch, Gesamtnote 2,6
1994-1996	Zivildienst in Göppingen, Baden Württemberg
1996-2001	Studium der Chemie an der Friedrich Schiller Universität in Jena, Thüringen
09/1998- 08/1999	Studium der Chemie und Umweltchemie an der Northumbrian University of Newcastle, England, Great Britain
05/2001	Diplomarbeit am Max-Planck-Institut für Biogeochemie in Jena, Thüringen
11/2001	Diplom der Chemie (Fachrichtung Umweltchemie) an der Friedrich Schiller Universität in Jena, Thüringen, Gesamtnote 1,6 (Gut)
11/2001-	Promotion zum Thema „Characterization of coexisting dissolved organic carbon and heavy metals in surface waters“ in der Arbeitsgruppe von Herrn PD Dr. Gleixner am Max-Planck-Institut für Biogeochemie in Jena, Thüringen, Deutschland

Jena, den 16. Juni 2005

Marcus Wengel

Marcus Wengel

Eigenständigkeitserklärung:

Hiermit erkläre ich, daß ich diese Arbeit selbstständig und ausschließlich unter Verwendung der angegebenen Literatur und Hilfsmittel angefertigt habe.

Jena, den 16. Juni 2005

Marcus Wengel

Marcus Wengel