Mobilization of metals as influenced by redox potential in slurries of floodplain soil material and biogeochemical fractionation of mercury in floodplain soil profiles

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„Ich bin immer noch verwirrt,
aber auf einem höherem Niveau.“

(Enrico Fermi, Physiker)

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AED	Atomic emission detection
As_i	Inorganic arsenic
AVS	Acid volatile sulfides
C_{HWE}	Hot water extractable carbon
C_{org}	Organic carbon
CV-AFS	Cold vapor atomic fluorescence spectrometer
DMAs	Dimethyl arsenic
DOC	Dissolved organic carbon
EDX	Energy dispersive X-ray spectroscopy
E_H	Redox potential normalized to the standard hydrogen electrode
Fe_d	Dithionite soluble iron
Fe_o	Oxalate soluble iron
GC	Gas chromatograph
Hg^0	Elemental Hg
Hg_{sum}	Sum of Hg fractions
Hg_t	Total Hg
ICP-MS	Inductively coupled plasma mass spectrometer
ICP-OES	Inductively coupled optical emission spectrometer
MC	Microcosm
Me_2Hg	Dimethyl mercury
MeHg	Methyl mercury
MMAs	Monomethyl arsenic
MMSb	Monomethyl antimony
n	Number of samples
N_t	Total nitrogen
PLFA	Phospholipid fatty acids
Sb_i	Inorganic antimony
SE	Sequential extraction
SEM	Scanning electron microscopy
SRB	Sulfate reducing bacteria
SUVA	Specific ultraviolet absorbance
$SUVA_{254}$	Specific ultraviolet absorbance at 254 nm
TOC	Total organic carbon
UV	Ultraviolett

Summary

The current thesis is based on the results of laboratory experiments which were conducted during a postgraduate training at the University of Wuppertal. This summary refers to chapter 4 of this thesis as well as to the following publications: Frohne et al. 2011; Frohne et al. 2012, Frohne and Rinklebe 2013, Frohne et al. 2014. These papers can be found in the appendix (Appendices 1 to 4).

Floodplain soils are often enriched with various metals mainly of anthropogenic origin. Mobilization of metals poses a severe threat to humans and the environment. Floodplain soils are characterized by fluctuating water tables and thus fluctuating redox potential (E_H). The E_H is an important factor influencing metal mobilization and methylation in floodplain soils.

The knowledge about redox-induced dynamics and methylation of metals in floodplain soils, particularly at low pH, is incomplete. Mechanistic experiments concerning the redox-related dynamics of methyl mercury (MeHg) as well as the knowledge about the biogeochemical fractions of Hg and the factors affecting the methylation rate of Hg in floodplain soils are rare. The respective underlying biogeochemical processes such as redox-induced changes in the binding of metals to dissolved organic carbon (DOC), pedogenic oxides, or sulfur compounds as well as the influence of changing E_H on metal methylation are still not fully understood. For a better understanding of these processes, it is crucial to elucidate the basic mechanisms responsible for metal dynamics in floodplain soil material at changing E_H.

For this purpose a biogeochemical microcosm (MC) system was used to simulate flooding of floodplain soil material under controlled E_H. Therefore, floodplain top soil material was mixed with water to achieve soil slurries. Moreover, a Hg-specific sequential extraction (SE) procedure was conducted with soil material originating from different floodplain soil profiles to assess the biogeochemical fractions of Hg. To sum up the results, Ba, Cd, Co, Cu, Ni, Sr, and Zn are immobilized under reducing conditions, likely due to the formation of hardly soluble sulfides, and can be mobilized by the dissolution of these sulfides at high E_H. In contrast, As, Cr, and V are mobilized under reducing conditions and can be immobilized when E_H increases, most likely due to the co-precipitation with newly formed Fe (hydr)oxides at high E_H. Additionally, a direct influence of E_H via speciation changes is supposable for As and V. The oxidized ions of these elements are assumed to be less soluble compared with their reduced counterparts. Dissolved organic carbon is an important factor for the dynamics of the studied metals. At low E_H, DOC is suggested to be more prone to complex metals and

the binding strength of metals to DOC is assumed to be higher compared with high E_H. The methylation of As and Sb is promoted under reducing conditions, most likely due to the activity of microorganisms.

The mobilization of Hg under changing E_H in the slurry is mainly influenced by DOC, with a high affinity of Hg to hot water extractable carbon (C_{HWE}). Sequential extraction of material originating from floodplain soil profiles revealed that Hg is mainly associated with the fractions accounting for elemental Hg (Hg^0), followed by organic-bound Hg and Hg sulfides in the soil material originating from the floodplain soil profiles. Mercury in the mobile fractions was less abundant. Differences in Hg binding forms between Wupper and Saale River floodplain soil profiles are partly attributed to the different industrial history of the sites. Mercury is likely associated with Fe (hydr)oxides in the soil profiles. Amorphous Fe (Fe_o) is suggested to exhibit a higher affinity for Hg compared with crystalline Fe (Fe_d). However, the relationship between Hg and Fe was less obvious in the soil slurry of the MC experiment. Methylation of Hg seems to be partly governed by the DOC/Hg ratio and the soil microbial community.

Results of the current thesis demonstrate that the mobilization of metals in floodplain soil slurries is largely affected by E_H changes and related factors such as DOC, sulfur cycling, Fe (hydr)oxides, and the microbial community. Since many floodplain soils are highly contaminated with metals that might be mobilized and transferred to humans, it is recommended to further study the solubility of those contaminants. For the future, the structure of DOC, the speciation of metals and sulfur as well as the structure of the microbial community should be further studied in floodplain soil slurries under changing E_H. For example, this can be achieved by the combination of biogeochemical MC experiments with synchrotron based techniques or energy dispersive X-ray analysis (EDX). Analysis of acid volatile sulfides (AVS) can help to gain deeper insights into S cycling in floodplain soils. The determination of phospholipid fatty acids (PLFA) might be promising to elucidate the role of microorganism for the methylation of metals in floodplain soils.

Zusammenfassung

Die vorliegende Arbeit basiert auf den Ergebnissen verschiedener Laborexperimente, die während meiner Doktorandenzeit an der Universität Wuppertal durchgeführt wurden. Diese Zusammenfassung bezieht sich auf das Kapitel 4 dieser Arbeit und auf die folgenden Publikationen: Frohne et al. 2011; Frohne et al. 2012, Frohne and Rinklebe 2013, Frohne et al. 2014. Diese Veröffentlichungen befinden sich im Anhang (Appendices 1 bis 4)

Auenböden sind häufig mit persistenten Metallen anthropogenen Ursprungs belastet. Die Mobilisierung von Metallen stellt eine ernsthafte Gefahr für Mensch und Umwelt dar. Auenböden sind durch schwankende Wasserstände und somit durch ein schwankendes Redoxpotenzial (E_H) gekennzeichnet. Das E_H ist ein wichtiger Einflussfaktor für die Mobilisierung und die Methylierung von Metallen in Auenböden.

Die Kenntnisse über die redoxbedingte Dynamik und Methylierung von Metallen in Auenböden ist unvollständig, besonders im Hinblick auf saure Böden. Mechanistische Experimente, die sich mit der redoxinduzierten Dynamik von Methylquecksilber (MeHg) befassen, sowie das Wissen über die biogeochemischen Fraktionen von Hg und die Faktoren, die die Methylierungsrate von Hg in Auenböden beeinflussen, sind rar. Die zugrunde liegenden biogeochemischen Prozesse wie z.B. die redoxinduzierte Änderung der Bindung von Metallen an gelösten organischen Kohlenstoff (DOC), pedogene Oxide oder Schwefel sowie der Einfluss eines sich ändernden E_H auf die Methylierung von Metallen sind noch nicht vollständig aufgeklärt. Für ein besseres Verständnis dieser Prozesse ist es sehr wichtig, die grundlegenden Mechanismen zu erforschen, die für die Dynamik der Metalle in Auenböden bei veränderlichem E_H verantwortlich sind.

Um dieses Ziel zu erreichen, wurde ein biogeochemisches Mikrokosmensystem verwendet, das die Überflutung von Bodenmaterial unter kontrollierten Redoxbedingungen ermöglicht. Hierbei wurde Auenoberbodenmaterial mit Wasser vermischt, um eine Bodensuspension zu erhalten. Außerdem wurde eine sequentielle Extraktion an Böden verschiedener Auenbodenprofile durchgeführt, um die biogeochemischen Fraktionen von Hg zu bestimmen.

Zusammenfassend kann man festhalten, dass Ba, Cd, Co, Cu, Ni, Sr und Zn unter reduzierenden Bedingungen immobilisiert werden, wahrscheinlich aufgrund der Entstehung von schwer löslichen Metallsulfiden. Unter oxidierenden Bedingungen findet eine Mobilisierung dieser Metalle unter Auflösung der Sulfide statt. Im Gegensatz dazu sind As, Cr und V unter reduzierenden Bedingungen mobil. Diese Metalle werden mit steigendem E_H

immobilisiert, da sie gemeinsam mit neu gebildeten Fe (hydr)oxiden ausfallen. Zusätzlich wird ein direkter Einfluss des E_H auf die Mobilisierung von As und V vermutet. Die oxidierten Ionen dieser Elemente könnten im Vergleich zu ihren reduzierten Partnern schwerer mobilisierbar sein. Gelöster organischer Kohlenstoff beeinflusste das Verhalten der untersuchten Metalle maßgeblich. Bei niedrigem E_H neigt das DOC vermutlich eher zur Komplexbildung mit Metallen. Außerdem ist die Bindungsstärke der Metall-DOC-Komplexe bei niedrigem E_H im Vergleich zu hohem E_H vermutlich größer. Die Methylierung von As und Sb wird durch reduzierende Bedingungen gefördert und ist wahrscheinlich auf die Aktivität von Mikroorganismen zurückzuführen.

Die Mobilisierung von Hg in der Bodensuspension wird bei veränderlichem E_H hauptsächlich durch DOC beeinflusst. Quecksilber zeigt eine hohe Affinität zu heißwasserlöslichem organischem Kohlenstoff (C_{HWE}). Die sequentielle Extraktion des Bodenmaterials der Auenbodenprofile verdeutlicht, dass das Hg in den Böden der Auenbodenprofile hauptsächlich als elementares Hg (Hg^0) vorliegt. Außerdem wurde es in den Fraktionen „organisch gebundenes Hg“ und „Hg Sulfide“ gefunden. Der Anteil an mobilisierbarem Hg war gering. Unterschiede zwischen den Bindungsformen von Hg in den Auenbodenprofilen der Wupper und der Saale lassen sich hauptsächlich durch die unterschiedliche Industriegeschichte der beiden Regionen erklären. Quecksilber ist in den Auenbodenprofilen wahrscheinlich mit Fe (hydr)oxiden assoziiert. Dabei scheinen amorphe Fe (hydr)oxide eine größere Rolle zu spielen als kristalline Fe (hydr)oxide. Die Beziehung zwischen Hg und Fe war in den Bodensuspensionen im Inkubationsversuch allerdings weniger augenscheinlich. Die Methylierung von Hg wird teilweise durch das Verhältnis DOC/Hg und durch die mikrobielle Gemeinschaft des Bodenmaterials gesteuert.

Die Ergebnisse der vorliegenden Arbeit zeigen, dass die Mobilisierung von Metallen in Bodensuspensionen stark durch E_H-Änderungen und den damit verbundenen Faktoren -wie z.B. DOC, Schwefelkreislauf, Fe (hydr)oxide und die mikrobielle Gemeinschaft- beeinflusst wird. Da viele Auenböden stark mit Metallen belastet sind, die mobilisiert werden und in den menschlichen Körper gelangen könnten, wird empfohlen, die Löslichkeit dieser Kontaminanten weiter zu untersuchen. Zukünftige Forschung sollte sich darauf konzentrieren, die Struktur des DOC, die Speziierung von Metallen und Schwefel sowie die mikrobielle Gemeinschaft in Suspensionen von Auenböden bei sich änderndem E_H vertieft zu untersuchen. Dies kann zum Beispiel durch die Kombination von biogeochemischen

Mikrokosmenexperimenten mit synchrotonbasierten Techniken oder energiedispersiver Röntgenspektroskopie (EDX) erreicht werden. Die Analyse von säureflüchtigen Sulfiden (AVS) kann dazu beitragen, den Kreislauf der Sulfide in Auenböden aufzuklären. Die Bestimmung von Phospholipidfettsäuren (PLFA) könnte dazu dienen, die Rolle der Bodenmikroorganismen für die Methylierung der Metalle näher zu beleuchten.

1 Introduction

1.1 Justification of the research

1.1.1 Metals

Pollution of the environment with trace elements such as (heavy) metals and metalloids (called “metals” in the following) is a serious problem worldwide. The origins of metals in the environment are numerous including geogenic and anthropogenic sources such as agriculture or industrial activities (e.g., Das et al. 2009; Antić-Mladenović et al. 2010; Acquavita et al. 2012). Pollutants enter soils, sediments, ground and surface water posing a risk for the environment and human health. Since industrialization started in Germany in the 19th century, large amounts of industrial wastes were discharged into the environment in liquid, solid, or gaseous form without proper filtering. These wastes contained various metals, which were fed into rivers, were adsorbed and transported by suspended material or in dissolved form, and accumulated during flooding with low flow velocity in floodplain soils (e.g., Devai et al. 2005; Du Laing et al. 2009a).

Floodplains are heterogeneous and vulnerable ecosystems characterized by a close interconnection between aquatic and terrestrial habitats. Many riparian areas around the world are intensively cultivated or urbanized (Lair et al. 2009). Floodplain soils are often multi-contaminated with persistent and potentially toxic trace elements including heavy metals (e.g., Cd, Co, Cr, Cu, Hg, Ni, V, Zn) and metalloids (e.g., As, Sb) (e.g., Overesch et al. 2007; Rinklebe et al. 2007; Schulz-Zunkel and Krueger 2009).

The risks emanating from those sites are numerous. Potentially toxic metals can be leached into groundwater or surface water, can evaporate, or can reach the human food chain via transfer into plants and animals (e.g., Wang and Mulligan 2006; Overesch et al. 2007; Han et al. 2012). During the last decades enhanced ecological awareness and tightened environmental legislation resulted in a decreased input of metals into the environment, for example due to improved waste water treatment techniques, but many metals are enriched in floodplain soils. Thus, many floodplain soils have turned from sinks to sources of metals during the last decades.

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Total amounts of metals have been identified in floodplain soils and floodplain soil material all over the world (e.g., Benson 2006; Vandeberg et al. 2010). However, total metal concentrations alone are insufficient indicators for an appropriate risk assessment of contaminated sites. Instead, the individual risk potential arising from every site is largely determined by the mobilization, release kinetics, and binding forms of the respective metals (Davis et al. 1997; Rinklebe and Du Laing 2011). For a better understanding of the dynamics of metals in contaminated soils, it is crucial to consider the underlying biogeochemical processes.

The dynamics of metals in floodplain soils are controlled by complex interactions of many factors, e.g., total metal content, adsorption/desorption processes, salinity, organic matter, S cycling, carbonates, pH, and redox processes (e.g., Du Laing et al. 2009b; Schulz-Zunkel and Krueger 2009). Redox processes can be identified by means of the **redox potential (E_H)**. The E_H is an electric potential measured in mV and arises from the transfer of electrons from electron donors to electron acceptors. It is a measure for the tendency of the soil to reduce or oxidize substances. Low E_H indicates prevailing reduction processes as they occur under O_2-poor conditions, whereas high E_H arises when oxidation processes prevail (Ponnamperuma 1972; Scheffer et al. 2010). Changing E_H due to fluctuating water table levels is characteristic for floodplain soils. As a result of water saturation during flooding, O_2 diffusion into soils is largely reduced. The O_2 remaining in the soil is consumed by microorganisms. After the depletion of molecular O_2, other compounds such as NO_3, Mn (hydr)oxides, Fe (hydr)oxides, SO_4^{2-}, and CO_2 act as electron acceptors for microorganism. As a consequence the E_H decreases. In turn, E_H increases when the soil is re-aerated (Ponnamperuma 1972; Fiedler and Sommer 2004; Du Laing et al. 2009b).

Changing E_H can have profound influence on metal dynamics in soils via different mechanisms. First, E_H can directly change the valence states of some metals such as As, Co, Cr, Cu, or V, leading to an enhanced or decreased mobilization of these elements (Wanty and Goldhaber 1992; Johnson et al. 2006; Mitsunobu et al. 2006; Du Laing et al. 2009b; Borch et al. 2010). Secondly, E_H influences the mobilization of metals indirectly via the following mechanisms. Redox potential and **pH** are directly related. Oxidation processes produce protons and consequently the pH is lowered, whereas reduction processes consume protons and the pH increases (Yu et al. 2007). In turn, pH influences metal mobilization, since many metal cations such as Cd^{2+}, Cu^{2+}, Ni^{2+}, or Zn^{2+} desorb from organic matter and other sorbents when pH decreases (e.g., Bourg and Gustav Loch 1995; Carbonell et al. 1999; Bisone et al. 2012).

The E_H largely determines the presence of **pedogenic (hydr)oxides** such as Fe- and Mn (hydr)oxides in soils. These (hydr)oxides precipitate at high E_H and dissolute at low E_H. They can act as important scavengers for metals. At high E_H, metals can co-precipitate with pedogenic oxides, at low E_H dissolution of these complexes can occur resulting in the release of associated metals (Charlatchka and Cambier 2000; Kumpiene et al. 2009; Rinklebe and Du Laing 2011).

The E_H can have an influence on dissolved organic matter. At low E_H, complex organic matter can be degraded to **dissolved organic carbon (DOC)** by microorganisms via degradation and hydrolysis. At high E_H, DOC can be microbially consumed leading to a decrease of DOC (Yu et al. 2007; Abel et al. 2012; Husson 2013). Dissolved organic carbon is an important factor affecting metal dynamics in soils with a high capacity to complex and adsorb cations due to the presence of many negatively charged groups (Grybos et al. 2007; Laveuf and Cornu 2009).

The dynamics of metals in floodplain soils can be additionally influenced by redox-induced **S cycling**. For example, low E_H can lead to the formation of hardly soluble metal sulfides resulting in metal immobilization (e.g., Carbonell et al. 1999; Koretsky et al. 2007; van der Geest and Leon Paumen 2008). These sulfides can be transformed to soluble sulfates at high E_H leading to a re-mobilization of associated metals (Du Laing et al. 2008).

Changing E_H has an impact on the **microbial community** in floodplain soils. For example, bacteria, fungi, and algae can be involved in the methylation of metals such as As, Sb, and Hg. Methylation can fundamentally enhance the mobilization, bioavailability, and toxicity of metals (Boening 2000; King et al. 2002; Duester et al. 2005).

The influence of E_H on metal dynamics is complex and often counteracting. Generally, redox reactions have not yet received the same attention as pH, which is often regarded as a master variable for metal dynamics in the soil/plant/microorganism system (Husson 2013). Although the dynamics of metals in flooded soils and sediments have been documented (e.g., van der Geest and Leon Paumen 2008; Weber et al. 2009), mechanistic experiments addressing the redox-induced mobilization or immobilization of metals in frequently flooded soils and floodplain soil material are still scarce up to date. The numbers of studies dealing with metal dynamics in floodplain soils or in floodplain soil material are limited and often focus on a restricted range of metals. For example, the dynamics of alkaline earth metals such as Ba and Sr are seldom studied in floodplain soils or floodplain soil material, despite their potential toxicity (Public Health Service Agency for Toxic Substances and Disease Registry 2004; Ohgami et al. 2012). Furthermore, the greater part of the studies dealing with soil solution concentrations of Co are more than 20 years old, and less attention has been paid to the partitioning behavior of this element in soils or soil slurries (Collins and Kinsela 2010). Moreover, most of the previous studies that address redox-induced mobilization and immobilization processes of metals focus on soil material or sediments that are neutral or alkaline (e.g., Buykx et al. 2000; Caetano et al. 2003; Herbel et al. 2007), whereas acidic soils and acidic soil material are rarely studied. Thus, the current state of knowledge concerning the redox-induced dynamics and methylation of different metals in floodplain soils and floodplain soil material, particularly under low pH conditions, is still deficient. The respective underlying biogeochemical processes are still not fully understood.

The current work aims to study the influence of controlled, changing E_H and related factors on metal dynamics in floodplain top soil slurries in microcosm experiments with regard to the related basic biogeochemical processes. There have been several approaches to control E_H in microcosm (MC) experiments in the laboratory to study the effect of varying E_H on metal dynamics in soil and sediment suspensions (Guo et al. 1997; Ackermann et al. 2010; Burkhardt et al. 2010; Johnson et al. 2010). However, the controlled regulation of E_H, the temporal resolution of measurements and the reproducibility of E_H remains a challenge. To partly overcome these deficiencies, an advanced biogeochemical MC system was used (Chapter 1.2).

1.1.2 Mercury

Mercury is one of the most hazardous and widespread metals with numerous adverse effects on different environmental compartments and human health, particularly in its methylated forms (e.g., Wolfe et al. 1998; Gibicar et al. 2006; Bergeron et al. 2011). Mercury is a ubiquitous element and can be found in many environmental compartments all over the world (e.g., Agusa et al. 2005; Bargagli et al. 2007). It is very persistent in soils and exhibits a complex biogeochemical behavior (Schuster 1991; Davis et al. 1997; Zheng et al. 2012). Today, many floodplain soils are contaminated with Hg caused by atmospheric deposition or transport from watershed (e.g., Boening 2000; Rinklebe et al. 2010).

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The risk arising from those contaminated floodplain sites is largely determined by the solubility and binding forms of Hg. Generally, the mobilization and methylation of Hg in frequently flooded soils is reported to be mainly affected by DOC, S, chloride (Cl^-), Fe, E_H, pH, soil texture, and total Hg (Hg_t) content (Skyllberg et al. 2003; DeLaune et al. 2004; Sunderland et al. 2006). Soil organic matter and Hg can form strong humic complexes, preferentially with the reactive S groups in the hydrophobic acid fraction of DOC (e.g., Ravichandran 2004; Khwaja et al. 2006; Feyte et al. 2010). Mercury can further form hardly soluble Hg sulfides under reducing conditions (e.g., Du Laing et al. 2009b; Skyllberg et al. 2003) or can be adsorbed on Fe (hydr)oxides at high E_H (Fiorentino et al. 2011; Harris-Hellal et al. 2011). Furthermore, methylation of inorganic Hg can change the mobilization, bioavailability, ecological and toxicological effects of Hg (Boening 2000; Ullrich et al. 2001). The methylation process is promoted by certain microorganisms, for instance sulfate reducing bacteria (SRB). During this methylation process lipophilic, highly toxic methyl mercury species such as dimethyl mercury [Me_2Hg] or the monomethyl mercury ion [$MeHg^+$] can be built. These compounds are neurotoxic and can accumulate in the food chain posing a threat to humans and wildlife (Wolfe et al. 1998; Boening 2000; King et al. 2006; Li et al. 2010).

The geochemical behavior of Hg in floodplain soils is not clearly understood yet. For a better understanding of Hg dynamics, it is important to determine the binding forms and the methylation of Hg (Davis et al. 1997; Wang et al. 2012). While the occurrence of Hg and MeHg in the environment has been frequently documented (e.g., Boening 2000; Devai et al. 2005; Gibicar et al. 2006), mechanistic experiments dealing with the redox-induced dynamics of Hg and MeHg as well as the knowledge about the biogeochemical fractions of Hg and the factors affecting the methylation rate of Hg in floodplain soils and floodplain soil material are still rare. The current work aims to study the influence of controlled, changing E_H and related factors on Hg dynamics and methylation in floodplain top soil slurries via microcosm experiments with regard to the underlying biogeochemical processes. Another aim of this work is to get a deeper insight into Hg binding forms and to quantify the impact of various factors on the biogeochemical fractionation of Hg by sequential extraction (SE) of soil material originating from floodplain soil profiles.

1.2 Methods

In the current work an advanced biogeochemical MC system was used for the assessment of the mobilization of metals (As, Ba, Cd, Co, Cu, Cr, Hg, Ni, Sb, Sr, V, and Zn) in multi-contaminated floodplain soil slurries under changing E_H (Chapters 2 to 5; Frohne et al. 2011, 2012, 2014; Appendices 1-3). The terms “soil slurry” and “slurry” are used in the following interchangeably for “slurry of soil material”. This technique allows establishing pre-definite redox conditions in soil slurries by adjusting the E_H automatically as described in detail by Yu and Rinklebe (2011). The MC system was successfully used by Yu et al. (2007) and Rinklebe et al. (2010) for the determination of gaseous emissions from floodplain soil material as well as by Antić-Mladenović et al. (2010) for the assessment of the influence of E_H on Ni dynamics in slurries of a serpentine soil. The MC system offers the great advantage of reproducible, defined, and quickly changeable E_H. Slurry samples can be taken at pre-set E_H values. Moreover, E_H, pH, and temperature values are recorded simultaneously in a very high temporal resolution every 10 minutes.

For a better understanding of Hg dynamics and to assess the impact of various factors on the biogeochemical fractionation of Hg in floodplain soils, a SE procedure was carried out with soil material originating from floodplain soil profiles (Chapter 6; Frohne and Rinklebe 2013; Appendix 4). Sequential extraction procedures are well established tools to assess the binding forms of metals in different environmental compartments (e.g., Tessier et al. 1979; Rao et al. 2008). However, most of the established SE procedures have turned out to be inapplicable for Hg. Hence, a Hg-specific SE procedure for sediments and soils was developed by Bloom et al. (2003) and used in this work (Chapter 6; Frohne and Rinklebe 2013; Appendix 4). This procedure permits the differentiation of Hg compounds into five biogeochemical fractions representing behavioral classes.

For the determination of Hg in soil solution, a cold vapor atomic fluorescence spectrometer (CV-AFS) optimized for the determination of mercury traces in solution (mercur duo plus, Analytik Jena, Germany) was used (Chapters 5 and 6; Frohne et al. 2012; Frohne and Rinklebe 2013; Appendices 3 and 4).

For the current work, representative soil profiles from the catchment areas of the Wupper River in North-Rhine Westphalia (Germany) and the Saale River in Saxony-Anhalt (Germany) were chosen (Chapters 2 to 6; Frohne et al. 2011, 2012, 2014; Frohne and Rinklebe 2013; Appendices 1 to 4). These soil profiles have been exposed to various intense industrial activities in the past. In the Wupper River catchment, mainly textile and metal industry contributed to elevated levels of metals, particularly As, Cu, Cr, and Hg, which are among the main pollutants in the soils and sediments of this area (Schenk 1994; Lacombe et al. 2000). The region around the Saale River was mainly affected by chemical and photochemical industry, for example in the well-known industrial region "Bitterfeld-Wolfen". Arsenic, Cd, Hg, Pb, and Zn are the predominant metals which can be found in elevated levels in the floodplain soils of this region (Kowalik et al. 2004; Zerling et al. 2004; Devai et al. 2005). For example, in previous studies, concentrations up to 17.5 ppm Hg were found in floodplain soils at the Saale River (Devai et al. 2005; Rinklebe et al. 2009), whereas up to 40 ppm were detected in floodplain soils of the Wupper River (Rinklebe unpublished). These high amounts of Hg are alarming and clearly exceed the action value of 2 ppm for top soils set by the German Soil Protection Law (Bundesbodenschutzverordnung 1999).

1.3 General and specific objectives

The general objective of this work is primarily to study systematically the diverse impacts of changing E_H and related factors on metal dynamics in floodplain top soil slurries and to gain a deeper insight into the underlying biogeochemical processes. Furthermore, this work aims to quantify the impact of various factors on the biogeochemical fractionation of Hg in soil material originating from floodplain soil profiles. A deeper knowledge about these processes can help understanding metal dynamics at contaminated floodplain sites. A wide range of metals was covered since many floodplain sites are multi-contaminated.

The soil material originating from the Wupper and Saale River floodplain soils was selected since it represents typical contaminated soils from these areas. In the Wupper River floodplain top soil slurries the dynamics of frequently found metals typical for the wastes of metal, textile, and chemical industry (As, Cd, Cr, Cu, Hg, Ni, and Zn) were examined (Chapters 2 to 5; Frohne et al. 2011, 2012, 2014; Appendices 1 to 3) as well as the less studied metals Ba, Co, Sb, Sr, and V (Chapters 2 to 4; Frohne et al. 2011, 2014; Appendices 1 and 2). In the Saale River floodplain soils, the focus was on Hg binding forms. Moreover, Wupper and Saale River floodplain soil profiles with different pollution history were compared with regard to total amounts and binding forms of Hg (Chapter 6; Frohne and Rinklebe 2013; Appendix 4).

The specific objectives of this work are:

- Assessing the impact of pre-definite redox conditions and related factors on the mobilization of As, Ba, Cd, Co, Cu, Cr, Ni, Sb, Sr, Zn, and V as well as on the methylation of As and Sb in slurries from contaminated floodplain top soil material with regard to the underlying biogeochemical processes
- Assessing the impact of pre-definite redox conditions and related factors on the dynamics and methylation rate of Hg in slurries from contaminated floodplain top soil material with regard to the underlying biogeochemical processes
- Identifying and quantifying the biogeochemical fractions of Hg in soil material originating from two distinct floodplain soil profiles in Germany with different pollution history and different Hg contamination levels

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2

Impact of controlled redox variations on the mobilization of Cd, Cu, Ni, and Zn as well as on the biomethylation of As and Sb in a floodplain soil slurry of the Wupper River

2.1 Introduction and significance of the chapter for the overall context

This chapter summarizes the main findings of a biogeochemical MC experiment that was carried out in 2009. This experiment was conducted to lay a basis on the understanding of metal dynamics under controlled changing redox conditions in floodplain soil slurries. The results of this experiment are published in Geoderma (Frohne et al. 2011; Appendix 1). In this experiment, the dynamics of selected toxic metals and metalloids that are known to be frequent in contaminated soils (As, Cd, Cu, Ni, Sb, and Zn) were studied. The soil material was taken from the floodplain of the Wupper River. This soil material is slightly acidic and had turned out to be highly contaminated with several metals before (Rinklebe unpublished). This soil material was chosen, since studies dealing with redox-induced mobilization of metals and metalloids under low pH conditions in frequently flooded soils or soil material were rare as previous studies mainly focussed on neutral or alkaline soils and alkaline soil material, respecively (e.g. Caetano et al. 2003; Herbel et al. 2007).

The experiment was performed in the laboratory at the Department of Soil- and Groundwater-Management at the University of Wuppertal under the supervision of Prof. Jörg Rinklebe. Prof. Rinklebe and I generated the research idea. He assisted me in the MC experiment, and corrected the manuscript before submission. The majority of studied elements were analyzed at the laboratory in Wuppertal by inductively coupled optical emission spectrometry (ICP-OES). The detection of As and Sb species requires special laboratory equipment that was not present at the laboratory in Wuppertal. Therefore, As and Sb species were analyzed by Dr. Roland Diaz-Bone at the University of Duisburg-Essen (Department of Microbiology). The analytical experience of Dr. Diaz-Bone helped to assess particularly the methylated As and Sb species in soil solution with hydride generation followed by purge-and-trap gas chromatography and detection via inductively coupled plasma mass spectrometry (HG-P&T-GC-ICP-MS). Dr. Diaz-Bone further gave me analytical advice, helped me with the quality control of measured data, and proofread the manuscript before submission. Prof. Gijs Du Laing from Ghent University (Belgium) gave me scientific advice, since he is very experienced in this research field and has published a lot of papers. He helped to correct the manuscript before submission and assisted in English editing.

2.2 Abstract

In the study of Frohne et al. (2011) (Appendix 1), the effect of E_H on the mobilization of Cd, Cu, Ni, Zn as well as on the methylation of As and Sb in contaminated and slightly acidic soil slurries from the Wupper floodplain was assessed. For this purpose, an automated biogeochemical MC system was used. The advantage of this system is the possibility of controlled adjustment of E_H in soil slurries. The E_H was varied stepwise from reducing (- 300 mV at pH 5) to oxidizing (+600 mV at pH 5) conditions (Frohne et al. 2011; Appendix 1).

In this study, it was found that redox conditions have a large influence on the dynamics of studied compounds and elements. Concentrations of soluble Cd, Cu, Mn, Ni, and Zn were low at reducing conditions and increased with increasing $E_{H;}$ this is attributed to the interaction with DOC and sulfide precipitation (Frohne et al. 2011; Appendix 1). Soluble Fe concentrations were high at low E_H and decreased at E_H >350 mV at pH 5 because of the formation of Fe (hydr)oxides (Frohne et al. 2011; Appendix 1). The acidic conditions and other factors might have inhibited co-precipitation of other studied metals with Fe (Frohne et al. 2011; Appendix 1). Concentrations of soluble inorganic arsenic (As_i), inorganic antimony (Sb_i), monomethyl arsenic (MMAs), monomethyl antimony (MMSb), and dimethyl arsenic (DMAs) decreased significantly with increasing E_H; it was assumed that low E_H might enhance the transfer of these compounds to soil solution (Frohne et al. 2011; Appendix 1).

Keywords: biogeochemical microcosm system; redox potential; trace elements; wetland soil

2.3 Conclusions

The E_H significantly controlled the mobilization of metals in the slightly acidic floodplain soil slurries in the study of Frohne et al. (2011) (Appendix 1). Under oxidizing conditions, the transfer of Cd, Cu, Mn, Ni, and Zn to soil solution was enhanced, what is attributed to the dissolution of less soluble sulfides to more soluble sulfates and the resulted release of associated metals (Frohne et al. 2011; Appendix 1). An interaction of metals with DOC was supposed and might have contributed to the strong mobilization of Cd, Cu, Ni, and Zn at high E_H in the study of Frohne et al. 2011 (Appendix 1). The formation of Fe (hydr)oxides under high E_H and their dissolution under low E_H controlled the solubility of Fe, but the adsorption of metals (Cd, Cu, Mn, Ni, and Zn) on Fe and/or a co-precipitation with Fe (hydr)oxides seemed to be hindered (Frohne et al. 2011; Appendix 1). There was no evidence for the formation of Mn (hydr)oxides at high E_H presumably due to the low pH (Frohne et al. 2011; Appendix 1). Reducing conditions promoted the formation of some methylated As and Sb species (MMAs, MMSb, and DMAs) (Frohne et al. 2011; Appendix 1). The results suggest that flooding of the studied soil material decreases the transfer of Cd, Cu, Ni, and Zn to soil solution, whereas the degree of mobilization of As and Sb is higher at the same time (Frohne et al. 2011; Appendix 1).

However, although the metal concentrations measured in the soil solution might be considered close to field conditions in the biogeochemical MC experiment, a confirmation of the observed metal dynamics and the redox induced processes at various scales (e.g. Rennert et al. 2010; Rupp et al. 2010) including in situ investigations can be useful in future. Moreover, it is important to conduct similar studies with further acidic soil material originating from the Wupper River and also with a variety of frequently flooded soil material worldwide to verify the results of Frohne et al. 2011 (Appendix 1). Furthermore, future studies should include further toxic elements.

2.4 References

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3

Contamination of a Wupper River floodplain top soil material with As, Co, Cu, Ni, Sb, and Zn and the impact of pre-definite redox variations on the mobilization of these elements

3.1 Introduction and significance of the chapter for the overall context

In this chapter, the main results of a biogeochemical MC experiment are presented, that was conducted in 2010. The objectives of this study were firstly to validate the findings of the previous study from chapter 2 (Frohne et al. 2011; Appendix 1), secondly to include a new, scarcely studied element of environmental interest (Co), and thirdly to gain a deeper insight into the role of DOC on metal dynamics, since DOC seemed to be an important parameter for metal dynamics in the previous study presented in chapter 2 (Frohne et al. 2011; Appendix 1). A special focus was on the E_H-dependence of metal/DOC ratios as an indicator of the binding strength of metals with DOC. The results of this study are published in Soil and Sediment Contamination: An International Journal (Frohne et al. 2014; Appendix 2).

For this experiment, I incubated another top soil material with similar properties from a different study site of the Wupper River floodplain and studied the release kinetics of As, Co, Cu, Ni, Sb, and Zn.

The experiment was carried out in the laboratory of the Department of Soil- and Groundwater-Management at the University of Wuppertal under the supervision of Prof. Jörg Rinklebe, who generated the research idea together with me, assisted in the MC experiment, and corrected the manuscript before submission.

The DOC in soil solutions was analyzed at the laboratory in Wuppertal. Arsenic and metals in solution were determined by Dr. Roland Diaz-Bone at the University of Duisburg-Essen (Department of Microbiology) with inductively coupled plasma mass spectrometry (ICP-MS) technique. This technique is particularly suitable for the detection of metal traces in solution with a very high precision. In this context, I benefitted from Dr. Diaz-Bone’s analytical experience. He offered analytical advice and helped me with the quality control of obtained data. Furthermore, he proofread the manuscript before submission.

3.2 Abstract

The study of Frohne et al. (2014) (Appendix 2) reveals that floodplain top soils of the Wupper River are critically contaminated with several metals. An automated biogeochemical MC system allowing controlled variation of E_H was used to evaluate the impact of changing redox conditions on the dynamics of As, Co, Cu, Fe, Mn, Ni, Sb, and Zn in acid floodplain soil slurries of the Wupper River in this study. The solubility of Co, Cu, Mn, Ni, Sb, and Zn was

low at low E_H, most likely due to the precipitation of metal sulfides, and rose with increasing E_H; this was attributed to the oxidation of sulfides to sulfates and subsequent release of associated metals, and secondly to the association of metals with DOC (Frohne et al. 2014; Appendix 2).

A significant positive correlation was found between metal/DOC-ratio and E_H, indicating that the binding of the metals to DOC shifted from stronger to weaker with rising E_H (Frohne et al. 2014; Appendix 2). Soluble As concentration decreased with rising E_H in the study of Frohne et al. (2014) (Appendix 2), indicating the co-precipitation of As with Fe (hydr)oxides and/or oxidation of more soluble As(III) to less soluble As(V). The other studied elements did not appear to co-precipitate with Fe (hydr)oxides at oxidizing conditions, what was attributed to the prevailing low pH in the experiment (Frohne et al. 2014; Appendix 2).

Keywords: biogeochemical microcosm system; redox potential; trace elements; wetland soil; heavy metals; metalloids

3.3 Conclusions

The study of Frohne et al. (2014) (Appendix 2) illuminates that the impact of E_H on the mobilization of metals and metalloids is complex and diverse. Copper, Co, Mn, Ni, Sb, and Zn are more mobile under oxidizing compared with reducing conditions, possibly due to the oxidation of sulfides to sulfates and subsequent release of metals (Frohne et al. 2014; Appendix 2). The solubility of Fe was mainly controlled by the generation of Fe (hydr)oxides at high E_H and their reductive dissolution at low E_H in the study of Frohne et al. (2014) (Appendix 2). The results of this study indicate that As was strongly associated with Fe (hydr)oxides, whereas the adsorption of Cu, Co, Mn, Ni, Sb, and Zn was obviously hindered. The prevailing acidic conditions in the studied Wupper soil material might have prevented Mn from precipitation as Mn (hydr)oxides at high E_H (Frohne et al. 2014; Appendix 2). Dissolved organic carbon likely influences the dynamics of the studied metals; for example the binding of metals to DOC seems to be stronger at low E_H compared with high E_H (Frohne et al. 2014; Appendix 2).

The results show that inundation of the studied soil material might decrease the degree of mobilization of Co, Cu, Ni, Sb, and Zn while enhancing the transfer of As to soil solution at the same time (Frohne et al. 2014; Appendix 2). For a better understanding of the dynamics of metals at contaminated floodplain sites, studies in an analogous manner should be conducted with further frequently flooded soil material worldwide and further elements should be included. Particularly, the role of DOC and sulfur cycling on metal dynamics needs further investigation.

3.4 References

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Frohne, T., Rinklebe, J., Diaz-Bone, R.A., 2014. Contamination of floodplain soils along the Wupper River, Germany, with As, Co, Cu, Ni, Sb, and Zn and the impact of pre-definite redox variations on the mobility of these elements. Soil Sediment Contam. 23. 779-799.

4

Impact of systematic change of redox potential on the dynamics of Ba, Cr, Sr, and V in a floodplain soil slurry of the Wupper River

4.1 Significance of the chapter for the overall context

This chapter focuses on the dynamics of Ba, Cr, Sr, and V in the same Wupper floodplain soil material that was studied in chapter 3 (Frohne et al. 2014; Appendix 2). The objectives of the study in this chapter were to further elucidate the complex role of DOC for metal dynamics in soil slurries and to include further metals of environmental importance (Ba, Cr, Sr, V). These metals are scarcely studied concerning their dynamics under controlled E_H. Particular attention was paid to the influence of the aromaticity of DOC, expressed by the specific ultraviolet adsorption at 254 nm ($SUVA_{254}$), on metal dynamics under changing E_H, since DOC has turned out to be an important factor for metal dynamics in chapter 2 (Frohne et al. 2011; Appendix 1) and chapter 3 (Frohne et al. 2014; Appendix 2).

The experiment was set up and conducted in the laboratory of the Department of Soil- and Groundwater-Management at the University of Wuppertal under the supervision of Prof. Jörg Rinklebe, who generated the research idea together with me and assisted in the experiment. Dissolved organic carbon in the soil solution was determined at the laboratory in Wuppertal. Barium, Cr, Fe, Mn, Sr, and V in soil solution were analyzed by Dr. Roland Diaz-Bone at the University of Duisburg-Essen (Department of Microbiology) with ICP-MS technique. This technique is very precise and thus particularly suitable for the detection of metal traces in solution. Dr. Diaz-Bone offered helpful analytical advice and supported me with the quality control of the data.

4.2 Abstract

An automated biogeochemical MC system allowing controlled variation of E_H in soil slurries was used to evaluate the impact of pre-definite redox conditions on the leaching of Ba, Cr, Sr, and V from a riverine soil. Chromium and V were negatively correlated with E_H possibly due to co-precipitation of these metals with Fe (hydr)oxides at high E_H. Vanadium dynamics might additionally be influenced directly by E_H via changes in its speciation. Barium and Sr were positively correlated with E_H, which might be attributed to their association with DOC. The influence of pH on trace element dynamics seemed to be of minor importance in the current study. A significant negative correlation was observed between $SUVA_{254}$ and E_H indicating that oxidizing conditions favored the removal of aromatic DOC molecules from solution via binding to Fe (hydr)oxides. Perspectively, similar studies should be performed with further wetland soil material worldwide to elucidate the role of DOC and S chemistry on the dynamics of Ba, Cr, Sr, and V. Furthermore, it will be a challenge for the future to determine V species in wetland soil material under pre-definite redox conditions.

Keywords: biogeochemical microcosm system; floodplain soil; wetland soil; alkaline earth metals; heavy metals

4.3 Introduction

Floodplains are dynamic ecosystems which often act as sinks for contaminants originating from anthropogenic as well as from geogenic sources. River water and particulate matter can carry potentially toxic metals such as V, Cr, Ba, and Sr. These metals are deposited in floodplain soils during flooding, where they can persist for many decades (Du Laing et al. 2009b; Rennert and Rinklebe 2009). The floodplain soils of the Wupper River are contaminated with various pollutants since large amounts of waste water from textile, metal, and chemical industry were discharged into the river during the last centuries. Thus, even though the river water is pristine today, sediments and floodplain soils of the Wupper River have accumulated several contaminants (e.g. Schenk 1994; Frohne et al. 2011, Appendix 1).

When floodplain soils are inundated, the water table fluctuates largely and the balance of chemical and biological processes is altered, triggering a series of chemical, physical and biological changes. For example, in floodplain ecosystems, drastic changes in the water table levels result in frequent changes in E_H. Redox-sensitive processes can affect the dynamics of metals directly via changes in their speciation or indirectly through related changes in pH, DOC, and the redox chemistry of Fe, Mn, and S (Du Laing et al. 2009b; Husson 2013). During flooding of a soil and subsequent decrease of E_H, Fe- and Mn (hydr)oxides are reduced to Fe^{2+} and Mn^{2+}. These ions occur as soluble metals and organic complexes in soil solution (Reddy and DeLaune 2008). Metals which are fixed to Fe- and Mn (hydr)oxides are transformed into more mobile forms and released to soil solution under reducing conditions, whereas oxidation is followed by co-precipitation or adsorption and subsequent immobilization of these metals. Furthermore, E_H has an impact on the transformation and the mobilization of organic matter. Metals which are associated with organic matter can be mobilized particularly under reducing conditions, when organic matter is degraded to soluble DOC (Yu et al. 2007; Abel et al. 2012). Metals are preferentially associated with aromatic DOC rather than with aliphatic DOC and the aromaticity of DOC is E_H-dependent (Fiedler and Kalbitz 2003; Grybos et al. 2009). Thus, low E_H can lead to a higher metal mobilization due to the presence of more aromatic DOC.

Chromium and V are widespread redox-sensitive contaminants. The degree of Cr mobilization and toxicity in soils depends on its oxidation state and on its adsorption on soil and sediment components (e.g. Richard and Bourg 1991; Agnieszka and Barbara 2012). Whereas Cr(III) is relatively immobile in soils and essential for plants, animals, and humans, Cr(IV) is highly mobile and exhibits adverse effects on human health and environment (Kotas and Stasicka 2000; Rifkin et al. 2004; Johnson et al. 2006). Contamination with V is an important environmental issue. In future, V will be paid more and more attention due to its potential toxicity. Threshold values for V in soils will be included in the revised version of the German Soil Protection Law (Bundesbodenschutzverordnung 1999). Vanadium mainly exists in the oxidation states +3 to +5 in soils and water (Wanty and Goldhaber 1992). In each oxidation state, V can form various complexes, ion pairs, polymers, and solids with different mobilization potential, geochemical behavior, and toxicity. For example, the reduced V(IV) is considered to be relatively mobile. In turn, oxidized V(V) species might be less mobile (Amrhein et al. 1993). However, many of these mechanisms are not clearly understood until now (Mukherjee et al. 2004; Korbecki et al. 2012).

The release kinetics of Ba and Sr in floodplain soils and floodplain soil material are rarely studied, regardless of the potential toxicity of these elements (Public Health Service Agency for Toxic Substances and Disease Registry 2004; Ohgami et al. 2012). Recent studies dealing with the geochemical behavior of Ba mainly focus on marine environments (e.g. Santos et al. 2011; Henkel et al. 2012). Studies referring to the geochemical behavior of Sr in soils/soil material often concentrate on radioactive isotopes rather than stable forms (e.g. Wang and Staunton 2005; Dimović et al. 2012).

A deeper insight in the geochemical behavior of the potentially toxic elements Ba, Cr, Sr, and V in floodplain soils is essential to estimate their environmental behavior. The objective was to assess the impact of pre-definite E_H on the dynamics of Ba, Cr, Sr, and V in a floodplain soil slurry. Therefore an automatic biogeochemical MC system was used allowing computer-assisted regulation of E_H in order to study the effect of E_H mechanistically. With this system, redox conditions are reproducible and defined, and can be modified rapidly.

4.4 Materials and Methods

4.4.1 Study site

Soil samples were collected from a grassland site at the floodplain of the Wupper River close to the confluence into the Rhine River (RW 2568987, HW 5659539). The study site is located about 20 km to the north of Cologne, Germany. The climate in this area is Atlantic with mild summers and winters and the study site is characterized by a long term annual precipitation of approximately 774 mm and a long term annual air temperature of 10.8 °C (Deutscher Wetterdienst 2011). The study site is flooded periodically by the Wupper River, particularly in springtime (Wupperverband 2011). The Wupper River has got a length of approximately 115 km; the discharge averages 15.4 $m^3 s^{-1}$ and the average gradient is 0.4 %. The compass of the catchment area of the Wupper River is 814 km^2. The geological parent material is sediment of the Rhine River ("Niederrheinische Bucht") dominated by Devonian shale ("Rheinisches Schiefergebirge").

4.4.2 Bulk soil: Sampling, pre-treatment, and characteristics

The soil material was sampled from the genetic A-horizon at a depth of 0 to 10 cm. Four replicates of 1 kg each were collected and pooled to one composite sample. The sample was homogenized, air-dried and sieved to < 2 mm and ground using an agate disc mill. The soil is classified as Eutric Fluvisol according to IUSS-FAO (2006). Basic properties of the soil material and (pseudo)total concentrations of studied elements in the bulk soil material are given in Table 4-1.

Table 4-1 Soil texture and total concentrations of elements in the bulk soil material; C_{org} = organic carbon; N_t = total nitrogen

Depth	**Texture**			**C_{org}**	**N_t**	**C_{org} N_t^{-1}**	**Fe^a**	**Mn^a**	**S^a**	**Cr^a**	**V^a**	**Ba^b**	**Sr^b**
[cm]	**[%]**			**[%]**			**[g kg^{-1}]**		**[mg kg^{-1}]**				
	Sand	Silt	Clay										
	0.063-2 mm	0.002-0.063 mm	< 0.002 mm										
0-10	44	48	8	6.2	0.4	15.6	34	1.3	915	423	58	569	86

[a] microwave digestion

[b] X-ray fluorescence

Sand and silt dominate the texture of the soil material which is typical for soils along the Wupper River (Schenk 1994). The content of organic carbon (C_{org}) in the soil material is relatively high. Concentrations of Cr exceed the action value of 400 ppm for residential areas set by the German Soil Protection Law (Bundesbodenschutzverordnung 1999).

4.4.3 Analysis of bulk soil material

The basic soil properties of the bulk soil material were determined according to standard methods after Blume et al. (2011) in the following way: Total carbon and total nitrogen (N_t) were measured with dry combustion and thermal conductivity detection using a C/N/S-Analyser (Vario EL Heraeus, Analytik Jena, Jena, Germany). Inorganic carbon was quantified by dry combustion and IR-Detection with a C-MAT 550 (Stroehlein). Organic carbon in the soil material was calculated as the difference between total and inorganic carbon. The pipette sampling technique with wet sieving and sedimentation according to Blume et al. (2000) was used to determine the particle-size distribution in the soil material. Total Ba and Sr were measured by energy dispersive X-ray spectroscopy (EDX) after compressing the soil material to pellets (XLAB 2000, Spectro, Germany). Total Ba, Cr, Sr, V, and S concentrations of the soil material were quantified after microwave digestion using 37 % HCl and 65 % HNO_3 (ratio 1:3) in dependence on the U.S. Environmental Protection Agency (2007), followed by inductively coupled plasma atomic emission detection (Ultima 2, Horiba Jobin Yvon, Unterhaching, Germany).

4.4.4 Setup of the microcosm experiment with controlled redox variations

An automated biogeochemical MC system was used to simulate flooding of the soil material in the laboratory. A detailed description of this system can be found in Yu et al. (2007), Yu and Rinklebe (2011), and Frohne et al. (2011), respectively. Four independent biogeochemical MCs were used as replications. The vessels were wrapped with Al foil to protect them against daylight, preventing algae growth and photo-oxidation. Every MC was filled with 200 g air-dried soil sample and deionized water in a 1:8 ratio. The achieved slurry was continuously stirred to reach homogeneous conditions. Redox potential, pH, and temperature in each MC were automatically monitored every ten minutes. The E_H values of MC 1 after approximately 820 hours incubation could not be monitored due to an error of the electrodes. The measured redox potential values were normalized to pH 5, since the pH median was 5.2 between the first and the last sampling point during the experiment (Table 4-2). These values will be referred to as “$E_{H \text{ at pH } 5}$”. Straw (5 g after 31 days) and 5 g glucose (twice after 37 and 39 days respectively) were added to each MC to provide an additional source of organic matter for microorganisms. Levels of $E_{H \text{ at pH } 5}$ were decreased to values between approximately -181 and -34 mV by continuously flushing the MCs with N_2 for a period of 5 to 7 days . Thereafter, E_H-values were increased in steps of 100 mV by adding oxygen. Redox potential was kept within E_H-windows of approximately 30-40 mV around the set E_H-values by automated supply of O_2 or N_2. The E_H was kept for approximately 24 h within each window and subsequently set to the next value. The slurry was sampled (55 mL) approximately 24 h after reaching each new E_H-window. The soil material/water ratio remained the same during the experiment. After achieving the highest $E_{H \text{ at pH } 5}$ levels between approximately 490-540 mV, N_2 was added to decrease E_H again. Total incubation period was 61 days.

4.4.5 Microcosm experiment: Sample preparation, subsampling, and storage

The slurry samples were centrifuged for 15 min at 3000 rpm and the supernatants were immediately filtered under N_2-atmosphere through a 0.45 µm Millipore membrane (Whatman Inc., Maidstone, UK). The filtrate was thereafter aliquoted to different subsamples of 10 mL each for subsequent analysis. The first subsample was taken for analysis of Ba, Cr, Fe, Mn, Sr, and V. A second subsample was used to determine S. These samples were both stabilized by adding 0.1 mL 65 % HNO_3. Another subsample was used for determination of UV absorbance, DOC and SO_4^{2-}.

4.4.6 Chemical and spectrometric analysis

Concentrations of Ba, Cr, Fe, Mn, Sr, and V in the samples were determined by the use of ICP-MS (7500ce, Agilent Technologies, Yokogawa, Japan) optimized for hot plasma conditions. Before analysis, eluates were diluted 1:10 in 1 % HNO_3 (65 %, subboiled, J.T. Baker, Phillipsburg, USA). Afterwards, 10 µg L^{-1} Li, Ga, Y, In and Tl were added for internal standardization. Whereas Ba and Sr were measured in no gas mode, V, Fe as well as Cr were analyzed in helium collision mode. For external calibration of Ba, Cr, Fe, Sr, and V Merck VI multielement solution was used (Merck, Darmstadt, Germany). Barium, Cr, Sr, and V were calibrated in the range from 0.01 µg L^{-1} to 200 µg L^{-1}, Fe in a range from 0.1 to 2000 µg L^{-1} with a 14 point calibration. Yttrium served as internal standard recovery with a drift below 10 % over the measurement time. To exclude drift and carry-over effects, samples were analyzed twice in randomized order. Sulfur was determined by ICP-OES (Ultima 2, Horiba Jobin

Yvon, Unterhaching, Germany). All samples were measured in three replicates after 4-point calibration with standard solutions (CertiPur, Merck, Germany) diluted in deionized water.

The relative standard deviations of replicate analyses were below 5 % with very few exceptions. Blank levels and sensitivity were controlled every ten samples to monitor for drifts in instrument background and sensitivity.

An analyzer for total organic carbon (TOC-V_E, Shimadzu, Kyoto, Japan) was used to quantify DOC. Each sample was measured twice after 2-point calibration with a detection limit of 1 mg L^{-1}. Sulfate was determined using an ion chromatograph (Personal IC 790, Metrohm, Filderstadt, Germany) with a Metrosep A Supp 4 - column (Metrohm, Filderstadt, Germany). A 2-point calibration was conducted. The detection limit was 0.03 mg L^{-1}. The UV absorbance at 254 nm wavelength was measured by a UV/VIS spectrophotometer and a 1 cm path length cell (CADAS 200, Dr. Lange, Germany). This wavelength was chosen since natural organic matter absorbs strongly at 254 nm and shows a strong correlation with the aromatic carbon content of organic matter (Weishaar et al. 2003). The specific ultraviolet absorbance ($SUVA_{254}$) serves as an indicator of the chemical composition of DOC and was calculated as the UV absorbance measured at 254 nm divided by the DOC concentration and reported in units of cm^{-1} mg^{-1} L as described by Weishaar et al. (2003).

4.4.7 Statistics

Since E_H and pH were measured in the soil slurry every 10 min during the experiment, a very large dataset was obtained (Table 4-2). Since concentrations of elements and compounds measured at the sampling points are supposed to be a result of the processes occurring a several time period before sampling, the mean values of E_H and pH data originated from 3, 6, 12, and 24 hours prior to sampling were calculated to achieve representative E_H and pH values. Thereafter Pearson’s correlation coefficients (r) between the calculated E_H / pH mean values and Ba, Cr, DOC, Fe, Mn, phosphate (PO_4^{3-}), S, SO_4^{2-}, Sr, $SUVA_{254}$, and V were calculated using IBM SPSS Statistics 21. The results 6 hours before sampling generally revealed the highest correlation coefficients and are therefore presented here. ORIGIN 6.0 was used for calculating the regressions equations and creating the figures.

Multiple regression analysis was performed stepwise to assess the combined impact of E_H, DOC, Fe, and SO_4^{2-} on the dynamics of soluble V, Cr, Ba, and Sr in the current experiment. For this purpose, IBM SPSS Statistics 21 was used. The most significant results of the multiple regression analysis were selected for further interpretation.

4.5 Results

Selected statistical values for the measured parameters are given in Table 4-2. The E_H ranges from -180-742 mV and the pH ranges from 4.1-7.2 (all data). A slightly positive correlation was found between pH and E_H, each measured every 10 min during the experiment ($R^2 = 0.12$; $p < 0.0001$; n = 29,826). Concentrations of Cr, DOC, $SUVA_{254}$, and V showed a significant negative correlation with E_H (Figure 4-1 and Figure 4-2). Total Ba and Sr were significantly positively correlated with E_H (Figure 4-1). Moreover, a positive relationship between Mn and E_H was found ($Y = 50611.274+32.544 X$; $R^2 = 0.40$; $p < 0.0005$; n = 30). Soluble Fe initially increased with rising E_H and the highest values were attained at about $E_{H \text{ at pH } 5}$ 100 mV and declined at about $E_{H \text{ at pH } 5}$ 350 mV (Figure 4-1). The correlations between SO_4^{2-} and S (data not shown) and E_H and between PO_4^{3-} and other parameters were insignificant. Chromium and DOC were weakly negative correlated with pH ($Y = -6068.382+2342.216 X-224.2014 X^2$; $R^2 = 0.26$, $p < 0.05$; n = 29 for Cr, and $Y = 3680.255-438.067 X$; $R^2 = 0.18$; $p < 0.05$; n = 30 for DOC, respectively). The relationships between pH and other parameters were less clear.

Table 4-2 Variations of concentrations of elements, compounds, pH, and redox potential (E_H) in the slurry

		n	**Mean**	**Minimum**	**Maximum**	**Percentiles**				
						10	**25**	**50**	**75**	**90**
E_H (6h)[1,3]	[mV]	30	270	-140	534	23	140	303	414	511
E_H (all data)[2,3]		29,830	355	-180	742	8	198	373	571	647
pH (6h)[1]		30	5.2	5.0	5.7	5.1	5.1	5.2	5.3	5.5
pH (all data)[2]		32,961	5.9	4.1	7.2	5.1	5.3	6.3	6.5	6.7
Cr	[µg L^{-1}]	29	43.4	18.8	76.8	20.4	31.9	36.7	62.3	70.0
V		30	6.7	1.7	12.8	2.1	2.6	6.2	11.2	12.4
Ba		30	3.0	1.6	4.4	2.1	2.6	3.0	3.5	3.9
Sr		30	0.9	0.6	1.3	0.8	0.9	0.9	1.0	1.1
Fe	[mg L^{-1}]	30	82.6	0.2	241.0	0.6	5.0	73.1	167.9	192.2
Mn		30	59.6	37.4	82.2	47.1	53.9	59.8	65.3	69.1
DOC		30	1384.3	1082.0	1692.5	1092.5	1276.3	1391	1486.4	1654.4
SO_4^{2-}		30	7.9	2.2	13.5	3.4	6.1	8.3	9.9	11.3
S		26	2.0	0.7	3.9	1.3	1.6	2.0	2.4	3.1

[1] means of data 6 hours before sampling; [2] data measured every 10 minutes during the experiment; [3] E_H corrected to pH 5

Multiple regression analysis revealed that E_H and Fe together explain 90 % of V dynamics (adjusted R² = 0.902) and 88 % of Cr dynamics (adjusted R² = 0.876), while E_H and DOC together explain 68 % of V dynamics (adjusted R² = 0.678) and 42 % of Cr dynamics (adjusted R² = 0.422). The combined impact of E_H together with DOC on Ba dynamics was moderate (adjusted R² = 0.545). Redox potential and SO_4^{2-} together are able to explain 33 % of Mn dynamics. Further results of the multiple regression analysis were of minor significance.

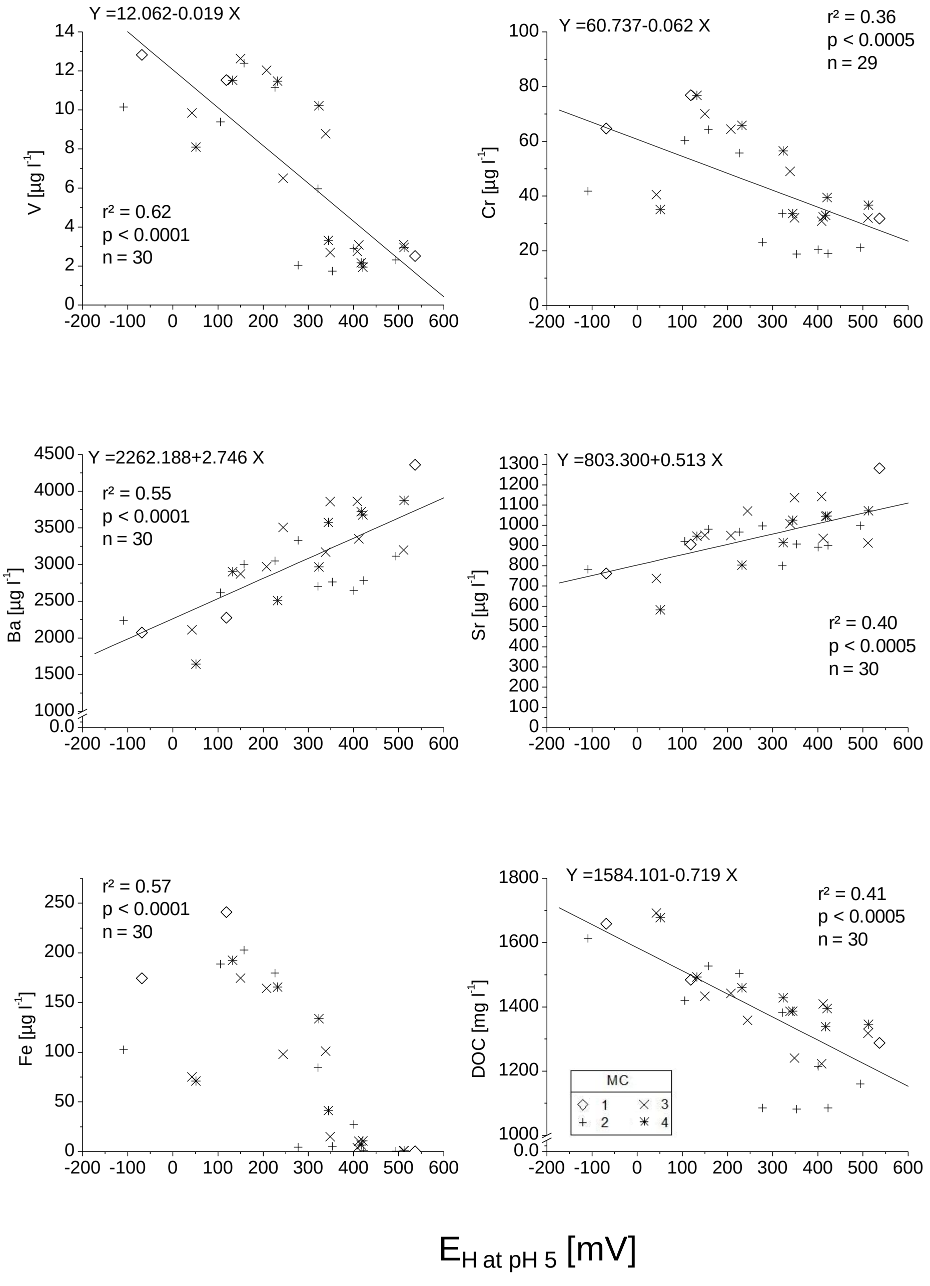

Figure 4-1 Dynamics of soluble V, Cr, Ba, Sr, Fe, and DOC during the experiment

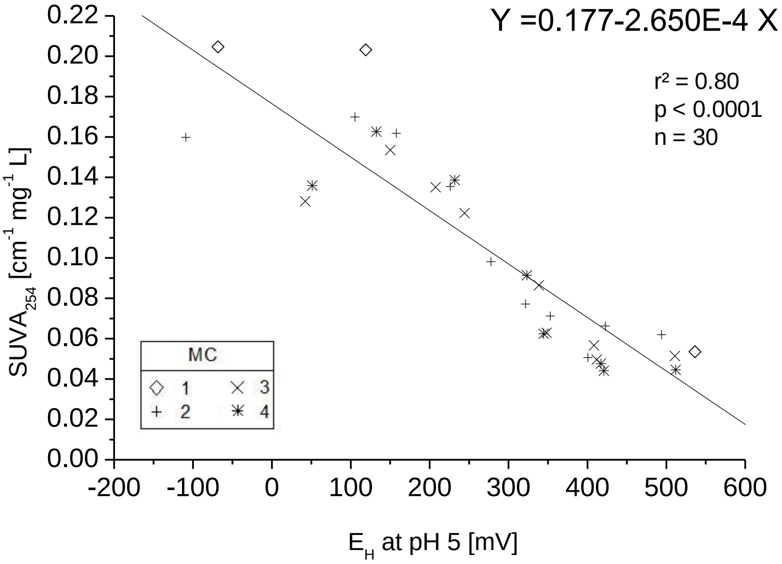

Figure 4-2 Dynamics of $SUVA_{254}$ (specific UV absorbance at 254 nm) during the experiment

4.6 Discussion

4.6.1 Dynamics of E_H and pH

Flooding of the soil material induced decreasing E_H in the current experiment as frequently reported (e.g. Yu et al. 2007; Frohne et al. 2011, 2012; Appendices 1 and 3). Generally, pH should decrease when E_H increases and vice versa, due to the formation of protons during oxidation processes (Zarate-Valdez et al. 2006; Frohne et al. 2011, 2012; Appendices 1 and 3). In contrast, the correlation between E_H and pH was slightly positive in the current study which is in accordance with the results obtained by Antić-Mladenović et al. (2010). However, various different processes in soils can have an impact on pH, for example buffer capacity, microorganisms, and organic matter transformation (e.g. Wang et al. 2012; Husson 2013). In the current experiment, an interaction of these processes might have occurred.

4.6.2 Behavior of Fe and Mn

High Fe concentrations in solution were observed at low E_H. A sudden decline of soluble Fe at E_H 350 mV strongly indicates the formation of Fe (hydr)oxides as frequently described before (e.g. Reddy and DeLaune 2008; Antić-Mladenović et al. 2010; Frohne et al. 2011; Appendix 1). Soluble Fe concentrations were relatively low at the first sampling points at the beginning of the experiment (Figure 4-1) as observed in a former MC experiment (Frohne et al. 2011; Appendix 1). The reduction of Fe (hydr)oxides is a process which is catalyzed by microorganisms. A certain time span is needed to re-establish facultative anaerobe and anaerobe microbial communities after inundation of a soil (Rinklebe and Langer 2006; 2008; Langer and Rinklebe 2009). Possibly the time period from the start of the incubation until the first sampling was insufficient to reduce the entire Fe (hydr)oxide pool.

Manganese concentrations in solution increased with increasing E_H (data not shown) as also found in a previous MC experiment (Frohne et al. 2011; Appendix 1). One might expect a decrease of soluble Mn with increasing E_H due to the oxidation of soluble Mn^{2+} to insoluble Mn (hydr)oxides at high E_H as observed in several other studies (e.g. Tack et al. 1998; Yu et al. 2007). These Mn(hyxdr)oxides act as important binding agents for many trace elements (e.g. Borch et al. 2010). However, an evidence for the presence of Mn(hdr)oxides is lacking in the current study. Possibly, E_H and pH were too low for the formation of Mn oxides. The generation of Mn oxides requires very strong oxic conditions (approximately 900– 1000 mV at pH 5) and is in addition pH-dependent (Takeno 2005). The slightly acidic pH in the current study might have inhibited the formation of Mn oxides. However, the relationship between Mn and pH was less clear (data not shown) indicating that the variability of pH might be too low to affect the mobilization of Mn noticeably. However, an indirect effect of E_H on Mn dynamics is possible. One might think of the formation of Mn sulfides as a plausible explanation for the low Mn solubility at low E_H and rising Mn solubility with increasing E_H. This might be confirmed by multiple regression analysis, where E_H and SO_4^{2-} together can explain 33 % of Mn dynamics.

4.6.3 E_H and pH dependence of DOC and $SUVA_{254}$

The very high concentrations of DOC in the current experiment can originate from the high C_{org} content of the bulk soil material (Table 4-1) and glucose and straw amendments. Concentrations of DOC were negatively correlated with E_H in the current study (Figure 4-1). Accordingly, higher amounts of DOC in solution under reducing compared with oxidizing conditions were observed in literature (e.g. Grybos et al. 2009; Antić-Mladenović et al. 2010). Decreasing DOC with increasing E_H can be explained by enhanced microbial carbon consumption under oxic conditions (Yu et al. 2007; Abel et al. 2012). Under reducing conditions, DOC concentrations are high since organic matter is degraded to DOC via hydrolysis and fermentation (Abel et al. 2012; Husson 2013). This could have happened in the current study as well as indicated by the high DOC concentrations in solution at low E_H in the current experiment (Figure 4-1). Additionally, mobilization of DOC might be influenced by soil reaction. One might expect a positive relationship between DOC and pH in solution because at low pH, mineral surfaces are more positively charged which promotes the removal of negatively charged DOC molecules from solution via adsorption and surface complex formation (Grybos et al. 2009). Instead, a negative correlation between DOC and pH was found ($R^2 = 0.18$; $p < 0.05$; $n = 30$). However, this correlation is very weak relative to the correlation between DOC and E_H (Figure 4-1). The current results indicate that pH might play a subordinate role for the dynamics of DOC in the current experiment in comparison to E_H.

$SUVA_{254}$ decreased significantly with increasing E_H in the current experiment (Figure 4-2). Low $SUVA_{254}$ indicates low levels of aromaticity, low molecular weight, and a high ratio of hydrophilic to hydrophobic DOC components, whereas high $SUVA_{254}$ indicates high aromaticity, high molecular weight, and a low ratio of hydrophilic to hydrophobic DOC molecules (Weishaar et al. 2003). Current results indicate that the proportion of aromatic DOC in relation to aliphatic DOC in solution is higher at low E_H than at high E_H. These findings are in good agreement with Grybos et al. (2009), where SUVA increased in a wetland soil material under reducing condition during an anoxic incubation experiment. Accordingly, Fiedler and Kalbitz (2003) found that aromaticity of DOC in soil solution is lower under oxic conditions than under anoxic conditions. They suggest that the aromatic part of DOC is sorbed to Fe (hydr)oxides and thus removed from solution at high E_H, leading to a higher relative proportion of aliphatic DOC in solution. Comparable results were reported by Mladenov et al. (2008) and McKnight et al. (2002). In the current study, aromatic DOC molecules might be removed from soil solution by sorption on Fe (hydr)oxides at high E_H. In turn, aromatic DOC might be released to solution at low E_H when Fe (hydr)oxides are dissolved.

4.6.4 Direct effects of E_H on trace element mobilization

In the current study, V concentrations in solution decreased with increasing E_H and the correlation was significantly negative (Figure 4-1). Similar results after simulated flooding of soils were obtained by Amrhein et al. (1993). There can be different reasons for this behavior. Changing E_H can have a direct impact on V speciation, which in turn can affect its mobilization largely. Under reducing soil conditions, V(IV) species dominate, whereas V(V) species prevail under oxidizing soil conditions. Vanadium (IV) is produced via reduction of V(V), e.g. after flooding of a soil. This reduction process requires the presence of electron donors such as organic compounds (Wanty and Goldhaber 1992). There are large amounts of

DOC present in the current study which can serve as possible reductants (Figure 4-1). Thus, the presence of V(IV) is likely under reducing conditions in the current study. The vanadyl ion (VO^{2+}) might be the dominant V(IV) species under reducing and moderately oxidizing conditions in the current experiment when considering pH (Breit and Wanty 1991; Takeno 2005).

Further reduction of V(IV) to V(III) requires strong reductants like sulfides (Wanty and Goldhaber 1992). Sulfides are generated from sulfates below E_H -50 mV at pH 7 (corresponds to E_H 68 mV at pH 5) (Brümmer 1974). On the one hand, the formation of sulfides and consequent reduction of V(IV) to V(III) was possible since $E_{H \text{ at pH } 5}$ was below 68 mV in all MCs at the first sampling points of the experiment and the soil material contains sufficient amounts of S (Table 4-1). On the other hand, the reduction process of V(IV) to V(III) is very slow and occurs only to a low extent under natural environmental conditions (Wanty and Goldhaber 1992). Thus the presence of V(III) under the experimental conditions in the current experiment is rather doubtful.

As E_H increases, oxidation of V(IV) to V(V) is likely to occur in the presence of O_2, air, or other oxidizing agents. Considering the slightly acidic pH in the studied soil slurry, one might expect vanadate oxoanions ($H_xVO_4^{-3+x}$) to be among the most abundant V species under oxidizing conditions in the studied soil slurry (Takeno 2005), which is in good agreement with literature (Wanty and Goldhaber 1992; Wehrli and Stumm 1989; Bell et al. 2004).

The results might indicate that changing E_H influences V mobilization directly. Since at low E_H soluble V concentrations are high and V is expected to be present as V(IV), it can be assumed that reduced V species are easily mobilized. In turn, at higher E_H soluble V concentrations are lower and V(V) species might prevail in the studied soil slurry, which should be less mobilized than V(IV). Accordingly, Amrhein et al. (1993) reported a higher solubility of V in sediments under reducing conditions versus oxidizing conditions in a laboratory incubation study, which they attributed to V speciation. They stated that the reduced tetravalent vanadyl ion was more soluble than the higher oxidized pentavalent $H_2VO_4^-$ ion.

Chromium in solution showed a significant negative correlation with E_H (Figure 4-1). Similar results after flooding of soil material were found by Du Laing et al. (2007). The mobilization of Cr in soils is partly affected by its speciation. Chromium occurs mainly in the oxidation states +3 and +6 in the environment, depending on E_H and pH conditions. At low E_H, relatively immobile Cr(III) species such as $Cr(OH)^{2+}$ and Cr_2O_3 (s) dominate due to reduction of Cr(VI) in the presence of reductants such as organic matter and/or Fe(II). During this reduction process, intermediate Cr(V) is produced, which decays into Cr(III) within a few days. This process is favored by acidic conditions and occurs within minutes (Richard and Bourg 1991; Graham and Bouwer 2010; Lee et al. 2013). The studied soil material is slightly acidic and contains high amounts of organic matter and Fe which can promote Cr reduction (Table 4-1). Thus, Cr(III) was expected to be present under reducing conditions.

As E_H increases, Cr(III) can be oxidized to highly mobile Cr(VI) (e.g. chromate (CrO_4^{2-})) in natural soils and waters (e.g. Rifkin et al. 2004). However, several authors observed that oxidation of anoxic soils containing Cr(III) did not result in Cr(VI) production (Masscheleyn et al. 1992; Whittleston et al. 2011). The same might be true for the current experiment due to several reasons. First, Cr(VI) species are only stable under neutral-alkaline conditions (Takeno 2005; Graham and Bouwer 2010). Instead, the slurry in the current experiment is slightly acidic making the presence of Cr(VI) species rather improbable. Secondly, Cr(III) is not readily oxidized to Cr(VI) with O_2 alone. Manganese oxides are most likely required as catalysts (Richard and Bourg 1991; Whittleston et al. 2011). An evidence for the formation of Mn oxides is lacking in the current study (see chapter 4.5.2) which might have inhibited Cr(III) oxidation. As a consequence, Cr(III) species are considered to be dominant rather than Cr(VI) species over the entire E_H range in the current experiment. Thus, direct effects of E_H on Cr speciation and mobilization might be less important in the studied soil slurry.

The soluble fraction contained Ba and Sr which increased with increasing E_H (Figure 4-1). Generally, Ba and Sr ions are rarely reduced or oxidized (e.g. Menzie et al. 2008; Kabata-Pendias 2011). Under the experimental conditions of the current study, it was expected that Ba^{2+} and Sr^{2+} ions occur over the whole range of E_H and pH (Takeno 2005). Thus, the direct effect of E_H on the mobilization of Ba and Sr is expected to be of minor importance. However, the observed dynamics of Ba and Sr can be induced by other mechanisms and might be explained by indirect effects of E_H on these elements.

4.6.5 Indirect effects of E_H on trace element mobilization

Total Cr and V concentrations in solution were high at low E_H and decreased with increasing E_H, which is coincident with the behavior of Fe in solution (Figure 4-1). Vanadium(IV) as well as V(V) can be associated with Fe (hydr)oxides in soil and water (Wehrli and Stumm 1989; Naeem et al. 2007; Baken et al. 2012). Moreover, Cr is reported to be adsorbed by Fe (hydr)oxides in soil and water (Richard and Bourg 1991; Kumpiene et al. 2009; Agnieszka and Barbara 2012). The high mobilization of Cr and V under reducing conditions in the current experiment can be attributed to the reduction of Fe (hydr)oxides and concurrent release of associated Cr and V. The decrease of Cr and V with increasing E_H indicates that these metals were immobilized by adsorption on the surfaces of Fe (hydr)oxides and/or incorporation in the structure of Fe (hydr)oxides as described before (e.g. Aide 2005; Hseu 2006). This is confirmed by multiple regression analysis, since E_H and Fe together explain 90 % of V dynamics and 88 % of soluble Cr dynamics.

Barium and Sr are reported to adsorb on Fe (hydr)oxides in different environmental compartments too (e.g. Carbonell et al. 1999; Ferris et al. 2000; Liszewski et al. 2000). However, opposite dynamics of Fe on the one hand and Ba and Sr on the other hand were observed (Figure 4-1). This development indicates that neither Ba nor Sr is associated with Fe (hydr)oxides under the experimental conditions of the current experiment, which is in agreement with Falkner et al. (1993), who studied Ba distributions in anoxic marine waters. They observed that Ba was not adsorbed on Fe (hydr)oxides. Furthermore, Menzie et al. (2008) found that Ba ions do not bind strongly to inorganic ligands. A possible explanation can be that Ba and Sr are rather associated with Mn (hydr)oxides than with Fe (hydr)oxides, which is also reported by Palumbo et al. (2001) for Fe-Mn nodules. The evidence for the formation of Mn (hydr)oxides is lacking in the current study (see chapter 4.5.2). Furthermore, adsorption of Sr on Fe (hydr)oxides can be hindered due to competition with other dissolved cations with a similar ionic radius such as Ca^{2+} und Mg^{2+} (Kamel 2010; Seliman et al. 2010). This could have happened in the current study as well.

Organic matter has a high adsorption capacity for cations due to the presence of many negatively charged groups (Laveuf and Cornu 2009) and might contribute to trace element dynamics in the current study. Vanadium is associated with organic matter in soils and sediments. This is reported for V(IV) as well as for V(V) (Beck et al. 2008; Gäbler et al. 2009). Since vanadyl (V(IV)) is a very hard Lewis acid, it forms stronger complexes with organic chelates than vanadate (V(V)). These V-organic complexes may lead to a high stability of vanadyl even at higher E_H (Wehrli and Stumm 1989; Breit and Wanty 1991). Accordingly, Cr tends to complex with organic matter in soil material and sediments, in particular Cr(III), which is a hard Lewis acid (Du Laing et al. 2007; Beck et al. 2008).

When organic matter is degraded, mainly under reducing conditions, Cr and V bound to this organic matter can be transferred to the DOC fraction leading to a mobilization of these metals by the formation of soluble DOC complexes (Beck et al. 2008; Huang et al. 2011). Considering the high amounts of DOC in the study (Figure 4-1), complexation of Cr and V by DOC might partly explain the high solubility of these metals at low E_H (Figure 4-1). Accordingly, multiple regression analysis revealed that E_H and DOC^- together can partly explain Cr and V dynamics (42 % and 68 %, respectively), which might be an indicator of the influence of DOC on the dynamics of soluble Cr and V. Generally, aromatic DOC has got a higher reactivity and a higher ability to complex metals in comparison to aliphatic DOC (McKnight et al. 1992; Grybos et al. 2009; Riscassi and Scanlon 2011). Since $SUVA_{254}$ and thus the proportion of aromatic DOC is higher at low E_H, one might assume that Cr and V are preferentially associated with these aromatic DOC molecules.

Barium and Sr ions are reported to be mobilized by DOC in soil material and sediments as well (Tsialtas et al. 2003; Santos et al. 2011). Thus a positive relationship between Ba and Sr and DOC might have been expected in the current experiment. Instead, an opposite behavior of Ba and Sr on the one hand and DOC on the other hand was observed (Figure 4-1). However, interactions of trace elements with various different DOC molecules can be complex and counteracting. Thus, it cannot be excluded that there was an impact of DOC on Ba and Sr dynamics. The multiple regression analysis might partly confirm those assumptions since E_H and DOC together can explain about 55 % of the dynamics of Ba.

In the current study, Cr correlated slightly negative with pH ($R^2 = 0.26$, $p < 0.05$; $n = 29$). Generally, metal cations desorb from soil components such as organic matter or the surface of clay minerals and other sorbents when pH decreases (Du Laing et al. 2009a). However, this effect is expected to be relatively weak for Cr relative to other metals (Bisone et al. 2012) and the relationship between Cr and pH in the current study is weaker compared with E_H (Figure 4-1). The relationship between Ba, Sr, and V and pH was insignificant. It can be assumed that the pH variations in the current experiment might be insufficient to affect the mobilization of studied trace elements significantly.

The most abundant Ba species in soils and sediments is reported to be barite ($BaSO_4$), which is nearly insoluble under common environmental conditions (Menzie et al. 2008). The dissolution of $BaSO_4$ increases only slightly at pH 4 and lower in combination with strongly reducing conditions (Carbonell et al. 1999; Menzie et al. 2008). The pH in the current experiment is > 4. Thus, dissolution of $BaSO_4$ and subsequent release of Ba into the soluble fraction was unlikely to occur under the experimental conditions of the current study. Accordingly, Sr can be deposited as solid celestite ($SrSO_4^{2-}$) in sediments (Kabata-Pendias 2011). It might be assumed that a part of Ba and Sr was present as $BaSO_4$ respectively $SrSO_4^{2-}$ in the experiment, but remained insoluble despite of E_H and pH changes. Vanadium can be present as soluble sulfates in oxic water (Wanty and Goldhaber 1992). However, a very low mobilization of V under oxic conditions was observed. According to Du Laing et al. (2009b), Cr concentrations in pore water are not significantly influenced by S compounds. Thus S cycling might be of minor importance for the mobilization of Cr and V in the current study.

4.7 Conclusions

Redox potential was found to be an important factor influencing metal dynamics in the current study. Chromium and V showed a negative correlation with $E_{H.}$ The relationships between Ba, Sr, Mn, and Fe versus E_H were positive. Iron dynamics were mainly controlled by the formation of Fe (hydr)oxides at high E_H and their dissolution under reducing conditions. A proof for the occurrence of Mn (hydr)oxides was missing at high E_H possibly due to the relatively low pH in the studied soil slurry. The results of the current study suggest a strong absorption of Cr and V to Fe (hydr)oxides. An interaction between Cr and V with DOC is also possible. Additionally, a direct effect of E_H on V via the formation of more soluble V(IV) under reducing conditions compared with less soluble V(V) at high E_H could have occurred. The direct effect of E_H on Cr is supposed to be low due to acidic experimental conditions. The adsorption of Ba and Sr on Fe (hydr)oxides was hindered and the direct influence of E_H on Ba and Sr speciation seemed to be negligible. The dynamics of Ba and Sr may be rather influenced by DOC. A negative relationship was observed between $SUVA_{254}$ and E_H indicating a higher relative amount of aromatic DOC under reducing than under oxidizing conditions.

In summary, inundation of the soil material favors the mobilization of Cr and V while diminishing the mobilization of Ba and Sr. For a better understanding of the mobilization of metals in wetland soils, similar studies should be conducted globally with a variety of frequently flooded soil material. In future, the role of DOC and S chemistry on metal dynamics should be elucidated more in detail. Moreover, a detailed knowledge of V speciation is essential for risk assessment of contaminated sites since E_H can affect the mobilization of V directly. In future it will be a challenge to determine V species in floodplain soil slurries under changing redox conditions.

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4.9 References

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5

Biogeochemical factors affecting Hg dynamics and methylation rate in two contaminated floodplain soil slurries of the Wupper River

5. Biogeochemical factors affecting Hg dynamics and methylation rate

5.1 Introduction and significance of the chapter for the overall context

This chapter deals with the mobilization and methylation of Hg in floodplain soil slurries. Previous studies of the Wupper River floodplain soils revealed that concentrations up to 40 ppm Hg can be detected in the bulk soil (Rinklebe unpublished). These high amounts of Hg are striking since they clearly exceed the action value of 2 ppm for top soils set by the German Soil Protection Law (Bundesbodenschutzverordnung 1999). Moreover, Hg is very toxic, particularly in its methylated form, and its geochemical behavior differs from that of other metals (Ullrich et al. 2001). For these reasons, the Hg dynamics under changing E_H in two contaminated soil slurries were examined. The MC technique (see chapters 2 and 3) was used to assess the impact of controlled variations in redox conditions on the mobilization and methylation of Hg in two soil slurries of the Wupper River floodplains. The results of this study were published in 2012 in Biogeosciences (Frohne et al. 2012; Appendix 3). Prof. Jörg Rinklebe and I generated the research idea together. Moreover, he advised me in the MC experiment, and corrected the manuscript before submission.

The ICP-OES and ICP-MS techniques that were used in the previous studies of chapter 2 (Frohne et al. 2011; Appendix 1) and chapter 3 (Frohne et al. 2014; Appendix 2) are suboptimal for the detection of Hg traces in solutions. Thus, I measured total Hg (Hg_t) in soil solution with a special and very precise Hg analyzer with CV-AFS technique (mercur duo pluas, Analytik Jena) at the laboratory of the Department of Soil- and Groundwater-Management (University of Wuppertal). Methylated Hg species in soil solution were determined via gas chromatography with atomic emission detection (GC-AED) at the Helmholtz Centre for Environmental Research (UFZ) in Leipzig by Mrs. Sibylle Mothes. Mrs. Mothes and Dr. Rainer Wennrich from the UFZ gave me scientific advice concerning Hg species and corrected the manuscript before submission.

The phospholipid acids (PLFA) in the bulk soil material could not be analyzed in the laboratory in Wuppertal, since we did not have suitable equipment at that time. Thus, PLFA in the bulk soil material were analyzed by Dr. Uwe Langer at the Helmholtz Centre for Environmental Research (UFZ) in Leipzig by gas chromatography interfaced to a mass spectrometer (GC-MS). Dr. Langer gave me scientific advice concerning PLFA in soils and proofread the manuscript before submission. Prof. Gijs Du Laing from Ghent University (Belgium) helped to correct the manuscript before submission and assisted in English editing.

5.2 Abstract

To assess the effect of a range of factors on the mobilization of Hg and on the methylation of Hg in two contaminated floodplain soil slurries with different Hg concentrations (approximately 5 mg Hg kg^{-1} and > 30 mg Hg kg^{-1}), an automated biogeochemical MC system allowing controlled changes of E_H in soil slurries was used (Frohne et al. 2012; Appendix 3). Redox potential varied between approximately -350 mV at pH 5 and approximately 600 mV at pH 5 in this study.

By the results of phospholipid fatty acids (PLFA) analysis, the occurrence of SRB such as *Desulfobacter* species (10Me16:0, cy17:0, 10Me18:0, cy19:0) or *Desulfovibrio* species (18:2ω6,9) can be expected (Frohne et al. 2012; Appendix 3), that are considered to promote Hg methylation (e.g. Compeau and Bartha 1985; Hines et al. 2012). The products of this methylation process can be harmful, lipophilic methyl mercury species such as the monomethyl mercury ion [$MeHg^+$], which is designated as MeHg here (Frohne et al. 2012; Appendix 3).

The net production of MeHg normalized to Hg_t concentration is assumed to be reflected by the ln(MeHg/Hg_t) ratio (Skyllberg et al., 2007). In the study of Frohne et al. (2012) (Appendix 3), the ln(MeHg/Hg_t) ratio rises with increasing dissolved DOC to Hg_t ratio (ln(DOC/lnHg_t) ratio) ($R^2 = 0.39$, $p < 0.0001$, $n = 63$); the relation between ln(MeHg/Hg_t) ratio and lnDOC was found to be weaker ($R^2 = 0.09$; $p < 0.05$; $n = 63$).

In summary, the DOC/Hg_t ratio is supposed to be a more important factor for the Hg net methylation compared with DOC alone in the study of Frohne et al. (2012) (Appendix 3). The biogeochemical behavior of dissolved inorganic Hg species and MeHg seems to be affected by redox changes mainly in an indirect way through related changes in DOC, S cycle, and the soil microbial community structure; E_H, pH, and concentration of dissolved Fe and Cl^- might rather play subordinate roles in Hg mobilization and methylation under the occurring experimental conditions (Frohne et al. 2012; Appendix 3).

Keywords: mercury; mercury methylation; floodplain soil; biogeochemical microcosm system

5.3 Conclusions

The ln(DOC/Hg_t) ratio is positively related to net MeHg production indicating that the ln(DOC/Hg_t) ratio might play an important role for the Hg net methylation in soil slurries (Frohne et al. 2012; Appendix 3). Additionally, the formation of soluble complexes between DOC and Hg or MeHg can mobilize these compounds (Frohne et al. 2012; Appendix 3). There seems to be a linkage between Hg methylation and S chemistry, but the influence of Fe and Cl^- on Hg methylation and speciation was found to be rather weak (Frohne et al. 2012; Appendix 3). Nonetheless, the methylation of Hg is suggested to be affected by the soil microbial community, for example Hg methylation might be facilitated by low E_H through enhanced microbial activity such as SRB bacteria, as indicated by the occurence of the respective PLFA biomarkers (Frohne et al. 2012; Appendix 3). However, low E_H can lead to the formation of hardly soluble HgS at the same time, what might contribute to a decline of MeHg production (Frohne et al. 2012; Appendix 3). Thus, inundation of the soil material can either enhance or decrease Hg methylation. In summary, the behavior of Hg was found to be complex making the prediction of Hg mobilization in floodplain soils difficult. Prospective studies on the behavior of Hg in floodplain soils should also cover silty and clayey soil material and should furher be focussed on the specific role of the soil microbial community structure. Furthermore, it might be promising to assess Hg binding forms in floodplain soils.

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6

Biogeochemical fractions of Hg in soil profiles of the Wupper and Saale River floodplains

6.1 Introduction and significance of the chapter for the overall context

The outcome of the study of chapter 5 (Frohne et al. 2012; Appendix 3) showed that the selected floodplain soil material originating from the Wupper River is highly contaminated with Hg and that the behavior of Hg differs from metals which were studied before (Chapters 2 to 4; Frohne et al. 2011, 2014; Appendices 1 and 2). Thus, more detailed information on Hg binding forms in frequently flooded soils was needed.

The study summarized in the current chapter was conducted in order to elucidate the binding forms of Hg in soil material originating from Wupper River floodplain soil profiles and to include soil material originating from a different ecosystem, the Saale River floodplain in Saxony-Anhalt. The soil profiles from the Saale River floodplain were chosen since this region is known to be highly contaminated with Hg (e.g. Devai et al. 2005) but exhibits a different industrial history due to the different locations of the two study areas. Thus, a comparison of the Hg binding forms in soil profiles from those areas may allow gaining new insights into the origin of Hg contamination of the floodplain sites. The study presented in this chapter was published together with my supervisor Prof. Jörg Rinklebe in the journal Water, Air, & Soil Pollution in 2013 (Frohne and Rinklebe 2013; Appendix 4).

In summary, the aim was to assess and compare the binding forms of Hg in soil originating from floodplain soil profiles of two different ecosystems. Therefore, a SE procedure was conducted to floodplain soil material originating from the Wupper and Saale River floodplains (Frohne and Rinklebe 2013; Appendix 4). This SE technique was developed by Bloom et al. (2003), taking into account the complex behavior of Hg. I carried out the SE procedure with the help of Mrs. Anja During at the laboratory of the Department of Soil- and Groundwater-Management (University of Wuppertal). Afterwards, Hg in the extractants was measured at the laboratory in Wuppertal with a very precise Hg analyzer with CV-AFS technique, which was successfully applied in chapter 5 (Frohne et al. 2012; Appendix 2). Prof. Rinklebe and I generated the research idea together and he corrected the manuscript before submission.

6.2 Abstract

A SE procedure particularly developed for the assessment of Hg binding forms was applied in order to determine biogeochemical fractions of Hg and their influencing factors in soil

material originating from four contaminated floodplain soil profiles from two distinct ecosystems, which differ in their Hg loads due to different industrial histories (Frohne and Rinklebe 2013; Appendix 4). The soil profiles from the first study area at the Wupper River (Western Germany) revealed sum of Hg (Hg_{sum}) concentrations up to 48 ppm, whereas the soil profiles at the second study area at the Saale River (Eastern Germany) exhibit Hg_{sum} concentrations up to 4.3 ppm (Frohne and Rinklebe 2013; Appendix 4).

The results from Frohne and Rinklebe (2013) (Appendix 4) revealed that the majority of Hg was found in fraction IV (FIV, Hg^0) for both study areas, that points towards an anthropogenic origin, followed by Hg in the fractions V (F V) and III (FIII). Since Hg in FV is mainly associated with Hg sulfides that are built at low E_H (Bloom et al. 2003), its occurrence indicates reduction processes which can be usually found during flooding of a soil (Frohne and Rinklebe 2013; Appendix 4). Mercury in FIII represents organo-chelated Hg with a moderate mobility and a high methylation potential (Bloom et al. 2003). There was a significant statistical relationship between Hg in FIII and C_{HWE}, which represents the easy degradable, labile part of soil organic matter (Breulmann et al. 2011), indicating that Hg in FIII might preferentially be associated with this labile organic matter (Frohne and Rinklebe 2013; Appendix 4). The results of Frohne et al (2013) (Appendix 4) further suggest that Hg_{sum} has a high affinity for C_{org}. Moreover, the concentrations of Hg in the mobile and exchangeable fractions FI and FII were found to be low in this study and the significant positive correlation between Fe and Hg in FIV points towards an interaction between Hg and Fe. In summary, the greater part of the Hg in the studied soil profiles can be considered relatively immobile (Frohne and Rinklebe 2013; Appendix 4). Nonetheless, oxidation or methylation processes might enhance mobilization and methylation of Hg in these immobile fractions. Moreover, the low Hg concentrations in mobile fractions must not be underestimated in view of their high mobilization potential and potential plant availability.

Keywords: binding forms of mercury; sequential extraction; hot water extractable carbon; wetland soils

6.3 Conclusions

The soil profiles of the Saale and Wupper floodplains are both seriously polluted with Hg, with the Wupper sites being more heavily contaminated compared with the Saale sites (Frohne and Rinklebe 2013; Appendix 4). This might be attributed to the different industrial history at the two sites (Frohne and Rinklebe 2013; Appendix 4). The SE procedure after Bloom et al. (2003) revealed that Hg in FIV is the dominating fraction at both study sites (Frohne and Rinklebe 2013; Appendix 4) which is characteristic for soils with anthropogenic Hg pollution (e.g. Ramasamy 2012). An association between Hg and C_{org} was indicated, for example C_{HWE} seems to have a high affinity for Hg in FIII (Frohne and Rinklebe 2013; Appendix 4). Moreover, the results of Frohne and Rinklebe (2013) (Appendix 4) point towards the binding of Hg in FIV and Hg_{sum} to Fe (hydr)oxides. In this context, the crystallinity of Fe (hydr)oxides seems to play a role, since amorphous Fe (hydr)oxides are supposed to be more prone to interact with Hg in FIV compared with crystalline Fe (hydr)oxides (Borggaard 1983; Frohne and Rinklebe 2013; Appendix 4).

Even though the major part of Hg in the studied soil profiles was found to be relatively immobile (Hg in FIV and FV), transformation processes such as oxidation by dredging or draining can lead to the formation of more mobile Hg species. Mercury in FIII has a moderate mobilization potential but poses an environmental threat due to its high methylation potential (Bloom et al 2003, Covelli et al. 2011). Even though the concentrations of Hg in FI and FII are relatively low in the studied soil profiles, the importance of Hg in these fractions should not be underestimated since this Hg is expected to be highly mobile and potential plant available. Future studies should focus on the interactions between Hg on one hand and Fe (hydr)oxides and soil organic carbon on the other hand in soils from frequently flooded sites worldwide.

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7 Main research results

In floodplain top soil slurries was found:

- Increasing E_H mobilizes Ba, Cd, Co, Cu, Ni, Sr, and Zn
- Increasing E_H immobilizes As, Cr, and V
- Dynamics of metals are significantly influenced by changing E_H mainly via transformations of DOC, Fe, and S
- Direct effects of E_H via speciation changes are suggested for As and V
- Metal/DOC ratio increases with increasing E_H, indicating a higher binding strength of metals to DOC at low E_H compared with high E_H
- SUVA decreases significantly with increasing E_H, indicating a higher affinity of metals for DOC at low E_H compared with high E_H
- Methylation of As and Sb is promoted under reducing conditions
- Studied materials originating from floodplain soil profiles of the Wupper River and Saale River are highly contaminated with Hg
- Mobilization of Hg is mainly influenced by DOC and S in floodplain top soil slurries
- Methylation of Hg in soil slurries is governed by DOC/Hg ratio and soil microbial community
- Biogeochemical MC setup is a suitable technique and helped to gain new insights into metal dynamics in soil slurries under controlled E_H

Sequential extraction of Hg in material from floodplain soil profiles reveals:

- Mobilization of Hg is mainly influenced by DOC, S, and Fe
- Fe_o/Fe_d ratio influences scavenging of Hg
- Applied SE technique is a useful tool for the assessment of Hg binding forms

8 Synthesis

8.1 Introduction

Contamination of floodplain soils with metals is a serious environmental issue, since the contaminants can reach the human food chain via ground or surface water, animals, or plants. For the prediction of the dynamics of metals in flooded soils, it is crucial to understand the basic mechanisms responsible for metal release under changing E_H. Therefore, a biogeochemical MC system was successfully used to incubate contaminated top soil material originating from the Wupper River floodplain in the laboratory in order to assess the influence of pre-definite E_H on the release kinetics of As, Ba, Cd, Co, Cr, Cu, Hg, Ni, Sb, Sr, and V as well as on the methylation of As, Hg, and Sb (**Chapters 2 to 5**; Frohne et al. 2011, 2012, 2014; Appendices 1 to 3). Furthermore, the binding forms of Hg in soil material originating from floodplain soil profiles were determined by conducting a SE procedure especially developed for Hg, and data from two different distinct floodplain ecosystems (Saale and Wupper River floodplains) were compared (**Chapter 6**; Frohne and Rinklebe 2013). The current chapter combines **Chapters 2 to 6** (Frohne et al. 2011, 2012, 2014; Frohne and Rinklebe 2013; Appendices 1 to 4) and provides a joint discussion of the single studies in order to elucidate the impact of pre-definite E_H on the dynamics of metals and to give

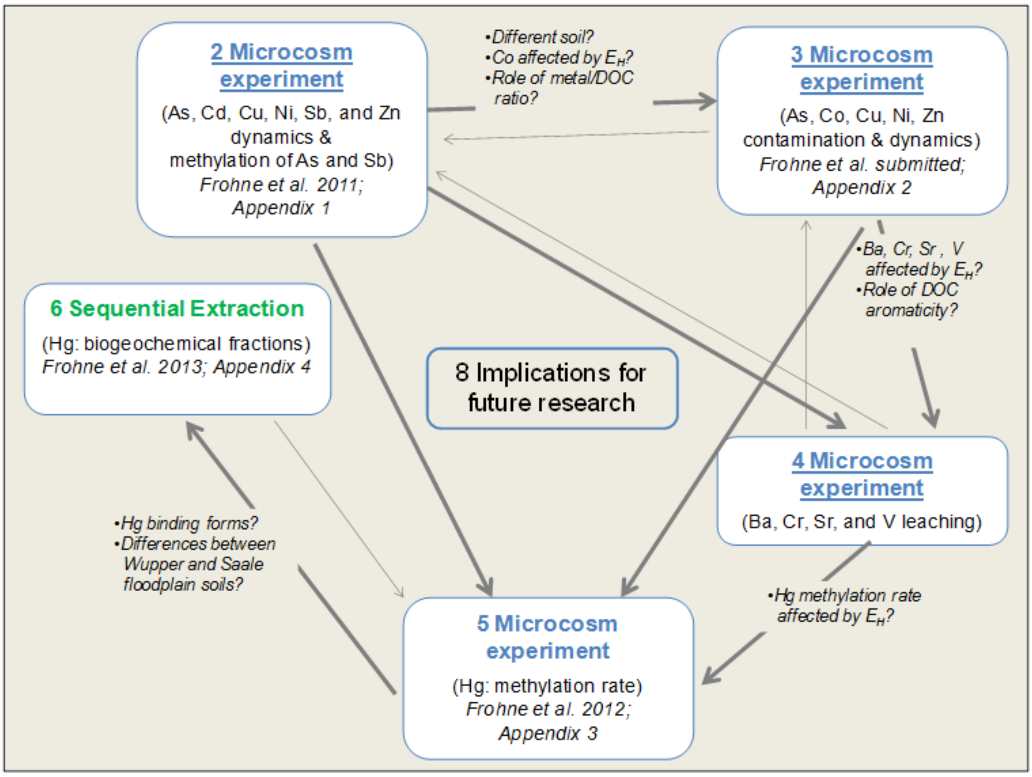

Figure 8-1 Overview over the linkages between the Chapters 2 to 6 and the structure of Chapter 8

recommendations for future research. An overview over the **Chapters 2 to 6** (Frohne et al. 2011, 2012, 2014; Frohne and Rinklebe 2013; Appendices 1 to 4) and their interrelations are illustrated in Figure 8-1. Every chapter represents a single study. The thick arrows show the links between the chapters, whereas the thin arrows highlight the feedback on previous chapters. Every single chapter and their interlinking allow providing implications for future research as illustrated by the grey area.

8.2 Dynamics of metals in slurries of floodplain top soil material as influenced by E_H

First, a fundamental basis for understanding metal dynamics under changing E_H was required. Therefore, a representative contaminated acidic top soil material originating from the Wupper River floodplain was incubated to study the influence of pre-definite E_H on the release kinetics of As, Cd, Cu, Ni, Sb, and Zn as well as on the methylation of As and Sb (**Chapter 2**; Frohne et al. 2011; Appendix 1). For a better understanding of metal dynamics in further temporarily flooded acidic soil material and for validation of the first results, another top soil material with similar properties from a different study site of the Wupper River floodplain was incubated and the release kinetics of As, Co, Cu, Ni, Sb, and Zn were studied (**Chapter 3**; Frohne et al. 2014, Appendix 2). Since DOC was suggested to be an important parameter influencing metal dynamics in **Chapter 2 (**Frohne et al. 2011; Appendix 1), a focus was laid on the E_H-dependence of metal/DOC ratios as an indicator of the binding strength of metals with DOC (**Chapter 3;** Frohne et al. 2014; Appendix 2). **Chapter 4** focuses on the leaching of Ba, Cr, Sr, and V from a floodplain top soil material into the water. To further elucidate the complex role of DOC for metal dynamics, special attention was paid to the influence of the aromaticity of DOC ($SUVA_{254}$) on metal dynamics under changing E_H.

In summary, different factors have turned out to be responsible for metal dynamics under pre-definite E_H in the studied acidic floodplain top soil slurries. Dissolved organic carbon is known to have a high affinity for the complexion of metals (e.g. Grybos et al. 2007). After incubation of the soil material microorganism activity was accelerated by the supply of straw and/or glucose. Therefore, concentrations of DOC in solution were high in all MC experiments (**Chapters 2 to 5**; Frohne et al. 2011, 2012, 2014; Appendices 1 to 3). Generally, DOC contributed to the dynamics of metals in this work. First of all, DOC can govern metal solubility via the formation of soluble metal-DOC complexes, as suggested in **Chapters 2 and 3** (Frohne et al. 2011, 2014; Appendices 1 and 2) for Co, Cd, Cu, Ni, and Zn, as well as in **Chapter 4** for Ba and Sr. Results of **Chapters 3** (Frohne et al. 2014; Appendix 2) **and 4** indicate that the characteristics of DOC are relevant for metal-DOC interactions. These characteristics are influenced by changes in the E_H. The positive correlations between metal/DOC ratios and E_H (**Chapter 3**; Frohne et al. 2014; Appendix 2) indicate that the binding of Co, Cu, Ni, and Zn to DOC might have shifted from stronger to weaker when E_H rose. Furthermore, the aromaticity of DOC decreases significantly with rising E_H **(Chapter 4)**. The aromaticity of DOC is expressed by $SUVA_{254}$. Aromatic DOC, indicated by high $SUVA_{254}$, is highly reactive and is more prone to complex metals compared with aliphatic DOC, indicated by low $SUVA_{254}$ (McKnight et al. 1992; Grybos et al. 2009; Riscassi and Scanlon 2011). Results of **Chapter 4** revealed that the relative proportion of aromatic DOC is higher under low E_H compared with high E_H. Consequently, the binding of metals to DOC should be stronger under reducing conditions compared with oxidizing conditions. This is consistent with the findings of **Chapter 3 (**Frohne et al. 2014; Appendix 2), where low metal/DOC ratios at low E_H indicated a stronger binding of metals to DOC at low E_H compared with high E_H.

Moreover, the cycling of S seemed to have an impact on metal dynamics in the slurry. The results of **Chapters 2 and 3** (Frohne et al. 2011, 2014; Appendices 1 and 2) partly confirmed each other and revealed that Co, Cd, Cu, Ni, and Zn were less soluble at low E_H compared with high E_H. This was attributed to the formation of hardly soluble sulfides at low E_H and the dissolution of these sulfides at high E_H, which is in good agreement with literature (Koretsky et al. 2007; Schröder et al. 2008; Du Laing et al. 2009a).

Pedogenic (hydr)oxides are frequently assumed to be important scavengers for metals in soils and soil material (Carbonell et al. 1999; Du Laing et al. 2009b; Borch et al. 2010). Arsenic concentrations in soil solution decreased with increasing E_H in the studies of **Chapters 2 and 3** (Frohne et al. 2011, 2014; Appendices 1 and 2). Accordingly, negative correlations of Cr and V with E_H were found (**Chapter 4**). These findings indicate that As, Cr, and V co-precipitated with Fe (hydr)oxides at high E_H as reported before (e.g. Wehrli and Stumm 1989; Kumpiene et al. 2009; Baken et al. 2012). However, co-precipitation of Ba, Co, Cd, Cu, Ni, Sr, and Zn with Fe (hydr)oxides seemed to be hindered possibly due to the low pH of the soil slurries, the nature of Fe (hydr)oxides, or due to interactions of metals with DOC (**Chapters 2 to 4**; Frohne et al. 2011, 2014; Appendices 1 and 2). For Sb, the findings are contradictory. Whereas Sb concentrations in soil solution decreased with increasing E_H in the study of

Chapter 2 (Frohne et al. 2011; Appendix 1), indicating co-precipitation of Sb with Fe (hydr)oxides, concentrations of Sb in soil solution slightly increased with increasing E_H in the concurrent study (**Chapter 3**; Frohne et al. 2014; Appendix 2). It is suggested that additional factors besides Fe (hydr)oxides such as DOC influenced the behavior of Sb. In summary, Fe (hydr)oxides seem to play an important role for the dynamics of As, Cr, and V, but were less important for the dynamics of other studied metals. Possibly, the relatively low pH in the studied soil slurries might have prevented co-precipitation of some metals with Fe (hydr)oxides. Moreover, interactions of Fe and/or studied metals with DOC should be taken into account. Furthermore, the relatively low pH might be the reason why Mn (hydr)oxides were obviously not built in the studies of **Chapters 2 to 4** (Frohne et al. 2011, 2014; Appendices 1 and 2) and are thus unavailable for co-precipitation with metals.

The methylation of As and Sb was found to be redox-dependent as well. Low E_H seems to promote the formation of MMAs, MMSb, and DMAs leading to an enhanced mobilization and toxicity of As and Sb under reducing conditions (**Chapter 2**; Frohne et al. 2011; Appendix 1). These results are partly inconsistent with the findings of Duester et al. (2008), who worked with sediment pore water. However, studies dealing with the redox-dependent methylation of As and Sb in floodplain soils and soil material are very scarce.

Direct effects of E_H on metal dynamics via changes in their speciation were suggested. The decreasing As solubility under oxidizing conditions (**Chapters 2 and 3**; Frohne et al. 2011, 2014; Appendices 1 and 2) might partly be attributed to the oxidation of more soluble As(III) to less soluble As(V) (Masscheleyn et al. 1991). Furthermore, soluble V(IV) might have been oxidized to less soluble V(V) when E_H increased (Amrhein et al. 1993) (**Chapter 4**). A similar behavior was not observed for Cr most likely due to the relatively low pH of the studied soil slurry (**Chapter 4**).

Soil reaction is often regarded as a master variable for metal dynamics in soils (Husson 2013). The pH range was small during all incubation experiments and the direct impact of pH on the dynamics of metals in the soil slurries seemed to be low (**Chapters 2 to 5**; Frohne et al. 2011, 2012, 2014; Appendices 1 to 3). As expected, the E_H decreased during the first days of incubation in all redox experiments and was systematically increased afterwards (**Chapters 2 to 5**; Frohne et al. 2011, 2012, 2014; Appendices 1 to 3). The pH is generally expected to show an opposite trend with E_H (Yu et al. 2007). However, E_H and pH were only very slightly negatively correlated in the study of **Chapter 2** (Frohne et al. 2011; Appendix 1). In

Chapters 3 to 5 (Frohne et al. 2012, 2014; Appendices 2 and 3), a slightly positive relationship between E_H and pH was observed.

8.3 Dynamics and binding forms of Hg

In **Chapter 5** (Frohne et al. 2012, Appendix 3), Hg dynamics in two top soil slurries of the Wupper River floodplain were studied with the help of a biogeochemical MC system. In contrast to other studied metals (**Chapters 2 to 4**; Frohne et al. 2011, 2014; Appendices 1 and 2), neither Hg nor MeHg concentrations in solution were directly correlated with E_H in the slurry, demonstrating that Hg exhibits a complex behavior and that a variety of factors might contribute to Hg dynamics in floodplain soil slurries (**Chapter 5**; Frohne et al. 2012; Appendix 3). Results revealed that organic matter influences Hg dynamics largely. Dissolved organic carbon can mobilize Hg and MeHg via the formation of soluble complexes (**Chapter 5**; Frohne et al. 2012; Appendix 3). This is in good agreement with literature (Feyte et al. 2010; Klerk et al. 2013; Wu et al. 2013). The outcome of the study of **Chapter 5** (Frohne et al. 2012; Appendix 3) emphasizes that a more detailed look on Hg binding forms is necessary to further elucidate the dynamics of Hg in floodplain soils.

Thus, a SE technique particularly adapted to Hg and developed by Bloom et al. (2003) was applied to soil material originating from the Wupper and Saale River floodplain soil profiles in **Chapter 6** (Frohne and Rinklebe 2013; Appendix 4). Results of this study demonstrate that the Hg contamination levels are serious in the Wupper River as well as in the Saale River floodplain soil profiles. Concentrations of Hg in some layers of the soil profiles are considerably high at the Wupper site compared to the Saale site, what might be due to the different industrial history at the respective sites (**Chapter 6**; Frohne and Rinklebe 2013; Appendix 4). Results of the SE procedure demonstrated that Hg was mainly found in the immobile fraction FIV (Hg^0) at both study sites, what is characteristic for soils with Hg pollution of anthropogenic origin (Bloom et al. 2003; Covelli et al. 2011), followed by the moderate mobile fraction FIII (Hg bound to organic matter) and the immobile fraction FV (Hg sulfides), whereas less Hg was found in the mobile fractions FI and FII (**Chapter 6**; Frohne and Rinklebe; Appendix 4). Even though the majority of Hg is strongly bound in the studied soil materials, the risk of methylation and re-mobilization should not be underestimated (**Chapter 6**; Frohne and Rinklebe; Appendix 4). This hypothesis is supported by the results of **Chapter 5** (Frohne et al. 2012, Appendix 3), since considerable amounts of soluble Hg and MeHg were detected in the soil solution.

A detailed look on the geochemical fractions of Hg showed that considerable amounts of Hg were found in FIII; this fraction is defined as Hg bound to organic matter (**Chapter 6**; Frohne and Rinklebe 2013; Appendix 4). A positive correlation of Hg_{sum} with C_{org} was found, indicating that Hg is associated with C_{org} (**Chapter 6**; Frohne and Rinklebe; Appendix 4). It was indicated that C_{HWE}, as a measure for the supply of decomposable part of organic matter, has a special affinity for Hg (**Chapter 6**; Frohne and Rinklebe 2013; Appendix 4). Interestingly, a high DOC/Hg ratio seems to promote net methylation and/or suppress demethylation of Hg in soil slurries (**Chapter 5**; Frohne et al. 2012, Appendix 3).

Considerable amounts of Hg were found in the immobile fraction FV at both study sites; these amounts were higher at the Wupper site compared with the Saale site indicating longer and more intense inundation periods at the Wupper site (**Chapter 6**; Frohne and Rinklebe 2013; Appendix 4). Generally, these findings point towards the immobilization of Hg by S as reported by Ravichandran (2004). Furthermore, methylation of Hg might be linked to the S cycle in soil slurries (**Chapter 5**; Frohne et al. 2012, Appendix 3). Analyses of PLFA in bulk soil material allow hypothesizing that the microbial community is a significant factor for the methylation of Hg (**Chapter 5**; Frohne et al. 2012, Appendix 3). For example, SRB might facilitate Hg methylation as reported by other authors (e.g. Ouddane et al. 2008).

Mercury is known to be associated with Fe (hydr)oxides in soils (Harris-Hellal et al. 2011). Positive correlations between Hg_{sum} and Hg in FIV with Fe indicate an association of Hg with Fe (hydr)oxides in the studied soil profiles (**Chapter 6**; Frohne and Rinklebe 2013; Appendix 4**)**. The nature of Fe (hydr)oxides might play a role in this context. Mercury may have a higher affinity for Fe_o than for Fe_d, possibly due to the higher surface area and thus a higher adsorption capacity of Fe_o compared with Fe_d (**Chapter 6**; Frohne and Rinklebe 2013; Appendix 4**)**. In contrast, the impact of Fe on Hg dynamics and methylation seems to be rather low in the MC experiment of **Chapter 5** (Frohne et al. 2012; Appendix 3). These inconsistent findings might partly be due to interactions of Fe with organic matter. The high amounts of DOC in the redox experiment might have inhibited co-precipitation as suggested for other metals in **Chapter 8.2**.

8.4 Conclusions

The studied floodplain soil materials are multi-contaminated with several metals (**Chapters 2 to 6**; Frohne et al. 2011, 2012, 2014; Frohne and Rinklebe 2013; Appendices 1 to 4). Information gained in this work is useful to elucidate basic mechanisms that might influence metal dynamics in contaminated floodplain soils. The advanced biogeochemical MC setup applied in this work has turned out to be an appropriate tool to assess the mobilization of metals in floodplain soil slurries under changing E_H systematically. Large E_H ranges were achieved in the MC studies, and provided new insights in metal dynamics (**Chapters 2 to 5**; Frohne et al. 2011, 2012, 2014; Appendices 1 to 3). In this work, a wide range of metals was covered including elements, whose mobilization has been rarely studied under controlled E_H in floodplain soil materials before (**Chapters 2 to 6**; Frohne et al. 2011, 2012, 2014; Frohne and Rinklebe 2013; Appendices 1 to 4).

Results revealed that Ba, Cd, Co, Cu, Ni, and Sr in soil solution are positively related to E_H, whereas As, Cr, and V in soil solution were negatively correlated with E_H (**Chapters 2 to 4**; Frohne et al. 2011, 2014; Appendices 1 and 2). The behavior of Sb in the slurry differed in the studies of **Chapters 2 and 3** (Frohne et al. 2011, 2014; Appendices 1 and 2). Generally, studied metals exhibit different geochemical behaviors in soil slurry depending on various factors such as, DOC, S cycling, Fe (hydr)oxides, speciation, and microorganisms. Some interactions with other elements and compounds are very intricate making a prediction of metal mobilization and immobilization processes difficult.

Even though the slightly acidic conditions of the studied soil material might have generally contributed to metal dynamics, since the pH is closely linked to many basic characteristics of the soil material, the pH appears to be less important for the variations in soluble metal concentrations in the current work compared with the E_H (**Chapters 2 to 5**; Frohne et al. 2011,2012, 2014; Appendices 1 to 3). Redox potential has turned out to be a key factor for metal mobilization in soil slurries mainly via related transformations of DOC, Fe (hydr)oxides, and S compounds; the role of DOC for metal dynamics is complex and interferes with other processes such as the formation of Fe (hydr)oxides (**Chapters 2 to 6**; Frohne et al. 2011, 2012, 2014; Frohne and Rinklebe 2013; Appendices 1 to 4).

For Hg, the biogeochemical MC experiment, the SE procedure after Bloom et al. (2003), and the sensitive and Hg-specific CV-AFS method (mercur duo plus, Analytik Jena) helped to increase the knowledge about the dynamics and methylation of Hg under pre-definite E_H in floodplain soil slurries and the binding forms of Hg in floodplain soil profiles. The mobilization of Hg was found to be largely determined by organic matter and in addition by Fe and S (**Chapters 5 and 6**; Frohne et al. 2012; Frohne and Rinklebe 2013; Appendices 3 and 4). Mercury methylation was likely influenced by the DOC/Hg ratio and the microbial community of the soil material (**Chapter 5**; Frohne et al. 2012; Appendix 3). The current work reveals high Hg contaminations in certain layers of the Wupper and Saale floodplain soil profiles, where Hg was mainly present as Hg^0, Hg sulfides, and Hg bound to organic matter (**Chapter 6**; Frohne and Rinklebe 2013; Appendix 4).

8.5 Implications for future research

Floodplain sites are often multi-contaminated with metals. The hydrological regime is an important factor influencing the water table level and thus the E_H in floodplain soils. Since the dynamics of metals depend on the E_H, changes in the hydrologic regime can affect metal dynamics in flooded soils. The hydrologic regime in floodplains is often altered by several anthropogenic activities such as riparian landuse, restoration, or remediation (e.g. Lair et al. 2009). In future, the risk of mobilizing even persistent metals by such interventions has to be considered.

Generally, obtained results from this work (**Chapters 2 to 6**; Frohne et al. 2011, 2012, 2014; Frohne and Rinklebe 2013; Appendices 1 to 4) should be verified with further flooded soil materials and soil profiles worldwide. Similar studies on the mobilization and binding forms of metals in floodplain soils should include silty and clayey as well as alkaline soil material in future. Moreover, even if the measured concentrations of metals in soil solution during MC experiments are considered as levels near to field conditions, a proof of the identified processes at different scales including field conditions is recommended in future studies.

Furthermore, the elucidation of the complex role of organic matter on metal dynamics is a challenging task. The structure of organic matter molecules in soils and soil solution is not exactly described yet due to its complexity. Particularly, the characteristics of DOC under changing E_H need to be studied, since the knowledge of DOC characteristic helps to understand the interactions between DOC and metals. The assessment of metal/DOC ratios and $SUVA_{254}$ in soil solution is useful and easily practicable and should be included in future MC experiments. Moreover, the determination of the molecular weight distribution of DOC by high performance size exclusion chromatography (HPSEC) can be a useful tool to characterize DOC in soil solution more fully (Kothawala et al. 2012) and can possibly be combined with biogeochemical MC experiments. Furthermore, pyrolysis-mass spectrometry can help to characterize organic matter in bulk material from floodplain soils (Leinweber et al. 1995).

The chemistry of S might be of high importance for metal dynamics in floodplain soil slurries and floodplain soil profiles (**Chapters 2 to 6**; Frohne et al. 2011, 2012, 2014; Frohne and Rinklebe 2013; Appendices 1 to 4). Thus, different S species such as sulfides and sulfates should be analyzed under changing E_H. For example, AVS are an approved measure to estimate the portion of the solid phase sulfide that reacts with free metals and serves as an indicator of metal availability (Ditoro et al. 1992). Thus, it would be of interest to combine AVS measurements with the biogeochemical MC experiments by determining AVS in soil solution.

Additionally, future research should focus on metal binding forms and the interactions between metals including Hg and Fe (hydr)oxides in floodplain soil material and profiles. The crystallinity of Fe (hydr)oxides seems to play an important role for scavenging Hg (**Chapter 6**; Frohne and Rinklebe 2013; Appendix 4) and presumably this is also true for other metals. Furthermore, interactions between metals on one hand and Fe (hydr)oxides and organic matter on the other hand should be studied in soils and soil slurries with a special attention on floodplain sites.

Geochemistry and microbiology are closely interlinked. Some groups of microorganisms are involved in the biomethylation of metals, enhancing their mobilization potential and toxicity. For example, bacteria, fungi and algae can methylate As, Sb, and other metals (Thayer 2002; Drewniak and Sklodowska 2013). Recent studies show that Hg methylation is a topic of ongoing scientific interest (e.g. Parks et al. 2013). Sulfate and Fe reducing bacteria can be involved in the methylation of Hg (Compeau and Bartha 1985; Kerin et al. 2006). Analysis of PLFA allows characterizing the microbial community in soils (Taylor and Parkes 1983; Frostegård et al. 2011). The presence of these microorganisms in the soil material is indicated in **Chapter 5** (Frohne et al., 2012; Appendix 3) via PLFA measurements. Prospective studies on the methylation of As, Hg, and Sb and the binding forms of Hg and other metals should further focus on the microbial community composition, for example via the determination and evaluation of PLFA in floodplain soils at changing E_H. In future, it is promising to combine biogeochemical MC experiments with PLFA analyses by determining PLFA in the solid phase of the soil slurry. One possibility would be to assess the micocrobial community structure and their changes at pre-definite E_H during the MC incubation experiment. These data will help to gain new insights into the methlyation of metals under changing E_H in floodplain soil slurries.

Moreover, a detailed knowledge of metal speciation changes under varying E_H is very useful for the prediction of the degree of mobilization of redox sensitive metals. For example, E_H is supposed to have significant direct impacts on V speciation and thus V mobilization (**Chapter 4**). Thus, future research should include the determination of V speciation changes in soil slurries under changing E_H. At the same time, V contamination of soils will attract more attention in future, because it is potentially toxic and new threshold values for V will be incorporated in the upcoming revised version of the German Soil Protection Law (Bundesbodenschutzverordnung 1999). New analytical tools such as synchrotron radiation based techniques allow investigating metal speciation in bulk soil material (e.g. Terzano et al. 2010; Khaokaew et al. 2012). Moreover, scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX) can contribute to elucidate distribution and binding forms of metals in bulk soil material (e.g. Jorgensen et al. 2005; Kreidie et al. 2011). These methods are promising and should be combined with the biogeochemical MC technique to assess metal speciation, the structure of DOC-molecules, and the speciation of S in samples under changing E_H in floodplain soils.

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Appendix 1

Controlled variation of redox conditions in a floodplain soil: impact on metal mobilisation and biomethylation of arsenic and antimony

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Abstract

An automated biogeochemical microcosm system allowing controlled variation of redox potential (E_H) in soil suspensions was used to assess the effect of E_H on the mobility of cadmium (Cd), copper (Cu), nickel (Ni), zinc (Zn), iron (Fe) and manganese (Mn) as well as on the methylation of arsenic (As) and antimony (Sb) in a contaminated and slightly acidic floodplain soil. The experiment was conducted under stepwise variation from reducing (approximately -300 mV at pH 5) to oxidizing (+600 mV at pH 5) conditions. The E_H was found to be an important factor controlling the dynamics of many studied compounds and elements. Concentrations of Cd, Cu, Mn, Ni, and Zn in solution were low at low E_H and increased with rising E_H what might attributed to the interaction with dissolved organic carbon (DOC) and precipitation as sulphides. Redox potential and pH correlate significantly with Cd, Ni, Cu, Zn, and Mn. Total Fe concentrations in solution were high at low E_H and dropped sharply at E_H -350 mV to lower values due to the formation of Fe (hydr)oxides. Other metals did not adsorb to or coprecipitate with Fe, which may be attributed to the low pH (between 4.4 and 5.3) amongst other factors. Concentrations of inorganic arsenic (As_i) and antimony (Sb_i), momomethyl arsenic (MMAS), monomethyl antimony (MMSb) and dimethyl arsenic (DMAs) in solution decreased significantly with rising E_H, indicating that low E_H promotes the mobility and bioavailability of these compounds.

Appendix 2

Contamination of floodplain soils along the Wupper River, Germany, with As, Co, Cu, Ni, Sb, and Zn and the impact of pre-definite redox variations on the mobility of these elements

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Abstract

This study demonstrates that floodplain soils of the River Wupper, Germany, are seriously contaminated with metal(loid)s. We used an automated biogeochemical microcosm system allowing controlled variation of redox potential (E_H) to assess the impact of pre-definite redox conditions on the dynamics of arsenic (As), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), antimony (Sb), and zinc (Zn) in an acid floodplain soil. The solubility of Co, Cu, Mn, Ni, Sb, and Zn was low at low E_H, possibly due to the precipitation of metal sulfides, and increased with rising E_H, possibly caused by their association with dissolved organic carbon (DOC). A significant positive correlation between metal/DOC-ratio and E_H indicated that the binding of the metals to DOC shifted from stronger to weaker when E_H rose. Decreasing As concentrations with increasing E_H indicated co-precipitation with Fe(hydr)oxides and/or oxidation of more soluble As(III) to less soluble As(V) during oxidation. The other studied elements seemed not to co-precipitate with newly formed Fe(hydr)oxides when E_H rose, possibly due to the prevailing low pH. In the future, the specific role of DOC and sulphur chemistry on metal(loid) dynamics should be elucidated more fully, and similar studies should be conducted with additional frequently flooded soils worldwide to verify these results.

Appendix 3

Biogeochemical factors affecting mercury methylation rate in two contaminated floodplain soils

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Biogeochemical factors affecting mercury methylation rate in two contaminated floodplain soils

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Abstract. An automated biogeochemical microcosm system allowing controlled variation of redox potential (E_H) in soil suspensions was used to assess the effect of various factors on the mobility of mercury (Hg) as well as on the methylation of Hg in two contaminated floodplain soils with different Hg concentrations (approximately $5\,\mathrm{mg\,Hg\,kg^{-1}}$ and $>30\,\mathrm{mg\,Hg\,kg^{-1}}$). The experiment was conducted under stepwise variation from reducing (approximately -350 mV at pH 5) to oxidizing conditions (approximately 600 mV at pH 5). Results of phospholipid fatty acids (PLFA) analysis indicate the occurrence of sulfate reducing bacteria (SRB) such as *Desulfobacter* species (10Me16:0, cy17:0, 10Me18:0, cy19:0) or *Desulfovibrio* species (18:2ω6,9), which are considered to promote Hg methylation. The products of the methylation process are lipophilic, highly toxic methyl mercury species such as the monomethyl mercury ion [$\mathrm{MeHg^+}$], which is named as MeHg here. The ln(MeHg/$\mathrm{Hg_t}$) ratio is assumed to reflect the net production of monomethyl mercury normalized to total dissolved Hg ($\mathrm{Hg_t}$) concentration. This ratio increases with rising dissolved organic carbon (DOC) to $\mathrm{Hg_t}$ ratio (ln(DOC/$\mathrm{Hg_t}$) ratio) ($R^2 = 0.39$, $p < 0.0001$, $n = 63$) whereas the relation between ln(MeHg/$\mathrm{Hg_t}$) ratio and lnDOC is weaker ($R^2 = 0.09$; $p < 0.05$; $n = 63$). In conclusion, the DOC/$\mathrm{Hg_t}$ ratio might be a more important factor for the Hg net methylation than DOC alone in the current study. Redox variations seem to affect the biogeochemical behavior of dissolved inorganic Hg species and MeHg indirectly through related changes in DOC, sulfur cycle, and microbial community structure whereas E_H and pH values, as well as concentration of dissolved $\mathrm{Fe^{3+}}$/$\mathrm{Fe^{2+}}$ and $\mathrm{Cl^-}$ seem to play subordinate roles in Hg mobilization and methylation under our experimental conditions.

1 Introduction

Mercury (Hg) is one of the most hazardous heavy metals, posing a risk to humans and environment (e.g. Wolfe et al., 1998; Gibicar et al., 2006; Bergeron et al., 2011). It is distributed widespread all over the world and can be found in various environmental compartments such as floodplains (e.g. Devai et al., 2005; Overesch et al., 2007; Rinklebe et al., 2009). Many floodplain soils have accumulated large amounts of Hg as a result of atmospheric deposition or through transport from the watershed (e.g. Boening, 2000; During et al., 2009; Rinklebe et al., 2010). Large floodplain areas along the Wupper River (Germany) are heavily polluted with Hg due to the discharge of waste originating from textile industry, particularly from dye factories, and metal industry during the last centuries.

The mobility, bioavailability, ecological and toxicological effects of Hg are strongly dependent on its chemical speciation (Ullrich et al., 2001). Methylation of inorganic Hg is an important process, which can fundamentally change its mobility, bioavailability, and toxicity (Boening, 2000). The

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products of this methylation process are lipophilic, highly toxic methyl mercury species such as dimethyl mercury [Me_2Hg] or the monomethyl mercury ion [$MeHg^+$], which is named as MeHg in the following. Both methyl mercury species exhibit a significant risk to humans and wildlife due to its neurotoxicity and tendency to accumulate in the food chain (Wolfe et al., 1998; Boening, 2000; King et al., 2002; Li et al., 2010).

Generally, the mobility and methylation of Hg in frequently flooded soils is determined by a range of factors, such as redox potential (E_H), pH, dissolved organic carbon (DOC), sulphur (S), chloride (Cl^-), iron (Fe), and total dissolved Hg (Hg_t) content (e.g. Skyllberg et al., 2003; DeLaune et al., 2004; Sunderland et al., 2006). Dissolved organic carbon interacts strongly with Hg by the formation of Hg-DOC complexes (e.g. Ravichandran, 2004; Khwaja et al., 2006; Feyte et al., 2010). The high affinity of Hg to DOC can partly be attributed to the binding of Hg with reactive sulfur groups in the hydrophobic acid fraction of DOC (Karlsson and Skyllberg, 2003; Shanley et al., 2008).

Mercury immobilization can be induced under anoxic conditions due to the formation of hardly soluble Hg sulfides (e.g. Skyllberg et al., 2003; Du Laing et al., 2009). Sulfides (S^{2-}) are generated through sulfate (SO_4^{2-}) reduction, which is mainly catalyzed by microorganisms. Sulfate reducing bacteria (SRB) (e.g. *Desulfovibrio desulfuricans, Desulfobulbus proprionicus*) mediate the formation of S^{2-} as a result of respiration processes that require SO_4^{2-} as a terminal electron acceptor (King et al., 2002). During Hg methylation, microorganisms increase their resistance to Hg by rendering the Hg^{2+} ion ineffective in disturbing the normal biochemical processes within the cell (Boening, 2000). This methylation process has been found to be mainly conducted by SRB and Fe reducing bacteria (Compeau and Bartha, 1985; Macalady et al., 2000; Fleming et al., 2006; Merritt and Amirbahman, 2009). Furthermore, it might be possible that other organisms such as aerobic bacteria, fungi, and seaweed may play a role in Hg methylation as suggested for tropical environments and mangrove wetlands (Coelho-Souza et al., 2006; Wu et al., 2011). The microbial community composition in soils and sediments can be characterized by the analysis of phospholipid fatty acids (PLFA) (e.g. Macalady et al., 2000; Rinklebe and Langer, 2006; Langer and Rinklebe, 2009). This method allows identifying the presence of SRB (Taylor and Parkes, 1985; Coleman et al., 1993; Macalady et al., 2000).

Iron can influence the dynamics of Hg in soils. For instance, Fe(hydr)oxides are able to adsorb Hg; thus, they can act as important Hg sinks (Fernandez-Martinez et al., 2006; Harris-Hellal et al., 2011). Moreover, the mobility and methylation of Hg can be influenced by Cl^-, for example through the formation of Hg chloride or MeHg-Cl complexes (Davis et al., 1997; Skyllberg et al., 2003).

Although the presence of Hg and MeHg in the environment has been frequently documented (e.g. van Faassen, 1975; Boening, 2000; Agusa et al., 2005; Devai et al., 2005; Gibicar et al., 2006), mechanistic experiments aimed to study the redox-induced mobilization and immobilization of Hg and MeHg as well as information on biogeochemical factors affecting the methylation rate of Hg in floodplain soils are very scarce up to date.

Thus, our aim was to assess the impact of E_H, pH, DOC, SO_4^{2-}, Fe, and Cl^- on the mobility and methylation of Hg in two floodplain soils with different Hg contamination levels (approximately 5 and >30 mg Hg kg^{-1}, respectively) under acidic to neutral pH conditions. Therefore, we used an automatic biogeochemical microcosm system allowing establishing definite, computer-controlled redox conditions in soil suspensions.

2 Materials and methods

2.1 Study site

Soil samples were collected from two floodplain soils (Wupper 1 = W1; Wupper 2 = W2) at the lower course of the Wupper River (Germany) close to the confluence into the Rhine River (Fig. 1). The study sites are located about 15–20 km to the north of Cologne, Germany, near the town Leverkusen, (W1: 51°4′0.48″ N, 6°59′0.77″ E; W2: 51°5′4.1″ N, 7°0′12.61″ E). The distance between the two study sites is about 2 km. The long term average annual precipitation is approximately 800 mm and the long term average annual air temperature is 10.8 °C (DWD, 2009). The geological parent material consists of sediments of the Rhine River ("Niederrheinische Bucht"), which is predominantly shale from Devonian origin ("Rheinisches Schiefergebirge"). The study sites are used as grassland and are flooded seasonally by the Wupper River, usually in springtime (Wupperverband, 2009). The Wupper River is approximately 115 km in longitude with an average gradient of 0.4 %. The discharge averages 15.4 m^3 s^{-1}. The catchment area of the Wupper River comprises 814 km^2. Both soils are classified as Eutric Fluvisols (IUSS-ISRIC-FAO, 2006).

2.2 Sampling, pre-treatment, and analysis of bulk soil

Soil samples were collected from the genetic A-horizons (0–10 cm for W1; 0–32 cm for W2). Soil sampling was performed in four replicates of about 1 kg each which were merged to one sample. For chemical analysis, soil material was homogenized, air-dried, and sieved to <2 mm. Subsamples were ground in an agate disc mill. Physico-chemical soil properties were determined according to standard methods (Schlichting et al., 1995). Total C (C_t) and total N (N_t) were determined via dry combustion and thermal conductivity detection using a C/N/S-Analyzer (Vario EL Heraeus, Analytik Jena, Germany). A C-MAT 550 (Stroehlein, Germany) was used to measure inorganic C by dry combustion and IR-detection. Soil organic C was calculated as the difference

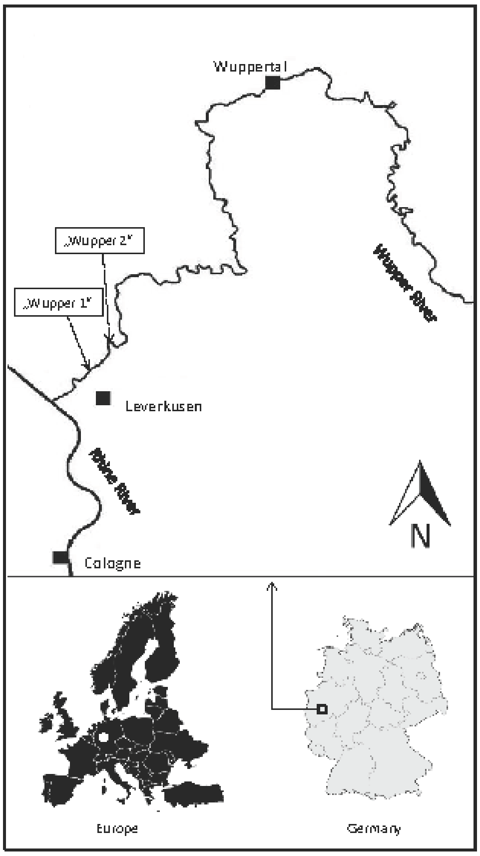


Fig. 1. Study site.

between C_t and inorganic C. Particle-size distribution was measured using the pipette sampling technique by wet sieving and sedimentation (Blume et al., 2000). Total metal concentrations of the soil were quantified after digestion using aqua regia (37 % HCl + 65 % HNO_3, volume ratio 3:1) ignoring the immobile silica-bound fraction. Total Hg was analyzed by a cold vapor atomic absorption spectrometer (FIMS 400, Perkin Elmer, USA). The calibration range was 0.5–20 $\mu g l^{-1}$. The dissolved samples were appropriate diluted for this calibration range using 0.01 M nitric acid. Analytical accuracy was achieved by the use of certified reference material (IAEA 405, IAEA 433, NIST 2709, and LGC-6139). Inductively coupled plasma atomic emission detection (Ciros CCD, Spectro, Germany) was used for determination of Fe. For the determination of total S, the soil was compressed to pellets and S was measured by energy dispersive X-ray spectroscopy (XLAB 2000, Spectro, Germany). The calibration (using wax pellets) was done in the concentration range 0.0150–15 $g kg^{-1}$. The following 15 reference materials had been used: GBW 7309–7312, GBW 7409–7411, LKSD 1, LKSD 4, NIST 2704, and NIST 2710.

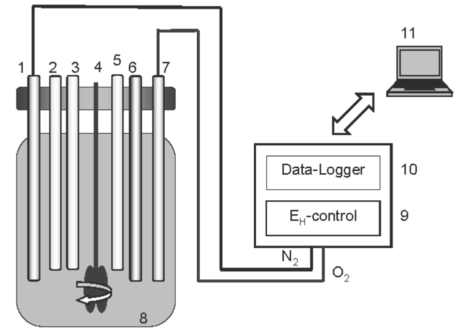
For the soil microbial analysis (PLFA), fresh soil samples were sieved to <2 mm and thereafter frozen at −20 °C. After storage, samples were allowed to thaw at 4 °C for one day and 4 h at 20 °C before analysis. Phospholipid extraction and PLFA analysis were performed following the standard procedure described by White et al. (1979) and Frostegård et al. (1991). Lipids were extracted with a modified single-phase mixture chloroform-methanol-citrate buffer (1:2:0.8 v/v/v) (Bligh and Dyer, 1959). The resulting lipid material was fractionated into neutral lipids, glycolipids, and polar lipids by a silica-bonded phase column. The polar lipids were transesterified to the fatty acid methyl esters by a mild alkaline methanolysis (Guckert et al., 1985). Samples were analyzed by gas chromatography/mass spectroscopy using a Hewlett-Packard 6890 series gas chromatograph with a HP-5MS column (60.0 m length, 0.25 mm internal diameter, coated with a cross-linked 5 % phenyl methyl rubber phase with a film thickness of 0.25 µm) interfaced to an Agilent 5973 mass selective detector. The resulting chromatograms were evaluated by mass spectra, retention times, and non-adecanoic acid methyl ester (19:0) as the internal standard (N-5377, Sigma Chemical, Inc.). The analytical quality was confirmed by the repeated analysis of a standard bacterial acid methyl ester mix and a 37-component FAME mix (47080-U and 47885-U, Supelco, USA). PLFA were designated using the nomenclature described by Feng et al. (2003). More details regarding the method of PLFA analyses can be found in Rinklebe and Langer (2006) and Langer and Rinklebe (2009).

2.3 Redox experiment

2.3.1 Biogeochemical microcosm system

Flooding events were simulated using an automated biogeochemical microcosm system in the laboratory (Fig. 2). This system allows establishing pre-defined redox conditions in soil suspensions by flushing them with nitrogen (N_2) or oxygen (O_2). Thus, it is possible to study the effect of E_H almost independent from other parameters. Recently this system was described in detail by Yu and Rinklebe (2011) and successfully used in previous studies for the investigation of trace gases (Yu et al., 2007), for the quantification of mercury emissions (Rinklebe et al., 2010), and for the determination of the dynamics of trace metals (Rupp et al., 2010; Frohne et al., 2011). The current study was conducted in four independent trials for each soil. The microcosms (MCs) were filled with 200 g air-dried soil and 1600 ml deionized water. Homogenous conditions were reached by stirring the slurry continuously. Redox potential, pH, and temperature in each MC were monitored every ten minutes by electrodes (Meinsberger Elektroden, Germany) and stored by a data logger. The pH values of MC 8 and the E_H values of MC 1 after approximately 800 h incubation could not be monitored due to an error of the electrodes. The measured redox potential values were normalized to pH 5, because the mean pH during the experiment was around 5 for both soils. Thus, the corrected values will be referred to as "E_{HatpH5}" in the following.

Straw and glucose were added to each MC to provide an additional source of organic matter for microorganisms. As a result, levels of E_{HatpH5} decreased (Fig. 4). This process was accelerated by continuously flushing the MCs with N_2 for several days. When lowest E_H values were reached, the first sample was taken from each MC. Thereafter, E_H-values were increased in steps of approximately 100 mV by adding O_2. Thereby, E_H was kept within the set E_H-windows $\pm$10–20 mV around the aimed value by supplying O_2 or N_2 automatically when the outer limits of the E_H-windows were exceeded. Redox potential was maintained for approximately 24 h within each window and afterwards set to the next window. Sampling was conducted approximately 24 h after reaching each new E_H-window (Fig. 4). The soil/water ratio remained the same during the experiment. After achieving the highest E_H levels, N_2 was added to lower E_H again.



The soil microcosm system:

(1) dispersion tube for N_2
(2) redox potential- (E_H) electrode
(3) pH electrode
(4) stirrer
(5) thermometer
(6) sampling tube
(7) dispersion tube for O_2
(8) glass vessel
(9) automatic E_H regulation by N_2 and O_2 valves
(10) data logger (E_H, pH, temperature)
(11) PC control for datalogger and valve system

Fig. 2. Biogeochemical microcosm setup.

2.3.2 Sample preparation, sub-sampling, and storage during the redox experiment

The slurry in the MCs was sampled using a plastic syringe connected with a PTFE tube. The slurry samples were immediately centrifuged for 15 min at 3000 rpm. Afterwards, the supernatants were filtered through a 0.45 µm Millipore membrane (Whatman Inc., Maidstone, UK) under N_2-atmosphere. Thereafter, the filtrate (defined as the soluble fraction) was aliquoted to subsamples for subsequent analysis. For measuring Hg_t, the first 10 ml subsample was preserved with 200 µl 0.2 M bromine monochloride solution (BrCl) and stored at 8 °C in bottles of acid rinsed borosilicate glass with PTFE-lined caps. A second subsample (8 ml) was stored in acid rinsed glass bottles at 8 °C for the analysis of MeHg. Another 10 ml subsample was stabilized by addition of 400 µl 65 % HNO_3 for analysis of total Fe and total S. Another subsample (10 ml) was stored at -20 °C and gradually thawed for the determination of DOC, Cl^-, and SO_4^{2-}.

2.3.3 Chemical analyses of the redox experiment samples

Total Hg was measured with cold vapor atomic fluorescence spectrometry (CV-AFS) (mercur duo plus, Analytik Jena, Germany). Mercury standard solutions were prepared by diluting mercury standard solution 1000 mg l^{-1}Hg (CertiPur, Merck) with deionized water. A 7-point calibration curve including a blank sample was used for sample analysis. An intern reference sample was analyzed after every 10 samples to check the instrument drift. The drift was satisfying for all measurements. The detection limit was 10 ng l^{-1}. The relative standard deviation of repeated measurements was below 3 % for all samples.

The analyses of MeHg in the subsample were conducted by gas chromatography with atomic emission detection (GC-AED). An amount of 2 ml of the sample was spiked with 4 ml buffer solution (pH 4.5) and 20 µl Na-propylborat solution (2 % in THF). The solution was stirred for 10 min. The Hg species were enriched from the aqueous phase by solid phase microextraction (SPME) in the headspace mode. Analytes were enriched onto a 100 µm polydimethylsiloxane (PDMS) – fibre for 30 min at a temperature of 30 °C. The prepared samples were stored at 15 °C until measurement. Samples were processed automatically by a multipurpose sampler (MPS2, Gerstel, Mülheim, Germany) combined with a gas chromatograph Hewlett-Packard 6890 (Agilent, Waldbronn, Germany) and a microwave-induced plasma atomic emission detector jas 2350 (jas GmbH, Moers, Germany). Thermal desorption was carried out directly in the injector of the gas chromatograph for 1 min at 200 °C. The analyses were carried out using an HP1 column (25 m × 0.32 mm × 0.17 µm) and He as carrier gas. Injection was performed in splitless mode and the oven was programmed from 40 °C (2 min) to 280 °C at 25 °C min^{-1}. Reagent gases for the AED were O_2 and H_2, the make-up gas flow (He) was 130 ml min^{-1}. The Hg emission line 254 nm was monitored. Only monomethyl mercury ($MeHg^+$) – and no dimetyhl mercury (Me_2Hg) – could be detected. The detection limit for $MeHg^+$ was 0.8 ng Hg l^{-1}. The calibration range was 1–100 ng Hg l^{-1}. Quality control was carried out using following reference materials: IAEA 405, IAEA 433, and CRM 462. Total Fe and S were quantified by inductively coupled plasma optical emission spectrometry (ICP-OES) (Ultima 2, Horiba Scientific, Unterhaching, Germany). A four-point calibration was conducted by diluting single standard and multi element solutions (CertiPur, Merck) with deionized water. Analysis was conducted in three replications. The relative standard deviation of replicate analysis was below 5 %.

Dissolved organic carbon was measured after 2-point calibration with a TOC-analyzer (TOC-V_E, Shimadzu, Kyoto, Japan). Measurement was performed in two replications for each sample. The detection limit was 1 mg l^{-1}. Sulfate and Cl^- were determined using an ion chromatograph (Personal IC 790, Metrohm, Filderstadt, Germany) with a Metrosep A Supp 4 - column. The detection limit was 0.03 mg l^{-1} for both ions.

2.4 Calculations and statistical analysis

Mean values of E_H and pH data measured every 10 min originating from 3, 6, 12, and 24 h periods prior to sampling were calculated. Values below the detection limit were excluded for the statistical analyses. Thereafter correlation and regression analyses between Hg_t and MeHg on the one hand and E_H, pH, DOC, SO_4^{2-}, Cl^-, Fe, and S on the other hand were conducted. Relations between MeHg/Hg_t and DOC/Hg_t on one hand and DOC and Hg_t on the other hand were also calculated. Mean E_H and pH values of the 6 h period prior to sampling were used because they revealed the highest regression coefficients in most cases. Correlation analysis was conducted by SPSS 19. ORIGIN 6.0 was used to calculate regressions and descriptive statistics. For regression analyses, the naturally logarithmised values (ln) of Hg_t, MeHg, DOC, Cl^-, SO_4^{2-}, and Fe were taken, because the range of the values differed for several orders of magnitude. According to Fowler et al. (2006), the strength of the correlations was categorized in our study as follows: $r < 0.20$ (corresponds to the coefficient of determination $R^2 < 0.04$) represent very weak correlations; r between 0.20 and 0.39 (R^2 between 0.04 and 0.15) weak correlations; r between 0.4 and 0.69 (R^2 between 0.16 and 0.48) modest correlations; and $r > 0.69$ ($R^2 > 0.48$) strong correlations.

3 Results

3.1 Properties of the bulk soils

Selected properties of the studied bulk soils are provided in Table 1. The soils of W1 and W2 mainly consist of sand and silt. The content of organic carbon is relatively high and the contents of inorganic carbon are 0.01 % (W1) and 0.005 % (W2). The pH is slightly acidic to neutral. Soil W1 is contaminated with approximately 5 mg kg^{-1} Hg, whereas soil W2 is higher contaminated and contains >30 mg Hg kg^{-1} (Table 1). For both soils, concentrations of Hg exceed the action value of 2 mg Hg kg^{-1} set by the German Federal Soil Protection Ordinance (BBodSchV, 1999).

The results of PLFA analysis of the bulk soil are shown in Fig. 3. A total number of 26 PLFA (W1) and 20 PLFA (W2), respectively, were found in the soils. Here, those fatty acids which were previously identified as possible biomarkers for SRB are of particular interest. The fatty acids 15:0, 10Me16:0, cy17:0, 18:2ω6,9, 10Me18:0, and cy19:0 can serve as biomarkers for SRB (Taylor and Parkes, 1983; Kohring et al., 1994; Macalady et al., 2000). The fatty acids 10Me16:0 and cy19:0 revealed the highest values whereas cy17:0 and 10Me18:0 showed intermediate values in both soils. The PLFA 15:0 and 18:2ω6,9 were low concentrated in the studied soils.

3.2 Redox experiment

The variations (mean, median, and range) of the measured parameters during the E_H experiment are provided in Table 2. The range was 0.09–8.27 µg l^{-1} for Hg_t and 1.3–101 ng l^{-1} for MeHg. The mean values were 0.99 µg l^{-1} for Hg_t and 14 ng l^{-1} for MeHg. The E_H ranges from −335–601 mV (all data). The pH ranges from 4.1–7.2 (all data) with mean values around 5 for both soils. During the experiment, E_H and pH reveal a very weak significant negative correlation ($R^2 = 0.02$; $p < 0.01$; $n = 47{,}941$). The development of E_{HatpH5} measured in the slurry every 10 min during the experiment and concentrations of Hg_t in the soluble fraction at the sampling time are given in Fig. 4. The lowest E_{HatpH5} levels were around −150 and 0 mV in MCs 1–4 (W1) and between −100 and −350 mV in MCs 5–8 (W2). The highest E_{HatpH5} levels were around 500 mV for W1 and 500–600 mV for W2. The development of pH measured in the slurry every 10 min and the values of MeHg in the soluble fraction at the sampling points during the experiment are given in Fig. 5. The mean initial pH was 7.0 ± 0.2 for W1 (MCs 1–4) and 5.7 ± 0.3 for W2 (MCs 5–8). The pH dropped rapidly in all MCs to values between 4 and 5. Afterwards, the pH slightly increased in all MCs when increasing the E_H stepwise. A relationship between Hg_t and E_H, or between pH and MeHg in the course of the experiment is not obvious (Figs. 4 and 5).

The ln(MeHg/Hg_t) ratio revealed a modest positive relationship with ln(DOC/Hg_t) ($R^2 = 0.39$; $p < 0.0001$; $n = 63$)

Table 1. Selected properties of the studied soils (C_{org}: organic carbon, N_t: total nitrogen) total metal concentrations (aqua regia soluble), and total sulfur (S) of the studied bulk soils Wupper 1 (W1) and Wupper 2 (W2).

Soil	Depth [cm]	Texture [%]			C_{org}	N_t	$C_{org}N_t^{-1}$	Fe	Hg	S
		Sand 0.063–2 mm	Silt 0.002–0.063 mm	Clay <0.002 mm	[%]			[g kg $^{-1}$]	[mg kg $^{-1}$]	
W1	0–10	44	48	8	6.2	0.4	15.6	34	5.2	2060
W2	0–32	55	36	9	7.9	0.4	19.8	49	31.5	2669

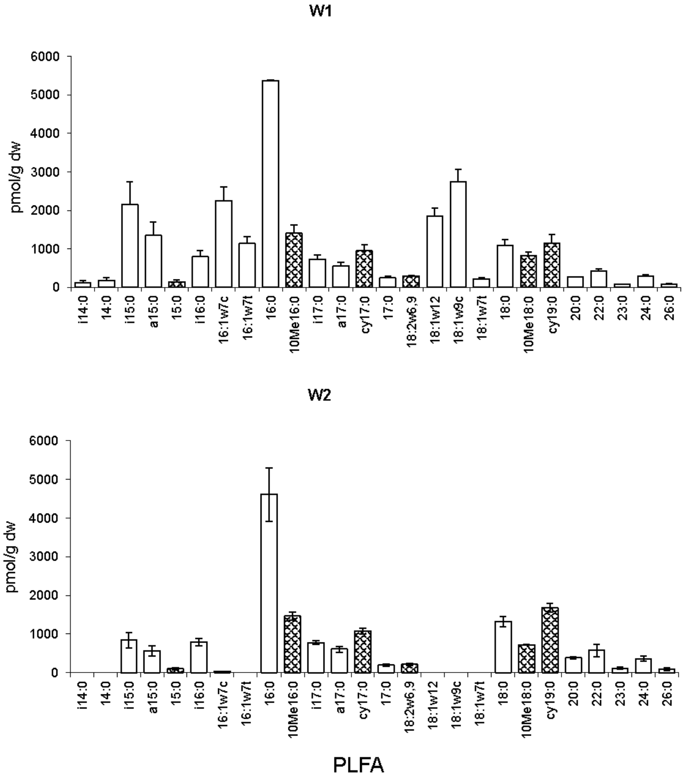


Fig. 3. Dry weight (dw) of phospholipid fatty acids (PLFA) in the bulk soils Wupper 1 (W1) and Wupper 2 (W2). Biomarkers for sulfate reducing bacteria are cross hatched.

(Fig. 6). There was a weaker negative relationship between ln(MeHg/Hg_t) and lnHg_t ($R^2 = 0.18$; $p < 0.001$; $n = 63$) and a weak positive relation between ln(MeHg/Hg_t) and lnDOC ($R^2 = 0.09$; $p < 0.05$; $n = 63$). LnHg_t values were correlated with lnMeHg values and regression analysis showed a modest positive relationship ($R^2 = 0.16$; $p < 0.005$; $n = 63$). Results of the regression analysis between lnDOC, $\ln Cl^-$, $\ln SO_4^{2-}$, E_H, lnFe, pH and lnHg_t on one hand and lnMeHg in the soluble fraction on the other hand are provided in Table 3. With the increase of Hg_t and MeHg, Cl^-, SO_4^{2-}, and

Table 2. Variations (mean, median, range) of concentrations of elements and compounds in the soluble fraction as well as pH, and redox potential (E_H) in the slurry.

		Mean	Median	Range	n
Hg_t	[$\mu g\,l^{-1}$]	0.99	0.49	0.09–8.27	65
MeHg	[$ng\,l^{-1}$]	14	5.8	1.3–101	65
$E_H(6h)^{1,3}$	[mV]	230	297	−332–577	67
$E_H(\text{all data})^{2,3}$		239	319	−335–601	53 553
pH $(6h)^1$		5.0	5.0	4.4–6.2	67
pH $(\text{all data})^2$		5.5	5.2	4.1–7.2	47 941
DOC	[$mg\,l^{-1}$]	2096	1989	1082–4463	67
SO_4^{2-}		32.0	8.6	2.2–223	67
Cl^-		584	144	72–3896	67
S		2.1	2.0	0.7–7.7	67
Fe		199	114	0.2–553	67

[1] means of data 6 h before sampling

[2] data measured every 10 min during the experiment

[3] E_H corrected to pH 5 (see Materials and Methods)

DOC in the soluble fraction increased. These correlations were strong for MeHg and DOC and modest for the other parameters. Iron was positively related to MeHg but not to Hg_t. Values of pH revealed a modest negative relation to MeHg and no correlation to Hg_t (Table 3). Values of E_H (Table 3) and S (data not given) are not related to Hg_t or MeHg. Values of Cl^- and SO_4^{2-} in the soluble fraction were weakly associated with E_{HatpH5} (linear relationship with $R^2 = 0.15$; $p < 0.01$; $n = 67$ for Cl^- and curved relationship $R^2 = 0.13$; $p < 0.05$; $n = 67$ for SO_4^{2-} respectively). Iron contents in the soluble fraction revealed a modest negative relationship to E_{HatpH5} ($R^2 = 0.33$; $p < 0.001$; $n = 67$). Contents of DOC did not have a significant relationship with E_{HatpH5} (data not shown).

4 Discussion

4.1 Direct impact of E_H and pH on the mobility and methylation of Hg

The biogeochemical behavior and the dynamics of Hg and MeHg under changing redox conditions are affected by various factors. Our original hypothesis was that systematic changes of E_H from anaerobic to aerobic conditions should have a considerable impact on the methylation of Hg since it has been reported that MeHg increases with decreasing E_H (DeLaune et al., 2004; Sunderland et al., 2006). On the other hand, Ullrich et al. (2001) stated that anaerobic conditions might favor the reduction from Hg^{2+} to hardly soluble Hg^0, which in turn may reduce Hg mobility and Hg methylation because of reduced bioavailability. However, in the current study, a direct impact of E_H on Hg_t or MeHg concentrations could not be detected (Figs. 4 and 5; Table 3). The variations of Hg_t values during the experiment seem to be almost independent from E_H variations (Fig. 4). Wallschläger et al. (1998) found consistent with our results, that the mobility of Hg is less influenced by changing redox conditions. Hintelmann and Wilken (1995) also reported that absolute E_H might not be the most important factor regulating Hg methylation activity in anoxic sediments. The results of the current study appear to confirm those assumptions.

Redox potential has also an effect on the pH. Generally it is well established that pH increases during reduction because reduction processes consume protons (e.g. Yu et al., 2007). Accordingly, a similar behavior was generally observed in our study. Literature data on effects of pH on the mobility and methylation of Hg are contradictory. Some authors found enhanced mobility and methylation of Hg at low pH (Boening, 2000; Ullrich et al., 2001; Wu et al., 2011). This was attributed to the fact that DOC is more positively charged at low pH and therefore has weaker tendencies to form complexes with Hg, enhancing its availability for methylating bacteria (Ravichandran, 2004). This process could have occurred in the current study as well and might contribute to explain the modest negative relationship between MeHg and pH (Table 3). In contrast, low pH can decrease Hg methylation in anoxic sediments, maybe due to the suppression of bacterial activity at low pH (Gilmour and Henry, 1991). However, the results presented here show a less clear effect of pH on Hg_t and on MeHg. Although the relationship between MeHg and pH is modest (Table 3), a mutual development between MeHg and pH is not obvious (Fig. 5) suggesting that additional factors are needed to explain MeHg variations. Accordingly, Wallschläger et al. (1996) have shown that the influence of pH on the solubility of Hg is relatively low compared to other metals (e.g. Cd, Ni, Co, Zn, Cu, Pb). The direct impact of E_H on the behavior of Hg and MeHg seems to be very weak in our study. Instead, indirect effects of E_H and pH on the mobility and methylation of Hg through E_H or pH related changes of other determining factors such as concentrations of DOC, Fe, Cl^-, and SO_4^{2-} should be more important under our experimental conditions.

4.2 Impacts of DOC, Fe, Cl^-, and SO_4^{2-} on the mobility and methylation of Hg

Total mercury and MeHg concentrations were positively related to DOC concentrations in the current experiment whereas this relationship is stronger between MeHg and DOC (Table 3). Similar results have been obtained by other authors (Covelli et al., 2009; Obrist et al., 2009; Feyte et al., 2010) since Hg and MeHg tend to form complexes with organic carbon. The interaction between DOC and Hg_t respectively MeHg can partly be attributed to the binding of Hg with reactive S groups in the dissolved organic molecules, especially in the hydrophobic acid fraction of DOC (Karlsson

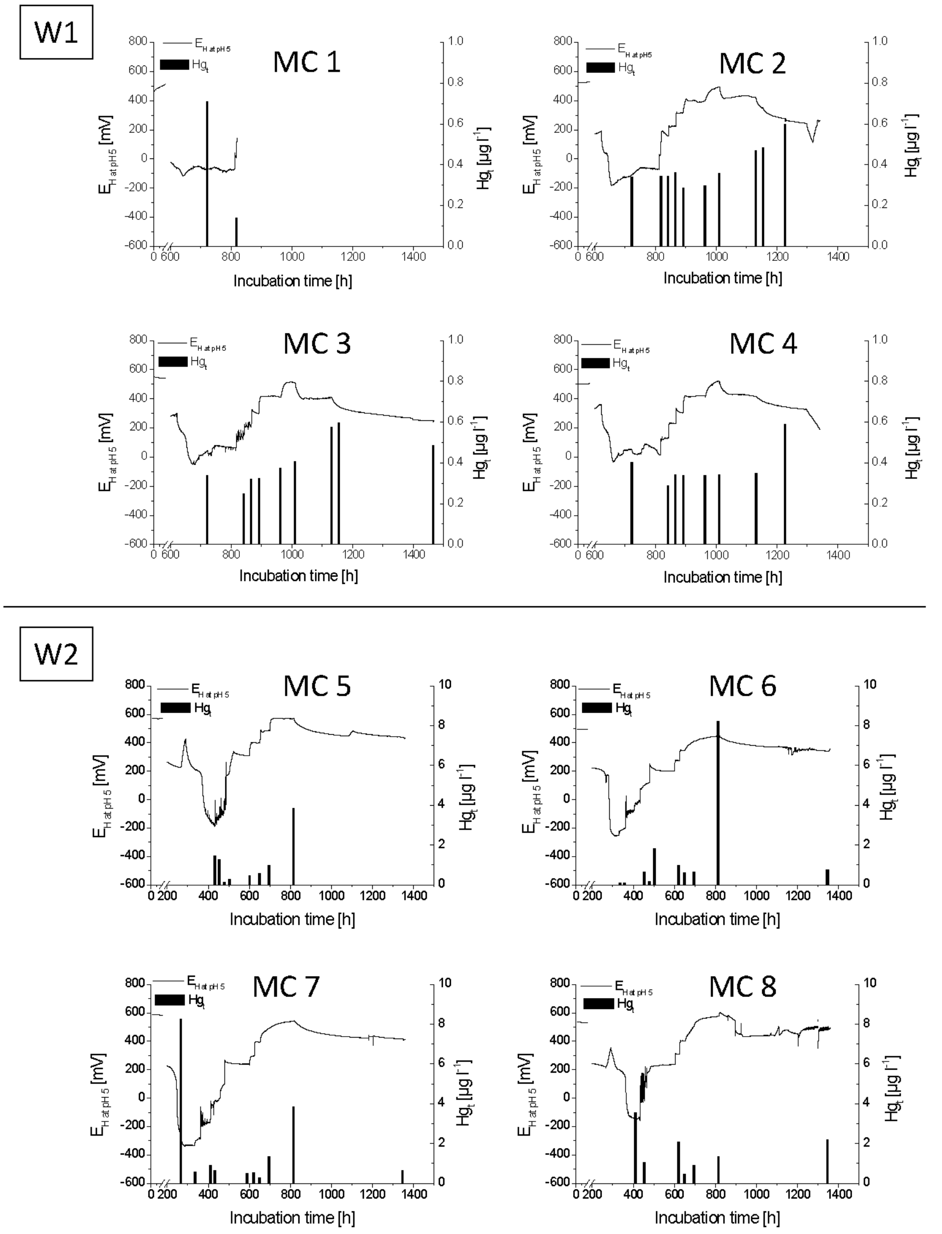


Fig. 4. E_{HatpH5} measured in the slurry every 10 min and Hg_t concentrations in the soluble fraction during the experiment for each microcosm (MC) (Wupper 1 = W1, Wupper 2 = W2).

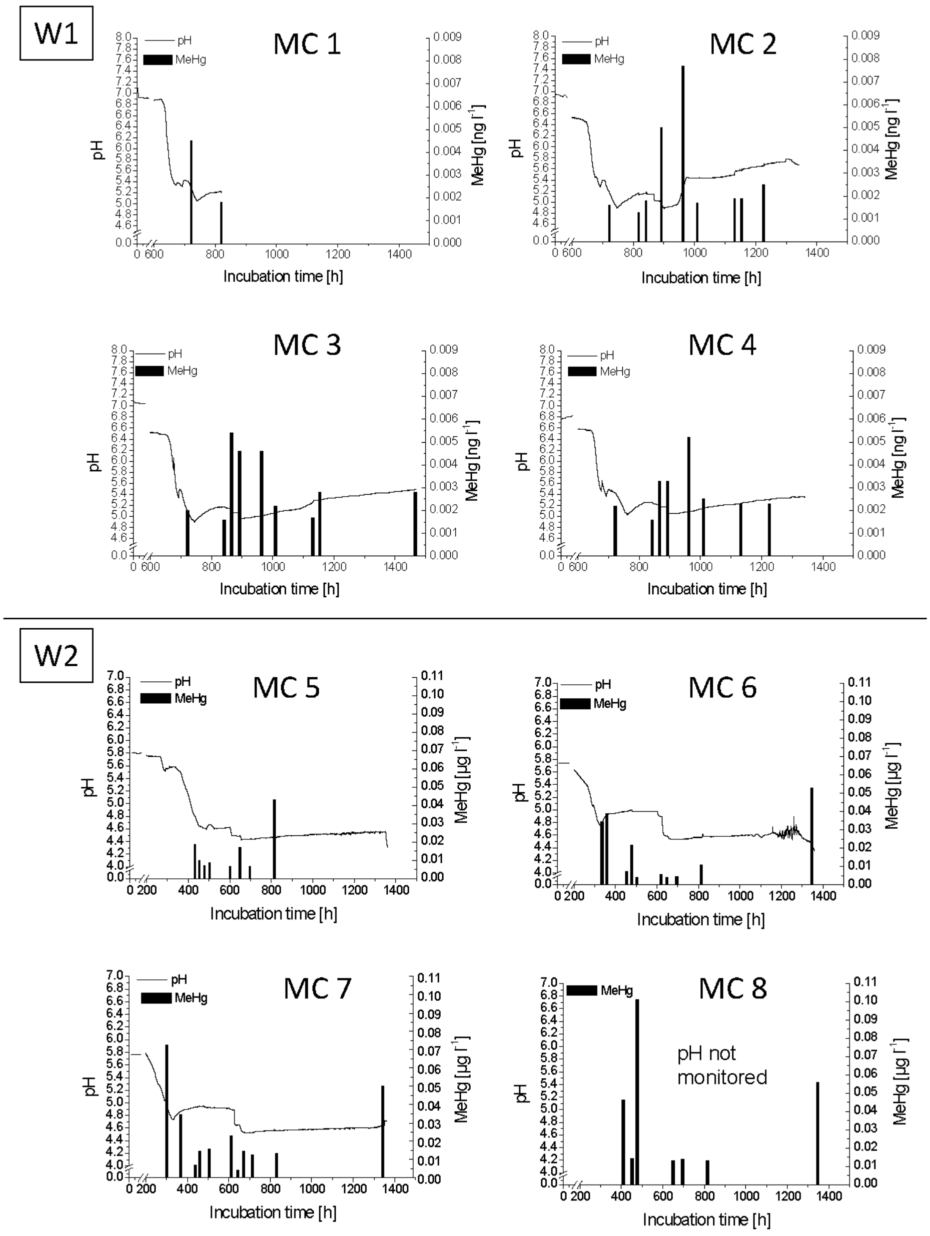


Fig. 5. Development of pH measured in the slurry every 10 min and MeHg concentrations in the soluble fraction during the experiment for each microcosm (MC) (Wupper 1 = W1, Wupper 2 = W2).

Table 3. Regressions between total mercury (Hg_t) resp. methyl mercury (MeHg) vs. anions (Cl^-, SO_4^{2-}), DOC, E_{HatpH5}, Fe, and pH in the soluble fraction. (+) positive relationship; (-) negative relationship; ns = not significant with $p \geq 0.05$.

		$\ln Hg_t$	lnMeHg
lnDOC	regression equation	Y = 7.674 + 0.209 X	Y = 8.739 + 0.236 X
	R^2	**0.26 (+)**	**0.53 (+)**
	p	<0.0001	<0.0001
	n	65	65
$\ln SO_4^{2-}$	regression equation	Y = 2.956 + 0.697 X	Y = 4.962 + 0.473 X
	R^2	**0.28 (+)**	**0.20 (+)**
	p	<0.0001	<0.0005
	n	65	65
lnCl-	regression equation	Y = 5.900 + 0.687 X	Y = 7.716 + 0.433 X
	R^2	**0.29 (+)**	**0.18 (+)**
	p	<0.0001	<0.001
	n	65	65
lnFe	regression equation	Y = 3.979–0.330 X	Y = 6.720 + 0.516 X
	R^2	0.02 (ns)	**0.08 (+)**
	p	0.236	<0.05
	n	65	65
pH	regression equation	Y = 4.957–0.0626 X	Y = 4.367–0.125 X
	R^2	0.03 (ns)	**0.17 (-)**
	p	0.19	<0.001
	n	65	65
E_{HatpH5}	regression equation	Y = 249.201 + 29.990 X	Y = −4.765–8.038E-4 X
	R^2	0.01 (ns)	0.03 (ns)
	p	0.36	0.19
	n	65	65

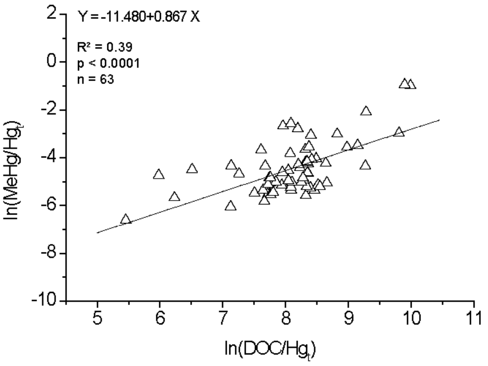


Fig. 6. Relation ln($MeHg/Hg_t$) vs. ln(DOC/Hg_t) in the soluble fraction.

and Skyllberg, 2003; Ravichandran, 2004; Khwaja et al., 2006; Shanley et al., 2008).

In addition to the impact of DOC due to a complexation of Hg_t and MeHg, a positive relationship between ln($MeHg/Hg_t$) and ln(DOC/Hg_t) was found in our study (Fig. 6). The $MeHg/Hg_t$ ratio is assumed to reflect the net production of MeHg normalized to the Hg concentration or the methylation efficiency respectively (Shanley et al., 2005; Skyllberg et al., 2007). Low $MeHg/Hg_t$ ratios can be due to low Hg methylation or to high MeHg demethylation rates (Remy et al., 2006). Thus, increasing DOC/Hg_t ratio might have favored Hg net methylation or decreased demethylation in the current study. One reason for rising DOC/Hg_t ratio might be increasing DOC concentrations. In this case, increasing DOC could have promoted Hg net methylation or depressed demethylation in our study. Other studies have shown that high DOC contents can promote Hg methylation by enhanced SRB activity (see below), since DOC can serve as an important carbon source for bacteria (Davis et al., 1997; Ullrich et al., 2001; Lambertsson and Nilsson, 2006). Furthermore, DOC can contribute to abiotic methylation of

Hg by donating methyl groups (Weber, 1993). However, abiotic methylation seems to be a process of minor importance compared to biological methylation (Avramescu et al., 2011). The positive relationship between ln(MeHg/Hg$_t$) and ln(DOC/Hg$_t$) seems to be important in our experiment. In contrast, the relationship between ln(MeHg/Hg$_t$) and lnDOC is weak, indicating that DOC alone might be a weaker factor in determining the Hg net methylation as also found by Skyllberg et al. (2003). Instead, the DOC/Hg$_t$ ratio seems to play a more important role for Hg net methylation.

We also observed a negative relationship between ln(MeHg/Hg$_t$) and lnHg$_t$. A positive relationship was found between lnHg$_t$ and lnMeHg as also reported by Sunderland et al. (2006) and Ouddane et al. (2008). Thus, increasing Hg$_t$ concentrations seem to have an inhibitory effect on Hg net methylation but may lead to higher total MeHg concentrations. High Hg concentrations can generally affect soil microorganisms in different ways. First, it is reported that high Hg$_t$ contents can exhibit toxic effects on methylating bacteria resulting in a depression of MeHg production (Ullrich et al., 2001). Secondly, microorganisms in Hg contaminated soils can be well adapted to Hg stress. This adaption can favor the selection of Hg tolerant bacteria in soils (Oliveira et al., 2010; Ruggiero et al., 2011). As a consequence, demethylation can be stimulated at high inorganic Hg concentrations by Hg tolerant bacteria which lead to reductive demethylation of MeHg (Schäfer et al., 2004). Bacterial Hg resistance is encoded by the mercury resistance (*mer)* operon encoding proteins that act amongst other factors in mercury detoxification. Most Hg resistant isolates contain *merB* (organomercury lyase), *merA* (mercuric reductase), *merP*, and *merR* genes (Lapanje et al., 2010; Ruggiero et al., 2011). Both toxic effects of Hg$_t$ on methylating bacteria and the occurrence of Hg tolerant bacteria might have occurred in the current study in parallel. However, the correlations between Hg$_t$, MeHg, and MeHg/Hg$_t$ are relatively low indicating that Hg$_t$ appears to have limited utility as a predictor of Hg net methylation and MeHg concentrations. This is in good agreement with Ouddane et al. (2008) who indicates that the production of MeHg is dependent on other parameters such as SRB in addition to total Hg concentrations in sediments with high Hg methylation activity.

Concentrations of MeHg and Hg$_t$ in the soluble fraction can also be influenced by the redox cycling of S, which is abundant in both soils (Table 1). The relationships between Hg$_t$ and SO_4^{2-}, and between MeHg and SO_4^{2-} are moderate (Table 3) what might indicate that both Hg$_t$ and MeHg may be linked to the sulfur cycle. Brümmer (1974) mentioned that sulfides are generated from sulfates below E_H −50 mV at pH 7 (corresponds to E_H 68 mV at pH 5). As the E_{HatpH5} values fell below 68 mV in all MCs during the incubation (Fig. 4), the formation of sulfides is most likely in our experiment. The soil slurries turned black and developed a typical odor with decreasing E_H, which also points towards the formation of sulfides. We did observe a correlation between E_{HatpH5} and SO_4^{2-}. However, this relationship is weak and not linear. Relationships between S$_t$ and E_{HatpH5}, Hg$_t$, or MeHg could not be found. One reason for that can be the rapid internal cycling of S, which makes SO_4^{2-} concentrations a poor indicator for SO_4^{2-} reduction rates (Koretsky et al., 2007; Yu et al., 2007). When the concentration of reduced inorganic S reaches a certain value the solubility and speciation of Hg^{2+} may be controlled by the precipitation of insoluble HgS or the formation of charged polysulfide Hg-complexes as previously reported by several authors (e.g. Davis, 1997; Benoit et al., 2001; Du Laing et al., 2009). This may result in decreasing Hg concentrations in the dissolved phase when sulfates are removed from the dissolved phase upon reduction to sulfides. On the other hand, this may also result in the solubilisation of mercury upon oxidation of sulfides to sulfates under oxic conditions. Both processes may explain the positive correlations we observed between dissolved SO_4^{2-} and Hg$_t$ and MeHg concentrations. The formation of HgS at low E_H can also decrease the availability of Hg^{2+} for methylation, consequently reducing MeHg production (Ullrich et al., 2001; Han et al., 2008). In contrast, reducing conditions can promote microbial mediated S reduction, which in turn can increase Hg methylation (Duran et al., 2008). In addition, high sulfide concentrations in marine environments containing organic matter seem to promote the uptake of Hg^{2+} by methylating bacteria such as sulfate reducing bacteria (SRB) maybe due to enhanced Hg bioavailability in mixed DOM-Hg-S complexes (Benoit et al., 2001; Sunderland et al., 2006). Sulfate reducing bacteria have been identified to be the principal methylators of inorganic Hg in sediments (Compeau and Bartha, 1985; King et al., 2002). The range of bacterial activity is large due to the variation in quantity and quality of organic matter, abundance of SRB, temperature, and SO_4^{2-} availability (Pallud and van Capellen, 2006). Various PLFA have been frequently used as biomarkers (e.g. SRB) to describe the microbial community structure in different environments (Taylor and Parkes, 1985; Coleman et al., 1993; Macalady et al., 2000; Wegener et al., 2008). The PLFA which might indicate the presence of *Desulfobacter* are 10Me16:0, cy17:0, 10Me18:0, and cy19:0 (Kohring et al., 1994). Those PLFA were abundant in both studied soils (Fig. 3). The fatty acids 10Me16:0 and 10Me18:0 might serve as indicators for *Desulfobacter* and additionally for actinomycetes (Taylor and Parkes, 1983; Frostegård, 1993). The polyunsaturated fatty acid 18:2ω6,9 might indicate the occurrence of *Desulfovibrio* (Macalady et al., 2000) or fungi (Frostegård, 1993). *Desulfobulbus* species are characterized by unbranched fatty acids such as 15:0 (Taylor and Parkes, 1983), which is widely distributed among different bacterial taxa (Macalady et al., 2000). Iron reducing bacteria such as *Geobacter* species are also able to methylate mercury in pure cultures at rates comparable to *Desulfobulbus* (Fleming et al, 2006; Kerin et al., 2006; Windham-Myers et al., 2009). Additionally, Avramescu et al. (2011) reported that

Fe reduction through Fe reducing bacteria might decrease demethylation. According to Kohring et al. (1994), *Geobacter metallireducens* mainly consists of the PLFA 16:1ω7c and 16:0, which are abundant in our soils (Fig. 3). These PLFA can also be found in many other organisms (Zelles, 1997). However, we might speculate that Fe reducing bacteria could be present in our soils and therefore might also contribute to Hg methylation in the current study. The soil W1 contains a higher quantity of PLFA compared to W2, both in numbers (W1 = 26; W2 = 20) and in total PLFA biomass (W1 $\approx$ 27 000 pmol g^{-1}; W2 $\approx$ 17 000 pmol g^{-1}). The number of PLFA which are related to SRB is 6 for both soils and the total biomass of these PLFA is approximately 5000 pmol g^{-1} for both soils. As measured by the total PLFA biomass, W1 contains 19 % PLFA related to SRB whereas W2 contains 29 % PLFA related to SRB. This indicates that SRB are more dominant at site W2. These values lead to the assumption that the relatively high Hg concentrations at the W2 site seem to have no direct toxic effect on SRB.

The statistical relationships between Hg_t and Cl^- as well as between MeHg and Cl^- are modest (Table 3) indicating that interrelations of these parameters might exist. Chloride can influence Hg speciation due to the competition of Cl^- with Hg_t and MeHg for binding sites of soil particles, which can reduce Hg adsorption onto soil particles and promote the release of Hg into the aquatic phase (Yin et al., 1997; Liu et al., 2009). This process probably occurred in our study. Moreover, the behavior of Hg in the soluble fraction can partly be affected by the formation of Hg-Cl-complexes, which is relevant at E_{HatpH5} >500 mV (Davis, 1997). Under the mostly acidic pH conditions which occurred in the current study, the formation of partly water soluble $HgCl_2$ as well as nearly water insoluble Hg_2Cl_2 is possible (Davis, 1997; Ullrich et al., 2001). Generally, in solution [MeHg]Cl is formed in the presence of Cl^- (Skyllberg et al., 2003). Thus, rising Hg_t and MeHg concentrations with increasing Cl^- concentrations in our study might indicate the formation of Hg-Cl compounds probably mostly under aerobic conditions (Davis et al., 1997; Takeno, 2005). The weak relationship between E_{HatpH5} and Cl^- reveals that Cl^- concentrations are not decisively influenced by E_H.

In general, Fe(hydr)oxides are able to adsorb Hg to a certain extent (Fernandez-Martinez et al., 2006; Liu et al., 2009; Harris-Hellal et al., 2011). Moreover, Mehrotra and Sedlak (2005) explained decreased mercury methylation in anoxic wetland slurries upon amendment of Fe(II) by reduced availability of Hg for methylation due to the formation of FeS which subsequently decreased the pool of bioavailable neutral mercury-sulfide species. Hollweg et al. (2009) indicate that Hg interacts with inorganic sulfur ligands in FeS complexes decreasing the bioavailability of Hg. In the current study, both soils contain considerable amounts of Fe (Table 1) and Fe(hydr)oxides should precipitate at high E_H which is confirmed by the negative relationship between Fe in the soluble fraction and E_{HatpH5}. However, no evidence was found in this study that Hg_t was linked to the Fe cycle whereas MeHg showed a weak relationship to Fe (Table 3). This may be attributed to the fact that DOC competes with Fe(hydr)oxides for binding Hg and MeHg (Feyte et al., 2010) and the DOC contents were high in our study. Additionally, pH might be an important factor in this context, because Hg is only preferentially sorbed to Fe(hydr)oxides in the neutral-alkaline pH-range (Ullrich et al., 2001). In summary, redox variations seem to affect the concentrations of dissolved Hg_t and MeHg indirectly through related changes in DOC, sulfur cycle, and microbial interaction and community structure whereas E_H and pH values, as well as concentration of dissolved Fe and Cl^- seem to play subordinate roles in Hg mobilization and methylation under our experimental conditions.

5 Conclusions

In our E_H experiment the ln(DOC/Hg_t) ratio is positively correlated to net MeHg production. This indicates that the ln(DOC/Hg_t) ratio seems to play an important role for the Hg net methylation. Dissolved organic carbon itself can mobilize Hg and MeHg due to the formation of soluble complexes. Mercury methylation also seems to be linked to the S chemistry while the influence of Fe and Cl^- on Hg methylation and speciation seems to be weak in our study. However, the methylation of Hg seems to be affected by the soil microbial community. On the one hand, Hg methylation might be favored by reducing conditions through enhanced microbial activity such as SRB bacteria, as indicated by the presence of the respective PLFA biomarkers. On the other hand, reducing conditions might lead to the formation of hardly available HgS what might contribute to a decrease of MeHg production. In conclusion, future studies on the fate of mercury in wetland soils should include silty and clayey soil material and should focus on the specific role of the soil microbial community structure.

Supplementary material related to this article is available online at: http://www.biogeosciences.net/9/493/2012/ bg-9-493-2012-supplement.pdf.

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Appendix 4

Biogeochemical fractions of mercury in soil profiles of two different floodplain ecosystems in Germany

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Abstract

A special sequential extraction (SE) procedure for mercury (Hg) was conducted to determine biogeochemical fractions of Hg and their controlling factors in four contaminated soil profiles located in two distinct floodplain ecosystems which differ in their industrial histories and thus in their Hg loads. The first study area is located at the Wupper River (Western Germany) and the soil profiles reveal sum of Hg (Hg_{sum}) concentrations up to 48 ppm. The second study area is located at the Saale River (Eastern Germany) and the soil profiles have Hg_{sum} concentrations up to 4.3 ppm. The majority of Hg was found in fraction IV (FIV, Hg^0) for both study areas, indicating its anthropogenic origin. Moreover we have detected Hg in fraction V (FV) and in fraction III (FIII). As Hg in fraction V (FV) is mostly associated with Hg sulfides being formed under reducing conditions, it indicates reduction processes which usually occurred during flooding. Mercury in fraction III (FIII) (organo-chelated Hg) exhibits a moderate mobility and a high methylation potential. Between Hg in FIII and hot water extractable carbon (C_{HWE}) as a measure of easy degradable, labile soil organic matter we found a significant correlation ($r^2=0.63$; $p<0.0001$; $n=18$). Sum of Hg seem to have a high affinity to organic carbon (C_{org}) ($r^2=0.45$; $p<0.005$; $n=16$). The concentrations of Hg in the mobile and exchangeable fractions FI and FII were low. Moreover the significant positive correlation between iron (Fe) and Hg in FIV indicate an interaction between Hg and Fe ($r^2=0.46$; $p<0.005$; $n=18$).

Proof of individual contribution

Manuscript included in the thesis	First author (T. Frohne)	Co-authors
Frohne, T.; Rinklebe, J.; Diaz-Bone, R.A.; Du Laing, G. (2011): Controlled variation of redox conditions in a floodplain soil: impact on metal mobilization and biomethylation of arsenic and antimony. Geoderma. 160(3-4), 414-424. **Chapter 2**	Generating research idea, generating and evaluating data, writing the manuscript. Conducting the microcosm experiment.	**Rinklebe, J.** (supervisor, generating research idea and correcting the manuscript, assistance in conducting the microcosm experiment, selecting the study site, soil description, soil classification) **Diaz-Bone, R.A.** (correcting the manuscript, measurement of inorganic and methylated As and Sb species, analytical advice) **Du Laing, G.** (correcting the manuscript, scientific advice)
Frohne, T.; Rinklebe, J.; Diaz-Bone, R.A. (2014): Contamination of floodplain soils along the Wupper River, Germany, with As, Co, Cu, Ni, Sb, and Zn and the impact of pre-definite redox variations on the mobility of these elements. Soil and Sediment Contamination: An International Journal. 23, 779-799. **Chapter 3**	Generating research idea, generating and evaluating data, writing the manuscript. Conducting the microcosm experiment.	**Rinklebe, J.** (supervisor, generating research idea and correcting the manuscript, assistance in conducting the microcosm experiment; selecting the study site, soil description, soil classification) **Diaz-Bone, R.A.** (correcting the manuscript, measurement of metals, analytical advice)
Frohne, T.; Rinklebe, J.; Langer, U.; Du Laing, G.; Mothes, S.; Wennrich, R. (2012): Biogeochemical factors affecting mercury methylation rate in two contaminated floodplain soils. Biogeosciences. 9, 493-507. **Chapter 5**	Generating research idea, generating and evaluating data, writing the manuscript. Conducting the microcosm experiment. Measurement of inorganic mercury.	**Rinklebe, J.** (supervisor, generating research idea and correcting the manuscript, assistance in conducting the microcosm experiment, selecting the study sites, soil description, soil classification) **Langer, U.** (correcting the manuscript, PLFA measurement, scientific advice) **Du Laing, G.** (correcting the manuscript) **Mothes, S.** (correcting the manuscript, measurement of

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		methylated mercury species, scientific advice) **Wennrich, R.** (correcting the manuscript, scientific advice)
Frohne, T.; Rinklebe, J. (2013): Biogeochemical fractions of mercury in soil profiles of two different floodplain ecosystems in Germany. Water, Air, & Soil Pollution. 224(6), 1-17. **Chapter 6**	Generating research idea, generating and evaluating data, writing the manuscript. Conducting the sequential extraction. Measurement of inorganic mercury.	**Rinklebe, J. (**supervisor, generating research idea and correcting the manuscript, selecting the floodplain soil profiles and horizons, soil description, soil classification)

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Declaration of primary authorship

Declaration of primary authorship

I hereby declare that I have written the present Ph.D. thesis entitled “Mobilization of metals as influenced by redox potential in slurries of floodplain soil material and biogeochemical fractionation of mercury in floodplain soil profiles” by myself and without the help of others. Other than the presented references were not used and quoted results were always marked with the relevant reference. The present thesis was never either abroad or in Germany submitted for examination in the present or a similar version.

Selbständigkeitserklärung

Ich erkläre hiermit, dass ich die eingereichte Dissertation mit dem Titel “ Mobilization of metals as influenced by redox potential in slurries of floodplain soil material and biogeochemical fractionation of mercury in floodplain soil profiles” selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe. Die vorgelegte Dissertation wurde bisher weder im Ausland noch im Inland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt.

Wuppertal, 25.06.2014