Hydrocarbon contamination induced changes of magnetic properties in soil and sediment

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“*If we knew what we were doing it wouldn't be research*.”

Albert Einstein

Summary

Environmental magnetism is an applied branch of rock and mineral magnetism. Mineral magnetic techniques have been extensively used during recent decades for the study of heavy metal pollution by analyzing soils, sediments, dust-loaded leaves, needles, tree barks as well as by collecting atmospheric dust particles with passive sampler devices. Environmental magnetic methods, in comparison to other analytical methods, are a fast, cost effective and non-destructive approach. Therefore, magnetic proxies are widely used for assessment of heavy metal pollution. However, the relationship between organic contamination, especially hydrocarbon contamination, and magnetic parameters is basically an unexplored research topic. The positive correlation between magnetic susceptibility (MS) and polycyclic hydrocarbons (PAHs) content mentioned in a few studies could also be an indirect effect because both heavy metals and PAHs were present together at the study sites.

The main goals of the present thesis were (1) to investigate changes of magnetic properties due to hydrocarbon contamination in soils and sediments, (2) to examine the relationship between non-magnetic parameters and magnetic properties of hydrocarbon contaminated samples, (3) to explore the role of microbial processes for the (trans)formation of magnetic iron minerals and (4) to determine if changes of the magnetic iron mineral content in hydrocarbon contaminated material can be detected with the currently available magnetic equipments and techniques. More importantly, the ultimate goal of this research was to examine if magnetic proxies can be used for the delineation of hydrocarbon contamination of soils and sediments. Additionally, the influence of physical soil parameters such as water content on soil magnetic properties was determined in the present study.

Two field sites, a former oil-field near Hänigsen (northern Germany) and a former military air force base at Hradcany (Czech Republic), were studied by using magnetic methods for mainly hydrocarbon contaminated soil and sediment samples from the surface and from shallow sub-surface. From the oil-field site, the important finding is a very good correlation between total non-polar hydrocarbon content and magnetic concentration dependent parameters, namely MS and anhysteretic remanent magnetization (ARM). In contrast, the correlation between total PAHs content and MS and ARM is lower. Even contaminated sediments that were lying more than a meter below the surface in water saturated condition revealed a 4-fold higher MS compared to nearby non-contaminated sediments. The most important finding from the military air force base site, according to my knowledge, has never been mentioned in the literature: a significant increase

of magnetic concentration dependent parameters appeared in hydrocarbon contaminated unconsolidated sediments that lay in the area of groundwater table fluctuations.

From both field site investigations, it was concluded that the enhancement of magnetic concentration parameters was mainly caused by magnetite. Magnetite was found to be predominantly present in a single domain grain size range rather than as ultrafine superparamagnetic particles. The related driving processes for the neoformation of magnetite in hydrocarbon contaminated soils and sediments are not yet clear. However, the role of microorganisms might be quite important at the oil-field site because both iron oxidizers and reducers were present in contaminated sediments as shown by the MPN (most probable number) method. Also, at the military air force base site an important role of microorganisms could be inferred, possibly together with other geochemical processes. The autochthonous bacterium *Comamonas acidovorans* which is a hydrocarbon degrading microorganism was detected at this site in a study of Masak et al. (2003).

The main processes and factors that led to the neoformation of magnetite minerals in the hydrocarbon contaminated groundwater table fluctuations zone at the former military site were: (1) hydrocarbon content, (2) extent of groundwater table fluctuations generated mainly by the remediation method of air sparging that modified static and stratified redox zonations into dynamic and non-stratified redox zonations, respectively, and (3) intrinsic enhanced bioremediation by amendment of nutrients that increased the activity of *Comamonas acidovorans*. Although *Comamonas acidovorans* was the only microbial species mentioned to be present at this site, both iron(III)-reducing and iron(II)-oxidizing microorganisms might also play a major role depending on geochemical factors, hydraulic properties and types and distribution of organic contaminants in the system. The main process was definitely related with groundwater table fluctuations because an increase of magnetic concentration dependent parameters was not observed at the sampling site without groundwater table fluctuations.

Besides the study of hydrocarbon contaminated sites under natural conditions, laboratory batch experiments were set up to determine controlling factors and driving mechanisms that can lead to changes of magnetic properties of soils and sediments with or without hydrocarbon contamination. In these batch experiments the role of microorganisms, different carbon sources including hydrocarbons, and physical soil properties such as water content were investigated. Laboratory studies were conducted on six clean soil samples and a hydrocarbon contaminated sediment, all used for microbial active and sterilized setups. The soils showed an average change in MS of 7% in microbial active setups whereas there was no significant change (<2.4%) in sterilized setups. Four soils showed an increase in MS, the other three a decrease. From this result, it was concluded that microorganisms play a significant role in the (trans)formation of magnetic iron minerals. Moreover, MS change in the setups to which hydrocarbons had been added was not as high as in case of samples amended with lactate and acetate. It was not possible to isolate any newly formed mineral from the soils that would have been generated during the incubation period of several months. However, the ferro(i)magnetic component which was measured after opening batch bottles at the end of experiments was increased in a soil that also showed an increase of MS during the monitoring period. Although, the main processes that are solely related with microorganisms or both geochemical and microbiological effects were not fully clarified, this measurement confirms that magnetic mineralogical changes happened during the incubation of the soil and that currently available magnetic methods are sensitive enough to monitor these changes.

MS monitoring of one soil with different water content in batch experiments revealed that MS increased until a soil to added water ratio of 3 g/ml. Above this ratio, MS had slightly decreasing trend with increasing amount of added water. It is likely that this points out that water content up to a certain extent (here soil to water ratio 3.0 g/ml) has a mobilization effect of soil carbon and soil minerals from the soil matrix, but a higher water content diminishes the effect on MS probably because dissolution of magnetic iron minerals dominates over the mobilization effect. Obviously, water content plays a versatile role for changes of MS in soils and sediments. The lower water content might correspond to conditions that caused an increase of MS in paleosols of the Chinese loess Plateau related to rainfall. This is an additional important aspect of the findings of this study.

Furthermore, studies of secondary mineral formation during ferrihydrite reduction by pure cultures *Shewanella oneidensis* MR-1 revealed that MS measurements can also provide information about ferro(i)magnetic mineral changes during biogeochemical processes. This result demonstrates that even for microbial cultures, MS monitoring can provide an alternative and fast technique that does not require any sampling, in contrast to other geochemical methods.

In the present study it is shown that magnetic methods can be used for the assessment and investigation of hydrocarbon contaminated field sites even though main driving factors and processes causing such changes on magnetic properties of contaminated soils or sediments could not be fully clarified. Microorganisms in fact play an important role in modifying the magnetic signal of soils and sediments both at contaminated and clean sites. Additionally, magnetic methods could also be utilized for the quality control of permeable reactive barriers (PRBs) which are often used during bioremediation of hydrocarbon contaminated sites.

Zusammenfassung

Der Umweltmagnetismus stellt einen angewandten Zweig des Gesteins- und Mineralmagnetismus dar. Mineralmagnetische Techniken wurden in den letzten Jahrzehnten intensiv zur Untersuchung von Schwermetallverschmutzungen genutzt, sowohl durch die Analyse von Böden, Sedimenten, staubbelasteten Blättern oder Nadeln sowie Baumrinden, als auch durch die Beprobung atmosphärischer Staubpartikel mit passiven Probenehmern. Im Vergleich zu anderen analytischen Methoden erlauben umweltmagnetische Methode eine schnelle, kosteneffiziente und zerstörungsfreie Herangehensweise. Daher sind magnetische Proxies für die Ermittlung von Schwermetallbelastungen weit verbreitet. Jedoch ist der Zusammenhang zwischen organischer Kontamination, insbesondere von Kohlenwasserstoffbelastungen und magnetischen Parametern ein weitestgehend unerforschtes Gebiet. Die positive Korrelation zwischen magnetischer Suszeptibilität (MS) und dem Gehalt an polyzyklischen Kohlenwasserstoffen (PAKs), die in wenigen Studien erwähnt wurde, könnte auch ein indirekter Effekt sein, weil an den Untersuchungsstandorten sowohl Schwermetalle als auch PAKs gemeinsam vorkamen.

Hauptziele der vorliegenden Arbeit waren (1) die Veränderungen der magnetischen Eigenschaften von kohlenwasserstoffkontaminierten Böden und Sedimenten zu ermitteln, (2) den Zusammenhang zwischen nicht magnetischen Parametern und magnetischen Eigenschaften der kohlenwasserstoffkontaminierten Proben zu untersuchen, (3) die Rolle der mikrobiellen Prozesse für die (Um)Bildung von magnetischen Eisenmineralen zu erforschen und (4) zu bestimmen, ob Veränderungen im Gehalt an magnetischen Eisenmineralen in kohlenwasserstoffkontaminierten Materialien mit den momentan zur Verfügung stehenden magnetischen Messinstrumenten und verfügbaren Techniken detektiert werden kann. Weitaus bedeutender war das übergeordnete Ziel dieser Forschungsarbeit, nämlich zu untersuchen, ob magnetische Proxies zur Ortung von Kohlenwasserstoffkontaminationen in Böden und Sedimenten genutzt werden können. Darüber hinaus wurde in der vorliegenden Studie der Einfluss von physikalischen Bodenparametern, wie z.B. Wassergehalt, auf die magnetischen Eigenschaften von Böden untersucht.

Zwei Feldstandorte wurden untersucht, ein ehemaliges Ölfeld bei Hänigsen (Norddeutschland) und ein ehemaliger Militärflughafen bei Hradčaný (Czech Republic), wobei magnetische Methoden vorwiegend bei kohlenwasserstoffkontaminierten Boden- und Sedimentproben direkt von der Erdoberfläche und aus dem oberflächennahen Untergrund angewandt wurden. Der Standort Hänigsen lieferte die wichtige Erkenntnis, dass eine sehr gute Korrelation zwischen dem

Gesamtgehalt an unpolaren Kohlenwasserstoffen und den konzentrationsabhängigen magnetischen Parametern MS und anhysteretische remanente Magnetisierung (ARM) besteht. Im Gegensatz dazu war die Korrelation zwischen dem Gesamtgehalt an PAKs und MS und ARM geringer. Auch kontaminierte Sedimente, die mehr als einen Meter unterhalb der Erdoberfläche unter wassergesättigten Bedingungen lagen, zeigten eine vierfach höhere MS verglichen mit benachbarten unbelasteten Sedimenten. Das wichtigste Ergebnis der Untersuchung am Standort Hradčaný, eine signifikante Zunahme der konzentrationsabhängigen magnetischen Parameter in kohlenwasserstoff-kontaminierten Lockersedimenten im Bereich von Grundwasserspiegelschwankungen, wurde nach meinem Wissen bisher noch nicht in der Literatur erwähnt. .

Aus den Untersuchungen beider Standorte kann gefolgert werden, dass die Zunahme der konzentrationsabhängigen magnetischen Parameter hauptsächlich durch Magnetit verursacht wird. Es wurde festgestellt, dass Magnetit vorwiegend in der Größenordnung von Einbereichsteilchen vorliegt und weniger in Form von ultrafeinen superparamagnetischen Partikeln. Die entsprechenden Steuerungsprozesse, welche die Neubildung von Magnetit in kohlenwasserstoffkontaminierten Böden und Sedimenten verursachen, sind noch weitestgehend unbekannt. Jedoch könnte die Rolle von Mikroorganismen am Standort Hänigsen sehr bedeutend sein, da sowohl eisenoxidierende als auch eisenreduzierende Mikroorganismen in den kontaminierten Sedimenten vorkamen, wie durch die MPN (most probable number) Methode gezeigt werden konnte. Auch die Ergebnisse vom Standort Hradčaný lassen auf eine wichtige Rolle von Mikroorganismen schließen, möglicherweise zusammen mit anderen geochemischen Prozessen. Das autochthone Bakterium *Comamonas acidovorans,* ein Kohlenwasserstoff abbauender Mikroorganismus, wurde in einer anderen Studie von Masak et al. (2003) an diesem Standort nachgewiesen.

Die wichtigsten Prozesse und Faktoren, die zu einer Neubildung von Magnetitmineralen im Schwankungsbereich kohlenwasserstoffbelasteten Grundwassers am ehemaligem Militärstandort Hradčaný führten, waren (1) der Kohlenwasserstoffgehalt, (2) das Ausmaß der Grundwasserspiegel-schwankungen, welche hauptsächlich durch die Sanierungsmethode der Bodenluftabsaugung verursacht wurden und statische bzw. stratifizierte Redox-Zonierungen in dynamische bzw. nicht-stratifizierte Redox-Zonierungen umwandelten und (3) intrinsisch verstärkte Biosanierung durch die Zugabe von Nährstoffen, welche die Aktivität von *Comamonas acidovorans* steigerten. Obwohl *Comamonas acidovorans* als einzige mikrobielle Art an diesem Standort erwähnt wurde, könnten sowohl Eisen(III)-reduzierende, wie auch Eisen(II)-oxidierende Mikroorganismen eine wichtige Rolle spielen, abhängig von den geochemischen Faktoren, hydraulischen Eigenschaften und Art und Verteilung der organischen Kontaminationen im System. Der Hauptprozess stand zweifellos mit den Grundwasserspiegelschwankungen im Zusammenhang, da die Zunahme konzentrationsabhängiger magnetischer Parameter an Standorten ohne Grundwasserspiegelschwankungen nicht beobachtet wurde.

Neben der Untersuchung von kohlenwasserstoffkontaminierten Standorten unter natürlichen Bedingungen wurden im Labor Batchexperimente durchgeführt, um die Kontrollfaktoren und Steuerungsmechanismen zu bestimmen, welche zu einer Veränderung der magnetischen Eigenschaften von Böden und Sedimenten mit und ohne Kohlenwasserstoffkontamination führen können. In diesen Batchexperimenten wurde die Rolle der Mikroorganismen, verschiedener Kohlenstoffquellen einschließlich Kohlenwasserstoffe und physikalische Bodeneigenschaften wie z.B. Wassergehalt, untersucht. Laborstudien wurden mit sechs nicht kontaminierten Bodenproben und einem kohlenwasserstoffbelasteten Sediment durchgeführt, wobei alle Proben für mikrobiologisch aktive und sterilisierte Proben genutzt wurden. In den mikrobiell aktiven Proben veränderte sich die MS der Böden durchschnittlich um 7%, wohingegen in den sterilisierten Proben keine signifikante Veränderung (<2.4%) auftrat. Vier Böden zeigten eine Zunahme der MS, die anderen drei eine Abnahme. Aus diesem Ergebnis wurde geschlossen, dass Mikroorganismen eine entscheidende Rolle bei der (Um)Bildung magnetischer Eisenminerale spielen. Darüber hinaus war die Veränderung der MS in Proben, denen Kohlenwasserstoffe zugesetzt worden waren, nicht so hoch wie in den Proben, die mit Lactat oder Acetat versetzt waren. Es war nicht möglich, ein neugebildetes Mineral aus den Böden zu isolieren, welches sich im Verlauf der Inkubationszeit von einigen Monate gebildet hatte. Jedoch war die ferro(i)magnetische Komponente, die nach dem Öffnen der Batch-Flaschen am Ende des Experiments gemessen wurde, in einem Boden, der auch eine Zunahme der MS während des Messzeitraums zeigte, erhöht. Obwohl die hauptsächlichen Prozesse, die entweder allein mit Mikroorganismen im Zusammenhang stehen oder sowohl mit chemischen als auch mikrobiologischen Effekten, nicht vollständig geklärt werden konnten, bestätigte dieses Messergebnis, dass Veränderungen der magnetischen Mineralogie während der Inkubation des Bodens stattgefunden haben und dass die momentan verfügbar magnetische Methodik sensitiv genug sind, diese Veränderungen zu detektieren.

Die UNtersuchung einer Bodenprobe mit verschiedenen Wassergehalten im Batch-Experiment zeigte, dass die MS bis zu einem erhältnis von Boden zu zugegebenem Wasser von 3.0 g/ml anstieg. Über diesem Verhältnis zeigte die MS einen leicht abnehmenden Trend mit zunehmender Menge an zugegebenem Wasser. Dies weist darauf hin, dass der Wassergehalt bis zu einer bestimmten Menge (hier ein Boden zu Wasser Verhältnis von 3.0 g/ml) einen Effekt auf die

Mobilisierung von Kohlenstoff und Mineralphasen aus der Bodenmatrix hat, wobei ein höherer Wassergehalt den Effekt auf die MS aber verringert, wahrscheinlich weil die Auflösung von magnetischen Eisenmineralen gegenüber dem mobilisierenden Effekt überwiegt. Offensichtlich spielt der Wassergehalt eine vielseitige Rolle für die Veränderung der MS in Sedimenten und Böden. Ein niedriger Wassergehalt könnte den Bedingungen entsprechen, welche den Anstieg der MS in Paläoböden des chinesischen Löss-Plateaus im Zusammenhang mit Niederschlägen bewirkten. Dies stellt einen weiteren wichtigen Aspekt in den Ergebnissen dieser Studie dar.

Darüber hinaus zeigten Untersuchungen der sekundären Mineralbildung während der Ferrihydritreduktion durch Reinkulturen von *Shewanella oneidensis* MR-1, dass MS Messungen auch Informationen über ferro(i)magnetische Mineralveränderungen während biogeochemischer Prozesse liefern. Dieses Ergebnis zeigt, dass sogar für mikrobielle Kulturen MS Monitoring eine alternative und schnelle Technik bereitstellen kann, die im Gegensatz zu anderen geochemischen Methoden keine Probennahme erfordert.

Aus der vorliegenden Studie geht hervor, dass magnetische Methoden für die Beurteilung und Untersuchung von kohlenwasserstoffbelasteten Standorten genutzt werden können, auch wenn die hauptsächlichen Faktoren und Prozesse, welche Veränderungen der magnetischen Eigenschaften von kontaminiertem Böden bzw. Sedimenten verursachen, nicht vollständig geklärt werden konnten. Mikroorganismen spielen zweifellos eine wichtige Rolle bei der Veränderung des magnetischen Signals von Böden und Sedimenten, sowohl an kontaminierten als auch an nicht kontaminierten Standorten. Weiterhin könnten magnetische Methoden auch für die Qualitätskontrolle von permeablen reaktiven Wänden genutzt werden, wie sie oft bei der Bioremediation von kohlenwasserstoff-kontaminierten Standorten verwendet werden.

Thesis content and publications

This thesis contains six chapters. The first chapter presents an overview of the research framework addressing main research questions and achievements. The chapters two and four contain articles that have been already published in international peer-reviewed journals. Chapter three represents a submitted manuscript while chapter four is a manuscript that is under preparation and chapter five is the draft version of a manuscript. The last chapter encloses conclusions and an outlook of the thesis.

List of publications

1. Change of magnetic properties due to fluctuations of hydrocarbon contaminated groundwater in unconsolidated sediments.

Moti L. Rijal, Erwin Appel, Eduard Petrovsky, Ulrich Blaha. ***Environmental Pollution, 2010, 158(5), 1756-1762.***

2. Magnetic signatures of hydrocarbon-contaminated soils and sediments at the former oil-field Hänigsen, Germany.

Moti L. Rijal, Katharina Porsch, Erwin Appel, Andreas Kappler. ***Studia Geophysica et Geodaetica (submitted)***

3. In-situ magnetic susceptibility measurements as a tool to follow geomicrobiological transformation of Fe minerals.

Katharina Porsch, Urs Dippon, **Moti Lal Rijal,** Erwin Appel, Andreas Kappler. ***Environmental Science & Technolology. 2010, 44, 3846–3852.***

4. Remediation processes driven changes on magnetic properties of hydrocarbon contaminated sediments from unsaturated zone, Hradcany, Czech Republic (in prep.)

Moti L. Rijal, Erwin Appel, Eduard Petrovský

5. Microbially induced changes of soil magnetic susceptibility caused by hydrocarbon contamination and mobilization of bioavailable organic Carbon - A microcosm study (Draft)

Katharina Porsch, **Moti L. Rijal**, Thomas Borch, Lyndsay Troyer, Sebastian Behrens, Erwin Appel, Andreas Kappler

Own contribution to the joint publications

This thesis and the publications presented here are my own work unless it is stated (see % indicated below). My supervisor, E. Appel contributed for scientific ideas, dogmatic discussion as well as editorial guidance in publications 1, 2, 3 and 4. C. My second supervisor A. Kappler has provided scientific ideas, ascetic discussion and editorial advice in publications 2, 3 and 5. K. Porsch has contributed for publication 2. Also, she has mainly contributed with ideas, data and elaboration for publication 3 and 5. U. Dippon has provided significant contribution for microbiological part of the publication 3.

Publication 1
Ideas: 50%
Data: 90%
Analyses: 90%
Elaboration: 80%

Publication 2
Ideas: 50%
Data: 90%
Analyses: 90%
Elaboration: 90%

Publication 3
Ideas: 20%
Data: 15%
Analyses: 10%
Elaboration: 10%

Publication 4
Ideas: 60%
Data: 90%
Analyses: 90%
Elaboration: 100%

Publication 5
Ideas: 30%
Data: 40%
Analyses: 20%
Elaboration: 20%

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CONTENTS

1

Introduction

1. Background and objectives

All earth's materials are magnetic and can be grouped in three categories of magnetism: diamagnetism, paramagnetism and ferromagnetism. Diamagnetism is a property of all materials, which arises from the applied magnetic field with the interaction of electrons orbiting the nucleus. Paramagnetism, on the other hand, arises from both orbital and spin magnetic moments. Ferromagnetism, the strongest of all, generates from strong interactions of electron orbitals in which magnetic moments of all atoms inside the crystal lattice are aligned. If the magnetic moments of two sub-lattices have an opposite direction and equal strength then this type is termed antiferromagnetism, for unequal strength of the magnetic moments from the sub-lattices ferrimagnetism is caused. Iron, the fourth most abundant element in the Earth's curst occurs mainly in many different oxides such as magnetite (Fe_2O_3), maghemite (γ-Fe_2O_3), hematite (α-Fe_2O_3); oxyhydroxides such as goethite (α-FeOOH) and also as sulfides such as pyrrhotite(Fe_7S_8) and greigite (Fe_3S_4).

Magnetic minerals in soils and sediments generate from different sources through various pathways: from weathering and erosion of host rocks, deposition into river catchments by water, eolian deposition such as loess, volcanic eruptions, chemical and biological processes, and from various anthropogenic activities. Atmospheric particulates produced from various anthropogenic sources such as coal-firing power plants and industrial smelters, are one of the most harmful pollutants, since they can cause cancer.

Magnetic properties of natural materials (soils and sediments) without any anthropogenic inputs are determined by their source rocks and soil forming processes. However, due to various anthropogenic activities (e.g. combustion of fossil fuel), magnetic properties measured in soils and sediments are not solely dependent on natural materials but also on constituents from anthropogenic sources. Magnetic methods are therefore used to delineate anthropogenic pollutions of soils and sediments. Magnetic methods can be used for monitoring air, water and soil pollution (Petrovský and Ellwood, 1999). The close association of magnetic particles and heavy metals can be explained by the fact that iron phases that are originally contained in industrial raw materials and traffic fuels are converted during combustion processes into iron oxides, mainly magnetite, which are released into the atmosphere together with the heavy metals. Therefore, magnetic proxies can be used for heavy metal contaminated soils and sediments.

Environmental magnetism is an applied aspect of rock and mineral magnetism that mainly focuses on environmental purposes using mineral magnetic parameters of environmental materials such as soils, sediments, dusts, tree leaves/needles. Magnetic methods, in comparison to other analytical methods, are rapid, mostly non-destructive, cost-effective and very sensitive. Therefore, environmental magnetism has become quite popular during recent decades for the study of soils (Mullins, 1977), sediments (Thomson and Oldfield, 1986), atmospheric particulates (Hunt et al., 1984; Morris et al. 1995), street dust (Xie et al., 1999), dust-loaded tree leaves (Matzka and Maher, 1999; Hanesch et al., 2003; Moreno et al., 2003), road- traffic (Gautam et al., 2005), steel making industries (Hanesch and Scholger, 2002) coal-firing power plants (Strzyszcz et al., 1996), and monitoring of urban air quality (Urbat et al., 2004) . The concept of using magnetic properties as proxy parameters and correlation of these parameters, mainly magnetic susceptibility (MS) with heavy metals are extensively used in many aforementioned studies.

There are a few studies which deal with the correlation of magnetic proxies, mainly MS, and polycyclic aromatic hydrocarbons (PAHs) content of soils and sediments. However, the effect of hydrocarbon contamination for changing magnetic properties in these studies is not clearly verified because heavy metals were present together with PAHs. Thus, the relationship between MS and PAHs, in fact, is poorly constrained. The studies that were done so far haven't discussed how organic contamination can drive changes of magnetic properties of soils and sediments. The change of magnetic iron minerals after hydrocarbon contamination of soils and sediments is a very substantial factor that can be measured with magnetic techniques. The measured magnetic properties are due to the result of several processes that are undergoing in soil and sediments. One of these important processes is the role of microbial iron redox cycling for the (trans)formation of magnetic iron minerals

It is very important to determine predominant factors which can cause a change in magnetic mineralogy of soil and sediments contaminated with hydrocarbons before using magnetic proxies for the delineation of these contaminations. Therefore, the present study mainly focuses on the understanding of driving processes, controlling factors, and leading mechanism which link the change of magnetic parameters of soils and sediments with hydrocarbon contamination. Therefore, the main goals of this research are: (1) Determination of the change of magnetic parameters of soils and sediments due to hydrocarbon contamination, (2) investigation of main controlling factors and mechanisms that can drive such changes of magnetic properties of hydrocarbon contaminated soils and sediments, (3) understanding the role of microbial iron redox cycling for changing iron magnetic mineralogy. The ultimate goal of this research is to work towards a possible use of magnetic proxies for the delineation of hydrocarbon contamination in soil and sediments, such as oil spills from pipelines etc.

2. Overview of the topics

2.1 Delineation of hydrocarbon contamination by magnetic methods

There are a few studies (Morris et al., 1994; Hanesch and Scholger, 2002; Martins et al., 2007) that report a relationship of magnetic proxies parameters and organic contamination, mainly PAHs. These studies noticed that there is a change of soil magnetic properties due to the presence of hydrocarbons in soils and sediments. Some other studies (Saunders et al., 1991; Constanzo-Alvarez et al., 2000; Liu et al., 2004; LeSchack and Van Alstine, 2002; Berger et al., 2002; Saunders et al., 1999; Elmore et al., 1987; Schumacher and Abrams, 1996) used magnetic parameters for exploration purposes. However, a correlation between MS and PAHs that found in contaminated soil/sediments could be just a secondary effect because the same samples also contained heavy metals. The distinct relationship between magnetic properties and hydrocarbons cannot be verified from the results mentioned in previous studies. In fact, there is only very little known about using magnetic proxies in relation with hydrocarbon contamination in soils and sediments. Focusing on this as a central goal, the current research investigates a relationship between hydrocarbon contaminated soils/sediments and their magnetic parameters, change of magnetic properties due to hydrocarbon contamination, driving processes and responsible factors that lead to changes of magnetic properties in the presence of hydrocarbons in both natural and laboratory conditions.

Furthermore, hydrocarbon contamination in soils and sediments can stimulate activities of certain microbes. These microbiological processes are responsible for iron redox changes and therby for the (trans)formation of iron minerals. Both iron(II)-oxidizing and iron(III)-reducing microbes can either produce magnetic iron minerals or remove them by dissolution under certain conditions (Lovley et al., 2004; Kappler and Straub, 2005). Microbes can use hydrocarbons as carbon source to reduce iron(III) to iron(II) and under certain environmental conditions even magnetite is formed (Lovley et al., 1989; Lovley and Anderson, 2000). Various microbially catalyzed processes were shown to play a key role in the precipitation, dissolution and transformation of iron minerals (Fortin and Langley, 2005). Therefore, it is very crucial to understand the iron cycling in soils and sediments and the role of microbes for the (trans)formations of iron minerals for the application of magnetic proxies for organic contamination determination.

2.2 Field investigation of contaminated sites

It is very important to study hydrocarbon contaminated soils and sediments from field sites that only contain organic contaminations excluding any contribution from other types of contaminants such as heavy metals. This gives a clue to understand differences in magnetic properties derived from organic contamination under natural field conditions. Furthermore, the comparison of magnetic properties of similar natural materials once with and once without contamination lying nearby each other, can provide the extent of change of magnetic properties due to hydrocarbon contamination. Differences in types of organic contaminants, history and sources of contamination, lithology and origin of contaminated materials and the time span of contamination may also play a key role to induce changes on magnetic properties of contaminated sediments.

Therefore, two field sites with different types of organic contaminants and different contamination history were investigated within this research. The first site, Haenigsen in northern Germany which is located about 100 km north of Hanover, is a former oil-field. For many years, this area was part of an oil mining site where oil was collected from open pits at shallow subsurface (Fig. 1.1 a). The site contains different types of contaminants that are all of natural origin, i.e. mostly heavy oils. Salt-tectonic is responsible for oil leakage to the surface from deep underground. Hence, soil and sediments in this area were severely contaminated during long-term oil-leakage up to the soil surface. As this is an area with long contamination history, aging of contaminants has happened at this site.

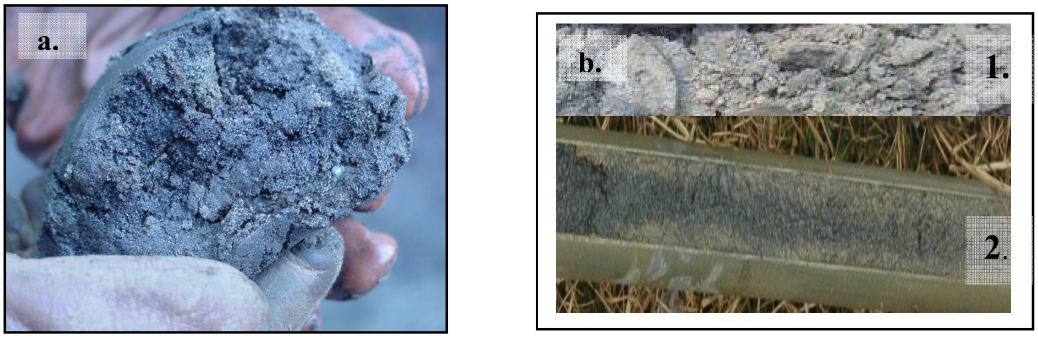

Fig. 1.1: Contaminated samples from field study sites: a. The former oil field, Haenigsen and b. The former military site, Hradcany. The two numbers in Fig. b indicate samples of the same site obtained by two different sampling methods.

The second field site, Hradcany, about 100 km northwest of Prague, is a former military air force base which was operated for more than 50 years (Fig. 1 b). The site was severely contaminated with jet fuel from fuel depots and oil-storage tanks. The area is one of the important water supply areas of Czech Republic. The area was under remediation from 1992 starting from the unsaturated zone and the remediation of both saturated and unsaturated zones was stopped in 2008 (Machackova, personal communication) Several contaminant remediation processes such as soil venting (SV), soil vapour extraction (SVE) and air sparging (AS) were used including natural attenuation and enhanced bioremediation with nutrients amendments. The contaminants from the unsaturated zone were considered as almost fully remediated in 2002 after achieving contaminants concentration below the clean up goal (total petroleum hydrocarbon content in soil/sediment 5000.0 mg/kg) Machackova et al., 2006). However, contaminants concentration below the targeted limit was still present in the sediments of the unsaturated zone. Furthermore, there were contaminants remaining in the saturated zone (Fig. 1.1 b).

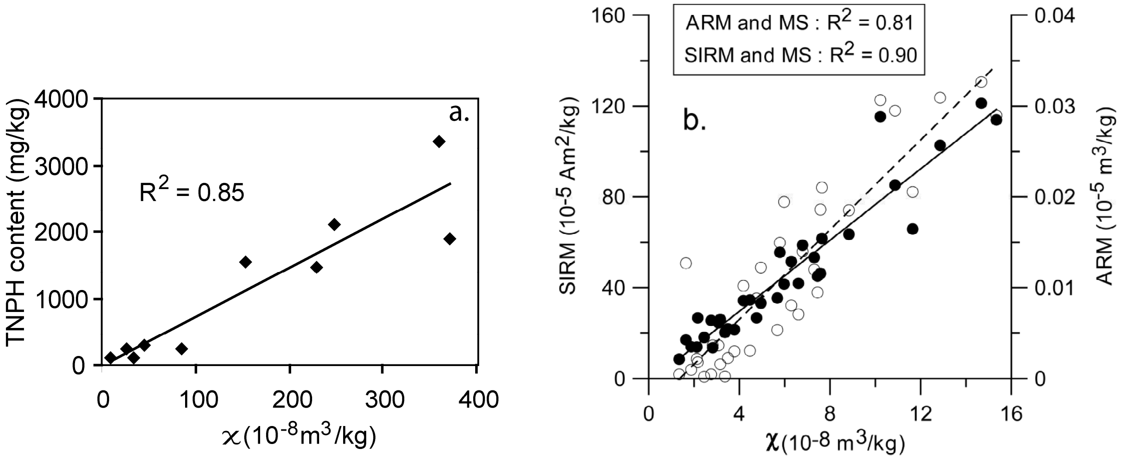

Fig. 1.2: a. Linear scatter plots between total non-polar hydrocarbon (TNPH) content and MS of hydrocarbon contaminated soil samples from the former oil-field Haenigsen. b. Linear scatter plots of magnetic concentration dependent parameters (ARM and SIRM) with MS of the samples from the groundwater table fluctuation zones of the former military site Hradcancy. The open and filled circles in Fig. b represent ARM and SIRM, respectively. R^2 denotes the coefficient of correlations.

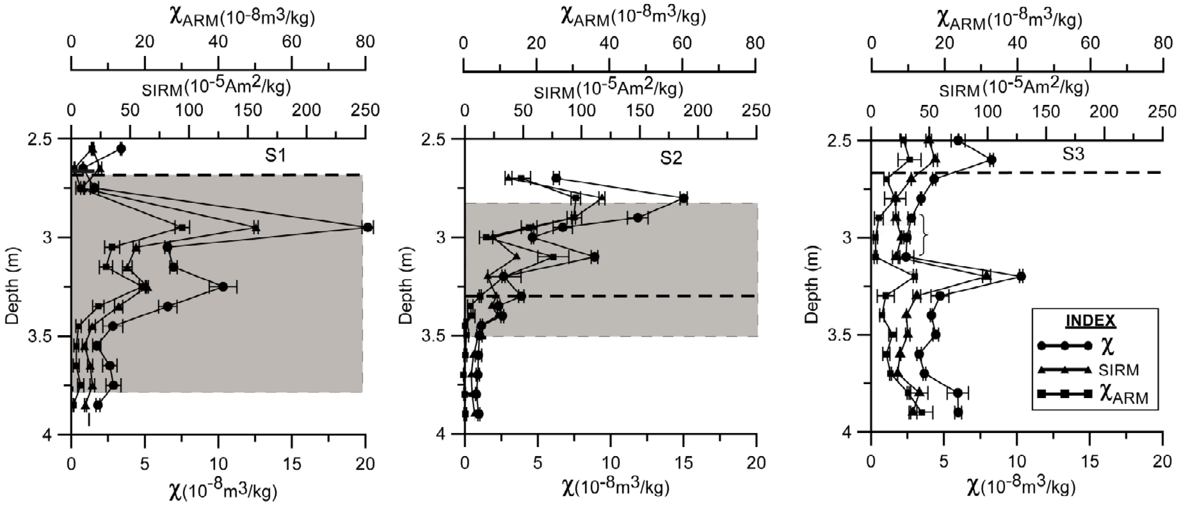

Fig. 1.3: Vertical variation of χ, χ_{ARM} and SIRM (mass specific) from three sampled sections at the Hradcancy site: S1 (left), S2 (middle), S3 (right). The shaded area shows the zone of groundwater table fluctuations and the dashed line is the groundwater table at the time of sampling. The variation of the data between the two sample sets is shown with horizontal bars.

During the remediation process of AS in the saturated zone, the groundwater table was continuously fluctuating during several years. Magnetic properties were systematically studied to 4 m depth by sampling from three different sites. The main focus of the study was the groundwater table fluctuation zone. Therefore, three sections from 2 to 4 m were systematically investigated for concentration and grain-size dependent magnetic parameters (e.g MS, ARM, SIRM and their ratios) and also for non-magnetic properties (e.g. pH and hydrocarbon content). There was a significant increase of magnetic concentration dependent parameters in the zone of groundwater table fluctuation of two contaminated sites (S1 and S2) where the groundwater table fluctuated due to continuous application of AS remediation process for many years (Fig. 1.2b and 1.3).

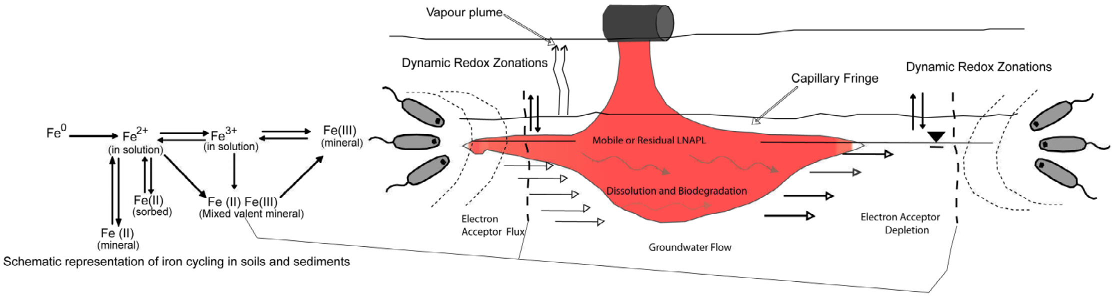

Fig.1.4: The conceptual model of changing magnetic signals in LNAPLs (jet fuel) contaminated groundwater table fluctuations zone. The hydrocarbon source zone, contamination release and schematic representation of iron cycling in soils and sediments, and dynamic redox zonations are shown. Small cartoons represent microbes.

A conceptual model of changing MS is introduced in the present thesis taking into account four major factors: release of hydrocarbon from the source zone, microbial degradation, dynamic redox zonation and groundwater table fluctuations (Fig. 1.4). Hydrocarbon degradation can happen during all redox processes of oxygen, nitrate, manganese(IV), iron(III), sulfate reduction and finally methanogenesis that develop after a hydrocarbon contamination around a contamination source zone (Baedecker et al., 1993; Eriksson et al., 1999; Grbic-Galic and Vogel, 1987; Kuhn et al., 1988; Lovley et al., 1989; Rueter et al., 1994; Borch et., 2010).

Although uncontaminated groundwater is commonly aerobic, anoxic conditions in unconfined aquifers usually develop when anthropogenic sources of labile organic matter reach the water table (Dobbins et al., 1992). During the remediation process of mainly AS, in combination with vacuum pumping, contaminants were brought six times a day approximately 0.2 to 0.7 m upward from their previous level, being flushed into the unsaturated zone by hydrocarbon contaminated groundwater and dispersed around. After switching AS off, the groundwater table slowly returned back to its original position but leaving behind dispersed contaminants in a very wide upper section above the original groundwater table. This periodic change of the groundwater level by AS happened in the background of natural groundwater table fluctuations.

The redox zonation in the hydrocarbon contaminated aquifer was continuously mixed due to remediation-driven continuous mixing processes, thereby creating an unstratified redox zonation. This unstratified redox zonation then led to enhanced microbial activities at this particular zone of the contaminated site (Skubal et al., 1999). The site naturally contains *Comamonas acidovorans* bacteria, which were actively degrading organic contaminants and converting them into non-hazardous carbon dioxide and water (Masak et al, 2003). In this remediation process, essential micronutrients such N, P and K were added in the contaminated site to enhance the bioremediation processes. By dispersing contaminants in the zone above the groundwater table, not only bioremediation activities were accelerated but the redox zonation boundaries were also continuously changing which created very favorable conditions for hydrocarbon oxidizing bacteria that were originally present in the sediments. However, at the interface of oxidizing and reducing zones different types of microbes live next to each other simultaneously playing a role for biodegradation of organic contaminants and iron mineral transformations which can stimulate microbial activities of both iron oxidizers and reducers.

Microbial iron reduction, an anaerobic process, is an important mechanism in the formation of magnetite deposits in a variety of sedimentary rocks and sediments (Blakemore et al., 1985; Moskowitz et al., 1993; Kim et al., 2005; Pan et al., 2005). More importantly, formation of biogenic magnetite was also observed in biodegraded oil (McCabe et al., 1987). More details of the field investigation of contaminated sites using magnetic methods are explained in chapter 2 (former military site) and 3 (former oil-field), respectively. Furthermore, the detailed magnetic characterization of sediments from 0.2 to 1.0 m of the unsaturated zone from the military site using variable magnetic parameters is discussed separately in chapter 5 of this thesis.

2.3 Magnetic susceptibility monitoring and determination of magnetic parameters controlling factors: A laboratory approach

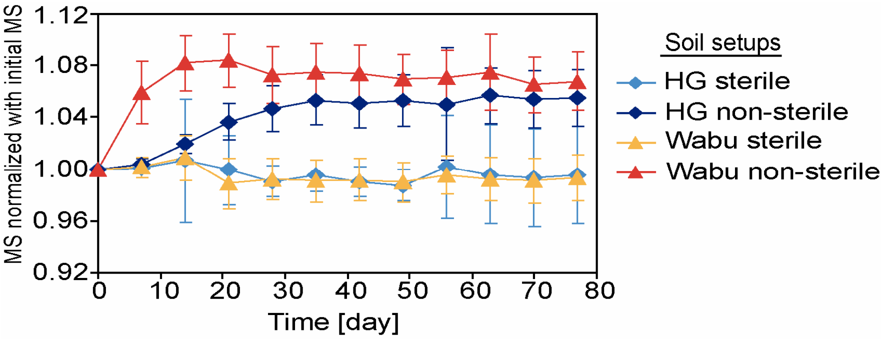

Fig.1.5: MS monitoring of batch setups of soils HG and Wabu are shown. The average initial normalized MS of three measurements of all parallels in sterile and non-sterile setups amended with *Shewanella oneidensis* MR-1 and *strain BoFeN* were plotted against time. Error bars show standard deviations.

Natural processes and mechanisms are often complicated because several processes/mechanisms are coupling together. In this natural scenario, it is almost impossible to figure out the role of individual controlling factors and processes that could play a significant contribution to cause changes in magnetic properties of hydrocarbon contaminated soils or sediments. Thus, laboratory batch experiments under controlled conditions were performed using different types of soils with and without hydrocarbon contamination. Mainly, the role of microbial processes was sought using two major (sterilized and microbial active) batch experimental setups and dividing them into several sub-setups with different carbon sources including hydrocarbons of different concentrations (Fig. 1.5). Furthermore, the role of other physical factors such as water content was also determined.

MS was monitored for several weeks using a KLY-3 Kappabridge (AGICO) in seven different soils. In microbial active setups the MS changed in average by 7%. For four soils an increase in MS was observed, whereas for three soils the MS decreased. One soil type revealed an exceptionally high decreasing trend of more than 60%. MS monitoring results of two soils are shown in Fig. 1.5. The increasing and decreasing behavior of MS for different soils could depend on original soil magnetic properties such as fine particles which can be detected by frequency dependent measurements of MS. It was observed that uncontaminated original soil samples with a frequency dependence varying from 7.3 to 9.5 % revealed a decrease of MS after addition of carbon sources under batch setups whereas other soil types with a frequency dependence less than 3.0 % displayed an increase of MS (Fig. 1.6 a). Nevertheless, both increase and decrease of MS only occurred in microbial active setups.

Magnetic hystereses were measured on eight sub-samples of sterilized and microbial active setups after opening the batch bottles. It was observed that the percentage of ferro(i)magnetic MS was enhanced in microbial active setups in comparison to sterilized setups of SB soil samples (Fig. 1.6 b). The wide distribution of MS values from the average value is mainly caused by a small amount of sub-samples and their magnetic heterogeneities. The change of ferro(i)magnetic components as obtained from magnetic hystereses measurements in microbial active setups also strongly supports the argument that microbial processes plays a significant role for transformation of magnetic iron minerals.

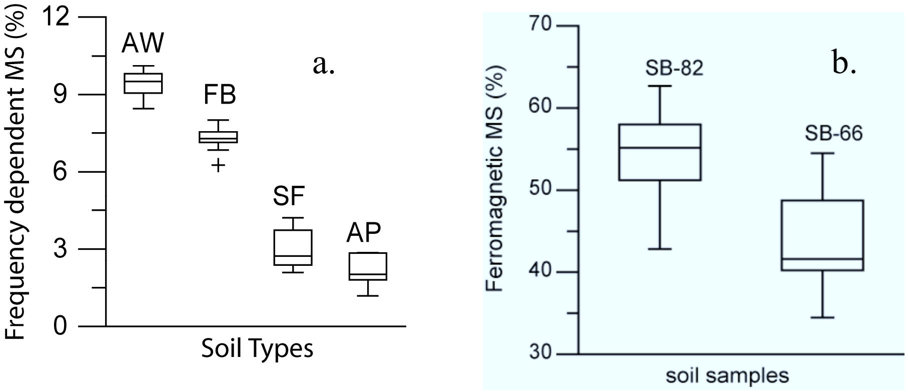

Fig.1.6: Frequency dependent MS (%) of four original soil samples AW, FB, SF and AP measured with Bartington MS2B dual frequency sensor is shown in a. The contribution of ferro(i)magnetic contribution obtained from hysteresis loops of SB soil; SB-66 (sterile) and SB-82 (microbial active) setups in Fig. b. The total 10 and 8 sub-samples from each of the sample were measured as shown Fig. a and b, respectively.

MS monitoring of different amount of water-added soil samples was investigated (Fig. 1.7). It was clearly observed that the amount of water-added to a certain extent (soil : 21.0 g and amount of water-added 7.0 ml giving soil to water ratio of 3.0 g/ml) has no effect .i.e. change of MS was equivalent to samples without any water addition. Interestingly, MS showed a decreasing trend with the amount of increasing water addition above that limit (3.0 g/ml).

A possible reason is that until a certain amount of water content, it plays a role to enhance the mobility of organic carbon and mineral phases from soil matrix whereas above that limit water creates a dissolution effect of these minerals. In the former case, it helps to increase microbial activities by increasing mobility of dissolved phases whereas in the latter case physical processes predominate over microbial processes leading to dissolution of minerals including magnetic minerals. The detailed description of MS monitoring of different soil types and its relationship with various non-magnetic parameters are postulated in chapter 6.

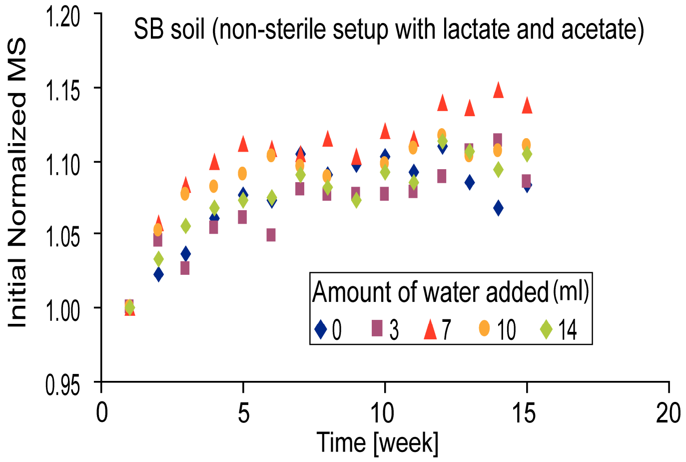

Fig. 1.7: MS monitoring of microbial active (non-sterilized) batch experimental setups. Lactate and acetate as well as different amounts of water were added.

In addition to soil batch experimental setups, some magnetic properties of magnetite minerals that were produced by the bacterium *Shewanella oneidensis* MR-1 in pure batch culture during the reduction of ferrihydride were studied. MS, temperature dependent MS, and frequency dependent MS were studied. It is evident that biogenic magnetite produced from *Shewanella oneidensis* MR-1 contained variable magnetite grains as depicted from these different measurements.

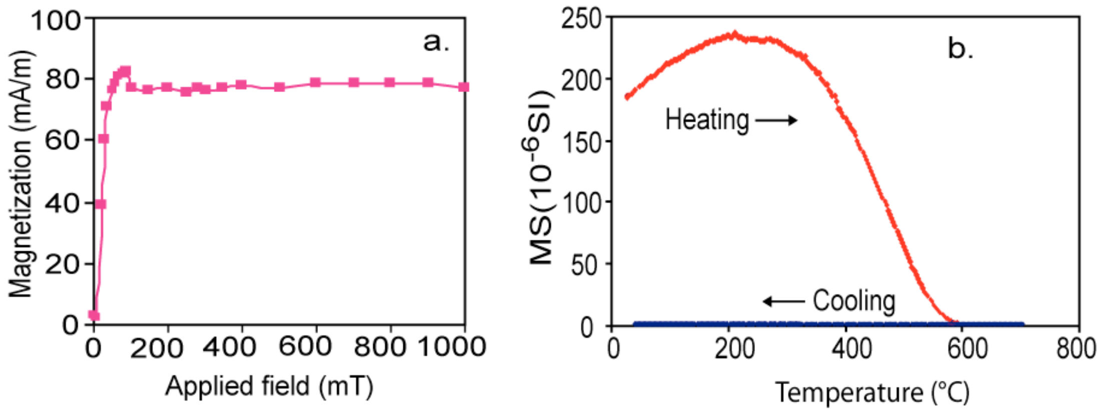

Fig. 1.8: a. An IRM acquisition curve and temperature dependentt MS of biogenic magnetite produced by *Shewanella oneidensis* MR-1 from ferrihydrite reduction

The frequency dependence (measured with a Bartington MS2B dual frequency sensor) of magnetite produced by *Shewanella oneidensis* MR-1 during the reduction of ferrihydrite was 5.0% in average. However, the average frequency dependence was 8.4% varying from 3 to 14% (measured with a MFK1 Kappabridge at two different frequencies 3904 and 15616 Hz). An isothermal remanent magnetization acquisition curve also suggested that the sample contained very wide range of grain sizes including SP grains (Fig. 1.8 a). Hysteresis parameters also showed that it contained a wide range of magnetic grain sizes as it is also supported by the temperature dependent MS (Fig. 1.8 b). The hard components in hysteresis loop are mainly related with some traces of original unreduced ferrihydrite that was present together with newly formed magnetite. More detailed description about microbiological analyses, magnetic properties, magnetic minerals (trans)formation in soil and in pure culture are described in chapter 4.

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2

Change of magnetic properties due to fluctuations of hydrocarbon contaminated groundwater in unconsolidated sediments

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Change of magnetic properties due to fluctuations of hydrocarbon contaminated groundwater in unconsolidated sediments

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Hydrocarbon contaminated groundwater together with its fluctuating water table lead to a change in magnetic properties of unconsolidated sediments.

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ABSTRACT

Sediments affected by fluctuations of hydrocarbon contaminated groundwater were studied at a former military site. Due to remediation, groundwater table fluctuation (GWTF) extends over approximately one meter. Three cores were collected, penetrating through the GWTF zone. Magnetic parameters, sediment properties and hydrocarbon content were measured. We discovered that magnetic concentration parameters increased towards the top of the GWTF zone. Magnetite is responsible for this enhancement; rock magnetic parameters indicate that the newly formed magnetite is in a single domain rather than a superparamagnetic state. The presence of hydrocarbons is apparently essential for magnetite to form, as there is clearly less magnetic enhancement in the core, which is outside of the strongly contaminated area. From our results we conclude that the top of the fluctuation zone has the most intensive geomicrobiological activity probably responsible for magnetite formation. This finding could be relevant for developing methods for simply and quickly detecting oil spills.

1. Introduction

Hydrocarbon contamination in soil, sediments and groundwater is a global environmental problem which it is very important to address if the Earth's resources are to be used in a sustainable manner. Technological advancements for detecting and monitoring hydrocarbon contamination in soils and sediments are required to quickly assess and select remediation strategies.

Magnetic methods can be efficiently applied to environmental purposes (Thompson and Oldfield, 1986; Evans and Heller, 2003), as they have a high potential for characterizing and monitoring soil, water and air pollution (Petrovský and Ellwood, 1999). Numerous studies of heavy metal pollution in soils caused by different pollution sources such as road traffic (e.g., Hoffmann et al., 1999; Gautam et al., 2005) or industrial pollution (e.g., Strzyszcz et al., 1996; Spiteri et al., 2005) have demonstrated how such methods can be applied.

In contrast, few studies have been carried out on the relation between magnetic properties and organic compounds. Hanesch and Scholger (2002) postulated a correlation between magnetic susceptibility (MS) and polycyclic aromatic hydrocarbon (PAH) content in soil near the Donawitz steel mill in Leoben (Austria). Furthermore, even in an area without industry, combined PAH and magnetic analyses provided a sensitive and discriminatory means of identifying outdoor and traffic-derived pollution (Halsall et al., 2008). Morris et al. (1994) analyzed a sediment core from Hamilton harbor in the area of Western Lake Ontario (Canada), and concluded that MS could be a reliable and less expensive empirical method for determining the extent of sediment contamination by PAH. Martins et al. (2007) verified a correlation between the distribution of MS and hydrocarbons in the Santos Estuary, mainly by comparing MS and PAH content. Although the aforementioned studies pointed to a significant correlation between magnetic concentrations and PAH content in soils or sediments, these studies did not exclude the presence of heavy metals, so that the correlation of MS with PAH may be just an artifact because the hydrocarbons often occur together with heavy metals.

Some studies (Aldana et al., 2003; Liu et al., 2006) investigated how magnetic properties could be used for oil prospecting and exploration. They noted a magnetic enhancement which was thought to be caused by hydrocarbon migration above oil fields in Venezuela and China. However, these studies focus on the effects caused by deep 'sources' and are not concerned with

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environmental pollution of soil or near-surface sediments caused, for example, by oil spills.

At the former military site in our study, the groundwater has been heavily contaminated by hydrocarbons. Strong fluctuations of the groundwater table (GWT) due to remediation by air sparging (AS) were frequently produced, thereby flushing contaminants into the sediments as the water level rose. At our study site aerobic biodegradation was used to remediate hydrocarbon contaminants from the saturated and unsaturated zones. The microbe *Comamonas acidovorans*, which actively mineralizes petroleum hydrocarbons, was found in sediments. To enhance bioremediation processes essential nutrients like N and P were amended and oxygen was supplied by soil vapour extraction (SVE) and AS.

Microbes play a significant role in the formation and transformation of iron minerals in hydrocarbon contaminated soil/sediments. They use hydrocarbons as a carbon source and transform them into non-hazardous carbon dioxide and water. Some iron (III)-reducing microbes can use hydrocarbons to reduce Fe (III) to Fe (II), and under certain conditions magnetite is even formed (Lovley et al., 1989; Lovley and Anderson, 2000). Depending on available iron species and essential nutrients for microbial growth, microbial Fe (III) reduction plays an important role for natural attenuation of organic pollutants.

Our paper focuses on magnetic changes observed due to fluctuations in the GWT in the presence of high amounts of hydrocarbons. To our knowledge this topic has not been addressed in the literature so far. The study could be relevant for developing methods to simply and quickly detect near-surface hydrocarbon contamination such as that caused by oil spills.

2. Research methodology

2.1. Study area

The study area, a former military air force base (AFB) at Hradcany (50°37′14″ N/14°44′17″ E), is situated about 100 km northeast of Prague (Fig. 1a). The site was heavily contaminated with petroleum hydrocarbons (Fig. 1a) due to leaks in petroleum storage tanks and jet fuelling stations over 25 years of active use by the Soviet Union, which closed the base in 1991. This site is one of the most important sources of high quality groundwater in the Czech Republic. The area consists of an upper layer of Quaternary river sediments (sand and gravel); the aquifer is composed of middle to fine grained Middle Turonian sandstone with a thickness between 67 and 75 m (Machackova et al., 2005).

Our study site belongs to the eastern part of the whole contaminated area, which covers an area of about 28 ha where most of the contaminants were released into sub-surface from oil storage tanks (Fig. 1a,b). Petroleum hydrocarbon is composed of jet fuel (70%) with an admixture of gasoline and diesel. The study site is located very close to the source zone (the storage tanks).

At the beginning of the clean-up in 1997, light non-aqueous phase liquids (LNAPLs) were frequently present in the wells below the GWT in layers over 0.5 m thick. The first phase of remediation was done using SVE and recovery of LNAPLs by vacuum pumping to remove the free oil phase and volatile compounds from the unsaturated zone. Afterwards, aerobic biodegradation was activated by supplying oxygen and amending nutrients to remove contaminants from the saturated zone in combination with SVE, AS and bioventing methods. Microbial screening had shown that the microorganisms *Comamonas acidovorans*, which were naturally present in the soils, were actively mineralizing petroleum hydrocarbons (Masak et al., 2003).

After the unsaturated zone had been completely remediated, the greatest levels of pollution were observed in the smear zone, which is the sediment zone influenced by fluctuations in the GWT. The average thickness of the contaminated zone at the top of the aquifer was found to be 1.75 m, located at depths between 3 and 10 m depending on surface elevation (Machackova et al., 2008). The total amount of hydrocarbon which had been released into soil and groundwater was estimated to be about 7,150 tons (Machackova et al., 2005).

2.2. Field sampling

Sediments samples were collected in July 2008 using an electrical hammer connected with a hollow pipe of 5 cm in diameter. Two cores (S1, S2) were taken at locations within the area directly influenced by groundwater fluctuations during the remediation process, whereas one core (S3) was taken from further upstream. Section S3 was less influenced by pumping during remediation measures, and groundwater fluctuations are probably due mainly to seasonal variation. Furthermore, section S3 is located near the storage place in the upstream direction and is thus less affected by contamination (Fig. 1b).

A cross-section of the sampled area is shown in Fig. 2. A common baseline is indicated by the dashed line. The surfaces of S2 and S3 lie at 1.3 m and 0.2 m below the baseline, respectively. Samples were collected from all three cores at 10 cm intervals down to a maximum depth of ca. 4 m. The samples were put into air-tight pre-leveled plastic bags and transported to a laboratory for measurements. The sampled zone extended from 1 to 4 m depths comprising the top of the aquifer, the zone of GWT fluctuation, and the unsaturated zone above. The unsaturated zone has a complex

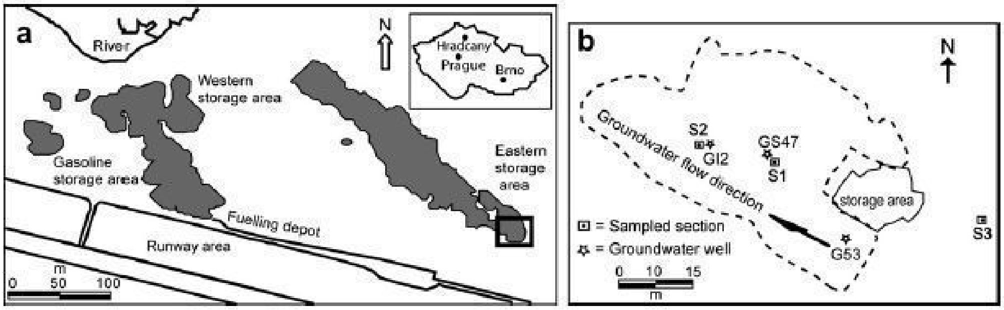

Fig. 1. a. The distribution of hydrocarbon contamination in the Hradcany study area is indicated in grey (state of the year 2000) along with source zones. The location of Hradcany is shown in the small map at the upper right corner. Our study site is indicated by the square at the lower right edge. b. Close up view of the field sampling layout at our study site. The sampled sections S1, S2 and S3 and nearest groundwater wells are shown. The hydrocarbon storage area as well as groundwater flow direction (towards NW) are also marked. The dashed line in b. is the area of contamination with a hydrocarbon concentration of 5000 mg/kg in the soil during the year 2000.

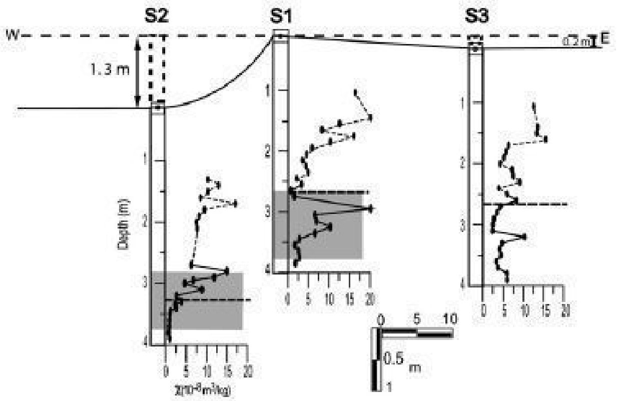

Fig. 2. Cross-section (E-W) of the location and surface elevations of the three sampled sections, S1, S2 and S3. The uppermost dashed line shows the ground surface elevation of S1 (baseline). The S2 and S3 sections are located, 1.3 m and 0.2 m below the baseline, respectively. The shaded area shows the minimum extent of the GWTF zone (especially the upper limits could be higher) and downhole curves show mass-specific magnetic susceptibilities vs. depths between ca. 1 and 4 m depth from the surface of the sections. Curves above the GWTF zone are plotted as dashed lines. The horizontal dashed lines indicate the position of the GWT at the time of sampling.

distribution of magnetic properties. Before remediation it was also heavily and very heterogeneously contaminated, leaving a complex fingerprint in the magnetic properties. Because of this complete different scenario this zone will be an issue for another paper and will not further be discussed here.

2.3. *Sample characterization methods*

2.3.1. *Magnetic properties*

Sediment core samples collected from the field were dried at room temperature and packed into 10 cm^3 plastic containers for magnetic measurements. Due to the coring procedure, the length of the depth intervals for individual samples was limited to a vertical resolution of 10 cm. Two sets from each sample interval were prepared. MS and anhysteretic remanent magnetization (ARM) were measured on both sample sets whereas all other magnetic measurements were done on only one set. Values for MS and ARM were very close to each other in parallel sets. It was not possible to prepare more than two sets of samples because of the limited amount of sample material obtained from the cores.

Bulk MS (κ) was measured with a KLY-3 Kappabridge (AGICO) and the value was normalized with the mass to obtain mass-specific MS (χ). ARM was induced with a steady biasing field of 40 μT and a peak alternating field of 100 mT using a Model 615 ARM magnetizer (2G Enterprises). Isothermal remanent magnetization (IRM) of the samples was imparted using a MMPM9 pulse magnetizer (Magnetic Measurements Ltd.) with a maximum field of 1 T (considered as SIRM) and a back field at 300 mT. Both ARM and IRM remanences were measured with a Minispin magnetomer (Molspin Ltd). Frequency-dependent MS ($\kappa_{fd\%}$) was measured with a MFK1 Kappabridge at two different frequencies of 3904 Hz and 15616 Hz in a peak field of 200 A/m. Temperature-dependent MS was obtained with a KLY-3 Kappabridge attached to a CS-3 heating unit.

2.3.2. *Sediment properties*

Selected samples were measured for their pH, total iron content (Fe_{tot}) and organic carbon content (C_{org}). The pH was determined 24 h after adding 0.01 M $CaCl_2$ solution according to ISO 10390 (1994). The total iron content of dried and fine-ground samples was quantified by X-ray fluorescence (XRF) using a Bruker AXS S4 Pioneer X-ray spectrometer (Bruker AXS GmbH). The total organic carbon was determined with a CN analyzer (Vario EL, Elementar). The inorganic carbon content was quantified by mixing soil with 10% HCl and measuring the released CO_2 from carbonates with a Scheibler apparatus. The organic carbon content was then calculated from the difference between total and inorganic carbon.

2.3.3. *Hydrocarbon analysis*

Ten grams of well homogenized wet samples were extracted with acetone after mixing with iso-hexane. A 10 ml solution was taken and 0.5 g of florisil was added to remove polar compounds, and the solution was extracted half an hour later. The solution was then removed and measured with a GC (HP 6890-II)/MS (MS 5973). The compounds were identified by matching retention times and ion mass fragments with results from standard mixtures of diesel and mineral oil. The sample concentration was calculated and calibrated to a standard mixture.

3. Results

3.1. *Groundwater table fluctuation*

The thickness of the GWTF zone in this study area is a crucial factor for our study. The GWTF is not only influenced by natural seasonal fluctuations, but also and especially by the remediation processes. In particular, AS caused frequent and rather large changes in the position of GWT, spreading contaminants over a broad section of the smear zone. During AS, compressed air was pumped 4 m below the GWT six times a day for one hour continuously over the last 8 years or so. This process induced GWTF six times a day, raising the GWT approximately 0.2 to 0.7 m above the previous level while the pumping was going on, though it then subsided when AS was stopped. This high frequent variation of the GWT is superimposed on the seasonal GWTF. The variation of the GWTF measured in wells nearest (1.0–1.5 m) to our sampling points S1 and S2 are 3.15–3.76 m (3 measurements) and 2.84–3.50 m (7 measurements), respectively (the measurements were carried out by the company performing remediation). There is no recording well close to S3 and thus no values are available for this section. However, as AS was never performed near S3, we assume that the GWTF at this location was always close to the natural seasonal variation which has been determined as 0.33 m (Fig. 3) at a well >100 m away from remediation measures. During our sampling we determined the GWT at the 3 sections (S1: 2.7 m, S2: 3.3 m, S3: 2.7, all data vs. surface). Including these data, we yield a minimum thickness for the GWTF zone of 1.06 m (S1) and 0.66 m (S2) which is illustrated in Fig. 2. It has to be noted that in S1 our own reading yields a higher GWT level than that determined by the remediation company. We have to take into account that especially the upper limits of the GWTF zone shown in Fig. 2 represent minimum extents. The higher level of the GWT at S1 compared to S3 (1.9 m

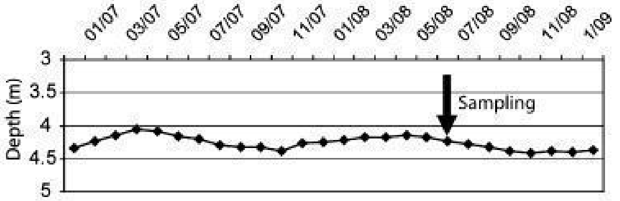

Fig. 3. Natural seasonal groundwater table fluctuations measured between 2007 and 2009 in well HJ556 located about 100 m NW from our study site where the GWT is not affected by remediation processes. The arrow indicates the GWT at the time of our sampling, which is a few cm below the maximum level.

higher in absolute elevation) is a consequence of AS which was going on close to S1 at the time of sampling.

3.2. Distribution of hydrocarbons

The total hydrocarbon content of some samples from all three sections shows a wide variation. Section S2 contains the highest amount of contaminants, almost reaching 300 mg/kg, whereas S1 and S3 have maximum concentrations of 47 mg/kg and 65 mg/kg, respectively (Fig. 4). In all sections the maximum concentration was observed about 0.5 to 1 m below the GWT at the time of sampling. Despite this low hydrocarbon content measured in S1 samples, we must assume a similar level of contamination at S1 locations as at S2 because both sites are within the downstream range of the groundwater flow with respect to the source (storage area). It has to be noted that the hydrocarbon values were measured on sediment samples and not directly in the groundwater.

3.3. Magnetic properties

Fig. 5 shows the relationship between χ and SIRM as well as ARM. All three parameters are indicators for the concentration of magnetic phases. While SIRM and ARM are only controlled by ferro(i)magnetic substances, MS is additionally dependent on the paramagnetic contribution. The coefficients of determination (R^2) for linear dependence between χ and SIRM, and χ and ARM are 0.90 and 0.81, respectively. This proves that MS is mainly dominated by ferro(i)magnetic phases; all three parameters therefore denote the concentration of the ferro(i)magnetic content.

3.3.1. Concentration-dependent parameters

Concentration parameters χ, χ_{ARM} and SIRM are shown in Fig. 6. The profiles show a more than 5 fold increase from bottom to top within the zone of the GWTF in sections S1 and S2, while this trend is much weaker in S3 (2–3 times increase, ignoring the outlier at a depth of 3.2 m), which is thus to be considered less contaminated. This indicates that new formation of ferro(i)magnetic phases occurred due to water level changes and the curves show that this effect is highest towards the top of the GWTF zone. In the lower part of the GWTF zone and below, where water is always or most time present, ferro(i)magnetic concentration is very low.

3.3.2. Magnetic mineral characterization

Further studies were conducted to identify the type and grain size of the newly formed magnetic phases. The temperature dependence of MS of two samples from the GWTF zone at S1 and S2 (position of samples indicated in Fig. 6) exhibits the presence of magnetite or a magnetite-near phase by a Curie temperature of about 580 °C (Fig. 7). IRM acquisition curves of samples from the GWTF zone were modeled to separate high and low coercivity phases using a two component model of log-Gaussian distribution (Kruiver and Passier, 2001). According to this method, the gradient of the total IRM acquisition curve is plotted against the log of the applied field (GAP curve) and decomposed into log-Gaussian distributions. The maximum values of the fitted log-Gaussian curves give the percentage contribution of different ferro(i)-magnetic mineral assemblages to IRM. The value $B_{1/2}$ is the field at which half of the SIRM is achieved given by the maximum of the log-Gaussian curves; it provides a measure of magnetic hardness. Two representative examples are shown in Fig. 8a. For the first sample (open symbols) it is evident that a soft magnetic component with $B_{1/2}$ of 40 mT (on average) dominates (relative contribution of ca. 95%,) whereas the hard magnetic phase has a $B_{1/2}$ value of ca. 630 mT. The second sample (full symbols) is from a zone with low ferro(i)magnetic concentration (Section 3, depth 3.45 m) and has a much higher contribution of the hard magnetic phase. Values of $B_{1/2}$ and the percentage contribution of the low coercive component from all analyzed samples are plotted vs. χ in Fig. 8b. Higher χ values can be related to larger amounts of newly formed magnetite. While $B_{1/2}$ shows no notable variation, the percentage contribution increases with χ. This suggests that the high coercive component (hematite or goethite) is of natural origin and its relative contribution gradually decreases as magnetite formations increases.

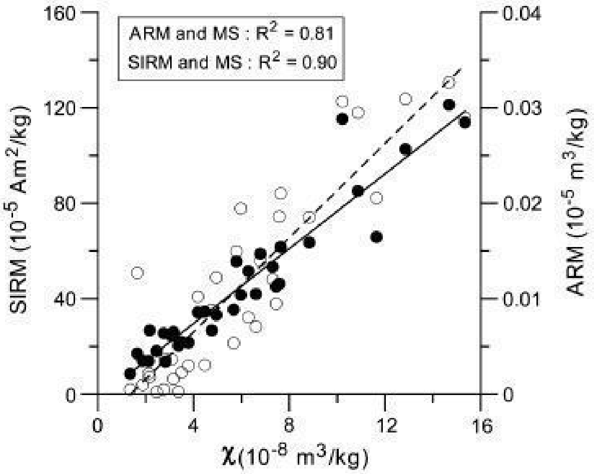

Fig. 5. Linear relationship between χ and SIRM as well as χ and ARM. Full circles/solid line and open circles/broken line are shown for χ vs. SIRM and χ vs. ARM, respectively. Coefficients of determinations are indicated.

S-ratios at $\text{IRM}_{-0.3\text{T}}$ as defined by Bloemendal et al. (1992) vs. χ are shown in Fig. 9a. The *S*-ratio is a measure of low coercive (e.g., magnetite) to high coercive (e.g., hematite) ferro(i)magnetic

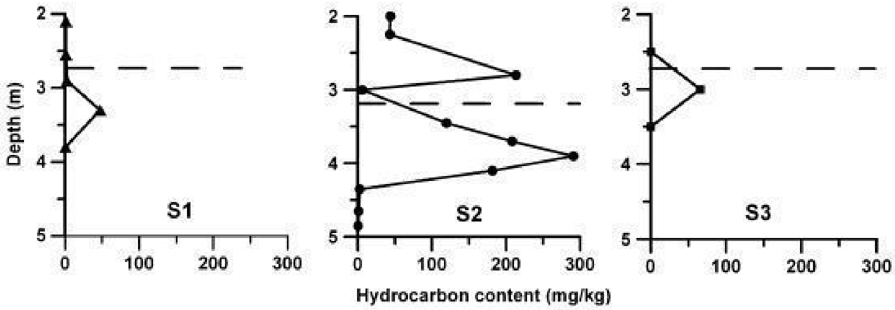

Fig. 4. Total hydrocarbon content of some selected samples from sections S1, S2 and S3. The dashed lines indicate the groundwater table at the time of sampling.

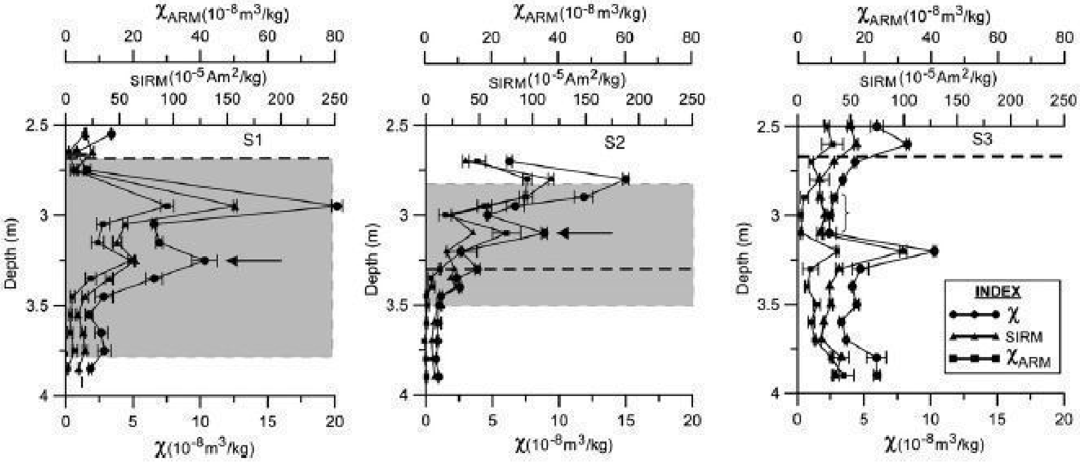

Fig. 6. Vertical variation of χ, χ_{ARM} and SIRM (all mass specific) from three sampled sections: S1 (left), S2 (middle), S3 (right). The shaded area shows the zone of groundwater table fluctuation; the dashed line is the groundwater table at the time of sampling. The variation of the data between the two sample sets is shown with horizontal bars. Arrows in S1 and S2 indicate the position of the samples shown in Fig. 7 (temperature-dependent MS). The bracket in S3 denotes the position of the samples which represent low *S*-ratio values shown in Fig. 9a.

phases. With increasing magnetite to hematite ratio the *S*-ratio can vary from 0 (no magnetite) to 1 (100% magnetite). Nearly all values from sections S1, S2 and S3 have *S*-ratios > 0.9. Like the trend of percentage contribution shown in Fig. 8, this reveals a relative increase of magnetite through new formations.

Values of $\kappa_{fd\%}$ against χ for sections S1, S2 and S3 are shown in Fig. 9b. Higher values are an indication for ultrafine particles in the superparamagnetic range (<30 nm). For most of the samples $\kappa_{fd\%}$ varies from 4 to 8% which means that all samples contain some superparamagnetic particles. There is no clear relationship between $\kappa_{fd\%}$ and χ; however, a slight trend of decreasing values can be noted. This trend may indicate that the newly formed magnetite is predominantly in a single domain range rather than superparamagnetic. Certainly one has to be very careful with such an interpretation as measurements of $\kappa_{fd\%}$ have a high uncertainty for such low χ values, although the used MFK1 instrument is at least one order more sensitive than the commonly employed Bartington MS2.

The most indicative result is the ratio of ARM/SIRM (Fig. 9c) which clearly increases with χ. ARM is particularly strong in single domain particles, therefore this trend of increasing ARM/SIRM ratios with χ is a good evidence for the conclusion that magnetite has been formed within predominantly a single domain state.

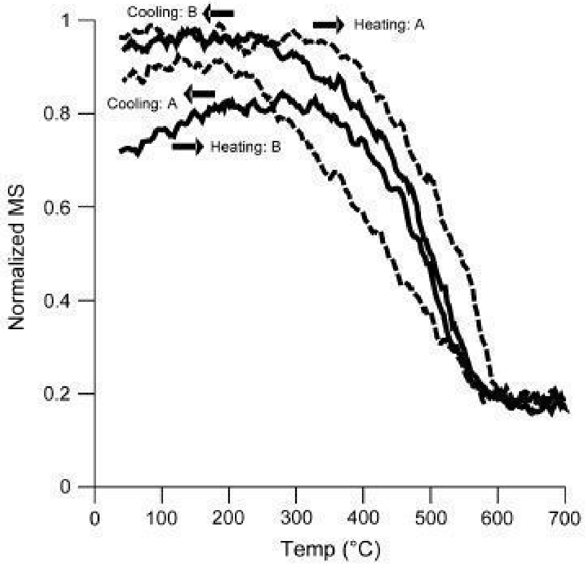

Fig. 7. Temperature-dependent normalized MS of two samples selected from S1 and S2 sections. Both the heating and cooling curves of the two samples are shown. Samples A and B are located within the GWTF zone at 3.25 m (S1) and 3.10 m (S2) (positions see Fig. 6).

3.4. Sediment properties

The sediments are mainly composed of sand-sized particles which vary from coarse to fine grained; about 80% of the samples are medium grained (2–0.63 mm). For some selected samples from all three sections pH, Fe_{tot} and C_{org} were measured (Fig. 10). Values of pH were within the acidic range (<6.0). Generally, Fe_{tot} is low (<1.5%) and organic C_{org} content is also very low (<1%). Low concentration of C_{org} leads to the conclusion that there is no significant influence of carbon from naturally occurring organic matter to control possible magnetic iron minerals transformation processes. None of the parameters Fe_{tot}, C_{org} or pH is correlated with χ. This supports the conclusion that new magnetite formation is not dependent upon these sediments being present, but on hydrocarbon contaminants and the GWTF.

4. Discussion

In this study of the former military AFB Hradcany, the contaminated groundwater and its fluctuating water table play the most important role in changing the magnetic property of the sediments. For several years AS created groundwater table fluctuations in the natural background of the GWTF. This process was responsible to carry LNAPLs repeatedly in the uppermost region of the GWTF zone, leaving parts of these contaminants there even after the GWT declined when the pumping was stopped. This type of processes was responsible in moving contaminants within the range of the GWTF zone. During the time of the sampling, the GWT was ca. 1.9 m higher at S1 compared to S2 due to AS going on close to S1. However, the width of the GWTF zone recorded by the remediation company over a longer time span (Fig. 2) also differs in sections S1

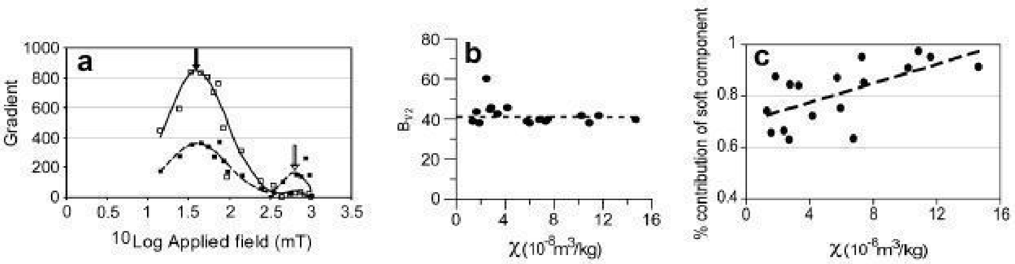

Fig. 8. a. Gradient acquisition plot (GAP) of IRM acquisition of two representative samples: one within the GWTF zone at a depth of 3.25 m in S1 (open symbols) and another at a depth of 3.45 m in S3 (full symbols). The full and open arrows indicate the $B_{1/2}$ values of the soft and hard components, respectively. b. Linear scatter plot of $B_{1/2}$ and c. Percentage contribution of the low coercive components obtained after Kruiver and Passier (2001) from all three sections within the GWTF zone. The dashed lines in b and c exhibit the linear trend lines.

and S2, although these sections are located only about 20 m apart from each other. One possible reason for such a difference is the limited number of data points for the GWT level, which may not cover the whole range of GWTF in the full cycle of AS. This became quite obvious as we observed the GWT in S1 at 2.7 m depth at the time of sampling, which is above the upper limit of the GWTF zone recorded by the remediation company. However, this does not explain why the absolute elevation of the GWTF zone in S2 is clearly lower than in S1, although it is determined by more data points ($N=8$) than in S1 ($N=4$). A slope of ca. 1 m cannot be explained by normal hydraulic conditions along the groundwater flow direction. A second reason could be a difference in the hydraulic conductivities of the sediments around S1 and S2 which might have created differences in how the groundwater rose at the two wells. Despite these uncertainties in establishing the extent of the GWTF zone and in explaining their different depths in S1 and S2, there is sufficient evidence to link the observed increase of magnetic concentration parameters to this smear zone.

Though the area had been undergoing remediation for several years, hydrocarbons were still present in high amounts at the top of the aquifer, contaminating the sediments in the GWTF zone. It seems clear that previous contamination in the unsaturated zone also left magnetic fingerprints in the sediments. This zone above the GWTF zone is not considered here and will be published in another paper as mentioned in Section 2.2.

The strong linkage between χ and ARM as well as SIRM proves that magnetic concentration signals are controlled by ferro(i)-magnetic phases. Temperature-dependent MS obtained from S1 and S2 sections clearly indicates that the main magnetic mineral is magnetite (or a magnetite-near phase). As demonstrated by increasing *S*-ratios and increasing contribution of a soft magnetic phase with higher χ values, magnetite is obviously formed in the GWTF zone. However, there is a difference in the type of magnetite. The newly formed magnetite within the GWTF zone is likely in a predominantly single domain state as indicated by decreasing $\kappa_{fd\%}$ as well as by increasing ARM/SIRM ratios with increasing χ. Furthermore, concentration-dependent magnetic parameters evidently increase strongly within the zone of GWTF of the S1 and S2 sections, which are in the downstream range of the pollution source (petroleum storage tanks), while the increase is relatively smaller in section S3, which is less contaminated due to its location with respect to the storage tanks. This result suggests that new formation of magnetite not only depends on the GWTF but also on the concentration of contaminants. Both parameters, i.e., hydrocarbon concentration and the GWTF seem to play a significant role in the formation and the stability of SD-type magnetite grains. The higher correlation coefficients between ARM/SIRM ratios and χ within S1 and S2 sections suggest that theses two sections contain more SD-type magnetite grains than the samples from section S3. It can be noted that the MS values of S1 and S1 below the GWTF zone are only about half compared to S3, however, it would be too speculative to assume an influence for the differences of magnetic enhancement towards the top of the GWTF zone.

No relationship between the formation of new magnetite and sediment parameters Fe_{tot}, pH and C_{org} could be detected. However, S3 section has lower Fe_{tot} than S1 and S2. This might be an indication that the bioremediation processes, which were implemented only in S1 and S2, increased the total iron content. All sections show pH values within an acidic range (<6.0) and C_{org} below 0.14%. This very low content of C_{org} reveals that the change of magnetic properties, and hence magnetic mineralogy, is controlled by bacterial oxidation and reduction of iron cycles using hydrocarbon from contaminated sediments rather than using natural organic matter.

The most interesting finding is the increase in new magnetite formations towards the top of the GWTF zone. The higher up in the GWTF zone, the less time water resides in the sediments. This seems to be a crucial controlling factor for the formation of magnetite. Hydrocarbon is available in the entire zone of GWTF as

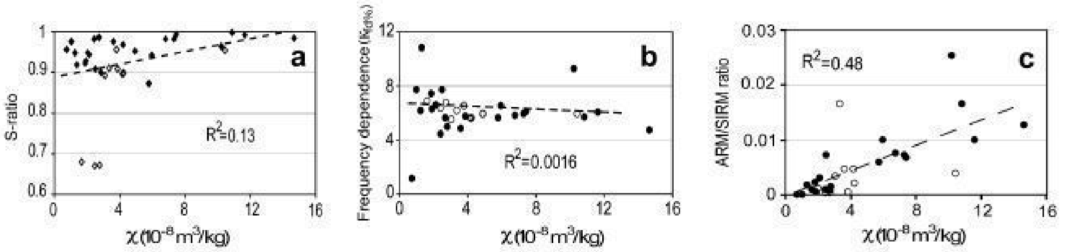

Fig. 9. Scatter plots between χ and *S*-ratio (a), χ and $\kappa_{fd\%}$ (b), χ and ARM/SIRM ratio (c) of samples in the GWTF zone of all three sections. The dashed lines indicate linear trend lines and R^2 denotes the coefficients of determinations. The three data points in figure a. have distinctly lower *S*-ratios (positions are indicated in Fig. 6). Open symbols denote samples from section S3.

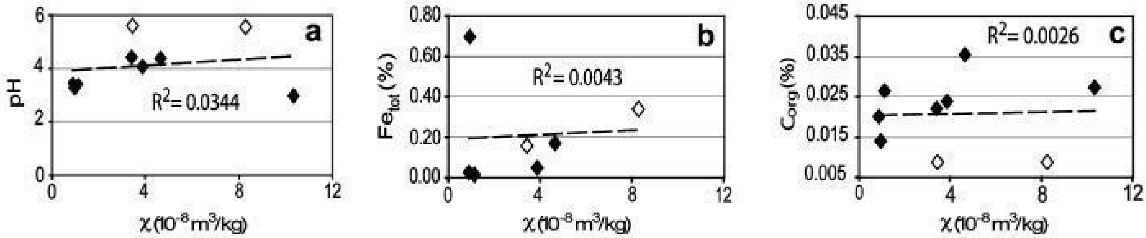

Fig. 10. Cross-plots of sediment parameters pH, Fe_{tot} and C_{org} vs. χ are shown in figure a, b and c respectively. The coefficients of determination of these parameters with χ are also indicated with respective trend lines. Open symbols denote the samples from section S3.

this is frequently flushed into the zone by changes in the water level. Comparison of concentration-dependent magnetic signals in S1/S2 to S3 indicates that the amount of hydrocarbon also plays an important role in the formation of new magnetite. Within the lower part of the GWTF zone, water, which is present for most time there, seems to prevent the formation of magnetite. At this stage we have identified, analyzed and discussed the phenomena of this effect.

There is no clear direct correlation between the amount of hydrocarbons measured in the sediment samples (Fig. 4) and the concentration-dependent magnetic parameters. However, it is evident from the remediation measures that in S1 and S2 the HC concentration in the water phase was much higher than in S3. Therefore certainly more HC was flushed into the GWTF zone during AS and increasing the HC availability for certain times. Future research will focus on studying the process and controlling factors of iron mineral transformation in the GWTF zone with and without hydrocarbon contamination. Bacterial activities are certainly the dominating factor for changes in iron mineralogy (Kappler and Straub, 2005) and the most active zone seems to be the topmost part of the GWTF.

5. Conclusions

Hydrocarbon contaminated groundwater together with its fluctuations derive a change in the magnetic properties of the sediments. Magnetite predominantly in the SD-range is produced in the GWTF zone. The amount of newly formed magnetite increases with increasing amounts of hydrocarbon and decreasing amounts of water over time. The zone at the top of the GWTF seems to be the zone of strongest bacterial activity leading to iron mineralogy changes. Our results provide a new approach for identifying and monitoring biogeochemical and remediation method-driven mineralogical changes using magnetic methods. The results could be important in developing new methods for detecting oil spills using simple and fast measurements.

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3

Magnetic signatures of hydrocarbon-contaminated soils and sediments at the former oil-field Hänigsen, Germany

Magnetic signatures of hydrocarbon-contaminated soils and sediments at the former oil-field Hänigsen, Germany

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Abstract

Magnetic properties of hydrocarbon (HC) containing soils and sediments from two sites of the former oil-field Hänigsen were analyzed in order to determine whether magnetic methods can be employed to delineate HC contamination of soils and sediments. Magnetic parameters such as magnetic susceptibility (MS) and induced isothermal remanent magnetizations, as well as soil properties, HC content and most probable number counts of iron-metabolizing microorganisms were determined. The magnetic concentration-dependent parameters were much higher (25 times) for HC contaminated soil samples from site A than for sediment samples from site B. However, at site B the MS was still four times higher in comparison to lithologically similar clean sediment samples from a third site C. Newly formed magnetite containing mainly single domain particles was responsible for the magnetic enhancement. There was only minor contribution of superparamagnetic grains. Site A had an acidic pH compared to neutral pH at site B, and a higher crystalline and bio-available total iron content. Nevertheless, site B samples contained significant numbers of both iron(II)-oxidizing and iron(III)-reducing microorganisms indicating that microbial iron cycling might have taken place at this site and potentially played a role for iron mineral transformation, including magnetite (trans)formation. The content of total non-polar hydrocarbons (TNPH) at site A was one order of magnitude higher than at site B. Only at site A MS correlated well with TNPH.

Our results demonstrate that HC contaminated samples had an enhanced magnetite content compared to non-contaminated soils and sediments. Therefore, magnetic methods may provide a quick and cost-effective way to assess HC contamination in soils and sediments. However, field site and laboratory investigations are needed to reveal the complex nature of the processes involved.

1. Introduction

Pollution of soils, sediments and groundwater by organic contaminants is one of the major global environmental problems. In order to assess HC contaminated sites and apply suitable remediation, rapid and cost effective assessment methods are needed. Environmental magnetic methods have a high potential to serve as such a screening and monitoring tool for soil, water and air pollution, since they are fast and highly sensitive (Petrovský and Ellwood, 1999). In recent years, these methods have been well established to study heavy metal pollution in soil from different anthropogenic sources (Evans and Heller, 2003). Specifically, MS has been successfully implemented to detecting anthropogenic pollution caused by fly ashes from power plants (Kapička et al., 2000; Spiteri et al., 2005), metallurgical dusts (Hanesch and Scholger, 2002) and urban airborne particulates (Muxworthy et al., 2001; Gautam et al, 2005). During combustion, the oxidation of material that contains a relatively large amount of iron and heavy metals (such as Pb, Cu and Zn) lead to the formation of magnetic particles explaining the significant relationship between enhanced MS of soils and their combustion-related pollution with heavy metals around industrial sites (Strzyszcz and Magiera, 1998; Kapička et al., 1999; Hanesch and Scholger, 2002).

However, there are only a few studies in which a relationship between magnetic properties and organic contaminants was studied in the environmental context. Hanesch and Scholger (2002) observed a correlation between MS and polycyclic aromatic hydrocarbons (PAHs) content in soil near Donawitz steel mill at Leoben, Austria. After analyzing sediment cores from Hamilton harbour, Canada, Morris et al. (1994) concluded that MS could be a reliable and less expensive empirical method for determining the extent of the contamination of sediments by PAHs. Martins et al. (2007) verified a good correlation between the distribution of MS and hydrocarbons in the Santos Estuary, Brazil, mainly by comparing MS and PAHs. Some studies have also been published investigating a possible use of magnetic properties for oil prospecting and exploration. Liu et al. (2006) reported magnetic enhancement that was caused by HC migration in the Mawagmiao Oil Field, Jianghan Basin, China. The authors observed a dramatic increase of some magnetic parameters such as MS and saturation magnetization of an oil-bearing formation and also observed the presence of secondary magnetite particles in association with the oil-bearing strata. A study done by Aldana et al. (2003) in oil reservoirs in Venezuela demonstrated that high-resolution aeromagnetic survey can be applied for exploring oil fields, suggesting that MS measurements in soils, sediments, and drill cuttings may be a complementary or alternative method for exploration and assessment of HC reservoirs. Díaz et al. (2006) used MS, electron paramagnetic resonance and extractable organic matter parameters in drilling fines from near surface levels of producer and non-producer wells to examine a possible causal relationship between magnetic anomalies and underlying hydrocarbons. Magnetic properties changes were also recognized in the groundwater table fluctuation zone of HC contaminated sediments (Rijal et al., 2009). In several of these studies, correlation of MS with organic compounds might be a secondary feature as heavy metal input appears together with enhanced MS and organic compounds are likely abundant as an additional contaminant. In any case, the controlling factors of magnetic property changes in soil and sediments in the presence of organic contaminants are still poorly understood.

Some iron(III)-reducing microorganisms can use hydrocarbons as a carbon source to reduce iron(III) to iron(II) (Lovley et al., 1989; Lovley and Anderson, 2000), which in turn can be oxidized by anaerobic and aerobic iron(II)-oxidizing bacteria (Kappler and Straub, 2005; Weber et al., 2006). Both, microbial iron(III) reduction and iron(II) oxidation can lead to the formation of magnetite (Bazylinski et al., 2007). HC contaminations in soils and sediments, therefore, have an influence on both iron(III)-reducing and iron(II)-oxidizing microorganisms and thereby on iron mineral (trans)formation and dissolution processes. These processes can lead to changes in the magnetic iron mineral content of soils and sediments. Magnetic properties, mainly MS, can be measured very quickly and in a cost-effective way to study or monitor such changes of magnetic iron minerals in contaminated soils and sediments.

Assuming that the change in magnetic iron mineralogy in HC contaminated soils and sediments can be caused by biological as well as abiological processes, our main hypothesis was that magnetic methods can be used to investigate and monitor such iron mineralogical changes. Therefore, the main goals of this study were (i) to determine if magnetic parameters of HC contaminated soils and sediments change due to HC contamination in comparison to non-contaminated areas and (ii) to investigate the controlling sediment and soil parameters such as pH and total iron content, which might also influence the microbial cycling of iron in presence of hydrocarbons and hence the change in magnetic properties. We reported results obtained from the HC contaminated field site Hänigsen, northern Germany, which represents an area where hydrocarbons are the only contaminants and anthropogenic heavy metal input can be neglected.

2. Material and Methods

2.1 Field sampling

The study area, Hänigsen (52°28’58’’N, 10°5’47’’E) is located approximately 100 km north of Hannover in northern Germany (Fig. 1a). Salt tectonics in the northern German basin has led to natural oil outcrops at the surface, which have been exploited for more than 400 years on a small scale by collecting oil in one to two meter deep pits. The area around Hänigsen led to intensive modern oil exploitation by drilling and later on also underground mining for oil-bearing sand during the oil boom in the 1920s. Oil production and processing around these areas continued until 1963 with about 2000 production wells.

Several HC contaminated samples of forest soil and of sediments from two different locations (sites A and B) were collected in the Hänigsen area. Besides these contaminated samples, two samples were collected from a non-contaminated site (site C) as background material for the comparison of its magnetic properties with those of the contaminated samples. The location of all three sites is shown in Fig. 1b and the depth of the samples is presented in Table 1.

The HC contaminated samples from site A were mainly soil samples taken from maximum 0.85 m depth, whereas at site B sediment samples were taken from 1.8 to 2.4 m below the soil surface. Site A is the dried oil-leaking surface while site B is the former oil-pit maintained by local people for historical demonstration purposes.

The samples from site B were water-saturated and taken from beneath the groundwater table. The individual samples from sites A and B were located within a lateral distance of 1.5 and 2 m to each other, respectively. Samples from the non-contaminated sediments at site C were collected from a maximum of 0.9 m below the surface. All samples were stored in plastic bags and kept at 4°C until further measurement.

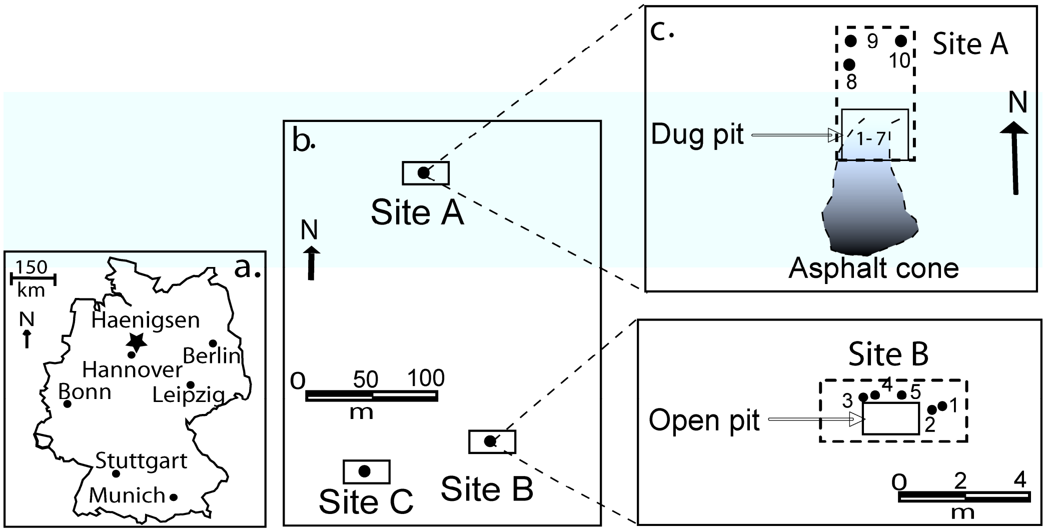

Fig. 1: (a) Location map of the study site; (b) Sampling layout of the three different sampling sites A, B, C; (c) distribution of sampling points within the two contaminated sampling sites A and B; non-contaminated samples were taken from two different depths at site C.

2.2 Laboratory sample characterization methods

2.2.1 Magnetic measurements

MS (given in mass-specific values χ), frequency dependent MS ($\kappa_{fd\%}$), temperature dependent MS, anhysteretic remanant magnetization (ARM) and isothermal remanent magnetization (IRM) were measured after drying the samples at room temperature. MS was measured with a KLY-3 Kappabridge (AGICO) at room temperature. $\kappa_{fd\%}$ was obtained with a Bartington dual frequency MS2B sensor at two different frequencies (470 and 4700 Hz). ARM was induced with a steady biasing field of 40 μT and a peak alternating field of 100 mT using a Model 615 ARM magnetizer (2G Enterprises). IRM was imparted with a MMPM9 pulse magnetizer by exposing samples to a series of successive larger field sizes starting from 10 mT to saturation IRM (SIRM) at 1 T, followed by successively larger backfields. Remanent magnetizations (ARM and

IRM) were measured using a Minispin magnetometer (Molspin Ltd.). S-ratios (after Bloemendal et al., 1992) for different reverse fields and ARM/SIRM ratios were calculated to distinguish magnetic mineral phases and their grain size (more precisely, magnetic domain state).

For identification of the magnetic mineralogy and magnetic grain size, low temperature remanence was recorded in some representative selected samples from sites A and B. The measurement was performed with a magnetic property measurement system (MPMS) by warming the samples from 10 to 300 K starting from an initial state after applying a saturating field of 2.5 T (zero-field cooling: ZFC). Furthermore, some samples were demagnetized by applying a 2.5 T field at room temperature (RT) and then cooling from 300 to 10 K (RT SIRM cooling). In addition, the mineralogical content of selected samples from site A was also examined by measuring temperature dependent MS with a KLY-3 Kappabridge by heating the samples to maximum 700°C starting from room temperature.

2.2.2 Soil and sediment characterization

Values of pH, water content (W_C), total crystalline iron (Fe_{cryst}) and bio-available iron (Fe_{bio}) content were determined to characterize the soil and sediment samples, and to compare these properties with their magnetic properties.

Before soil analysis, large particles (e.g. stones, roots) were removed by hand with tweezers, since some samples were highly contaminated with crude oil and therefore impossible to sieve. The soil was stored in plastic bags at 4°C in darkness until further use. The soil water content was determined and calculated after standard protocols (Blume et al., 2000) by drying the soil at 105°C until the soil weight was constant. The pH was measured 24 h after addition of 0.01 M $CaCl_2$ solution according to Blume et al., 2000. Two different iron fractions were extracted from the soil after (Moeslund et al., 1994; Roden and Zachara, 1996) with a soil:extractant ratio (w/v) of 1:50 and with at least 0.5 g of soil. Adsorbed iron, iron carbonates and poorly crystalline iron minerals, so called "bioavailable iron" (Fe_{bio}), were extracted with 0.5 M HCl for 1 h at room temperature on a shaker. Iron minerals with higher crystallinity (Fe_{cryst}) were extracted with 6 M HCl at 70°C in a water bath for 24 h and the extracts were cooled for 15 min at room temperature. Of all extracts 1.8 ml were centrifuged for 15 min at 20,817 *g* to remove soil particles. The total iron in the supernatants was quantified by the ferrozine assay (Stookey, 1970) as also described by Hegler et al. (2008) and the concentration was corrected by the water content of the respective samples. The metal content of dried and fine ground soil was quantified by X-ray fluorescence analysis (Bruker AXS S4 Pioneer X-ray spectrometer, Bruker AXS GmbH, Germany).

2.2.3 Hydrocarbon analysis

The HC content of samples from sites A and B was measured after extracting well homogenized 10 g of moist sample with two solvents: first with acetone and then florisil. The extract was analyzed by gas-chromatography mass-spectrometry (GC-MS) (HP 6890-II and MS 5973). HC were identified by matching retention times and ion mass fragments with results from standard mixtures of diesel and mineral oil. Polar compounds could not be measured and were removed by adding florisil. The remaining HC content can be referred as total non-polar hydrocarbon (TNPH) content including PAHs. The following types of PAHs were quantified with a GC-MS: Naphthalene, 2-Methylnaphthalene, 1-Methylnaphthalene, Acenaphtylene, Acenaphtene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo (a) anthracene, Chrysene, Benzo (b) fluoranthene, Benzo (k) fluoranthene, Benzo (a) pyrene, Indeno (1,2,3-cd) pyrene, Dibenzo (ah) anthracene and Benzo (ghi) perylene. The sum of these PAHs was calculated and this parameter was called total PAH (TPAH) content.

2.2.4 Most probable number (MPN) counts

Viable anaerobic, mixotrophic nitrate-reducing iron(II)-oxidizing microorganisms (FeOxM) and viable anaerobic iron(III)-reducing microorganisms (FeRedM) present in samples B1, B3 and B5 were quantified by the most probable number (MPN) method (Cochran, 1950). Dilution series of soil suspensions were set up with selective growth medium for the two metabolic groups of microorganisms (supporting information S1). Based on the number of parallels per dilution step showing growth after 17 weeks, the MPN of cells per gram soil present in the initial soil sample was calculated with the program “Most Probable Number Calculator“ version 4.04 (Environmental Protection Agency, USA) and corrected after Salama as described by Klee (1993). The cell numbers were corrected by the water content of the soil.

3. Results

3.1 Magnetic characteristics

3.1.1 Concentration of magnetic phases

Five sub-samples of each sample from all sites were packed into 10 cc plastic containers. First, as an important results, we found that contaminated soil samples from site A, which were all taken close to the soil surface (< 1 m depth), had a very high MS, i.e. ca. 25 times higher mean value in comparison to sediment samples that were taken from deeper depth at site B (Fig. 2a and b, Table 1). Furthermore, samples A1-A7 from site A showed a wider variation of MS than samples from site B. Samples A1-A7 which were sampled just below the asphalt cone had a higher MS in comparison to samples A8-A10 that were located further away from it. The sediment samples from site B exhibited a mass-specific MS of $\chi<10\times10^{-8}$ m³/kg; values were very similar to those indicated in Fig.2b. The average MS of all samples from site B, however, was still more than four times higher than the average MS of non-contaminated samples taken from site C (χ=1.06×10^{-8} ±0.046 m³/kg).

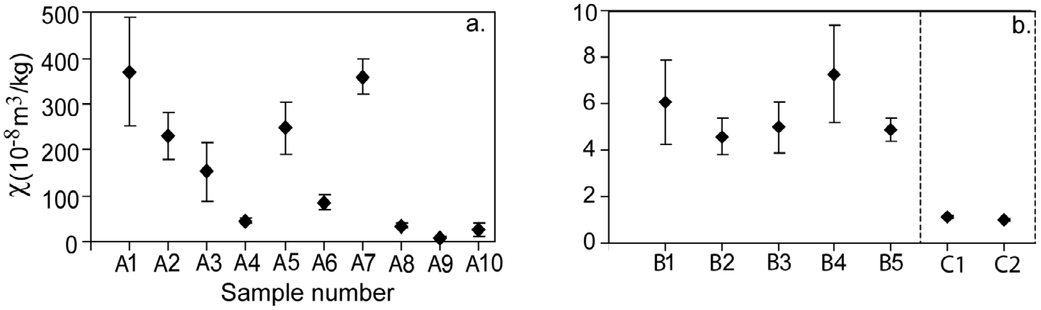

Fig. 2: Plots of χ (10^{-8} m³/kg) mean values of samples from sampling site A (a) and sites B and C (within two broken vertical lines) (b). Five sub-samples from each sample were measured. The bars denote the standard deviation. The sample name is shown along x-axis.

There was a strong linear relationship between χ and ARM, which showed that MS at both sites A and B is mainly controlled by ferro(i)magnetic particles (Fig. 3 a and b). The samples did not contain a large amount of superparamagnetic grains as depicted by the very low frequency dependence of MS (κ_{fd}% <3%) (Fig. 3 c and d). There was no significant relationship between χ and κ_{fd}% at both sites.

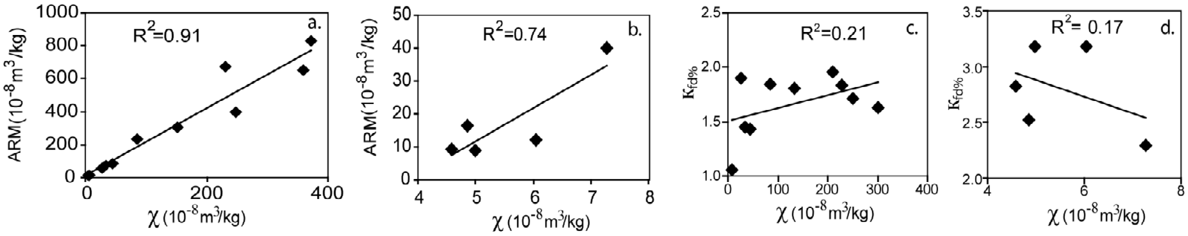

Fig. 3: Linear scatter plots between χ and ARM (10^{-8} m³/kg) of mean values from sites A (a) and B (b), as well as between χ and κ_{fd}% from sites A (c) and B (d). The solid lines show a linear relationship, R^2 denotes the coefficient of determination.

3.1.2 Magnetic mineral characterization

MS was measured during heating sample A6 up to 700°C to determine the type of ferro(i)magnetic minerals. New formation of magnetite during heating masks the results (Fig. 4). Nevertheless, the plateau-like behavior with significant positive values before the onset of mineral transformation around 400°C indicated the presence of magnetite in the original samples. The slight increase of MS around 250°C could be either due to the formation of maghemite and its destruction at 300-400 °C (slight decrease of MS) or due to fine particles which are single domain (SD) at room temperature and become superparamagnetic at increasing temperature.

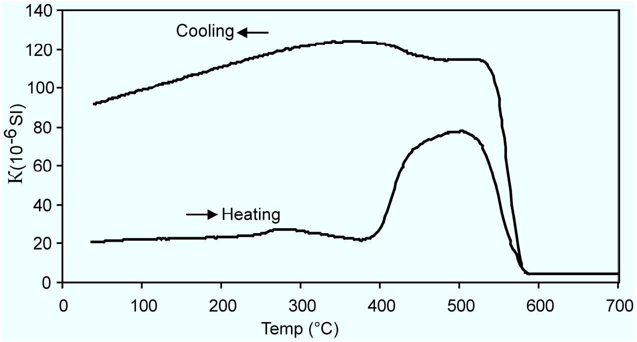

Fig. 4: Temperature dependent MS of sample A6. The sample was measured after hydrocarbon extraction.

Some representative samples from both the contaminated sites A and B and the non-contaminated site C were analyzed for zero-field cooling (ZFC) and room temperature SIRM (RT SIRM) cooling measurements. ZFC curves revealed a 65% loss of remanence between the Verwey transition (~120 K) and the magnetocrystalline isotropic point of magnetite (~130K for pure magnetite) for the contaminated sample A7, whereas for the non-contaminated sample C2 this loss was even 95% (Fig. 5a). For sample C2, the SIRM decreased 90% during warming to 30 K, which was probably due to phases that become paramagnetic at low temperature. In RT SIRM runs (Fig. 5b), samples from site A showed a clear Verwey transition and/or isotropic point; for samples B1 and B3 from site B, Verwey transition was also indicated but less clear. Remanence changes were also observed around the temperature of the Morin transition (~250 K) pointing out the additional presence of hematite. The non-contaminated sample C2 from site C showed a slightly different trend; the maximum remanence was observed below 50 K and then decreased with an intermediate bump between 110 and 150 K.

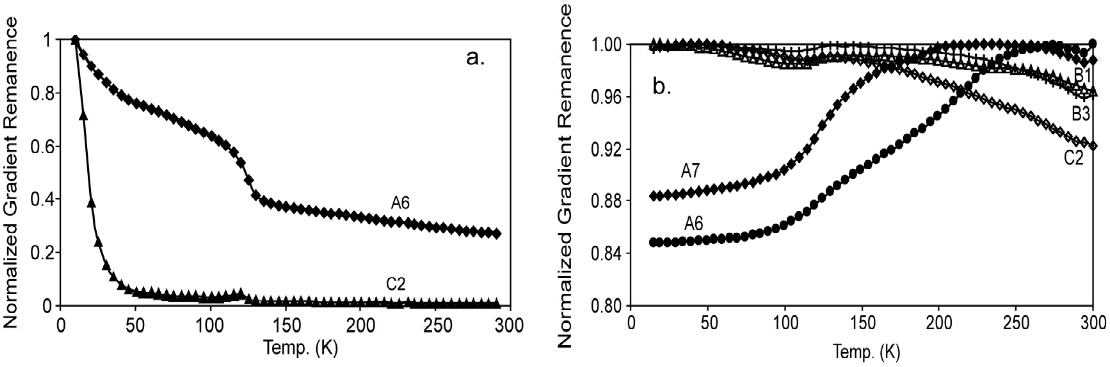

Fig. 5: Normalized plots of zero-field cooling (a) and room-temperature cooling (b) of SIRM remanences for representative samples from the contaminated sites A and B, and the non-contaminated site C.

The magnetic granulometry indicator ratio ARM/SIRM versus MS was plotted for both sites A and B (Fig. 6a and b). ARM/SIRM was positively correlated with MS for the samples from site B (R^2=0.79 for linear correlation) indicating new formation of predominantly SD magnetite particles in contaminated samples. There was no such relationship between ARM/SIRM and MS for the samples from site A, however, the ARM/SIRM ratios were similar to that of samples from site B. This indicates that also at site A a significant amount of SD magnetite has been formed. The results of S-ratios (Fig. 6c) depict soft magnetic magnetite dominates in all contaminated samples which almost saturated at 100 mT. All contaminated samples yield S-ratios above 0.95 at 300 mT indicating that these samples were dominated by magnetite, whereas clean samples showed a mean S-ratio at 300 mT below 0.90 suggesting hard magnetic phase-like hematite to be more predominate than magnetite.

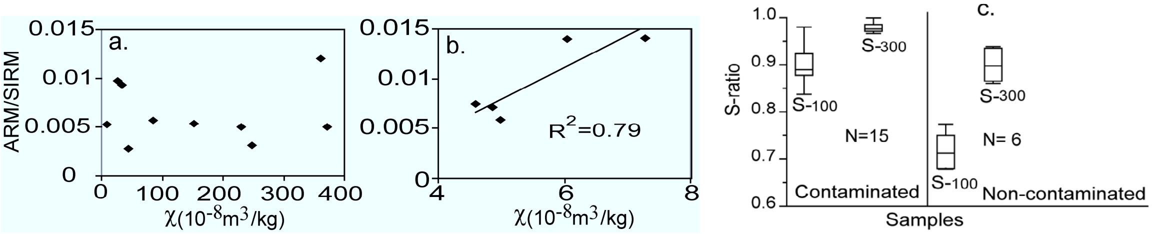

Fig. 6: Linear scatter plots of ARM/SIRM ratio vs. χ of samples from site A (a) and site B (b); the line in b indicates linear relationship between these two parameters and R^2 denotes the coefficient of determination. (c) S-ratios for backfields of 100 and 300 mT of all measured samples from contaminated (sites A and B) and non-contaminated (site C) sites calculated after Bloemendal et al. (1992); N shows the number of sub-samples measured from the respective sites.

The linear IRM acquisition depicts that the contaminated samples from both sites (A7, A8, B3, B5) saturated around 300 mT whereas the non-contaminated sample (C1) did not show saturation until 1 T (Fig. 7a). Log-normal Gaussian coercivity spectra (after Kruiver et al., 2001) were modeled for a two component system. The results showed that contaminated samples (Fig. 7b) were dominated by a soft magnetic component (contribution 98%, mean $B_{1/2}$ of 50 mT) whereas the non-contaminated samples (Fig. 7c) had a significant additional contribution (20%) of a harder coercive ($B_{1/2}$ of several 100 mT) phase probably related to hematite.

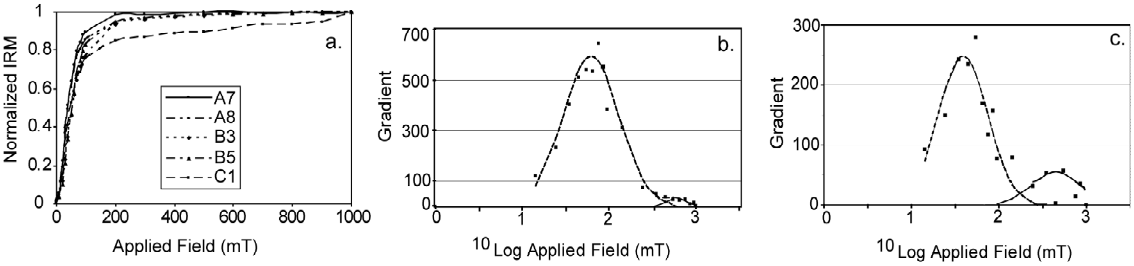

Fig. 7: Normalized IRM vs. applied field for selected samples from sites A, B and C (a). Coercivity separation of contaminated (b; sample B3) and non-contaminated (c; sample C1) samples after Kruiver et al. (2001); the dashed and the solid lines in b and c show the modeled cumulative log-Gaussian distribution curves for the soft and the hard components, respectively.

3.2 Soil and sediment properties

The mean water content (W_C) at site A was 17%, whereas at site B the samples were water saturated. The mean pH is almost neutral (pH≈7) at site B, whereas the samples from site A had an acidic pH value (pH≈4). The averaged Fe_{cryst} content of samples from site A wss about 1.75 times higher than for samples from site B while the averaged Fe_{bio} content was only slightly higher for A than for B samples (average of 13.1 and 7.8 wt.% for Fe_{cryst}, 1.7 and 1.1 wt.% for Fe_{bio}) (Table 1).

Table 1: Sampling depths, pH values, W_C, Fe_{cryst} and Fe_{bio} of the sampling.

Sampling site	Sample name	Depth (m)	pH	W_C (%)	Fe_{cryst} (%)	Fe_{bio} (%)
A	A1	0.25-0.30	2.8	19.6	13.86	3.49
	A2	0.30-0.35	4.6	15.7	14.36	1.26
	A3	0.40-0.50	6.7	9.87	11.12	1.36
	A4	0.00-0.05	3.9	11.7	17.18	0.82
	A5	0.05-0.10	3.8	34.8	16.98	2.58
	A6	0.10-0.15	4.5	10.7	11.88	0.87
	A7	0.80-0.85	3.3	32.2	10.00	0.92
	A8	0.00-0.05	3.4	7.5	3.36	0.65
	A9	0.05-0.10	3.8	10.7	31.70	2.83
	A10	0.01-0.20	6.7	15.6	7.09	2.59
B	B1	1.80-2.15	6.7	Water saturated samples	5.39	0.41
	B2	2.15-2.40	6.9		8.54	1.43
	B3	1.80-2.10	6.6		4.69	0.41
	B4	2.30-2.40	7.3		10.43	1.34
	B5	2.00-2.20	7.4		10.15	2.03
C	C1	0.50-0.70	not measured			
	C2	0.70-0.90				

3.3 Most probable numbers of Fe(II)-oxidizing and Fe(III)-reducing microorganisms

In order to determine how many microorganisms which are directly involved in the iron cycling under anoxic conditions were present at site B, the numbers of anaerobic FeRedM and FeOxM in sample B1, B3 and B5 were determined by the MPN method (Fig. 8).

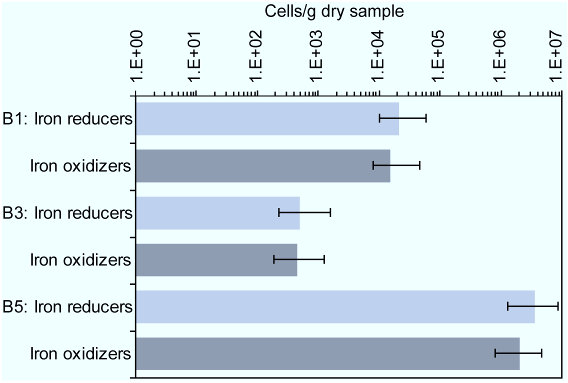

Fig. 8: Cell numbers of viable anaerobic iron(III)–reducing microorganisms (FeRedM) (light grey shading) and anaerobic, mixotrophic nitrate-reducing iron(II)-oxidizing microorganisms (FeOxM) (dark grey shading) in sample B1, B3 and B5 quantified by the most probable number method. Error bars indicate the 95 % confidence interval.

Both metabolic groups of microorganisms were found in all three samples. Among the samples the cell numbers of both groups varied between 4×10^2 and 4×10^6 cells per gram dry soil, with the lowest cell numbers being enumerated in sample B3 and the highest in sample B5. In all three samples, the number of FeRedM was similar to the number of FeOxM.

3.4 Relationship among magnetic and non-magnetic parameters

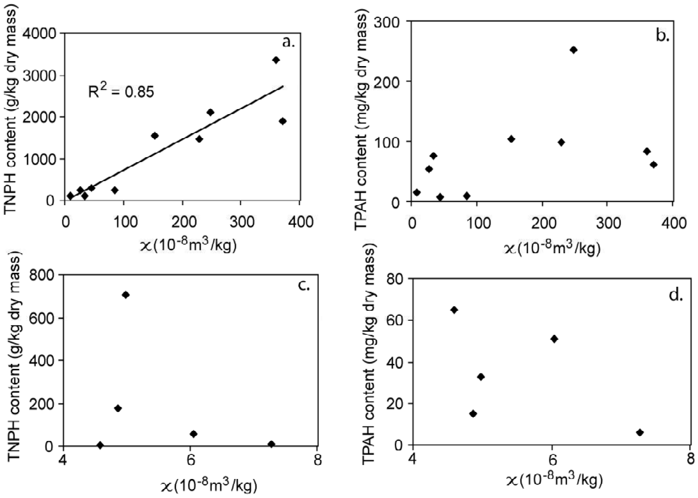

Fig. 9: Linear scatter plots of mass-specific MS and TNPH content of samples from sites A (a,) and B (c,) and of mass-specific MS and TPAH content of the same samples (b,d). The regression line in Fig. a indicates a linear relationship (R^2 denotes the coefficient of determination).

There was a very strong linear link ($R^2 = 0.85$) between MS and TNPH for site A (Fig. 9a), however, the relationship between MS and TPAH was very weak. Obviously, only those hydrocarbons which provided an easily available carbon source for bacteria led to new formation of magnetite. In contrast, the samples from site B showed neither a correlation between MS and TNPH nor between MS and TPAH content. The values of TNPH and TPAH were one order of magnitude lower than at site A.

The relationship among magnetic parameters, non-magnetic parameters and the HC content of all samples was defined by hierarchical cluster analysis using SPSS 11.5. The analysis was performed using the medians of nine different variables (MS, ARM, $\kappa_{fd\%}$, pH, W_C, TNPH, TPAH, Fe_{cryst}, Fe_{bio}) obtained for all samples from sites A and B. All nine variables were grouped together using Pearson correlation as a grouping criterion of different variables (Fig. 10). The dendrogram showed that the magnetic concentration parameters MS and ARM are closely related to the contents of TNPH. All other relationships are weak.

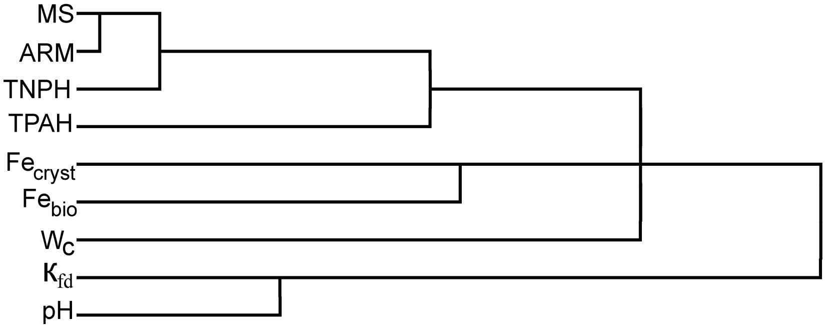

Fig. 10: Dendogram of nine different variables obtained from ten samples from site A and five samples from site B. Variables are grouped together using median method and Pearson correlation as grouping criterion.

5. Discussion

Soil samples from site A are mainly samples that were situated very close to a source of HC contamination. These samples were located close to the soil surface and had a significant higher MS than the samples from site B which were sampled from a contaminated water saturated zone. Differences of MS (Fig. 2) could be due to the variability of natural soil, differences of HC contents, redox zonations and/ or variable pH (2.8-7.4) conditions. It was noted that at low pH (3.0-4.0), dissolution of ferro(i)magnetic minerals is more likely than their neoformation (Grimley et al., 2004). Our results (high MS of samples from an acidic and contaminated environment, site A) did not support these findings, but indicated that the formation rather than the dissolution of magnetic minerals occurred when a large amount of carbon source in the form of hydrocarbons is available. It was also noticed by Grimley and Arruda (2007) that the dissolution of strongly magnetic minerals is a common and relatively rapid phenomenon in poorly drained soils, which results in low MS values.

Samples from site B, which were mainly sediment samples that lay about at least 1.8 m below the soil surface and remained water saturated throughout the year, had a much smaller MS in comparison to samples from site A. Maher et al. (1986) showed that soils that are seasonally saturated with water and iron-reduced have lower MS values than soils that are better drained and not iron-reduced. Depletion of magnetic concentration is especially prevalent in strongly reduced, anoxic soils in the presence of organic matter (Maher, 1986, 1998). Also, Grimley and

Arruda (2007) observed that at low E_h the reducing conditions are primarily responsible for magnetic mineral dissolution, a process likely mediated by FeRedM in the presence of soil organic matter. They also mentioned that although experiments indicate that reductive dissolution of magnetite can occur abiotically under extreme conditions, microorganisms likely play an important role in the natural environment. Their findings also supported our results because both FeRedM and FeOxM were present in samples from site B, where samples were water-saturated and the MS signal was lower than in site A. Nevertheless, samples from site B had more than three to four times stronger MS than the lithologically similar sediment samples from the non-contaminated site C, suggesting that HC contamination was responsible for the observed increase of MS.

In order to rule out a significant influence of atmospheric heavy metal input (and therefore magnetic enhancement as known from previous studies) in the surface-near samples at site A, the metal content was examined by X-ray fluorescence (XRF). The Pb and Zn contents in sample A5 were 82 and 50 ppm, respectively. This might reflect some anthropogenic contamination; however, the heavy metals content is clearly lower than observed at contaminated sites like those found around steel industry sites, leoben, Austria (Blaha U., University of Tuebingen, personal communication, 2004). The sediment samples from site B do not contain any significantly enhanced heavy metal levels. As these samples were taken from deeper depths, anthropogenic input by atmospheric deposition was unlikely. Magnetic enhancement in relation to anthropogenic heavy metal contamination might be responsible for part of the high MS values at site A, but certainly did not explain the increased MS values of samples from site B compared to the non-contaminated samples from site C. It is likely that the samples from site B had a lower magnetic mineral concentration than those of site A because these samples were completely water saturated which favors dissolution of magnetic grains or suppresses new formation of magnetite (Rijal et al., 2009).

Mineral magnetic parameters demonstrate that the magnetic enhancement is due to magnetite. Remanence parameters (ARM and SIRM) as well as MS are dominated by the magnetite phase. There were almost no ultrafine-grained superparamagnetic particles present as denoted by very low $\kappa_{fd\%}$ values of samples from both contaminated sites. For samples from site B the positive correlation of ARM/SIRM and MS indicates that the newly formed magnetite contains a significant portion of SD particles. Such correlation is missing in samples from site A, however, the ARM/SIRM ratios are similar to B site samples. Therefore, it can be concluded that also at site B newly formed magnetite is at least partly in the SD range. The presence of SD magnetite particles in ground water table fluctuation zones of HC contaminated areas was already demonstrated by Rijal et al. (2009).

A positive relationship between TNPH and MS is evident for site A. The TPAH content, however, did not correlate with MS ($R^2 = 0.20$). Water-saturated samples from site B did not show any clear relationship of HC content with MS. The TNPH content was about one order smaller at site B compared to site A. This could be explained by a larger mobility of HC phases within the groundwater leading to a faster removal from this zone. The residence time at site B, however, is probably long enough to enable new formation of magnetite due to microbial activity. Due to mobility, the TNPH concentration might have been much higher at an earlier time even leading to toxic effects for microorganism. These scenarios may explain why magnetic mineral concentration was enhanced at site B compared to the non-contaminated site C, but is without any noticeable correlation of MS with actual TNPH contents. It should be also noted that dissolution of magnetic minerals in water saturated soil samples (Grimley and Vepraskas, 2000) could contribute as well to the missing correlation.

The samples from site A had higher total crystalline and bioavailable iron contents than samples from site B. At site B, the water-saturated condition might have led to an outwash of fine iron particles from this site and hence to a lower total iron content. Furthermore, there might be a difference in iron mineral (trans)formation processes in HC contaminated soils/sediments with and without water saturation which might play an important role for creating such differences in the total bioavailable iron content. However, despite the lower iron content at the contaminated site B, MPN measurements showed that both FeOxM and FeRedM are present in samples from this site. Under iron(III)-reducing conditions, the degradation of organic contaminants such as aromatic hydrocarbons can be directly coupled to microbial iron(III) reduction (Lovley and Anderson, 2000). Similar cell numbers for FeRedM as for our site B samples were determined by the MPN method in the iron reduction zones of gasoline contaminated soils (10^3 cells/g soil; Kao et al., 2001) and in crude oil contaminated aquifer sediments (10^4-10^6 cells/ g sediment; Bekins et al., 1999). The similar cell numbers of FeOxM and FeRedM in our samples suggest that FeOxM were stimulated/ influenced by the geochemical conditions to the same extent as the FeRedM. Furthermore, both groups of microorganisms are linked to each other by the iron redox cycle. Microbial cycling of iron under anoxic conditions coupled to the complete oxidation of benzoate was shown by Straub et al. (2004). Aerobic Fe(II)-oxidizers could be coupled to Fe(III) reduction at oxic-anoxic interfaces.

6. Conclusions

The present study, which was done using HC contaminated soils and sediments from the former oil field in Hänigsen, revealed that there is a reasonable relationship between the enhancement of magnetite concentration and the content of hydrocarbons, in particular total non-polar hydrocarbons. FeOxM and FeRedM were present and might have played an important role in the (trans)formation of iron minerals in HC contaminated soils and sediments. Newly formed magnetite had a significant contribution in the single domain grain size range. The relationship between HC content and magnetic parameters was not quite clear in the case of HC contaminated sediment samples. Magnetite, however, was obviously formed as we detected a significant increase of magnetic concentration parameters compared to a clean nearby site with similar lithology. High mobility of hydrocarbons, as well as magnetite dissolution or suppression of magnetite formation due to water saturation, might explain the missing direct correlation between magnetic concentration and TNPH. We concluded from our results that HC contamination can be delineated by measuring magnetic proxy parameters, however, the processes are complex and more field sites and laboratory studies are required to evaluate the variability and general characteristics of the effects.

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Supporting information

Supporting information S1: Most probable number (MPN) enumeration of FeOxM and FeRedM

All labware used during this procedure was sterile, and all solutions were sterile and anoxic. For sample B1, B3 and B5, 1 g of soil was suspended in 2.5 mL (B5) and 5 mL (B1, B3) of 0.09 % NaCl solution in a 20 mL glass vial with N_2 atmosphere. Since sample B3 and B5 could not be suspended due to their very high crude oil content, two sterile glass beads were added to the vials to facilitate removal of cells from the soil and the samples were horizontally shaken for 6-7 days. The soil suspension was consecutively diluted eleven times 1:10 with freshwater medium (see below) to obtain a dilution series of 12 dilutions. Per targeted microorganism group and soil sample, a 96 well plate was filled with selective growth medium (see below) in a glovebox (100 % N_2) and from each of the 12 dilution 100 µL were added to each well of one plate column except the last well (Figure S1). By increasing the dilution from the left to the right of the plate, twelve 1:10 dilution steps each with seven parallels were obtained. The last row of the plate served as sterile negative control. The plates were sealed with a sterile polyester foil (Roth) and incubated in airtight plastic incubation bags with an oxygen-removing and CO_2-producing unit (Anaerocult®A mini, Merck) in the dark at 20 °C for 17 weeks. The MPN of cells in a sample is statistically calculated based on the number of parallels of a 1:10 dilution series showing growth. For plates containing FeOxM selective media, positive wells were identified by a color change from white to greenish-gray. For the FeRedM plates a ferrozine assay (Stookey, 1970) of one aliquot per well was performed and wells with twice as high iron(II) concentration than the average iron(II) concentration of the control wells were counted as positive.

The vitamins and trace elements containing freshwater medium (Straub et al., 2005) was buffered to pH 7.2 with 30 mM $NaHCO_3$ and used with a headspace of N_2:CO_2 (90:10). The selective growth medium of the FeOxM consisted of freshwater medium with 10 mM $FeCl_2$, 4 mM $NaNO_3$ and 0.5 mM Na-acetate. For the selective growth medium of the FeRedM the freshwater medium contained 5 mM ferrihydrite and the following electron donor mix: 5 mM Na-acetate, 5 mM Na-lactate, 10 mM Na-formate, 2 mM Na-propionate and 2 mM Na-butyrate. The ferrihydrite was synthesized according to (Raven et al., 1998), but was washed with water instead of NaCl solution.

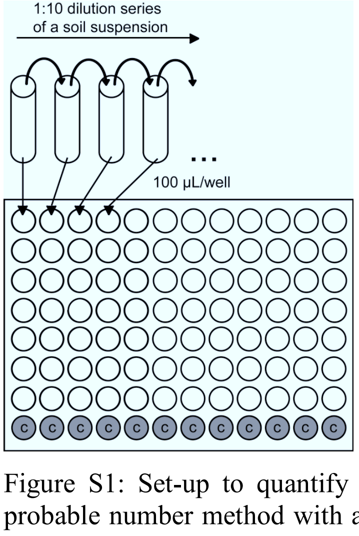

Figure S1: Set-up to quantify cell numbers of FeOxM and FeRedM in 96 well plates by the most probable number method with a dilution series of 12 dilutions and 7 parallels. The last row of the plate serves as sterile negative control. For details see supporting information S1.

4

In-situ magnetic susceptibility measurements as a tool to follow geomicrobiological transformation of Fe minerals

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In-Situ Magnetic Susceptibility Measurements As a Tool to Follow Geomicrobiological Transformation of Fe Minerals

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Fe minerals sorb nutrients and pollutants and participate in microbial and abiotic redox reactions. Formation and transformation of Fe minerals is typically followed by mineral analysis at different time points. However, in lab studies the available sample amount is often limited and sampling may even influence the experimental conditions. We therefore evaluated the suitability of in situ magnetic susceptibility (MS) measurements, which do not require sampling, as an alternative tool to follow ferro(i)magnetic mineral (trans-)formation during ferrihydrite reduction by *Shewanella oneidensis* MR-1, and in soil microcosms. In our experiments with MR-1, a large initial increase in volume specific MS (κ) followed by a slight decrease correlated well with the initial formation of magnetite and further reduction of magnetite to siderite as also identified by μ-XRD. The presence of humic acids retarded magnetite formation, and even inhibited magnetite formation completely, depending on their concentration. In soil microcosms, an increase in κ accompanied by increasing concentrations of HCl-extractable Fe occurred only in microbially active setups, indicating a microbially induced change in soil Fe mineralogy. Based on our results, we conclude that MS measurements are suitable to follow microbial Fe mineral transformation in pure cultures as well as in complex soil samples.

Introduction

Fe minerals are ubiquitous in the environment. Under anoxic and pH neutral conditions Fe(II) minerals such as Fe(II) carbonate (siderite), Fe(II) phosphate (vivianite), or mixed-valent Fe minerals (e.g., magnetite) are present. At neutral pH, Fe(III) exists mainly as Fe(III) (hydr)oxides and oxyhydroxides such as ferrihydrite (Fh), hematite and goethite (*1*). Fe(II) can be oxidized abiotically by MnO_2, nitrite and molecular O_2 (*2–4*) or biotically by anaerobic phototrophic and nitrate-reducing, as well as aerobic Fe(II)-oxidizing microorganisms (*5, 6*). Reduction of Fe(III) minerals can occur under anoxic conditions abiotically by sulfide and reduced humic substances (*7, 8*) or biotically by Fe(III)-reducing microorganisms (*9*). The type of Fe minerals formed during Fe redox reactions depends on geochemical parameters such as pH, rate of Fe^{2+} supply, and presence of other ions (*10–12*).

Abiotic and microbial Fe mineral (trans)formation processes are intensively studied due to their environmental relevance. For example, under Fe(III)-reducing conditions, the degradation of organic contaminants is directly coupled to microbial Fe(III) reduction (*13*). Furthermore, Fe minerals affect the mobility of nutrients and toxic compounds. Arsenic, for example, coprecipitates during Fe(III) mineral formation (*14*) and gets released when these minerals are dissolved (*15*).

Experimental studies aiming to understand the biogeochemical processes involved in Fe mineral (trans)formation face several problems. First, to follow Fe mineral (trans)formation over time, e.g. by X-ray diffraction, spectroscopy or by sequential Fe extraction, it is necessary to sample at different time points, which might disturb the experimental system due to shaking during the sampling procedure. Second, sampling removes significant amounts of the sample volume which may influence further mineral (trans-)formation. This is particularly relevant for Fe mineral identification by sophisticated techniques such as X-ray diffraction, Mössbauer spectroscopy, or X-ray absorption spectroscopy that require significant sample amounts (*16*). Third, in many cases, access to these techniques is limited and the measurements, including sample preparation and data analysis, are time-consuming. Fourth, the content of a formed Fe mineral might be under the detection limit for some of these techniques, especially when complex samples (e.g., soils, sediments) with a high content of other minerals (e.g., clay minerals, quartz) are studied. Therefore, it would be helpful to have an analytical technique that can follow Fe mineral (trans)formation and identify formed Fe minerals, even in small amounts, without sampling and large technical effort.

Magnetic susceptibility (MS) measurements might be such an alternative for systems in which ferro(i)magnetic Fe minerals are (trans)formed. MS describes how strongly a substance is magnetized in an external magnetic field. Diamagnetic materials (e.g., quartz, water) have a small, negative MS. Paramagnetic minerals (e.g., siderite, ferrihydrite) and antiferromagnetic minerals with spin-canting (hematite) or defect moments (goethite) have a small, positive MS, whereas ferromagnetic elements (e.g., metallic Fe) and ferrimagnetic minerals have a very high (e.g., magnetite, maghemite, greigite), or moderately high (e.g., pyrrhotite), positive MS (*17*). For simplicity the term ferro(i)magnetic minerals is used in this study and it refers to ferrimagnetic minerals and antiferromagnetic minerals with spin-canting or defect moments. Although MS of a sample is a bulk signal of all compounds, ferro(i)magnetic minerals dominate the MS or, if their concentration is very low, also paramagnetic Fe phases may be important. However, MS of a sample does not only depend on the concentration but also on the size (more precisely: magnetic domain state) and shape of these minerals (*18*).

Analysis of magnetic properties of dried minerals formed by microbial Fe(III) reduction has been used before to quantify the amount of magnetite formed (*19, 20*). Hence,

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this method might be also applicable for in situ measurements in cultures of Fe-metabolizing microorganisms or in soil microcosms. The aim of this study was to evaluate whether in situ MS measurements can be used (i) to monitor ferro(i)magnetic mineral formation in pure cultures of Fe(III)-reducing microorganisms, (ii) to determine how humic acids influence ferro(i)magnetic mineral formation in these cultures, and (iii) to follow Fe mineral (trans)formation in soils by MS measurements.

Materials and Methods

Ferrihydrite, humic acids. Fh was synthesized according to (*21*), washed four times with MilliPore water, deoxygenated by repeated evacuation and N_2-flushing, and autoclaved. Pahokee peat humic acids (PPHA) were purchased from the International Humic Substances Society. For a stock solution, 5 mg/mL PPHA were dissolved in 88.6 mM NaCl solution and the pH adjusted to 7.0 with NaOH. The solution was filtered (0.22 μm, mixed cellulose ester) into sterile anoxic (100% N_2) glass bottles.

Bacteria and Growth Media. *Shewanella oneidensis* MR-1 was kept as frozen stock at −80 °C. Cells streaked out on Luria−Bertani (LB) agar plates were incubated oxically for 24 h at 28 °C and stored for up to 10 days at 4 °C. One colony was transferred into 10 mL anoxic freshwater medium containing 20 mM Na-lactate and 40 mM fumarate. After 72 h at 28 °C, 200 μL of this culture were transferred to 10 mL fresh anoxic freshwater medium and incubated for 48 h at 28 °C. LB agar contained per L 10 g tryptone, 5 g yeast extract, 5 g NaCl and 12 g agar. Freshwater medium was prepared as described in ref *22*. LML medium modified from that in ref *23* contained per L 0.2 g yeast extract, 0.1 g peptone and 20 mM Na-lactate. Freshwater and LML medium were prepared anoxically, buffered at pH 7.0−7.1 with 30 mM $NaHCO_3$, and used with a headspace of N_2:CO_2 (90:10).

Experimental Set-up with MR-1. Experiments with MR-1 were performed in 60 mL serum bottles containing 25 mL LML medium, 15 mM Fh and 2 × 10^5 cells/mL. Bottles containing PPHA (final concentration of 210 and 630 mg/L) were equilibrated for 48 h prior to inoculation. The cultures were incubated at 28 °C in the dark.

Experimental Set-up with Soil. Top soil (~20 cm) from the Schoenbuch forest (Sbu) and Fraeulinsberg (Fb), both southwest Germany, was sampled. Selected soil properties are given in Supporting Information (SI) Table S1. Microcosms consisted of 21 g of soil Sbu in 60 mL serum bottles. For sterile set-ups, the soil was autoclaved twice with 2 days of incubation at room temperature in between. Two different set-ups were prepared: addition of (i) 10 mL water, no additional carbon source, and (ii) 10 mL of a lactate/acetate solution (15 mM each). The headspace was flushed with N_2:CO_2 (90:10). The microcosms were incubated at 28 °C in the dark and mixed once per week.

Analytical Techniques. *Fe extraction.* For Fe quantification in MR-1 cultures, 100 μL of culture suspension was extracted in 900 μL of 0.5 M HCl for 2 h at room temperature. After centrifugation (15 min, 20 817*g*), Fe(II) and total Fe (Fe_{tot}) were quantified by the ferrozine assay (*24*) as described in ref *22*. Fe from soil Sbu was extracted before incubation (original soil without amendment) and after incubation. Prior to extraction, the microcosm bottles were centrifuged (10 min, 2000 rpm) and the supernatant was stored in 15 mL sterile plastic cups at −28 °C for dissolved organic and inorganic carbon (DOC, DIC) analysis (see below). Fe extractions from three subsamples of the original soil Sbu and three subsamples of the soil pellets collected after incubation were performed after (*25, 26*) with a soil:extractant ratio (w/v) of 1:50. The first subsample was extracted with Na-acetate (pH 5) for 24 h and the second subsample with 0.5 M HCl for 1 h, both at room temperature on a shaker. The third subsample was extracted with 1 M HCl at 70 °C in a water bath for 24 h and the extracts were cooled for 15 min at room temperature. From all extracts, 1.8 mL was centrifuged (15 min, 20,817 *g*) to remove soil particles. Fe(II) and Fe_{tot} in the supernatants were quantified by the ferrozine assay. In the Na-acetate extracts only Fe_{tot} was quantified.

MS Measurements. Low-field MS of the experimental bottles was measured with a KLY-3 Kappabridge (AGICO, Czech Republic) at room temperature with a peak magnetic field intensity of 300 A/m and a frequency of 875 Hz. The medium and soil in the bottles were entirely placed within the homogeneity range of the pick-up coil. Each bottle was measured three times and the values were averaged. Results in this study are given in volume specific MS (κ) for a reference volume of 10 cm^3. Values of κ are only relative as the effective sample volume cannot be determined. This, however, does not affect the significance of MS results for measurements performed on the same sample over time. In the case of liquid cultures, κ of each bottle measured directly after setting up the experiment was subtracted from all following κ values in order to correct for the bottle, stopper, and medium.

Quantification of Dissolved PPHA. Since the LML medium had a high organic carbon background concentration, dissolved PPHA could not be quantified by DOC analysis in MR-1 cultures. Therefore, 2 mL of sample was centrifuged (2 min, 20 817*g*) and the absorbance of the supernatant was measured at 465 nm in polystyrene microtiterplates with a microplate reader (FlashScan 550, Analytik Jena, Germany). A calibration curve was obtained in the range of 0−500 mg PPHA/L with a quantification limit of 10 mg PPHA/L.

μ-XRD. Fe minerals were identified by μ-X-ray diffraction (μ-XRD) (Bruker D8 Discover XRD instrument, Bruker AXS, Germany). The minerals from one serum bottle were successively centrifuged (2 min, 9700*g*) into a 2 mL plastic cup, washed twice with anoxic water, and dried at room temperature in an anoxic glovebox (100% N_2). Dried minerals were ground in an agate mortar, resuspended in anoxic ethanol, and transferred to a silicon wafer that was covered with a polyethylene foil to prevent oxidation of the minerals during measurements under oxic conditions.

Dissolved Carbon Measurements. The supernatant sampled from the soil microcosms was thawed, centrifuged (10 min, 5000*g*) and filtered (0.22 μm, mixed cellulose esters). The DOC and DIC contents were determined with a carbon analyzer (high TOC, Elementar, Germany).

Results and Discussion

Magnetic Mineral Formation by *Shewanella oneidensis* MR-1. In order to determine if MS measurements can be used to follow ferro(i)magnetic mineral formation in pure cultures of Fe(III)-reducing microorganisms, reduction of 15 mM Fh by *S. oneidensis* MR-1, an Fe(III)-reducer known to be able to produce magnetite during Fh reduction, was followed over time by Fe(II) quantification, MS measurements and μ-XRD mineral analysis. The amount of extractable Fe(II) increased strongly during the first 5 days, followed by a slower increase to a value of 66.0 ± 0.9% Fe(II):Fe_{tot} at day 18 (Figure 1A), demonstrating Fh reduction by MR-1. During incubation with MR-1, the orange-brown color of the Fh turned dark brown to black (SI Figure S1) and in contrast to Fh, these minerals did not dissolve completely in 0.5 M HCl, indicating that transformation of Fh to another less soluble Fe-phase took place. The volume specific MS (κ) increased strongly to 7803 ± 26 μSI between days 2 and 5 (Figure 1B), indicating the formation of a ferro(i)magnetic mineral phase. The maximum κ of 7910 ± 173 μSI was measured after 7 days. The following slow decrease until day 18 to 6896 ± 170 μSI suggested that the formed magnetic mineral was probably further reduced by MR-1 and transformed into another Fe(II)-phase. This is supported by the accompanying slow increase

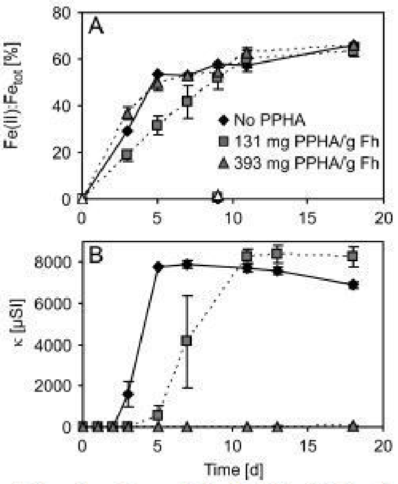

FIGURE 1. Reduction of 15 mM ferrihydrite (Fh) by *Shewanella oneidensis* MR-1. (A) Changes in the Fe(II):Fe$_{tot}$ ratio determined by 0.5 M HCl extraction and (B) changes in volume specific magnetic susceptibility (κ) in absence of PPHA (◆), in presence of 131 mg PPHA/g Fh (all PPHA sorbed, gray solid square), and in presence of 393 mg PPHA/g Fh (PPHA sorbed and in solution, gray solid triangle). Open symbols in (A) represent sterile controls. Results are means of duplicates. Bars bracket the range of duplicates.

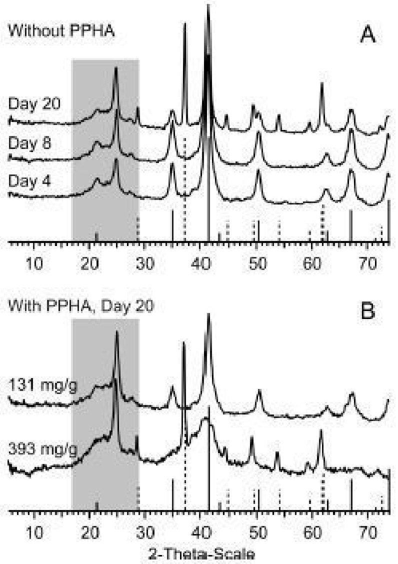

FIGURE 2. μ-XRD patterns of minerals formed during reduction of 15 mM ferrihydrite (Fh) by *Shewanella oneidensis* MR-1. (A) Minerals formed in absence of PPHA after 4, 8, and 20 days of incubation. (B) Minerals formed after 20 days of incubation in presence of 131 mg PPHA/g Fh (all PPHA sorbed) and 393 mg PPHA/g Fh (PPHA sorbed and in solution). Reference lines represent magnetite (solid lines) and siderite (dashed lines). The gray bar indicates the range where signals appear from the foil used to cover the samples to prevent oxidation by oxygen.

in Fe(II) concentration and siderite formation (see below). However, it has to be noted that the decrease in κ might also be caused by an increase of grain size; if part of the magnetite particles grow above the critical volume of the superparamagnetic to single domain transition, the overall κ value of the sample could decrease because of the lower κ of single domain particles compared to the smaller superparamagnetic grains.

In abiotic controls, neither the formation of Fe(II) (Figure 1A) nor an increase in κ was observed (data not shown). Control experiments with MR-1 cultures subjected seven times to MS measurements in comparison to cultures which were subjected only twice to MS measurements revealed that MS measurements did not influence Fh reduction by MR-1 and hence are noninvasive for the microorganisms (SI Figure S2).

Fe(II) and κ data of bottles that were set up in parallel for μ-XRD analysis were similar to the data shown in Figure 1 (data not shown). For μ-XRD mineral analysis, bottles were sacrificed either after 4 days (during the strong increase in κ), 8 days (approximately maximum κ) or 20 days (after the slight decrease of κ) of incubation, respectively. μ-XRD measurements revealed that at day 4 and 8 only magnetite was present as crystalline Fe mineral phase, whereas at day 20, siderite was also detected (Figure 2A). Depending on the Fe(III) reduction rate and the presence of other ions, microbial Fh reduction can also lead to the formation of other Fe minerals such as goethite and hematite (*10*). However, besides magnetite and siderite we did not detect any other Fe minerals by μ-XRD measurements (Figure 2A). Hence, the initial increase in κ was due to the formation of magnetite (a ferrimagnetic Fe(II)–Fe(III)-mineral with a high κ). Magnetite formation during microbial amorphous Fe(III) mineral reduction was first shown by Lovley et al. (*27*). Analysis of magnetic properties of magnetite formed by the Fe(III)-reducing bacterium *Geobacter metallireducens* revealed that the magnetite particles covered a broad grain size distribution (*28*). Since the κ value of ferro(i)magnetic minerals depends on their size and shape (*17*), analysis of the content of these minerals in microbial cultures using MS data is only semiquantitatively possible (see example for such a calculation in the SI S1, Figure S3). In order to determine the exact amount of magnetite formed, further analyses using different methods such as measurements of the saturation magnetization are necessary, as illustrated in SI S1.

The detection of siderite (a paramagnetic mineral with a low κ) by XRD at the end of the experiment (Figure 2A) confirmed that part of the formed magnetite was further reduced, as indicated by the decrease of κ at day 18. This is in line with previous research showing reduction of magnetite by *S. oneidensis* MR-1 (*29, 30*). Microbial formation of magnetite and siderite in bicarbonate buffered medium was also observed by Fredrickson and co-workers (*12*) during reduction of 45 mM hydrous ferric oxide by *Shewanella putrefaciens* CN32. However, since the authors identified the minerals only after 20 days of incubation, they could not determine if magnetite was first produced and then further reduced to siderite or if siderite was an initial reaction product. In a follow up study Dong et al. (*29*) showed that *S. putrefaciens* CN32 can indeed reduce biogenic magnetite to some extent and that siderite is formed during this reduction in bicarbonate buffered medium. However, Zachara et al. (*10*) also performed reduction experiments with Fh (20 mM) and *S. putrefaciens* CN32 and observed that depending on the N_2:CO_2 ratio either only magnetite (up to 95:5 N_2:CO_2), a mix of Fe minerals (90:10), or only siderite (80:20) was formed. They concluded that siderite is not formed via magnetite, but that high bicarbonate concentrations prevent Fe(II) adsorption on Fh, a process that is necessary for Fh conversion into magnetite. In contrast, our κ values and μ-XRD data indicated that magnetite was formed at the beginning of the Fh reduction and was then further reduced to siderite. Overall, this experiment showed that MS measurements are suitable to follow microbial magnetite (trans)formation in pure cultures of *S. oneidensis* MR-1 and, in combination with other techniques (e.g., measurements of the saturation magnetization), can give insights into Fe mineral (trans)formation pathways on even a quantitative basis.

Influence of PPHA on Microbial Magnetite Formation. In order to follow ferro(i)magnetic mineral formation by MS measurements in more complex systems such as soils, geochemical factors influencing magnetite formation need to be evaluated. Humic substances are ubiquitous in the environment. They influence microbial Fe(III) reduction and Fe mineral transformation by complexation of Fe(II) and Fe(III), by facilitating electron transfer from the cells to Fe(III) minerals (electron shuttling), and by sorption to Fe minerals (*31–34*). We therefore determined the influence of PPHA on Fh reduction and magnetite formation by *S. oneidensis* MR-1. Two concentrations of PPHA were chosen in order to have one set-up where the PPHA were virtually completely sorbed to the Fh (131 mg PPHA/g Fh) and one where a significant fraction of the PPHA remained in solution (393 mg PPHA/g Fh with 152 ± 34 mg dissolved PPHA/g Fh). The two PPHA concentrations were selected based on a sorption isotherm of PPHA to Fh (SI Figure S4).

The extent of Fh reduction after 18 days of incubation was similar in set-ups without PPHA addition, with all PPHA sorbed, and with PPHA sorbed but also present in solution (ratio of Fe(II):$\mathrm{Fe_{tot}}$ of 63.4 ± 2.2% to 66.0 ± 0.9%, Figure 1A). However, in the set-up with only sorbed PPHA, the Fh reduction rate was slower than in the absence of PPHA, whereas in the system containing both sorbed and dissolved PPHA, the Fh reduction rate was similar to that without PPHA. In set-ups with only sorbed PPHA (131 mg PPHA/g Fh), a black mineral was formed within 25 days (SI Figure S1). The supernatant in set-ups containing 393 mg PPHA/g Fh was initially dark brown due to the presence of dissolved PPHA. During Fh reduction, the medium in these set-ups turned light brownish indicating significant removal of PPHA from solution and after 25 days of incubation a black mineral was formed.

Although the extent of Fh reduction (Figure 1A) and the color of the formed minerals were similar independent of the added PPHA concentration (SI Figure S1), MS measurements revealed significant mineralogical differences between the different set-ups. In the case of only sorbed PPHA, the κ value started to increase 2 days later and the increase was slower than in the absence of PPHA (Figure 1B). The maximum κ of 8373 ± 439 μSI measured after 13 days was only slightly higher than in set-ups without PPHA, indicating that a similar amount of magnetite was formed in both set-ups. However, in contrast to the set-ups without PPHA, no significant decrease in κ occurred after the maximum κ was reached. In set-ups where both sorbed and dissolved PPHA were present, κ increased by only 34 ± 4 μSI over 18 days, indicating that no ferro(i)magnetic mineral (i.e., no magnetite) was formed. μ-XRD analysis of minerals formed in parallel set-ups supported the κ data. In set-ups containing only sorbed PPHA, only magnetite and no siderite was present after 20 days of incubation confirming the κ values that remained stable after the initial increase and indicating that no further reduction of magnetite occurred within the incubation time. Since the experiments were terminated after 18 days, the set-ups containing only sorbed PPHA were incubated for only another 5 days after reaching the maximum κ, in contrast to 11 days for set-ups without PPHA (Figure 1B). Thus, a potential further transformation of the magnetite into siderite in the presence of only sorbed PPHA after a longer incubation cannot be ruled out. When both sorbed and dissolved PPHA were present, only siderite was identified as a crystalline Fe mineral phase, which supports the small increase in κ. The dark color of the minerals in these set-ups was probably due to sorption of the PPHA to the siderite, which is usually white in color.

In our experiments, the addition of PPHA affected the Fh reduction rate, but not the extent of reduction (Figure 1A). It is known that the addition of humic and fulvic acids can increase Fe reduction rate by electron shuttling (*31, 33, 35*). Jiang and Kappler (*35*) determined that a minimum amount of ~10 mg/L dissolved PPHA must be present to stimulate Fh reduction by *S. oneidensis* MR-1, whereas Wolf et al. (*36*) showed that already 1 mg/L of groundwater aquifer humic acids was sufficient to stimulate Fh reduction by *Geobacter metallireducens*. In our experiments with 131 mg PPHA/g Fh, the amount of dissolved PPHA was <0.5 mg/L and therefore no electron shuttling and stimulation of Fh reduction was expected. The sorption of PPHA to Fh even had a negative effect on the reduction rate. In set-ups where a significant amount of PPHA was initially present in solution (152 ± 34 mg dissolved PPHA/g Fh = 236 ± 66 mg dissolved PPHA/L), the inhibitory effect of the sorbed PPHA was overcome and the reduction rate was as high as without PPHA added (Figure 1A). This suggests that *S. oneidensis* MR-1 used the dissolved PPHA to at least some extent as electron shuttle. However, despite the high concentration of initially dissolved PPHA, no stimulation of Fh reduction occurred in comparison to set-ups without PPHA. This is probably due to the fact that the initially dissolved PPHA were to a large extent removed from solution during Fh reduction as indicated by the color change of the solution from dark to light brown (SI Figure S1). This indicates that less electron shuttling molecules were available and therefore the stimulating effect of dissolved PPHA was reduced.

As indicated by both μ-XRD and MS analysis, the two different concentrations of PPHA had different effects on secondary mineral formation. In set-ups with only sorbed PPHA the Fe(II):$\mathrm{Fe_{tot}}$ ratio on the day at which the κ value started to increase was approximately 29.2 ± 0.0%. A similar Fe(II):$\mathrm{Fe_{tot}}$ ratio (31.6 ± 4.1%) was found in set-ups without PPHA when the increase in κ started. These Fe(II):$\mathrm{Fe_{tot}}$ ratios are comparable to that of stoichiometric magnetite (Fe(II):$\mathrm{Fe_{tot}}$ = 33%) suggesting that a threshold Fe(II):$\mathrm{Fe_{tot}}$ ratio of around 30% must be reached to initiate magnetite formation. Since Fh reduction in set-ups with only sorbed PPHA was slower than in PPHA-free set-ups, the Fe(II):$\mathrm{Fe_{tot}}$ threshold ratio was reached later, leading to a later start of magnetite formation and a slower magnetite formation rate (Figure 1B). In the case where both sorbed PPHA and dissolved PPHA were present, no magnetite formation occurred at all, suggesting that the humic acids completely inhibited magnetite formation from Fh. Magnetite forms from Fh either via dissolution-reprecipitation or solid state conversion. For both pathways, adsorption of Fe(II) to Fh is necessary (*37, 38*). In contrast to the experiments without PPHA and with only sorbed PPHA (where part of the Fh was not covered by PPHA and therefore available for the conversion into magnetite), all sorption sites were obviously blocked when PPHA were present in excess, and therefore no transformation of the remaining Fh into magnetite was possible.

Fe mineral Transformation in Soil Monitored by MS Measurements. After the experiments with pure cultures of *S. oneidensis* MR-1 demonstrated that MS measurements are suitable to follow microbial Fe(III) mineral transformation, in particular magnetite formation and transformation, we determined whether this technique can also be used to monitor changes in the ferro(i)magnetic Fe mineralogy of soils. To this end, microcosm experiments with soil Sbu were set up either without the addition of organic carbon or with the addition of lactate/acetate. Lactate and acetate are easily degradable by microorganisms and were added to determine if Fe mineral (trans)formation in the soil Sbu was limited by the bioavailable organic carbon content.

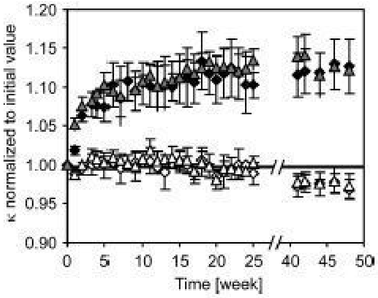

FIGURE 3. Changes in volume specific magnetic susceptibility (κ) over time of microbially active soil microcosms amended with 10 mL of water (◆) and 10 mL of 15 mM lactate/acetate solution (gray solid triangle). Open symbols represent sterile microcosms. The κ values measured at each time point were normalized by the κ value measured directly after setting up the microcosms. Solid horizontal line indicates κ without any change over time. Results are means of four replicates. Bars indicate the standard deviation.

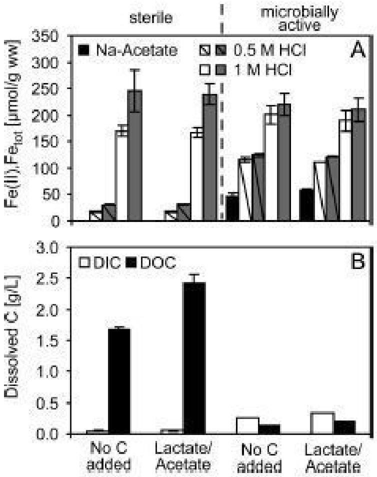

FIGURE 4. (A) Fe extracted with Na-acetate (pH 5, room temperature), 0.5 M HCl (room temperature) and 1 M HCl (70 °C) from pellets obtained after centrifugation of sterile and microbially active soil microcosms amended with 10 mL of water (no C added) and 10 mL of 15 mM lactate/acetate solution. Open bars represent Fe(II), filled bars $\mathrm{Fe_{tot}}$. Results are means of triplicate measurements of one bottle per set-up. Bars indicate the standard deviation. (B) Dissolved inorganic (DIC) and organic carbon (DOC) in the supernatants taken from the same bottles as in (A) after centrifugation. Results are means of duplicates. Bars bracket the range of duplicates.

The κ value of sterile microcosms did not change considerably over time (Figure 3). In contrast, the κ value of the microbially active microcosms increased significantly within the first 5−6 weeks followed by a slower increase until week 20 when the increase in κ leveled off. The extent of κ increase was not statistically different between microbially active set-ups either with or without organic carbon amendment, that is, 12.2 ± 2.9% for microcosms without carbon addition and 12.9 ± 1.6% for microcosms with lactate/acetate (calculated by averaging the last five κ measurements). Before the microcosms were set up, different Fe fractions were extracted from the field wet soil Sbu with Na-acetate ($\mathrm{Fe_{tot}}$: 1.1 ± 0.1 µmol/g wet weight (ww)), 0.5 M HCl ($\mathrm{Fe_{tot}}$: 27.9 ± 1.4 µmol/g ww, Fe(II): 4.0 ± 0.1 µmol/g ww) and 1 M HCl at 70 °C ($\mathrm{Fe_{tot}}$: 231.3 ± 1.0 µmol/g ww, Fe(II): 161.3 ± 4.3 µmol/g ww). After approximately one year the microcosms were opened and the different Fe fractions of the incubated soil were determined. For each extracted fraction, the $\mathrm{Fe_{tot}}$ and Fe(II) contents of the sterile microcosms were similar to the content of the original soil Sbu (Figure 4A), indicating that autoclaving, as well as incubation for approximately one year at 28 °C did not change the soil Fe redox speciation and mineralogy significantly. The $\mathrm{Fe_{tot}}$ extracted with 1 M HCl at 70 °C was similar in both sterile and microbial active microcosms showing that the total Fe content was similar in all bottles. However, with the weaker extractants (Na-acetate and 0.5 M HCl) that quantify sorbed and poorly crystalline (bioavailable) Fe, more $\mathrm{Fe_{tot}}$ was extracted from soil Sbu of microbially active set-ups than from sterile ones. Furthermore, the Fe(II):$\mathrm{Fe_{tot}}$ ratio of the Fe fractions extracted with 0.5 M HCl and 1 M HCl at 70 °C was much larger in the microbially active set-ups than in the sterile ones. Both MS measurements and Fe extraction data showed that (i) the changes in κ were due to a change in the Fe mineralogy of the soil and (ii) that these changes were microbially driven. Since in the microbially active set-ups the κ values increased over time, the concentration of ferro(i)magnetic Fe minerals in the soil Sbu must have increased. The most important ferro(i)magnetic minerals in soils are magnetite ($\mathrm{Fe_3O_4}$) and maghemite (γ-$\mathrm{Fe_2O_3}$) (*18*) and one or both of these minerals might have been formed in the microbially active microcosms. In order to determine the sensitivity of our method, in an additional experiment soil from Fraeulinsberg (SI Table S1) was mixed with varying amounts of synthetic magnetite and κ was determined. We found that an increase in the amount of Fe present in the form of magnetite relative to the total amount of Fe in the soil by only 1 wt.%, increased the soil κ value by about 55% (not shown). The κ of the microbially active microcosms changed by only ~10% (Figure 3), showing that MS measurements are a very sensitive tool able to detect even very small changes in the ferro(i)magnetic mineral content of soils.

Changes in κ and Fe speciation/mineralogy were similar in microbially active set-ups without organic carbon addition and with lactate/acetate addition, suggesting that organic carbon was bioavailable even without addition of lactate/acetate. Dissolved organic matter represents the most bioavailable organic carbon source in soils (*39*) and was probably leached from soil Sbu in the microcosms by the added water. In order to evaluate this hypothesis, we quantified DOC and DIC in the soil microcosms. In sterile microcosms amended with lactate/acetate, the DOC was 0.8 g/L higher than in sterile microcosms without carbon addition (Figure 4B). Lactate/acetate were both added at a concentration of 15 mM yielding a theoretical DOC of 0.9 g/L, thus 89% of the added carbon was recovered in the soil−water after one year (the rest was probably sorbed to soil particles such as Fe minerals (*40*)). In both organic carbon amended and nonamended microbially active set-ups the DOC was lower than 0.2 g/L, indicating efficient consumption of the bioavailable organic carbon by the microorganisms. Organic carbon mineralization was also evidenced by the DIC present in nonsterile compared to sterile set-ups. The DIC in the water of both the sterile set-ups was 0.05 ± 0.01 g/L, whereas in the microbially active set-ups it was much higher, varying between 0.25 ± 0.01 and 0.33 ± 0.00 g/L (Figure 4B).

The DOC, DIC, and κ values suggest that even without lactate/acetate addition, a large amount of bioavailable organic carbon was present. Although more DOC was initially present in set-ups with added lactate/acetate, this did not lead to more Fe mineral (trans)formation, indicating that either the bioavailable amount of Fe was limiting, or that the addition of lactate/acetate also stimulated microorganisms which were not involved in Fe redox reactions.

Application and Environmental Significance of in Situ MS Measurements. Fh reduction experiments with *S. oneiden-*

sis MR-1 showed that in contrast to wet-chemical Fe extraction data, in situ MS measurements were able to precisely detect the beginning of magnetite (trans)formation. Since MS measurements are fast, do not influence microbial activity, and can be done in situ and therefore do not require sampling, a high time resolution can be obtained. This allows the identification of sampling points for more detailed analyses that require more analytical effort (e.g., sequential Fe extraction, XRD, synchrotron-based analysis). The combination of MS measurements with these techniques can help to determine biogeochemical conditions for ferro(i)magnetic mineral (trans)formation and to identify the minerals present at different time points. Furthermore, the influence of environmentally relevant factors (e.g., humic substances) on ferro(i)magnetic mineral (trans)formation can easily be determined.

MS measurements over time of soil microcosms revealed that changes in κ can be used to follow microbial Fe mineral transformation processes even in complex environmental systems. The results showed that microbial Fe mineral transformation is accompanied by a consumption of bioavailable organic carbon. The mobilization of organic carbon by water as observed in our soil microcosms is unlikely to happen in the environment in well drained soils as well as in organic carbon poor soils. However, the input of organic carbon to these soils, for example, in form of hydrocarbons or other organic contaminants, might induce a similar microbially driven change in the ferro(i)magnetic soil mineral content to that caused by the mobilization of organic carbon. Field studies in hydrocarbon-contaminated soils and areas with natural oil reservoirs have indeed shown differences in the soil mineralogy compared to uncontaminated areas, including differences in the ferro(i)magnetic mineral content (for a review see ref *41*). Since surface MS measurements in the field can be performed quickly and easily, large areas can be screened with a high spatial resolution. We therefore believe that MS measurements can serve as a fast and inexpensive tool to localize hydrocarbon contaminated areas in the field.

Acknowledgments

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Supporting Information Available

Soil properties and the methods for their determination (Table S1), images of *S. oneidensis* MR-1 culture bottles over time (Figure S1), influence of MS measurements on Fh reduction by MR-1 (Figure S2), a description of the quantification of magnetite formed during Fh reduction by MR-1 (S1), temperature dependent MS measurement of magnetite (Figure S3) and sorption of PPHA to Fh (Figure S4). This material is available free of charge via the Internet at http://pubs.acs.org.

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Remediation processes driven changes on magnetic properties of hydrocarbon contaminated sediments from unsaturated zone, Hradcany, Czech Republic (manuscript in preparation)

Remediation processes driven changes on magnetic properties of hydrocarbon contaminated sediments from unsaturated zone, Hradcany, Czech Republic (manuscript in preparation)

Moti L. Rijal, Erwin Appel, Eduard Petrovský

Abstract

This study was conducted in the area of the former military site, Hradčaný, which lies about 100 km northwest of Prague, Czech Republic. Sediments were severely contaminated with jet fuel and the area was under remediation more than 10 years. The present paper focuses on remediation processes-derived changes on magnetic properties of sediments from 20 cm to maximum 1 m depth of the vadose zone by investigation of three sediment sections; two from the remediated area and one from the non-remediated area. Samples were measured for magnetic susceptibility (MS), frequency dependent MS ($\kappa_{fd\%}$) isothermal remanent magnetization (IRM), anhysteretic remanent magnetization (ARM), temperature dependent MS and hydrocarbon content.

The results demonstrate that MS is high in those samples that still contain a comparatively high amount of hydrocarbons. Both mass-specific MS (χ) and ARM (χ_{ARM}) profiles are almost the same suggesting that MS is mainly controlled by single domain particles and the remediation process has least influence on $\chi_{ARM.}$ However, $\kappa_{fd\%}$ revealed an increase of superparamagnetic particles in comparison to the non-remediated site and also show an enhancement of S-ratios obtained at reverse fields of 100 and 200 mT suggesting a relative increase of relatively harder coercive magnetic minerals in the remediated sites. Samples contained magnetite as the main carrier of the magnetic signal at the remediated site. A positive correlation with a second order parabolic fitting between χ and the total hydrocarbon content of the samples from all three sites shows that the relationship between these two parameters is quite complicated. Some possible reasons of having such a complicated relationship between these two parameters are due to coupling effect of several processes such as soil venting, air sparging, soil vapour extraction in combination with phase transformation, biodegradation and biotransformation. All these processes possibly contributed to derive such magnetic mineralogical changes. Thus, there is an indication of differences of magnetic properties of hydrocarbon remediated sediments and non-remediated sediments where magnetic proxies can be used for sire investigation. Furthermore,

the relationship between MS and hydrocarbon content could also provide an alternative way of modeling mineralogical changes in the vadose zone for hydrocarbon-contaminated and remediated sites.

1. Introduction

Environmental pollution of the geosphere by anthropogenic activities is an important issue, which is directly related with health issues of human and other living organisms. There are several types of contaminants such as heavy metals, radiogenic elements and organic contaminants. Hydrocarbons are one important type of contaminants. They are very hazardous due to their ubiqueous presence in the environment, high water solubility, degradation pathways, persistence for a long time period and modification due to their biogenic transformations and chemical reactions. There are several thousands of hydrocarbon-contaminated sites around the world which were caused by different types of anthropogenic activities such as oil exploration, oil pipeline leakage, underground oil storage tank leakage etc. This causes pollution of soil, sediments and water resources of surrounding areas.

Magnetic methods have been widely used for monitoring of soil, water and air pollution in different environmental settings (Petrovský and Ellwood, 1999). They provide a very fast, reliable, non-destructive assessment (Thompson and Oldfield, 1986). There are several studies which have been conducted using environmental magnetic methods for roadside pollution of soils from traffic-related sources (Gautam et al., 2005), soil pollution by coal burning power plants (Kapička et al., 1999) and metallurgical industries (Hanesch and Scholger, 2002). All these studies are related with heavy metal pollution of soils from different sources.

There are very few magnetic studies focusing on organic contaminants and their relation with magnetic properties. Morris et al. (1994) analyzed sediment cores from Hamilton harbour and obtained a correlation between magnetic susceptibility (MS) and polycyclic aromatic hydrocarbon (PAH) content. Hanesh et al. (2002) showed a correlation between MS and PAH content from soil samples near Donawitz steel mill. Several studies such as by Aldana et al. (2003) have been done to characterize soils, sediments and drill cuttings over oil fields in Venezuela. Liu et al. (2006) reported magnetic enhancement which had been caused by hydrocarbon migration in the Mawagmiao Oil Field, Jianghan Basin China. A recent study by Martins et al. (2007) verified a good correlation between the distribution of MS and hydrocarbons in the Santos Estuary, Brazil.

The present study focuses on a new aspect of applying magnetic methods which has not been attempted before, at least in our knowledge. The study area is a former military site, Hradčaný, where sediments were previously contaminated with petroleum hydrocarbons released into the sub-surface from oil storage tanks. The site was undergoing remediation for 10 years to remove contaminants from the unsaturated zone .The remediation of the unsaturated zone was stopped in 2002 (Machackova et al, 2006). The main hypothesis of our research is that the presence of hydrocarbons, their remediation and biogeochemical processes associated with remediation and hydrocarbons might have caused changes on magnetic properties of the sediments and these changes can be detected with magnetic methods. Therefore, three sediment sections were systematically investigated for different magnetic parameters and their relation with the hydrocarbon content of the sediments. Therefore, our study presents an integral impact of this long term contamination and remediation processes on magnetic properties of sediments from shallow subsurface i.e., from 20 cm to maximum ca. 1.0 m depth.

2. Remediation method

The site was remediated using pump and treat and soil venting techniques until 1997 (Machackova et al., 2008). Then, complex remediation was started combining both physical and biological methods for in situ remediation of petroleum hydrocarbons. The clean up was done in two stages. In the first stage, soil vapour extraction (SVE) was used to remove free oil phase and light non-aqueous phase liquids (LNAPLs), which were recovered by using vacuum pumping, while volatile compounds were removed by venting.

The main in situ remediation process was aerobic bioremediation using indigenous aerobic microbes like *comamonas acidovorans* which was found in soils from this area (Masak et al., 2003). This microbe has an ability to degrade hydrocarbons into biomass, water and carbon dioxide that are harmless substances. Later in the second phase of remediation, air sparging (AS) and main essential nutrients N, P and K which were present naturally in low concentration in the sediments, were added to enhance microbiological activity (Machackova et al., 2005). In this way the nutrient limiting factor for bioremediation which was observed in laboratory batch experiments was overcome (Masak et al., 2003). By combining both SVE (unsaturated zone) and AS (saturated zone) a sufficient amount of oxygen was supplied both into unsaturated and saturated zones to activate bioremediation processes along the whole profile of contaminated sediments. The clean up of unsaturated zone ended in 2002 by reaching the clean-up goal (total petroleum hydrocarbon content in soil/sediment <5000 mg/kg) and since that time SVE has been operating only periodically for measuring respiration intensity in saturated and unsaturated zones (Machackova et al., 2006).

3. Research methodology

3.1. Study area and field sampling

Our study area is located within a former military site which was actively used for military purpose from 1940 to 1991. Due to intensive use of the site for jet fuel storage and fuelling depots, the shallow sub-surface soil and sediments within an area of 28 ha, including the groundwater underneath, was contaminated with petroleum hydrocarbons consisting of 70% of jet fuel (Machackova, 2005). At the study site the upper 4 m consist of Quaternary sandy gravel which is underlain by cretaceous marine sedimenst. Samoles were taken from the eastern part of the contaminated area where contaminants were released into sub-surface due to storage facilities and oil fuelling depots that lasted for about 50 years (Fig. 1). Three different sections A, B and C were sampled. Sediments were sampled by electrical hammering up to maximum 1 m depth. Sub-samples were taken at every 10 cm interval and collected into pre-leveled air-tight plastic bags which were carried to the laboratory for further measurements and analysis.

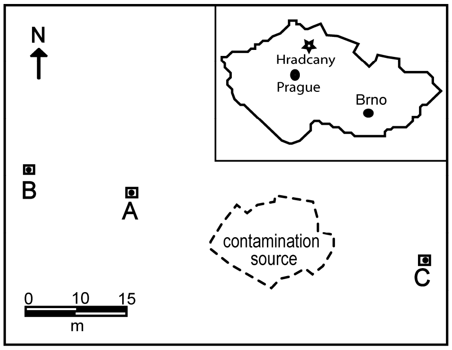

Fig. 1: The map in the inset shows the location of the study area within Czech Republic; the study area, Hradčaný, is indicated by a star. The three sampling sites A, B and C together with one nearby contamination source (within broken line) are indicated. A and B are remediated sites whereas C is the non-remediated site.

3.2 Laboratory measurements

3. 2.1 Magnetic measurements

Sediment samples that were collected from the field were dried at room temperature and packed into 10 cm^3 plastic containers for magnetic measurements. All samples were measured according to the procedures defined by Walden et al. (1999). Volume-specific MS (κ) was measured with a KLY-3 Kappabridge (AGICO), then mass-specific MS (χ) was calculated by normalizing to their corresponding sample mass. Frequency dependent MS ($\kappa_{fd\%}$) was measured with a MFK1 Kappabridge (AGICO) at two different frequencies and a field of 200 A/m field. Afterwards, ARM was induced by aModel 615 ARM magnetizer (2G enterprises) with a peak alternating field of 100 mT and a steady biasing field of 40 μT. IRM was obtained with a Molspin spinner magnetometer after magnetizing samples with a MMPM9 pulse magnetizer

(Magnetic Measurements Ltd.) in successive steps of increasing magnetic field up 1000 mT. IRM at different applied reverse fields were also measured to calculate different S-ratios.

3. 2.2 Analysis of hydrocarbon content

Hydrocarbon content was measured after wet extraction. Hydrocarbon extraction was done with isohexane after adding acetone to well homogenized sediment samples. After extracting with isohexane, florisil was added to remove polar compounds then further extraction was done. Then, the extracted solution was measured with a GC (HP 6890-II)/MS (MS 5973). The compounds were identified by matching retention times and ion mass fragments with results from standard mixture of diesel. Their respective concentrations were calculated with the calibration obtained from the standard diesel. The net hydrocarbon content was determined by dry wet basis using the water content of the respective samples.

4. Results and discussion

4.1 Magnetic parameters

4.1.1 Concentration dependent magnetic properties

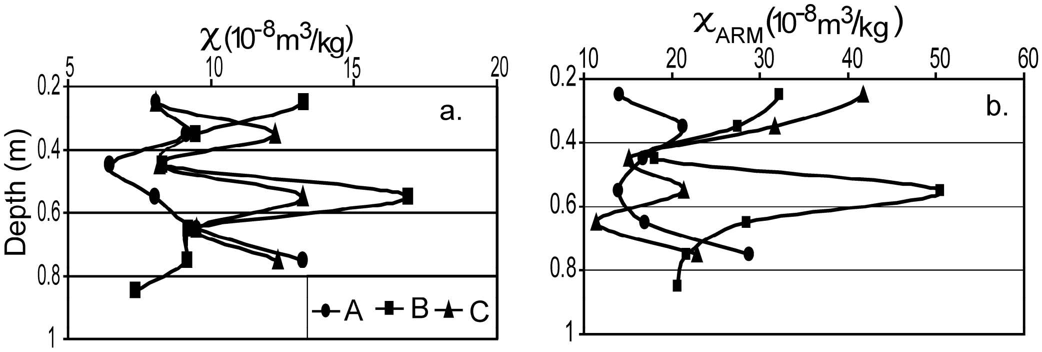

Fig. 2: Mass-specific MS (χ) and mass-specific ARM (χ_{ARM}) of three sections A, B and C are plotted against sample depth in Fig. a and b, respectively. The sites A, B were remediated, whereas site C was the non-remediated.

The samples from section A have the lowest χ from 40 to 60 cm depth whereas from the other two sections, B has the maximum (18 m^3/kg) and C has an intermediate value (13 m^3/kg) χ at around 55 cm (Fig.2a). This indicates that there is a threefold difference in χ values of samples between A and B at the depth of 55 cm. All χ profiles converge at the depth of 65 cm but they significantly deviate below this depth due to an increase in both C and A, and a decrease in B. There is very a similar trend of χ_{ARM} profiles from all three sections. χ_{ARM} of sections B and C is maximum at 55 cm depth except section A, which exhibits the least value(Fig. 2b). The most remediated section A has the the lowest χ and χ_{ARM} values at the depth at which the other two sections reveal high values (Fig. 2a and b). Both χ and χ_{ARM} correspond with each other. This indicates that the content of single domain particles is also equivocal with respect to MS suggesting that MS is mostly contributed by single domain particles.

This difference in χ of samples from nearly the same depth of the three sections A, B and C might be due to the fact that several different remediation processes were active at the remediated sections A and B, but not in the non-remediated section C. Another possible reason could be that the hydrocarbon content differs in different samples. Even after declaring the area fully remediated, hydrocarbons were still present in the sediments but the total hydrocarbon content was below the clean-up limit of 5000 mg/kg (Machackova et al., 2008). There is a difference in hydrocarbon content among the samples of the three sections. This suggests that the amount of hydrocarbon content, their remediation as well as their transformation coupled together to derive changes in magnetic mineralogy which reflect the current situation that was observed from the samples of sections A and B. However, section C shows a variation of both χ and χ_{ARM} which was only caused by contamination without any effect of remediation processes.

4.1.2 Magnetic minerals and their grain size characterization

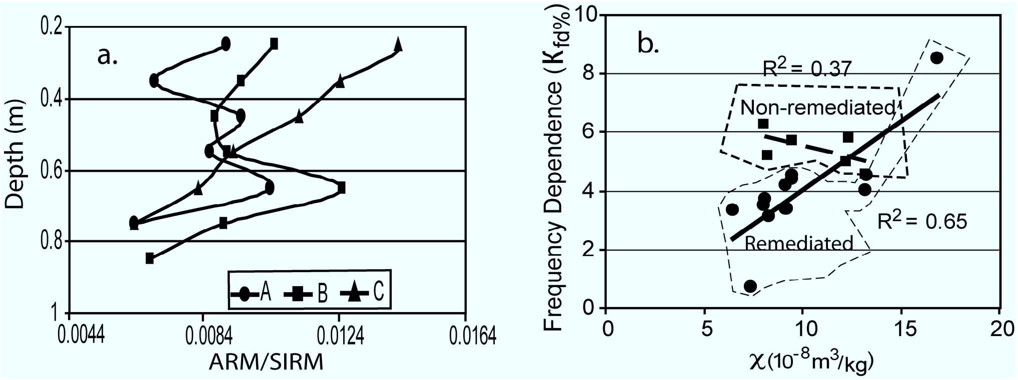

Fig. 3: Profiles of ARM/SIRM ratio (a.) and linear scatter plots between χ and $\kappa_{fd\%}$ from both the remediated sites A and B, and the non-remediated site C (b.). R^2 denotes the coefficient of correlation between χ and $\kappa_{fd\%}$ of samples from both remediated and non-remediated sites in Fig. b.

In general, ARM/SIRM ratio is low in all sections (Fig. 3a). However, this ratio varies more than three orders of magnitudes between the samples of the uppermost and the lowermost part in section C. The ratio lies with a very narrow range at the depth of 65 cm which is about 0.0088 and 0.0124 in A and B, respectively. The scatter plots of $\kappa_{fd\%}$ with χ demonstrate that there is a negative linkage between $\kappa_{fd\%}$ and χ in the samples of the non-remediated section C, though values of $\kappa_{fd\%}$ lay within a very narrow range (5-7 %) (Fig. 3b). In contrast, there is a positive correlation between samples from remediated sections A and B as indicated by filled dots and a solid line.

The ARM/SIRM ratio is a granulometry indicator of ferro(i)magnetic minerals in a sample. The higher the ratio, the more single domain (SD) magnetic mineral dominate in a sample. Samples that had still retained a higher amount of hydrocarbons showed a higher ARM/SIRM ratios below 60 cm which could be either directly due to their higher hydrocarbon content or to the remediation processes that had taken place there or both of these processes. This means that occurrence of SD particles is linked either with a high hydrocarbon content or with the variable remediation processes.

The negative correlation between χ and $\kappa_{fd\%}$ of the samples from the non-remediated section and the positive correlation between the same parameters of the remediation sections further emphasizes that remediation might have had an impact on the change of the magnetic mineralogy. Here it seems that the higher the impact of remediation, the better the positive correlation between these parameters. It suggests that remediation processes can produce more superparamagnetic (SP) grains, which is not the case in non-remediated site.

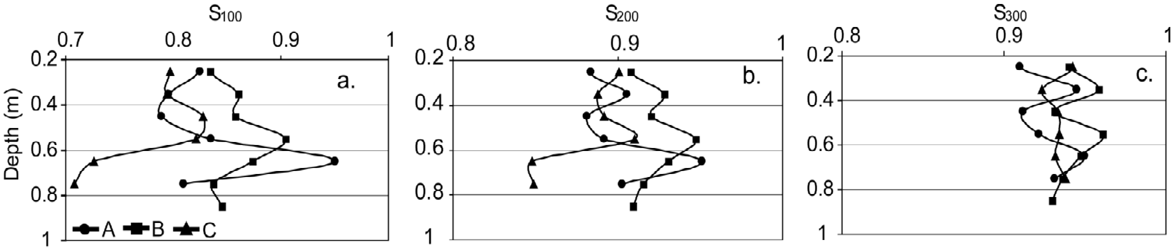

Fig. 4: Profiles of S-ratios of all three sections obtained at 100 (S_{100}), 200 (S_{200}) and 300 (S_{300}) mT of applied reverse field, respectively. The S-ratio is calculated as described by Bloemendal et al. (1992).

The S-ratio describes the relative contribution of high coercive to low coercive magnetic phases. S-ratios are are calculated as defined by Bloemendal et al. (1992) obtained at 100, 200 and 300 mT (Fig. 4). The ratio S_{100} is higher than 0.80 in most of the samples of the sections A and B, whereas, it varies from 0.85 to 0.70 in section C. The range of S_{200} in sections A and B is almost the same. However, the value is even higher in the sample at 65 cm depth. Furthermore, both of the sections have S_{200} more than 90% suggesting soft magnetic phases are dominating. But, most of S_{200} values in section C are below 90%. S_{300} is nearly equal ~0.95 for all the sections and in all samples. Moreover, section C shows the most stable values of around 0.93.

The variation of S-ratios indicate that magnetite is equally represented in all sections (equal S_{300} values), but that samples from the remediated sections A and B obviously contain more soft magnetite whereas then samples from the non-remediated site C. The soft magnetic phases are most likely related to the processes of remediation.

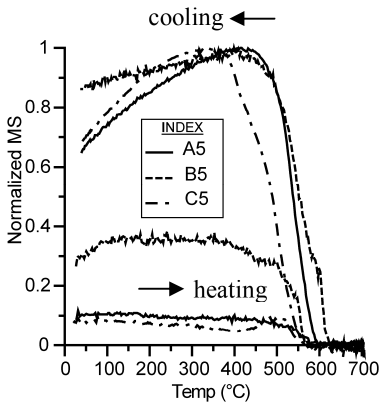

Fig. 5: Temperature dependent MS measured with a KLY-3 Kappabridge selecting three samples from the same depth of sections A, B and C. Both heating and cooling curves are shown.

Magnetic minerals were identified by using temperature dependence of MS. Samples from the same depth (~55 cm) of all three sections were measured with a KLY-3 Kappabridge (Fig.5). All samples revealed magnetite as the main carrier of the magnetic signal carrier. Following heating above 580°C, the cooling curves showed some neo-formation of magnetite.

4.2 Relationship between magnetic susceptibility and hydrocarbon content

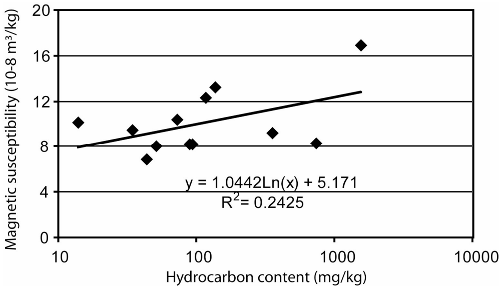

Fig. 6. Semi-logarithmic scatter plot between χ and total hydrocarbon content (mg/kg of dry wt.) of samples from all three sites A, B and C.

A very weak positive correlation between χ and the total hydrocarbon content (R^2= 0.24 for linear fit) is indicated, but the relationship between these two parameters could be also not linear (Fig. 6). A parabolic realtionship could have been generated because of the mixture of remediated and one non-remediated samples. The hydrocarbon analysis points out that even in the remediated sections hydrocarbons were still present in sediments. This presence of hydrocarbons could still have an effect for magnetic mineral transformation with or without the involvement of microbes.

4.3 Relevance of this study in relation with remediation of the unsaturated zone

From both sites A and B, LNAPLs were removed by SVE, bio-venting and enhanced in situ bioremediation in a form of a gas phase. The remediated sites underwent a variable scale of diffusion processes which are coupled together. Ostendorf and Kampbell (1991) observed that if an assumption is made for uniform diffusion then the net movement of a stoichiometrically summed surrogate can be modeled by an equation which has a solution of a linear equation. However, Davis et al. (2005) observed a nonlinear nature of measured data when the same equation was used for modeling of a vadose zone in an area of gasoline leakage. They postulated that the nonlinear nature of measured profiles reflect that other transport or reaction processes or processes with variable diffusion coefficients are responsible for creating such non-linearity of the profile which is very important to be considered in the vadose zone for modeling equations.

There were several diffusion processes happening in both sections A and B. These various diffusion processes were related either with remediation processes or natural processes. The remediation-related processes and natural diffusion processes were coupling together. Therefore, the relation between MS and hydrocarbon content is non-linear. The remediation process-driven diffusions have a minimum effect at site C. Due to the limited number of samples that were analyzed for hydrocarbon content, all samples were plotted together (Fig. 6).

Therefore, changes in iron mineralogy can also be affected by different diffusion, chemical reaction and/or transportation processes that are mainly related with remediation processes occurring in the field. Alternatively, the relationship between MS and hydrocarbon content can also be used to model such biogeochemical transformation processes in shallow sub-surface, especially in the vadose zone, which could be a topic of further research. Nevertheless, some more field sites are required to be investigated implementing similar research methodologies and analytical techniques so that necessary fundamental data for such modeling purpose can be achieved.

5. Conclusions

From this study it was observed that there were differences in magnetic parameters of sediments from remediated and non-remediated sites. The correlation between χ and the total hydrocarbon content show a non-linear fitting. However, the obtained magnetic parameters are the result of an integrated process such as the presence of contaminants, remediation processes such as bioventing, pump and treat and biodegradation as well as biogeochemical processes that happened after release of contaminants during the last several years. Moreover, there might have been also an effect of hydrocarbon contaminated groundwater table fluctuations, mainly created by AS remediation processes that modified magnetic signatures at the lower part of unsaturated zone (Rijal et al., 2010). This remains an important open question that needs to be investigated in future research both in the field sites and also in controlled laboratory experiments.

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6

Microbially induced changes of soil magnetic susceptibility caused by hydrocarbon contamination and mobilization of bioavailable organic carbon -A microcosm study (Draft)

Microbially induced changes of soil magnetic susceptibility caused by hydrocarbon contamination and mobilization of bioavailable organic Carbon-A microcosm study

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Abstract

Microorganisms are known to couple the degradation of hydrocarbons to Fe(III) reduction and thus to the (trans)formation of Fe minerals including ferro(i)magnetic Fe minerals such as magnetite. Therefore screening of soil magnetic properties, in particular of soil magnetic susceptibility (MS), has the potential to assist in localizing and assessing hydrocarbon contaminations in the environment. In order to evaluate this potential, hydrocarbon contamination was simulated in soil microcosms using seven different soils. Changes in MS of in average 7% of the initial MS values occurred in microbially active microcosms, whereas in sterile ones the changes were less than 2.5% demonstrating that microbial metabolism plays a major role in the (trans)formation of ferro(i)magnetic minerals. In presence of hydrocarbons no significant correlation was observed between soil properties and changes in MS suggesting that microbially induced (trans)formation of ferro(i)magnetic Fe minerals after hydrocarbon input is a general phenomenon and does not depend on general soil properties such as pH, organic carbon or Fe content. The microcosms reached stable MS values after a few weeks to months. While further addition of hydrocarbons after stable MS values were reached did not lead to further changes in MS, addition of Fe(III) minerals triggered a further increase in MS suggesting that the changes in MS were limited by bioavailable Fe but not by bioavailable organic carbon. Similar to the effects observed after hydrocarbon contamination, mobilization of organic carbon from the soil matrix by addition of water (0.14-0.67 mL/g soil) led to a microbially driven change in MS. This study shows that changes in MS after hydrocarbon input or mobilization of organic carbon can occur in a variety of soils suggesting that measurements of soil MS in the field have the potential to serve as a fast, efficient and cost effective tool to assess hydrocarbon contaminations in the environment.

1. Introduction

Crude oil and its products such as gasoline and diesel fuel are worldwide used and soil contaminations by these compounds, for example by leaking pipelines and storage tanks, and accidental spills at former industrial and military sites, represent a severe environmental threat (Masak et al., 2003; Readman et al., 1992; Zachara et al., 2004). For effective remediation of such sites, the contaminated area must be localized and its spatial extent must be assessed. For this purpose, soil samples are usually taken from the potentially contaminated area and analyzed in the lab. Since the early 1990s, passive samplers that also have to be analyzed in the lab became more and more popular (Koester and Moulik, 2005). Both methods are time consuming and cost intensive, especially when a large area has to be surveyed because of the unknown localization of the contamination. Therefore, rapid and inexpensive methods are desired to localize hydrocarbon contamination in the field.

The localization of heavy metal contaminations of soils faces principally the same problems as described above for hydrocarbon contamination. Analysis of soil magnetic susceptibility (MS) is used since more than a decade ago to localize heavy metals in soils which were released during combustion processes (for review see (Petrovský and Ellwood, 1999)). MS describes how strong a substance is magnetized in an external magnetic field. Diamagnetic materials (e.g. quartz, water) have a small negative MS. Paramagnetic minerals (e.g. siderite, ferrihydrite) and antiferromagnetic minerals with spin-canting (hematite) or defect moments (goethite) have a small positive MS, whereas ferromagnetic elements (e.g. metallic Fe) and ferrimagnetic minerals have a very high (e.g. magnetite, maghemite, greigite) or moderately high (e.g. pyrrhotite) positive MS (Dunlop and Özdemir, 1997). For simplicity the term ferro(i)magnetic minerals is used in this study in order to include ferrimagnetic minerals and antiferromagnetic minerals with spin-canting or defect moments. The localization of heavy metals by MS measurements is based on the fact that during the combustion process magnetite forms from pyrite present in fossil fuel and coal and is released to the environment together with the heavy metals as part of the fly ash. Since MS can be measured within seconds in the field, this parameter can be used for fast and cost effective surveys of large areas. The measurement of the soil MS as proxy for heavy metal input due to combustion processes was applied for assessment of small areas as roadside pollutions (Hoffmann et al., 1999) as well as for mapping of heavy metal loadings of a whole country (Hanesch and Scholger, 2002).

Although crude oil and its products such as gasoline and diesel have a low MS, the measurement of soil MS has also the potential to serve as a proxy for the presence of hydrocarbons. Crude oil components like n-alkanes and polycyclic aromatic hydrocarbons can be degraded by autochthonous soil microorganisms (Hamamura et al., 2006). Different Fe(III)-reducing microorganisms can use aromatic compounds and hydrocarbon degradation products as electron donors (Lovley and Anderson, 2000; Lovley et al., 1989). Fe(III)-reducing microorganisms were shown to be able to reduce poorly crystalline ferrihydrite as well as more crystalline Fe(III) minerals such as goethite, hematite, and magnetite (for reviews see Kappler and Straub, 2005; Weber et al., 2006) Depending on the environmental geochemical conditions present during Fe(III) reduction (e.g. pH, presence of carbonate, sulfide and phosphate), different Fe(II) minerals can form (e.g. Fe(II) carbonate, mono- and disulfides, phosphate), as well as mixed Fe(II)-Fe(III) mineral such as magnetite (Benner et al., 2002; Borch et al., 2007; Fredrickson et al., 1998; Hansel et al., 2005; Roden and Zachara, 1996). In turn, Fe(II) can be oxidized by anaerobic and aerobic Fe(II)-oxidizing bacteria (Kappler and Straub, 2005; Weber et al., 2006) which can also lead to the formation of magnetite (Chaudhuri et al., 2001; Jiao et al., 2005). On the other hand, Fe(II) present in magnetite can be also microbially oxidized (Brown et al., 1997). The input of hydrocarbons into soils and sediments therefore has a direct influence on Fe(III)-reducing but indirectly also on Fe(II)-oxidizing microorganisms and therefore on the Fe mineralogy (Fortin and Langley, 2005) including the (trans)formation of ferro(i)magnetic minerals.

So far only few studies tried to correlate the amount of hydrocarbons in the soil with soil MS (see references in Schumacher, 1996)) but no detailed studies exist which determined the role of microbial activity in changes of soil MS after hydrocarbon addition. Therefore we simulated hydrocarbon contaminations in soil microcosm experiments and followed the change of MS over time. The objectives of this study were i) to determine the importance of microbial processes for changes in MS as a response to hydrocarbon contamination, ii) to quantify the extent and kinetics of the changes in MS, and iii) to determine the influence of soil properties such as water content, concentrations of bioavailable carbon and Fe on MS changes.

2. Materials and Methods

Field site and soil sampling: Seven soil samples from six different field sites were collected. The field sites were chosen due to their minimal anthropogenic influence especially regarding combustion pollution that is known to release ferro(i)magnetic particles. The sampling sites Holzgerlingen (HG), Waldenbuch (Wabu), Fraeulinsberg (FB), Allemendwald (AW) (all four grass land) and Schoenbuch (Sbu) (forest) are located in Southwest Germany. At the grass land sites the top ~20 cm of soil were sampled including the sward, whereas the forest soil Sbu was sampled without litter. Soil samples were also collected in the Haenigsen area (Northeast of Hannover, Northern Germany), a region where crude oil is leaking naturally to the surface and oil on top of the the groundwater was exploited in shallow pits for several hundred years. We collected crude oil containing soil (Hoil) next to one of these pits with a soil corer from 80-100 cm depth. For comparison, a sample was taken from an uncontaminated surface soil of farm land (Hclean) less than 500 m away from the pit.

Experimental setup of microcosm experiments: In order to quantify changes of soil MS after hydrocarbon input, diesel and gasoline contaminations were simulated in the lab in three soil microcosms experiments (experiment I-III) under anoxic conditions (Table 1). All solutions added to the microcosms were sterile and anoxic. The microcosms were set up in 60 mL glass bottles with two (experiment I) and four (experiment II-III) parallels per setup, respectively. Each experiment consisted of two sets of microcosms, one with sterile soil and one with non-sterile soil (autochthonous microbial population). For setups with sterile soil, the soil was autoclaved (121°C, 25 min, 1 bar) in the glass bottles once (experiment I-II) or twice (experiment III) with 2-3 days of incubation at room temperature in between.

Either freshwater medium or MilliPore® water was added to the autoclaved and non-autoclaved soil in different soil:liquid ratios. The medium contained per liter: 0.6 g KH_2PO_4, 0.3 g NH_4Cl, 0.025 g $MgSO_4*7H_2O$, 0.4 g $MgCl_2*6H_2O$, 0.1 g $CaCl_2*2H_2O$, 1 mL vitamin solution, 1 mL trace element solution and 1 mL selenite-tungstate solution (Straub et al., 2005). The medium was buffered with 20 mM sodium bicarbonate and had a pH of 7.0. After addition of medium or water to the incubation bottles, the headspace of the bottles was exchanged with a N_2:CO_2 (90:10) gas mixture.

Microcosms with four different carbon amendments were set up: i) no carbon source added, ii) lactate and acetate (each 15 mmol/L added medium or water), iii) unleaded gasoline (0.36-17.90 μL/g wet soil) and iv) diesel (0.20 or 2.00 μL/g wet soil). Unleaded gasoline and diesel were obtained from a gasoline station. Bottles containing gasoline or diesel were closed with viton stoppers, whereas the other bottles were closed either with viton or butyl stoppers. Microcosms were homogenized directly after their preparation and weekly during the experiment. The microcosms were incubated at 28°C in the dark and their MS was measured weekly, or later, when the MS did not change anymore, every second week. In order to determine whether carbon limitation was responsible for cessation of changes in MS, lactate/acetate (each 15 mmol/L added medium or water), 0.36 μL unleaded gasoline/g wet soil and 0.20 μL diesel/g wet soil were added to corresponding setups of experiment I after 14.5 weeks of incubation.

Microcosms with soils Hclean and Hoil containing medium (experiment II) were amended with ferrihydrite and magnetite after 51 weeks of incubation to determine whether Fe transformation processes in the microcosms were Fe limited. To half of the parallels of one setup, 2 mL of a 0.5 M ferrihydrite suspension were added, to the other half 1 mL of a 0.5 M ferrihydrite suspension and 1 mL of a 0.25 M magnetite suspension were added. Ferrihydrite was synthesized according to Raven et al. (1998) and the precipitated mineral was washed with MilliPore® water and suspended in it. Commercial magnetite (Lanxess Deutschland GmbH, Germany) was suspended in MilliPore® water to obtain a 0.25 M magnetite suspension.

After incubation, usually one microcosm of each setup and experiment was opened and samples for the quantification of Fe(II) and Fe_{tot} of different Fe fractions (adsorbed, poorly crystalline and more crystalline Fe) and for pore water analysis (dissolved carbon, organic acids) were taken. .

Table 1: Experimental setup of microcosm experiments

Experiment (parallels per setup)	Soil	Incubation time [weeks] (before + after 2nd C and Fe addition)	Ratio Soil : Liquid	Carbon source added to sterile and microbially active microcosms									
				no carbon		lactate and acetate (15 mM each)[a]		gasoline [µL/ g wet soil]				Diesel [µL/ g wet soil]	
				medium	water	medium	water	0.36, medium	3.60, medium	3.60, water	17.90, water	0.20, medium	2.00 medium
I (2)	HG	14.5 + 12	15 : 5	x		X, 2nd C		x, 2nd C	x			x, 2nd C	x
	Wabu	14.5 + 12	15 : 5	x		X, 2nd C		x, 2nd C	x			x, 2nd C	x
II (4)	FB	51	15 : 7	x	x				x				
	AW	51	15 : 7	x	x				x				
	Hclean	51 + 73.5	15 : 7	x, Fe	x				x, Fe				
	Hoil	51 + 73.5	15 : 7	x, Fe	x				x, Fe				
III (4)	Sbu	48	21 : 0		x		x			x	x		
		48	21 : 3		x		x			x	x		
		48	21 : 7		x		x			x	x		
		48	21 : 10		x		x			x	x		
		48	21 : 14		x		x			x	x		

A - 15 mM refers to the amount of liquid added, in exp. III 200 µL of a 750 mM lactate/acetate solution were added to setups without water addtion.

2nd C - after 14.5 weeks (κ values of microcosms were stable) the same carbon source as before was added a 2nd time.

Fe - after 51 weeks (κ values of microcosms were stable) 2 mL of 0.5 M ferrihydrite suspension were added to two parallels per setup and 1 mL of 0.5 M ferrihydrite suspension plus 1 mL of 0.25 M magnetite suspension were added to the other two parallels.

Analytical methods:

Soil analysis. Before soil analysis, stones and large organic particles (e.g. roots) were separated from the soil either with tweezers (HG, Wabu, FB, AW, Hoil, Hclean) or by pressing the soil through a 2 mm sieve (Sbu). If necessary small amounts of sterile water were added to the soil during the sorting procedure to avoid drying which could potentially harm the microorganisms. The soil was stored in plastic bags at 4°C in the dark until further use. The water content of the soil samples was determined after standard protocols (Blume et al., 2000) by drying the soil at 105°C until the soil weight was constant. The pH was measured 24 h after addition of 25 mL of 0.01 M $CaCl_2$ solution to 10 g of soil according to Blume et al. (2000). Total organic carbon and total nitrogen was determined from dried and fine ground soil with a CN analyzer (Vario EL, Elementar, Germany) after carbonate removal with 1 M HCl. The $CaCO_3$ content was quantified by mixing dried and fine ground soil with 1 M HCl and determining the consumed HCl by titration with 1 M NaOH. The total Fe content of dried and fine ground soil was quantified by X-ray fluorescence analysis (Bruker AXS S4 Pioneer X-ray spectrometer, Bruker AXS GmbH, Germany). Fe extractions from three aliquots of the soil before and after incubation were performed after Moeslund et al. (1994) and Roden and Zachara (1996) with a soil:extractant ratio (w/v) of 1:50. Prior extraction microcosms with soil Sbu containing 7, 10, and 14 mL of water were centrifuged (10 min, 2000 rpm) and the supernatant was removed for dissolved carbon and organic acids analyses (see below). The first aliquot of soil was extracted with Na-acetate (pH 5) for 24 h and the second aliquot with 0.5 M HCl for 1 h, both under oxic conditions at room temperature on a shaker. The third aliquot was extracted with 6 M HCl at 70°C under oxic conditions in a water bath for 24 h and the extracts were cooled for 15 min at room temperature. For soil Sbu 1 M HCl was used for the third aliquot after recognizing that Fe(II) is oxidized by molecular O_2 in 6 M HCl. 1.8 mL of all extracts were centrifuged (15 min, 20,817 *g*) removing soil particles. Fe(II) and Fe total of the supernatant were quantified by the ferrozine assay (Stookey, 1970) in microtiterplates as described by Hegler et al. (2008). The determined soil properties are given in Table 2.

Table 2: Selected properties of soils used for microcosms experiments

Soil properties	HG	Wabu	FB	AW	Sbu	Hclean	Hoil[f]
pH[a]	5.4	7.1	5.2	3.6[b]	7.0	4.8	(6.6)
Water content [wt.%][b,d]	32	40	23	30	48	15	(8)
$CaCO_3$ [wt.%][a,d]	4.7	1.5	1.2	0.2	12.0	0.2	n.d.
TOC [wt.%][a,d]	2.8	4.1	3.3	4.6	3.5	2.0	n.d.
N_{total} [wt.%][a,d]	0.3	0.4	0.3	0.3	0.2	0.1	n.d.
Fe_{total} 0.5 M HCl [wt.%][b,d]	0.1	0.1	0.1	0.2	0.2	0.1	(0.0)
Fe_{total} 6 M HCl [wt.%][b,d]	3.4	2.0	1.7	1.8	1.8	0.4	(0.5)
Fe_{total} XRF [wt.%][a,d,e]	3.8	2.2	2.1	2.0	2.6	0.6	(0.9)[c]
Mass specific MS [10^{-8} m^3/kg][b]	19.9	17.3	27.1	45.5	13.9	11.6	(4.6)

a - average of duplicates; b - average of three or more replicates; c - single measurement; d - wt.% refers to 105°C dried soil; e - XRF = x-ray fluorescence analysis; f - high crude oil content might have influenced the determination of soil properties

Magnetic susceptibility measurements. Low-field MS of the microcosm bottles was measured with a KLY-3 Kappabridge (AGICO, Czech Republic) at room temperature (peak magnetic field 300 A/m, frequency 875 Hz). The soil sample was entirely placed in the homogeneity range of the pick-up coil. Each bottle was measured three times and the values were averaged. Values of MS are only relative as the effective sample volume cannot be determined. However, this does not affect the significance of measurements performed on the same sample over time. Results in this paper are given in % change of MS. In order to determine the mass specific MS of the original soil samples, 5 to 10 sub-samples of each soil were packed in 10 cm^3 plastic containers, measured as described above and the reading for the volume specific MS (κ) was divided by the overall density of the container content.

Dissolved carbon and organic acids. Microcosm bottles with soils FB, AW and Sbu were centrifuged (10 min, 2000 rpm) and the supernatant was stored in sterile plastic cups at -28°C for up to one year for dissolved organic/inorganic carbon and organic acid analyses. The supernatant was thawed and remaining soil particles were removed either by centrifugation (FB, AW, 10 min at 20,817 *g*) or by centrifugation and filtration (Sbu, centrifugation for 10 min at 5000 *g* followed by filtration with a 0.22 µm mixed cellulose ester filter). Dissolved organic and inorganic carbon (DOC, DIC) contents were determined using a carbon analyzer (high TOC, Elementar, Germany). Organic acids (acetate, butyrate, formate, lactate and propionate) were quantified by high pressure liquid chromatography using a diode array detector (absorption at 210 nm) and a refractive index detector. The acids were separated on a Bio-Rad Aminex HPX-87H Ion Exclusion Column (300*7.8 mm) with two pre-columns, a Bio-Rad Micro guard Cation H Cartridge and a Dionex IonPac NG1 Guard column (2*50 mm). 5 mM H_2SO_4 was used as eluent with a flow rate of 0.6 mL/min. The oven temperature was 60°C and the running time per sample 30 min.

Mössbauer spectroscopy. Preparation of samples from microcosms for Mössbauer spectroscopic analysis was done anoxically in a glovebox (100% N_2), whereas the original soil was prepared under oxic conditions. Soil samples were sealed between two layers of Kapton tape. Mössbauer spectra were recorded at room temperature for up to 19 days and analyzed as described by Hohmann et al. (2010).

Extended X-ray absorption fine structure. Dry samples for X-ray absorption spectroscopic analyses were packed in teflon sample holders and sealed with a Kapton polyimide film to prevent oxidation while minimizing X-ray absorption. The structural environment of Fe was determined using extended X-ray absorption fine structure (EXAFS) spectroscopy at the Stanford Synchrotron Radiation Lightsource (SSRL) on beamlines 11-2 (26-pole wiggler) and 4-1 (20 pole wiggler), running under dedicated conditions. The storage ring was operated at 3.0 GeV and at currents between 60 and 100 mA. The Fe EXAFS analytical procedures used here were similar to those described previously by Borch et al. (2007). Energy selection was accomplished with a Si (220) monochromator and spectra were recorded by fluorescent X-ray production using a Lytle-detector. A set of Fe reference compounds was used to perform linear combination k^3-weightened EXAFS spectral fitting using the SIXPACK interface to IFEFIT (Webb, 2005). Linear combinations of the reference compounds were optimized and the only variable parameters were the fractions of each reference compounds. Reference compounds were chosen based on their likelihood of being present in the soil or a reaction product (including, for example, criteria such as elemental composition), and were included in the fit only if they contributed with a fraction of 0.05 or more. Mössbauer spectroscopy was used to constrain the LC-XAFS fitting. Detection limit for minor constituents is approximately 5%. Mössbauer spectroscopy, with a detection limit of approximately 5-10 wt%, was used to confirm the EXAFS analysis as described earlier by Borch et al. (2007).

Statistical analyses. For statistical analyses the software package PASW statistics 18.0.0 from SPSS Inc. (Chicago, USA) was used.

3. Results

In order to determine if addition of organic carbon, especially of hydrocarbons, leads to changes in MS of soils, soil microcosms with seven soils, four different carbon amendments and different amounts of water and medium were set up (Table 1). Changes in MS of soils is due to the (trans)formation of ferro(i)magnetic soil minerals. Different soils were used to determine whether soil properties (Table 2) have an influence on the rate and the extent of MS changes. In order to determine how fast and to which extent a change in MS can occur in soils after hydrocarbon contamination, mainly non-contaminated soils were used for the experiments. The naturally oil-containing soil Hoil was used to determine whether the change in MS of soils containing microbial populations that are already adapted to the presence of hydrocarbons differs from changes in soils that contain non-adapted microbial populations. Unleaded gasoline and diesel were used as hydrocarbon compounds. Setups amended with lactate/acetate served as positive control since both organic acids are known to be used by many Fe-metabolizing microorganisms, whereas setups without organic carbon addition served as negative control. Microcosms were set up with sterile (autoclaved) and microbially active (non-autoclaved) soil in order to differentiate between abiotic and microbial processes.

Changes in MS of sterile vs. microbially active soil microcosms.

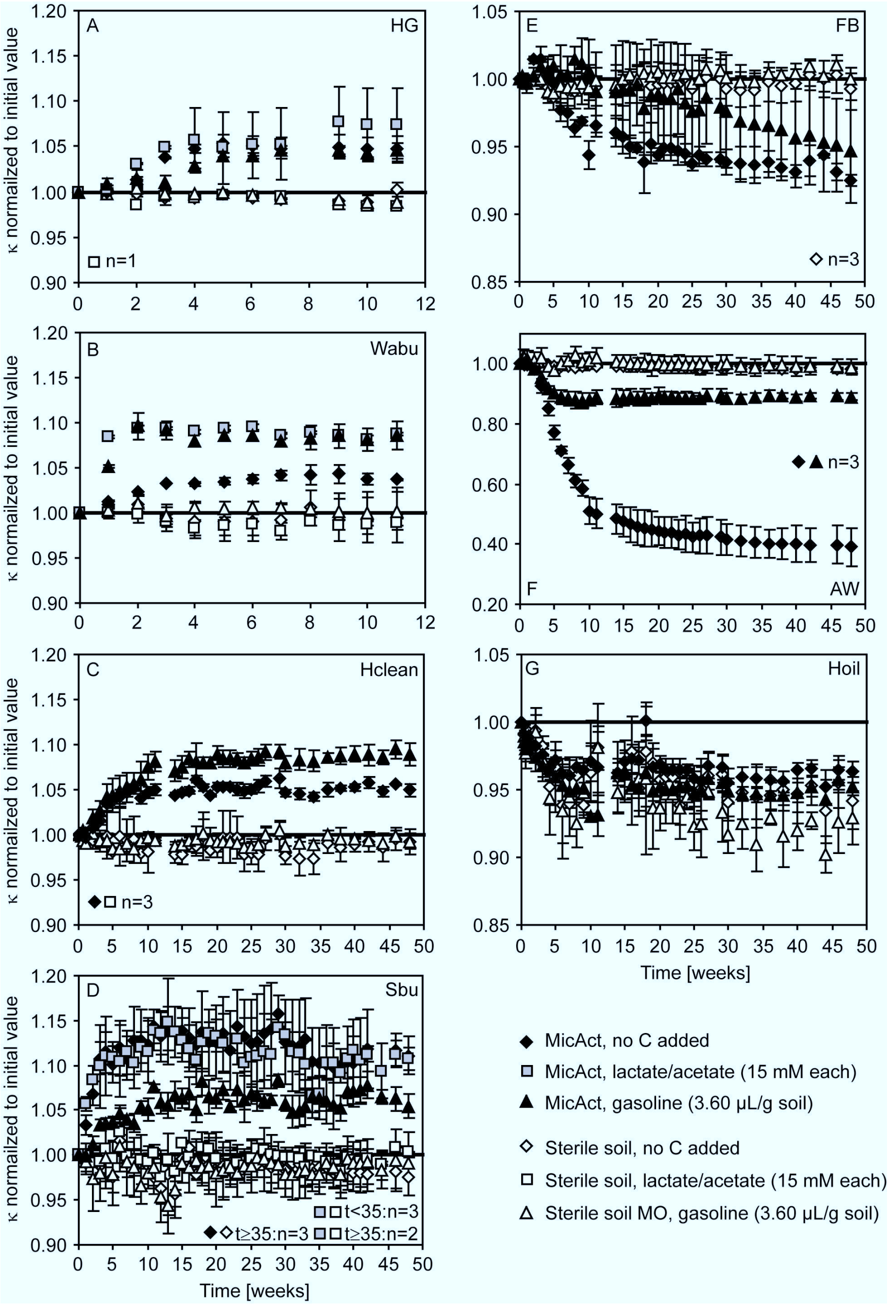

Figure 1: Changes of magnetic susceptibility (MS) over time of microbially active soil microcosms (MicAct) either without additional carbon (◆), amended with 15 mM lactate/acetate (■) or amended with 3.60 µL gasoline/g wet soil (▲). Open symbols represent sterile microcosms. Lactate/acetate was only added to soils HG, Wabu and Sbu. Water (7 mL) was added to microcosms with soil Sbu, freshwater medium to all other microcosms. The MS values measured at each time were normalized to the MS values measured directly after setting up the microcosms (time t=0). Results are means of two (n=2, A-B) or four (n=4, C-G) replicates, except those noted in the graph. One parallel of sterile and microbially active setups of soil Sbu without carbon amendment and with lactate/acetate were harvested in week 35 for EXAFS measurements. Bars bracket the range of duplicates or indicate the standard deviation of three and four replicates.

In setups without carbon addition and after addition of lactate/acetate and gasoline (3.60 µL/g soil) MS of most microbially active soil microcosms increased or decreased significantly, whereas for sterile microcosms only minor or no changes of MS occurred (Figures 1A-G, Table S1). For soil Hoil the change in κ of sterile microcosms was similar to the change of microbially active ones (Figure 1G) suggesting that some microorganisms survived the autoclaving procedure (probably encased in clumps of the crude oil-containing soil). The extent of MS change (average of the last four to five measurements) of the microbially active microcosms (including autoclaved setups with soil Hoil) varied between 3.8±0.8% (soil Hoil, microbially active, no carbon added) and 11.3±3.2% (soil Sbu, no carbon added). For microcosms with soil AW and no carbon amendment, MS changed even by 60.3±6.0%. On average, not considering the very high change of >60% observed with soil AW, MS of microbially active setups changed by 6.9±2.4%. The change in MS of the sterile microcosms, excluding setups with soil Hoil, varied between 0.6±0.3% (soil FB, gasoline) and 2.4±0.9% (soil AW, gasoline) with an average change of 1.4±0.6%.

The increase and decrease in MS values of the non-sterile microcosms over time depend on the soil (but not on the carbon source) (Figures 1A-G). Microcosms with soils HG, Wabu, Hclean and Sbu showed an increase in MS, whereas in case of soils FB, Hoil and AW a decrease of MS occurred. Furthermore, the time needed until stable MS values were reached also mainly depended on the soil and only to a small extent on the added carbon source. Microcosms with soil Wabu and carbon amendment reached stable MS values within 2 weeks, whereas microcosms with soil FB needed around 30 weeks.

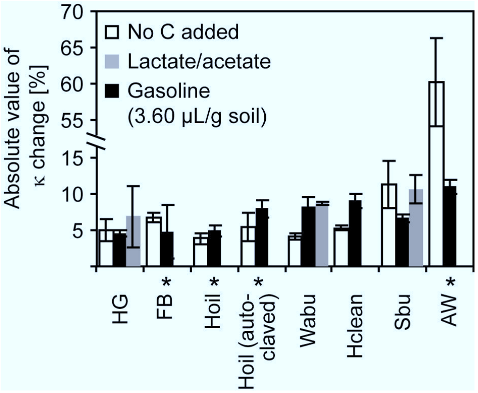

Figure 2: Values of maximum magnetic susceptibility (MS) changes in % of microbially active soil microcosms without carbon addition, with 3.60 µL gasoline/g soil or with 15 mM lactate/acetate (soils HG, Wabu and Sbu only). Autoclaved soil Hoil was incubated either without carbon addition or with 3.60 µL gasoline/g soil. Soils with decreasing MS over time are labeled with an asterisk. Changes of MS were calculated as average of the last four to five time points in Figure 1. Results are means of two (HG, Wabu) or four (other soils) replicates, except those setups noted in Figure 1. Bars bracket the range of duplicates or indicate the standard deviation of three and four replicates.

No carbon addition and addition of lactate/acetate or gasoline (3.60 µL/g soil) affected the extent of MS change of microbially active microcosms differently (Figure 2, Table S2). Depending on the soil three different groups of microcosms could be distinguished. First, for microcosms with soils HG, FB and Hoil (autoclaved and microbially active) the extent of MS change was similar for all three carbon treatments. For the second group, consisting of soils Wabu and Hclean, MS changed to a significant larger extent in setups with gasoline than without carbon addition. The addition of lactate/acetate to soil Wabu led to a similar change in MS as the addition of gasoline. In contrast, in the third group of microcosms with soils Sbu and AW changes of MS were significantly less in setups with gasoline than in setups without carbon addition. The addition of lactate/acetate to soil Sbu had the same effect on MS as no carbon addition.

Influence of medium and water addition on changes in MS of soil microcosms

The fact that the MS of most microbially active setups without carbon amendment changed to the same extent or even more than of setups with carbon amendment (Figures 1A-G, 2) indicates that the microbial activity did not depend on the addition of organic carbon. Freshwater medium was added to all soils except soil Sbu, and we therefore initially hypothesized that the vitamins and trace elements supplied with the medium might have stimulated the soil microorganisms. In order to evaluate the influence of the medium components, soils AW, FB, Hoil and Hclean were incubated (without carbon amendment) either with water or with freshwater medium (Table 1).

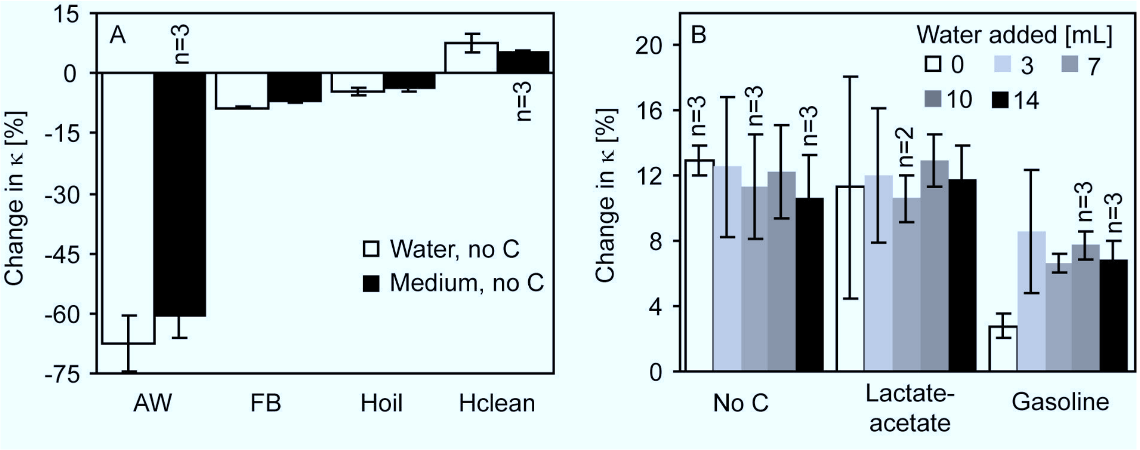

Figure 3: Maximum change in magnetic susceptibility (MS) of microbially active microcosms. (A) Microcosms with soils AW, FB, Hoil and Hclean were set up either with water or freshwater medium without carbon addition (no C) and incubated for 51 weeks. (B) Microcosms with soil Sbu without carbon addition (no C), with 15 mM lactate/acetate and with 3.60 µL gasoline/g soil were set up with different amounts of water (0-14 mL) and incubated for 48 weeks. (A-B) Changes of MS were calculated as average of the last four to five time points. Results are means of four replicates (n=4), except for those setups noted in the figure. Bars indicate the range of duplicates or the standard deviation of three and four replicates.

We found that after 48 weeks of incubation, the change in MS of microbially active microcosms containing only water was similar to the change of microbially active microcosms containing freshwater medium (Figure 3A), suggesting that the trace metals and vitamins in the medium did not have a stimulating effect on microbial activity and related MS changes of the soils. These results furthermore suggest that even in the microcosms without carbon amendment enough organic carbon was bioavailable to stimulate the (trans)formation of ferro(i)magnetic minerals. We therefore quantified the concentrations of organic acids, dissolved inorganic (DIC) and organic (DOC) carbon in the pore water of microcosms with soil Sbu after 48 weeks of incubation (Figures 4A-B) and with soils Fb and AW after 85 weeks (Figures S1A-D).

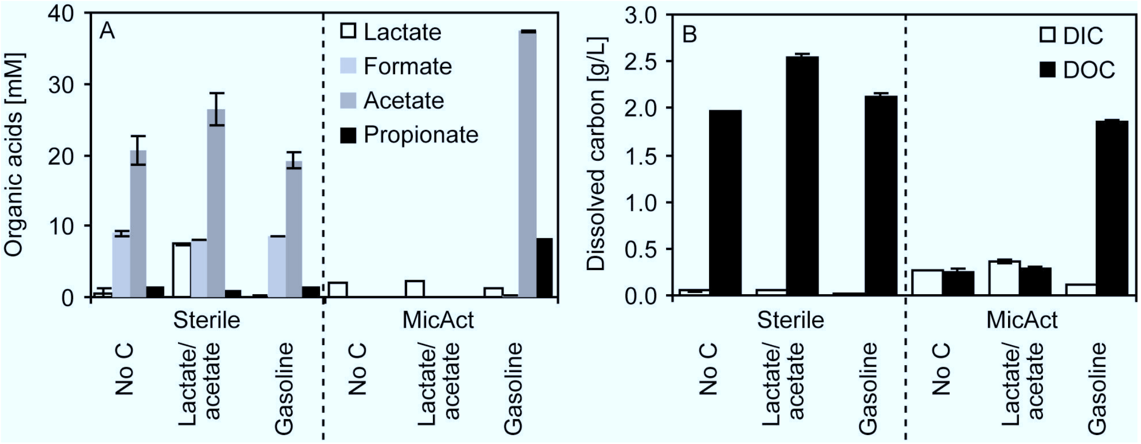

Figure 4: (A) Concentration of organic acids in the pore water sampled after 48 weeks of incubation from sterile and microbially active (MicAct) microcosms of soil Sbu with 7 mL water and without additional carbon (no C), 15 mM lactate/acetate or 3.60 µL/g gasoline. (B) Dissolved inorganic (DIC) and organic carbon (DOC) in the same pore water as in (A). Results are means of duplicate measurements of one bottle per setup, propionate was measured only once. Bars bracket the range of duplicates.

We found that formate (~8.5 mM) and propionate (<2 mM) concentrations were similar in all sterile setups. Additionally, lactate (<1 mM) and acetate (~20 mM) concentrations were similar in setups that were not amended with organic carbon and in the ones amended with gasoline (Figure 4A). In setups with lactate/acetate the lactate concentration was 6.9±0.4 mM and the acetate concentration 5.8±0.2 mM larger than in the sterile microcosms without carbon amendment yielding a recovery of the added lactate and acetate of 46% and 39%, respectively. In contrast, no organic acids besides small amounts of lactate (<2.5 mM) were detected in the microbially active setups without carbon amendment and with lactate/acetate addition. In the microbially active setups with gasoline, the lactate and formate concentrations were also low (below 1.2 mM). However, the acetate (37.4±0.1 mM) and propionate (8.2 mM) concentrations in these setups were higher than in all other sterile and microbially active setups.

The DIC in sterile microcosms was <0.10 g/L, whereas in microbially active ones the DIC was slightly higher (0.12-0.36 g/L) (Figure 4B). The DOC in sterile setups without carbon addition and with gasoline was similar (~2 g/L), whereas in the setup with lactate/acetate it was 0.58 g/L higher than in setups without carbon addition. Addition of lactate/acetate at a concentration of 15 mM each is expected to yield a DOC of 0.90 mg/L. Hence, based on DOC analyses, the lactate/acetate recovery was 65%. Organic acids, DIC and DOC concentrations of pore water obtained from microcosms with soils FB and AW were similar to those presented here for soil Sbu (Figures S1A-D).

These results show that bioavailable organic carbon was leached from the soils by the added water/medium and was metabolized by microorganism in the microbially active microcosms. In order to investigate this mobilization of organic carbon in more detail, we intended to determine whether and to which extent the soil water content affects the (trans)formation of ferro(i)magnetic minerals. To this end, a microcosm experiment with soil Sbu, different carbon sources (none, 15 mM lactate/acetate, 3.60 µL gasoline/g soil) and different soil:water ratios (21:0 to 21:14 w/v) was set up (Table 1). These experiments revealed that for microbially active microcosms without additional carbon and with lactate/acetate amendment the changes in MS after 51 weeks of incubation did not depend on the amount of water added (Figure 3B). Independently of the water amount added (including setups without water addition) the increase in MS of these setups varied between 10.6±1.4% and 12.9±1.6%. In contrast, MS of setups with gasoline increased less than in setups without carbon and lactate/acetate addition, and the extent of MS increase was affected by the addition of water. While in water amended setups MS increased by 6.6±0.6% to 8.6±3.7%, in setups without water amendment the increase was only 2.8±0.7%.

Influence of different hydrocarbons on change in MS of soil microcosms

In order to determine if other hydrocarbons than gasoline as well as concentrations of hydrocarbons lower or higher than 3.60 µL/g soil affect the changes in MS, gasoline and diesel were added at different concentrations to soils HG, Wabu and Sbu (Table 1).

Gasoline and diesel each added in two concentrations (0.36 and 3.60 µL gasoline/g soil and 0.20 and 2.00 µL diesel/g soil) showed very similar effects on the change in MS of microbially active microcosms with soils HG and Wabu (Figure 5). The extent of MS increase varied for soil HG for the four hydrocarbon amendments between 4.6±0.4% and 6.7±1.7% and for soil Wabu between 6.7±1.0% and 8.3±1.3%. In contrast, in sterile setups MS changed only slightly (<2%) except for setups with soil HG amended with 3.60 µL diesel/g soil (4.1±1.4%) and with soil Wabu amended with 0.36 µL diesel/g soil (2.3±0.6%). If five times more gasoline (17.90 µL/g soil) was added to soil Sbu the change in MS (3.9±3.6%) was similar to MS changes in setups with 3.60 µL gasoline/g soil (6.6±0.6%).

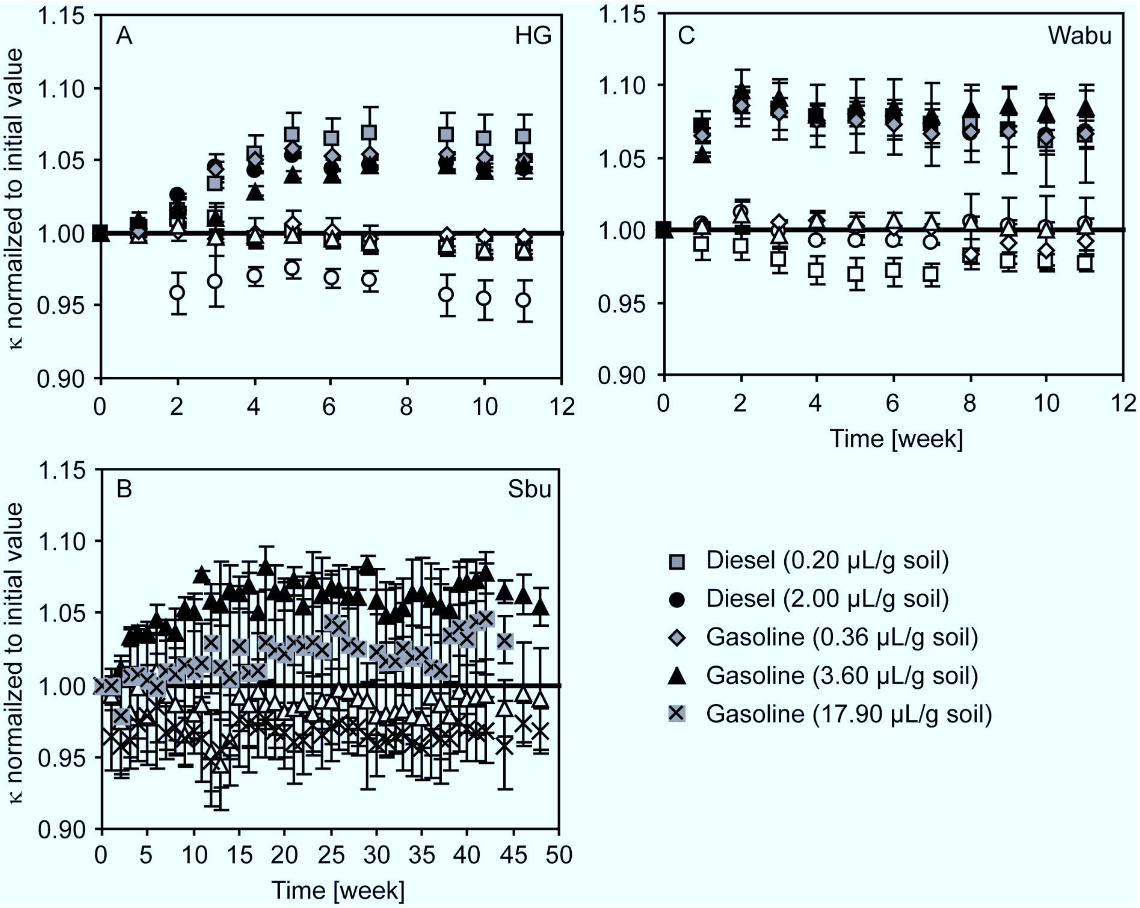

Figure 5: Changes in MS over time of soil microcosms amended with different amounts of gasoline (0.36, 3.60 or 17.90 mL/g) and diesel (0.20 or 2.00 mL/g). Diesel at both concentrations, 0.36 and 3.60 µL gasoline/g soil were added to soils HG and Wabu. 3.60 and 17.90 µL gasoline/ g soil were added to soil Sbu. Closed symbols represent microbially active microcosms, open symbols sterile ones. Freshwater medium was added to soils HG and Wabu, water (7 mL) to soil Sbu. The MS values measured at each time point were normalized to the MS values measured directly after setting up the microcosms (time point t=0). Results are means of two (HG, Wabu) or four (Sbu) replicates. Bars bracket the range of duplicates or indicate the standard deviation of four replicates.

Influence of organic carbon and Fe bioavailability on change in MS of soil microcosms

For setups with and without carbon addition, all microbially active soil microcosms reached stable MS values after a few weeks to several months (Figures 1A-G). This suggests that either the Fe mineral transformation reached a steady state (formation of ferro(i)magnetic minerals equaled their transformation) or that Fe mineral transformation ceased. This might be due to a limitation either in bioavailable organic carbon or in bioavailable Fe. In order to determine whether the amount of bioavailable organic carbon was limiting for further mineral transformation, we added lactate/acetate, gasoline (0.36 µL/g soil) and diesel (0.20 µL/g soil) a second time to the respective setups of soils HG and Wabu. The carbon sources were added after 14.5 weeks of incubation when the MS values of the setups had reached a plateau (Table 1). Due to the carbon addition itself, no significant MS changes of the microcosms setups were observed (changes <1%, calculated from the difference between the last measurement before and the first measurement after the second carbon addition). In the following 10 weeks, the MS values of both the sterile and microbially active setups changed in almost all setups by less than 2.6% (Figures 6A-B), indicating that the amount of bioavailable carbon was probably not a main limiting factor for ferro(i)magnetic mineral transformation. As an exception the microbially active setups of soil Wabu amended with lactate/acetate showed an increase in MS of 5.7±0.5% during the last three weeks of incubation (Figure 6B).

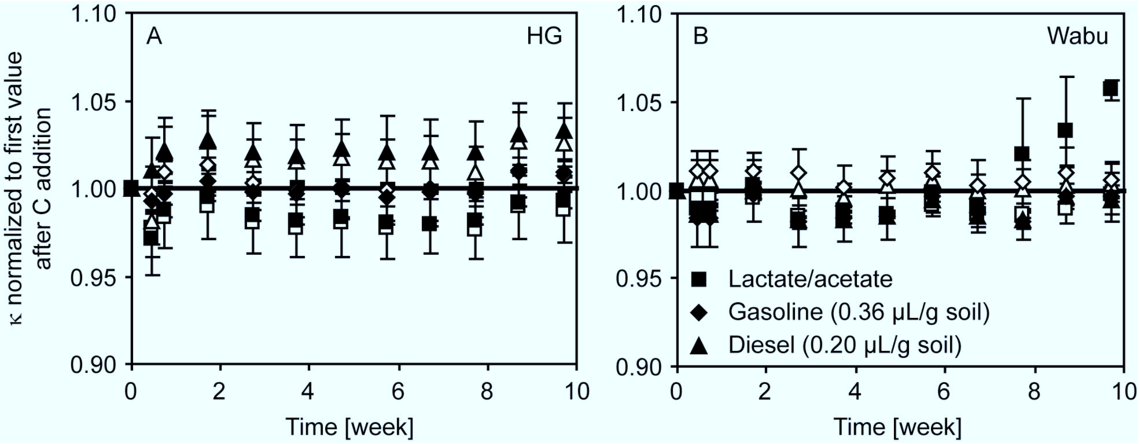

Figure 6: Changes in magnetic susceptibility (MS) over time of microbially active soil microcosms amended a second time with lactate/acetate (15 mM, ■), gasoline (0.36 µL/g soil, ◆) or diesel (0.20 µL/g soil, ▲). The second carbon addition was performed after 14.5 weeks of incubation when MS of the setups had reached a plateau (Figures 1A-B). Open symbols represent sterile microcosms. The MS values measured at each time point were normalized to the MS values measured directly after the second carbon addition. Results are means of duplicates. Bars bracket the range of duplicates.

In order to determine if bioavailable Fe in the soils was limiting and responsible for the observed cessation of the change in MS (Figures 1A-G), either ferrihydrite or a ferrihydrite-magnetite mixture was added to microcosms with soils Hclean and Hoil after 51 weeks of incubation (Table 1). Ferrihydrite is a poorly crystalline Fe mineral with a low positive MS which can easily be reduced by Fe(III)-reducing microorganisms. Magnetite is a crystalline Fe(II)-Fe(III) mineral, which is ferrimagnetic and has a very high MS. Magnetite was shown to be formed during microbial Fe(III) reduction and Fe(II) oxidation (for review see (Bazylinski et al., 2007)), but was also shown to be microbially reduced (Kostka and Nealson, 1995) and oxidized (Brown et al., 1997). In some soils including soil Hoil we observed a decrease in MS during incubation (Figure 1G) indicating that these soils contained microorganisms capable of transforming ferro(i)magnetic minerals such as magnetite. We therefore added synthetic magnetite in order to determine if it could be also microbially transformed.

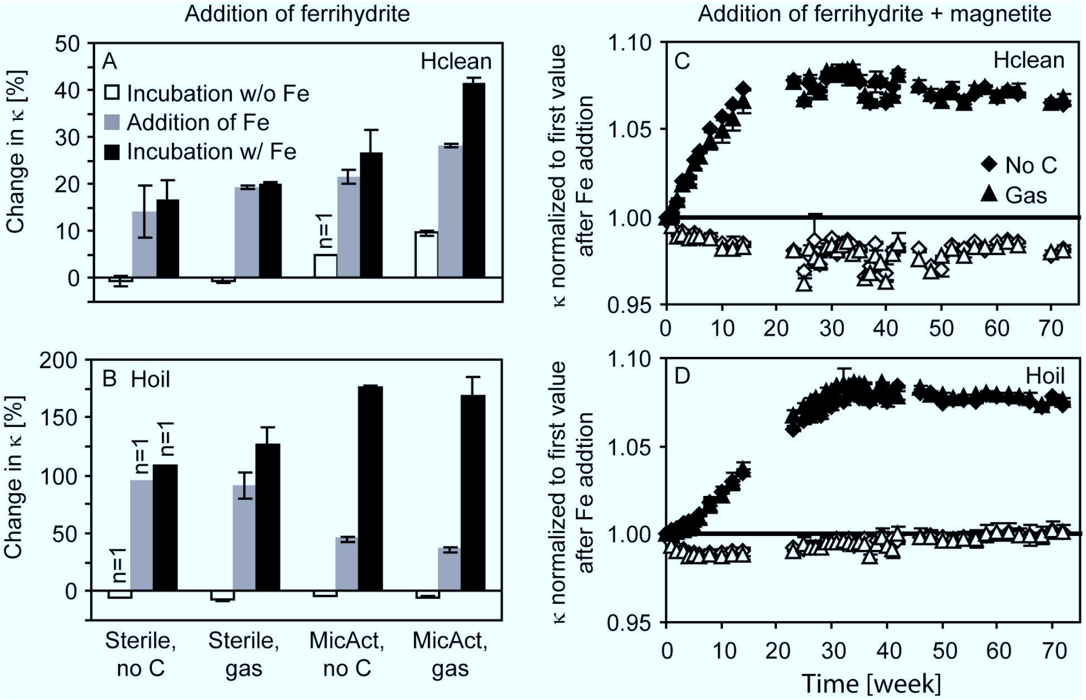

Figure 7: (A-B) Change in magnetic susceptibility (MS) of sterile and microbially active (MicAct) microcosms with soils Hclean and Hoil without carbon addition or with gasoline (3.60 µL/g soil) after 51 weeks of incubation (□, Figures 1C and 1G). After 51 weeks 2 mL of a 0.5 M ferrihydrite suspension was added to the microcosms leading to an immediate increase in MS (■) and to a slower MS increase in the following 73.5 weeks of incubation (■). The results refer to MS measured directly after setting up the microcosms (time point t=0). Results are means of duplicates, except those marked in the figure (n=1). Bars bracket the range of duplicates.

(C-D) Changes in MS over time of microcosms without carbon amendment (◆) or gasoline addition (3.60 µL/g soil, ▲) which were amended with 1 mL of 0.5 M ferrihydrite plus 1 mL of 0.25 mM magnetite suspension after 51 weeks of incubation (samples shown in Figures 1C and 1G). Open symbols represent sterile microcosms. The MS values measured at each time point were normalized by the MS values measured directly after the Fe addition. Results are means of duplicates. Bars bracket the range of duplicates.

The addition of ferrihydrite to soils Hclean and Hoil led to an immediate increase in MS of 10.6-20.1% for microcosms with soil Hclean and of 38.8-108.4% for microcosms with soil Hoil (Figures 7A-B). In the following 73.5 weeks, MS in microbially active microcosms with soil Hclean and ferrihydrite increased by another 5.4±3.4% (no carbon addition) and 13.4±1.6% (gasoline addition), respectively; in the corresponding sterile setups the increase was only minor

(2.4±1.2% and 0.8±0.0%). In contrast, in microbially active microcosms with soil Hoil and ferrihydrite, MS changed even by >130%, whereas in microcosms with autoclaved soil Hoil the increase of MS was <40%. It has to be noted, however, that as mentioned above, the autoclaved soil Hoil was probably still microbially active. Due to the high MS of magnetite, the addition of ferrihydrite/magnetite led to a huge increase in MS of the microcosms. During incubation with ferrihydrite/magnetite MS values of microbially active setups of both soils further increased by 6.7±0.2% (Hclean, no carbon) and 7.6±0.1% (Hoil, gasoline), respectively, whereas in sterile setups changes were <2% (Figures 7C-D).

Fe mineralogy changes in soil microcosms

Increasing and decreasing κ values of soils indicate the formation and transformation of ferro(i)magnetic iron minerals. In order to determine if MS changes in our microcosms were caused by changes in soil Fe mineralogy, adsorbed Fe and Fe carbonates, poorly crystalline Fe minerals and more crystalline Fe minerals were extracted from sterile and microbially active soils at the end of the experiment. In Figures 8A-B we show the Fe extraction data of microcosms with soil Sbu and different carbon treatments (Figure 1D) as an example. For comparison the Fe content of the three extracted Fe fractions of the original soil Sbu (no amendments) are also shown.

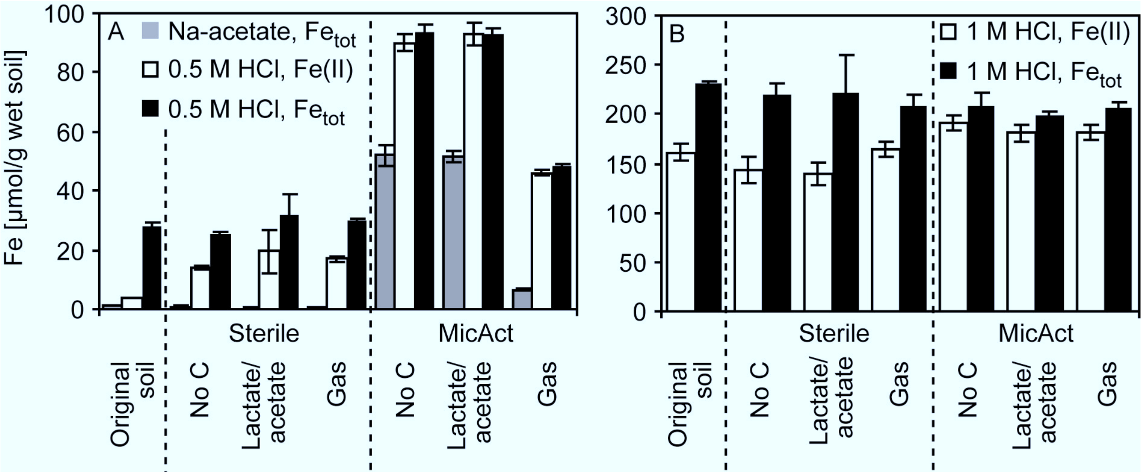

Figure 8: (A) Fe$_{tot}$ (■) extracted with Na-acetate, Fe(II) (☐) and Fe$_{tot}$ (■) extracted with 0.5 M HCl from original soil Sbu and from sterile and microbially active (MicAct) soil Sbu after 48 weeks of incubation with 7 mL water and without carbon addition (No C), with 15 mM lactate/acetate or with 3.60 µL/g gasoline (Gas). (B) Fe(II) (☐) and Fe$_{tot}$ (■) extracted with 1 M HCl (70°C) from the same soil as in (A). Results are means of triplicate measurements of one bottle per setup or the original soil, except for the original soil which was extracted with 1 M HCl in duplicates. Bars indicate the range of duplicates or the standard deviation of triplicates.

The Fe_{tot} concentration extractable with 1 M HCl at 70°C was similar for the original soil Sbu and for the soil Sbu incubated for around one year in sterile and microbially active microcosms (Figure 8B), showing that the Fe_{tot} content was similar in all bottles. However, the sterile setups differed significantly from the microbially active setups regarding the Fe redox speciation and the Fe fractions extractable with the weaker extractants. Fe_{tot} extractable with Na-acetate and 0.5 M HCl was similar for the sterile and the original soil, whereas for the microbially active setups, especially for those without carbon and with lactate/acetate addition, Fe_{tot} extracted by Na-acetate and 0.5 M HCl was significantly higher (Figure 8A). The Fe(II):Fe_{tot} ratio of the 0.5 M HCl fraction was similar among the sterile and among the microbially active setups, independent of the carbon treatment. The Fe(II):Fe_{tot} ratio was 14.3% in the original soil, 57.0±2.4% on average in the sterile setups and even 96.8±2.3% on average in the microbially active setups. In the 1 M HCl (70°C) fraction the Fe(II):Fe_{tot} ratio of sterile setups (70.6±7.3%) was similar to the ratio of the original soil (69.7%), whereas in microbially active setups the ratio was around 20% higher (89.3±2.55%).

The Fe extraction data indicated a change in soil Fe mineralogy and Fe redox states in microbial active microcosms in comparison to sterile ones (Figures 8A-B). However, such extraction data does not generally allow an identification of Fe minerals. Although extractions with 0.5 M and hot 1 M HCl allow an estimation of the contents of Fe minerals with lower crystallinity (e.g. ferrihydrite, siderite) and of Fe minerals with higher crystallinity (e.g. hematite, goethite, magnetite), we were interested in constraining or even identifying the Fe minerals that were (trans)formed in the soils during incubation. To this end we performed Mössbauer spectroscopy and synchrotron-based EXAFS analysis of soil Sbu from sterile and microbially active microcosms without carbon amendment and with lactate/acetate addition after several months of incubation and for comparison also of the original soil Sbu (Figures 1D, 8A-B).

Mössbauer spectroscopy revealed that the original soil Sbu and soil from sterile setups contained more Fe(III) phases (82-92%) than the microbially active setups (71% and 65%) (Table 3, Figures 9A-B). The main fraction of Fe(III) in all four setups was a paramagnetic Fe(III) phase which might have been ferrihydrite, nano-goethite, lepidocrocite, Fe(III) in clays and/or Fe(III) associated with humic substances. Additionally, hematite (<10%) was present in the original soil and in the microbially active setup with lactate/acetate (Figure 9B). Hematite was maybe also present in the sterile setups but was not detected because the amount of hematite was too small (<5-10%). In the microbially active setup without carbon amendment hematite could not be modeled since the signal to noise ratio was too low. The amount of Fe(II) phases varied between

29 and 35% in the microbially active setups, whereas it was 12 and 19% in the sterile setups and only 8% in the original soil. In the microbially active setups Fe(II) was present as siderite and an associated Fe(II) phase. In the original soil and in sterile soil without carbon amendment Fe(II) was present in silicates, which were probably phyllosilicates including clays and micas. The Fe(II) phase in the sterile setup with lactate/acetate is unknown and was maybe a mixture of siderite and the associated Fe(II) phase.

Table 3: Relative abundance and fitting data of different Fe phases determined by Mössbauer spectroscopy in the original soil Sbu and in soil Sbu from sterile and microbially active (MicAct) microcosms with soil Sbu, 7 mL water and without additional carbon or with 15 mM lactate/acetate after several months of incubation.

Sample (incubation time [weeks])	Reduced χ^2 of fit	Fe phases	Abundance[f] [%]	CS[f] [mm/s]	QS[f] [mm/s]	H[f] [T]
Original soil	3.14	Paramagnetic Fe(III)[a]	83	0.36	0.64	-
		Hematite	9	0.37	-0.20	51.3
		Fe(II) in silicates[b]	8	1.08	2.73	-
Sterile microcosm, no carbon (99)	0.56	Paramagnetic Fe(III)[a]	82	0.36	0.60	-
		Fe(II) in silicates[b]	12	1.14	2.67	-
		Fe(II) in silicates[b]	7	1.10	1.94	-
Sterile microcosm, lactate/acetate (93)	0.82	Paramagnetic Fe(III)[a]	88	0.35	0.65	-
		Unknown Fe(II) phase 1[c]	12	1.31	2.33	-
MicAct microcosm, no carbon (62.5)	1.35	Paramagnetic Fe(III)[a]	71	0.35	0.63	-
		Hematite	n.d.[e]	-	-	-
		Unknown Fe(II) phase 2[d]	13	1.21	2.49	-
		Siderite	16	1.20	1.80	-
MicAct microcosm, lactate/acetate (40)	1.29	Paramagnetic Fe(III)[a]	59	0.31	0.65	-
		Hematite	6	0.38	-0.20	51.2
		Unknown Fe(II) phase 2[d]	18	1.21	2.49	-
		Siderite	17	1.23	1.80	-

a - Paramagnetic Fe(III) might be ferrihydrite, nano-goethite, lepidocrocite, Fe(III) in clays, Fe(III) associated with humic substances or a mix of these.

b - Silicates are probably phyllosilicates such as clays and micas. Differences in the modeling parameters indicate Fe(II) present either in different silicates or in different sites of one silicate.

c - Maybe a mixture of siderite and unknown Fe(II) phase 2.

d - Unknown Fe(II) phase associated with siderite.

e - Hematite could not be modeled, since the signal to noise ratio was too low.

f - Abundance [%] = absorption of γ-ray [%]. CS = center shift, QS = quadrupol splitting, H = hyperfine field.

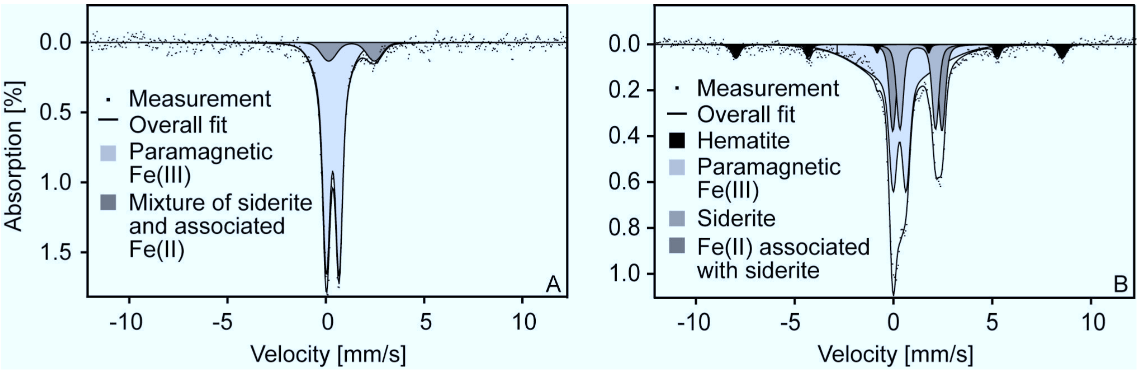

Figure 9: Mössbauer spectra at room temperature of soil Sbu from (A) sterile and (B) microbially active microcosms with 15 mM lactate/acetate amendment after 93 and 40 weeks of incubation, respectively. Fitting parameters are given in Table 3.

EXAFS analyses of soil Sbu microcosms confirmed the results of Fe extraction and Mössbauer spectroscopy. The Fe composition of the original soil was similar to the sterile setups regarding all Fe-containing phases identified by EXAFS (data not shown). The EXAFS analyses showed that in microbially active setups without carbon addition and with lactate/acetate the Fe(III) minerals goethite and hematite were reduced and the Fe(II) mineral siderite was formed (Table 4). The amount of Fe present as Fe(III) minerals in sterile setups decreased in microbially active setups by around 10%, which is slightly less than the amount of Fe found as Fe(II) in siderite in the microbially active setups. However, the main fraction of Fe (71-74%) identified by EXAFS in sterile and microbially active setups without carbon amendment and with lactate/acetate was present in augite- and ferro-smectite-like minerals and in association with humic substances. There were almost no differences in the amount of Fe associated with humic substances in sterile and microbially active setups. In contrast, the amount of Fe present in ferro-smectite like phases decreased significantly in microbially active setups, whereas Fe present in the augite-like phase increased in comparison to the sterile setups.

Table 4: Relative amounts of Fe phases (in % on a per mol basis) resulting from linear combination fits of k^3-weighted EXAFS spectra (the data are fit to k = 11 and the detection limit is approximately 5 mol% Fe). The data represents original soil Sbu and sterile and microbially active (MicAct) microcosms of soil Sbu incubated with 7 mL of water without carbon amendment (no C) or with addition of 15 mM lactate/acetate (lac/ac).

Sample	Goethite [a%]	Hematite [a%]	Siderite [a%]	Augite [a%]	Ferro-Smectite [a%]	Fe – HS[d] [a%]	Reduced χ^2 of fit
Sterile, no C[a]	20	5	-	28	15	31	0.026
Sterile, lac/ac[a]	20	6	-	28	14	31	0.037
MicAct, no C[b,c]	(16)	(-)	(14)	(19)	(6)	(46)	0.031
MicAct, lac/ac[a]	15	-	14	38	-	33	0.021

a - Measured at beamline 11-2 at the Stanford Synchrotron Radiation Lightsource.
b - Measured at beamline 4-1 at the Stanford Synchrotron Radiation Lightsource.
c - Uncertainties regarding the fit.
d - Fe associated with humic substances.

4. Discussion

After addition of gasoline, lactate/acetate or mobilization of soil organic carbon a significant change in MS occurred in almost all of the microbially active soil microcosms, but only in a few sterile microcosms (Figures 1A-G, Table S1). Among the sterile microcosms, those with autoclaved soil Hoil turned out to be also microbially active, since microorganisms in clumps of oily soil were obviously not killed by autoclaving (Figure 1G). These results show that during incubation of soil microcosms for about one year, mainly microbially mediated processes were responsible for the changes in MS of the microcosms. MS of microcosms with the crude oil-containing soil (Hoil) did not change faster or to a larger extent than the MS of microcosms with non-contaminated soil (Figures 1A-G), indicating that microorganisms adapted to hydrocarbons did not lead to an enhanced change in MS. Furthermore a change in MS of the microcosms was obvious for all soils within the first five weeks, indicating that microbial communities of non-contaminated soils adapted within this time span to the gasoline contamination and suggesting that even in the field changes in soil MS might occur within a few weeks after a contamination. The increase and decrease in MS of the soil microcosms indicated the formation and transformation of ferro(i)magnetic minerals in the soils.

Microbial (trans)formation of ferro(i)magnetic minerals especially magnetite

The most important ferro(i)magnetic minerals in soils are magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) (Mullins, 1977). In sulfur-rich environments also the ferrimagnetic minerals greigite (Fe_3S_4) and pyrrhotite (Fe_7S_8) may form (Farina et al., 1990; Stanjek et al., 1994). Regarding the microbial formation of ferro(i)magnetic minerals microbially controlled and microbially induced formation are differentiated.

Microbially controlled formation of magnetite and greigite is known from magnetotactic bacteria (Frankel et al., 1979; Mann et al., 1990) which produce intercellular chains of well-ordered, ferrimagnetic crystalls, so called magnetosomes. Although most of the known magnetotactic bacteria were isolated from aquatic environments (e.g. (Farina et al., 1990; Frankel et al., 1979; Mann et al., 1990)), they were also found in soil (Fassbinder et al., 1990). However, the cell numbers of magnetotactic bacteria determined in soils were too low to contribute significantly to the soil magnetic properties (Dearing et al., 1996; Fassbinder et al., 1990). Based on these results we therefore assume that the contribution of magnetotactic bacteria to the increase in MS of our microcosms with soil HG, Wabu, Hclean and Sbu was minor.

Microbially induced formation of ferro(i)magnetic Fe minerals refers to extracellular, secondary mineral formation during microbial Fe(III) reduction and Fe(II) oxidation, whereby especially the formation of the mixed-valent, ferrimagnetic Fe mineral magnetite was widely studied in lab experiments. Magnetite formation was observed for example during microbial reduction of ferrihydrite (Lovley et al., 1987) and hematite (Behrends and Van Cappellen, 2007). Ferrihydrite, hematite and goethite are common Fe minerals in soils (Cornell and Schwertmann, 2003). The paramagnetic Fe(III) phase identified in the present study by Mössbauer spectroscopy in original soil Sbu as well as in soil Sbu from sterile and microbially active microcosms could have been among other Fe(III) phases ferrihydrite and nano-goethite. Goethite and hematite were also detected by EXAFS measurements in the original and sterile soil Sbu. In microbially active soil Sbu, these minerals were less abundant (goethite) or not present (hematite) (Table 4), indicating microbial reduction of these minerals.

Besides microbial Fe(III) reduction also microbial Fe(II) oxidation can lead to the formation of magnetite (Chaudhuri et al., 2001; Jiao et al., 2005). In the present study the Fe(II) concentration of soil Sbu determined after incubation by 0.5 M HCl and hot 1 M HCl extraction and by Mössbauer spectroscopy was higher in microbially active microcosms than in sterile ones (Figures 8A-B, Table 3). For the other six soils of the present study the Fe(II) concentration extracted with 0.5 M HCl was also higher in microbially active microcosms (data not shown). These results indicate a net reduction of Fe(III) in the soils. However, the Fe(II) present in the original soils (e.g. soil Sbu 162.3±4.3 µmol/g, 1 M HCl (70°C) extraction) as well as the Fe(II) formed during microbial Fe(III) reduction, might have been used by Fe(II)-oxidizing microorganisms. Since the microcosms were anoxic and incubated in the dark, the activity of aerobic or phototrophic Fe(II)-oxidizing microorganisms can be excluded, whereas nitrate-reducing Fe(II) oxidizers (Straub et al., 2004) might have been present in the soil. Hence, both Fe(III) reduction as well as Fe(II) oxidation potentially led to the formation of ferro(i)magnetic minerals in soil microcosms showing an increase in MS.

In contrast to soils HG, Wabu, Hclean and Sbu, MS of microbially active microcosms with soils FB, AW and Hoil decreased over time indicating a microbially mediated decrease in the ferro(i)magnetic mineral content of the soils. As for the formation of ferro(i)magnetic minerals, most lab studies regarding the transformation of these minerals were performed with magnetite. Since magnetite is a mixed-valent Fe mineral it can serve as electron acceptor and electron donor for Fe(III)-reducing and Fe(II)-oxidizing bacteria (Brown et al., 1997; Kostka and Nealson, 1995).

Microbial (trans)formation of non-ferro(i)magnetic Fe-containing soil minerals

Soils contain also minerals in which Fe is only a minor component such as different silicates (micas, pyroxenes, amphiboles, olivine, clays) (Cornell and Schwertmann, 2003), which are paramagnetic and have only a small positive MS (Mullins, 1977). In original soil Sbu as well as in soil Sbu from sterile and microbially active microcosms a paramagnetic Fe(III) phase with unknown composition was identified by Mössbauer spectroscopy (Table 3). Additionally Fe(II) present in silicates was identified by Mössbauer spectroscopy in original soil Sbu and in soil Sbu from sterile microcosms without carbon amendment. EXAFS measurements of sterile and microbially active soil Sbu also revealed that most of the Fe (71-74%) was present in augite-like and ferro-smectite-like phases and in association with humic substances (Table 4). Augite $[(Ca,Mg,Fe,Na)(Mg,Fe,Al)\ (Si,Al)_2O_6]$ is a Fe(II)-containing pyroxene, which forms during cooling of magmas. Since augite forms at high temperature and has therefore a low silicium and oxygen content, it weathers quite easily at earth surface conditions (Perkins, 2002). Weathering of augite in the soil microcosms would have led to a decrease in the amount of Fe present in this phase. However, EXAFS measurements revealed an increase in the augite-like phase in microbially active soil Sbu in comparison to sterile soil. This was unexpected and might be due to the formation of an unknown Fe phase which gives similar EXAFS spectra as augite.

In contrast, the Fe-containing ferro-smectite-like phase in microbially active soil Sbu was lower than in the sterile soil. Kostka et al. (1999) showed that structural Fe(III) of smectites can serve as electron acceptor for Fe(III)-reducing microorganisms. The authors also showed that in presence of both microorganisms and organic ligands part of the reduced Fe was removed from the mineral structure and was present in the dissolved Fe fraction. Pore water from microbially active microcosms of soil Sbu sampled at the end of the experiments still contained significant amounts of dissolved organic carbon (0.28-1.87 g/L) (Figure 4B) despite microbial consumption. Hence, the combination of microbial activity and presence of organic ligands might be the reason for the decrease in Fe present in the ferro-smectite-like phase in microbially active soil Sbu. Royer et al. (2002) showed that humic substances can complex Fe(II) formed during Fe(III) mineral reduction, which would lead to an increase in the amount of Fe present in organic matter. However, we did not observe such an increase in humic substances-associated Fe in the microbially active soil Sbu, suggesting that either no additional Fe complexation by humic substances occurred or the release of Fe from humic substances and its complexation was balanced.

Besides magnetite also other secondary minerals can form during microbial Fe(III) reduction and Fe(II) oxidation. Depending on the geochemical conditions such as the presence of other anions, nucleation sites and humic substances and depending on the Fe reduction and oxidation rate also the formation of dissolved Fe(II), goethite, green rust, vivianite, siderite or amorphous Fe(III) phases was observed (for reviews see Fortin and Langley, 2005; Konhauser, 1998)). Since these Fe minerals have a low MS in comparison to ferro(i)magnetic minerals such as magnetite, they contribute only minor to the MS of soils. However, they might serve as electron donor or acceptor for secondary microbially catalyzed Fe redox processes and might limit or increase the amount of Fe available for the formation of ferro(i)magnetic minerals during these processes. Mössbauer spectroscopy and EXAFS measurements revealed that microbially active soil Sbu contained more siderite ($FeCO_3$) than the sterile and the original soil. The carbonate necessary for the siderite formation might originate from the soil Sbu itself, which had the highest $CaCO_3$ content (12.0±1.0 wt%) of the soils used in the present study, or from the microbial degradation of organic matter. CO_2 production during microbial activity was indicated by higher dissolved inorganic carbon contents in the pore water of microbially active soils Sbu and FB in comparison to the respective sterile soils (Figures 4B, S1), suggesting that also in $CaCO_3$ poorer soils siderite formation might have been possible.

Microbial metabolisms in soils indirectly linked to the Fe-cycle

Besides the direct microbial (trans)formation of Fe-containing minerals, also other microbially processes can lead indirectly to Fe redox changes under anoxic conditions and thereby potentially also to a (trans)formation of ferro(i)magnetic minerals in soils. Reduced sulfide species formed during microbial sulfate reduction reduces Fe(III) abiotically (Afonso and Stumm, 1992). Humic substances which can be reduced by various different microorganisms (Benz et al., 1998; Cervantes et al., 2002; Lovley et al., 1996) can in turn reduce poorly crystalline and crystalline Fe minerals (Bauer and Kappler, 2009). On the other hand nitrite a product of microbial nitrate reduction oxidizes Fe(II) (Moraghan and Buresh, 1977). If these microbially initialized abiotic processes also lead to the (trans)formation of magnetite or other ferro(i)magnetic minerals need to be determined in future experiments. However, independent of the processes leading to the (trans)formation of ferro(i)magnetic minerals in our soil microcosms over time, the results showed that these processes must be microbially mediated, since in sterile microcosms no or only minor changes in MS occurred in comparison to the microbially active ones.

Bioavailable carbon sources in soil microcosms

Mobilized soil organic carbon

The change in MS of microcosms amended only with growth medium or water without an additional carbon source (Figure 2) indicated that bioavailable carbon must have been also present in these setups. Pore water analyses revealed that dissolved organic carbon and organic acids were mobilized from the soil matrix and were consumed in microbially active microcosms (Figures 4A-B, S1A-C). Since dissolved organic carbon is one the most important carbon source in soils (Marschner and Kalbitz, 2003) it can be assumed that the microbial community was adapted to the mobilized. The fact that the microbial (trans)formation of ferro(i)magnetic minerals was not limited by the availability of organic carbon was supported by the experiment in which a second amendment of organic carbon to microcosms with soils HG and Wabu did not lead to a further change in MS (Figures 6A-B).

Gasoline as organic carbon source

The addition of gasoline led in all soils to a change MS, indicating that the gasoline was not toxic for all autochthonous soil microorganisms. Microbial communities of various soils were shown to adapt to the addition of hydrocarbons such as crude oil and gasoline and were able to degrade them partly (Bundy et al., 2002; Hamamura et al., 2006). In the present study, freshwater medium and water added to gasoline-containing soils mobilized similar concentrations of organic carbon from the soils as in setups without carbon addition (Figures

4B, S1). However, less dissolved organic carbon was consumed in microbially active setups of soils Sbu and AW with gasoline than in setups without carbon addition. Furthermore, the acetate and propionate concentrations in gasoline amended microbially active setups were even higher than in sterile microcosms of these soils (Figures 4A, S1) indicating the formation of these organic acids. Cozzarelli et al. (1994) determined the concentration of different aliphatic, alicyclic and aromatic organic acids in hydrocarbon contaminated aquifers. The organic acids including acetic acid and propanoic acid were identified as intermediates of microbial hydrocarbon degradation and their concentrations were higher near the contaminant source than in uncontaminated areas of the aquifers. One of the most abundant acids observed by the authors was acetic acid, which accumulated in regions in which the hydrocarbon degradation shifted from Fe reduction to methanogenesis. They suggested that acetoclastic methanogenesis was the rate limiting step leading to the accumulation of acetic acid. These processes might have been also responsible for the increase in acetate and propionate in microcosms with soil Sbu and AW, suggesting that the microorganisms degraded at least part of the added gasoline. The microbial degradation of hydrocarbons can be directly coupled to the reduction of Fe(III) (Lovley and Anderson, 2000; Lovley et al., 1989). However, also nitrate reducers, sulfate reducers and methanogens are able to degrade hydrocarbons under anoxic conditions (Grbic-Galic and Vogel, 1987; Kuhn et al., 1988; Rueter et al., 1994). As discussed above compounds formed during these processes might influence the formation of ferro(i)magnetic minerals as well.

Influence of soil properties and gasoline addition on the change in volume specific magnetic susceptibility of soils

For the microcosm experiments seven soils with different properties (Table 2) were used. In order to determine if soil properties had an influence on changes of MS, linear correlations between the extent of MS changes as well as the time needed to reach stable MS values and soil properties were assessed for microbially active microcosms (including autoclaved soil Hoil). Since lactate/acetate was added to only three soils, only setups without additional carbon and with gasoline amendment were considered. MS of soil AW without additional carbon decreased by >60% in contrast to the other microbially active microcosm with an average MS change of 6.9±2.4%. The calculations were therefore performed once with and once without soil AW without carbon addition (Table 5).

Table 5: Correlation coefficients after Pearson for the linear relationship between magnetic susceptibility (MS) parameters and soil properties. Soil parameters from microbially active microcosms (including autoclaved soil Hoil) without carbon addition (no C) and with gasoline addition (gas) were analyzed. The setup with soil AW without carbon addition showing an extreme decrease in MS (Figure 1F) was ones included and ones excluded from the calculations. Coefficients are only shown for reasonable and significant correlations (level of significance 5%).

Soil sample	MS parameter	Correlation with soil properties
Original soil	Soil MS	pH: -0.752
Microcosms without carbon addition including soil AW	MS change^a [%]	pH: 0.713 Soil MS: -0.783
	Maximum MS change^a [%]	$Fe_{0.5\ M\ HCl}$: 0.709 Soil MS: 0.834
	Time stable MS^b [weeks]	None
Microcosms without carbon addition excluding soil AW	MS change^a [%]	None
	Maximum MS change^a [%]	$Fe_{0.5\ M\ HCl}$: 0.909
	Time stable κ^b [weeks]	None
Microcosms with gasoline addition	MS change^a [%]	None
	Maximum MS change^a [%]	None
	Time stable κ^b [weeks]	None

a – A decrease of MS was represented by negative values and an increase by positive values

b – Time within stable κ values were reached

Influence of pH and initial ferro(i)magnetic mineral content.

The MS values of the soils were not linearly correlated with most of the soil parameters tested (Table 5). This might have several reasons. Either the correlation between the parameters was not linear, the crucial soil parameters were not tested or the sample number (here seven to eight) was too small to observe any correlation. Besides these limitations of the analysis, a significant negative correlation was found between the mass specific magnetic susceptibility (χ) of the original soils and the soil pH. χ is also an indicator for the amount of ferrimagnetic minerals present in the soil, and the results therefore suggested that with increasing pH the content of ferro(i)magnetic minerals decreased. This was unexpected, since magnetite formation is favored at higher pH values (Bell et al., 1987), whereas the lowest soil pH in the present study was pH 3.6 for soil AW (Table 2). Interestingly the decrease in MS of microbially active microcosms with soil AW without carbon addition was the highest observed in the present study, suggesting a relationship between the extent of the MS change of microcosms and the pH or χ of the original soils. And indeed such correlations were found for microbially active microcosms without carbon amendment (Table 5). However, when soil AW was excluded from the calculations because of its extreme decrease in MS, no correlation between the change in MS and the two soil parameters pH and χ occurred, suggesting that these two parameters do not determine the MS changes of the soils.

Influence of the bioavailable Fe content.

For microcosms without carbon amendment a positive correlation between the absolute value of the MS change and the Fe_{tot} extractable with 0.5 M HCl was observed. This correlation was even stronger when the setup with soil AW was excluded (Table 5). The Fe_{tot} fraction extractable with 0.5 M HCl is oftentimes considered as the "bioavailable" Fe fraction. Hence, the results suggests that the higher the concentration of the bioavailable Fe fraction in a soil the higher is the change in MS after carbon mobilization independent if MS decreases or increases. That the bioavailability of Fe might be a crucial factor is supported by the experiment in which ferrihydrite or ferrihydrite/magnetite was added to microcosms with soils Hclean and Hoil after they reached stable magnetic susceptibility values (Figures 7A-B). The Fe addition led to a further change in the magnetic susceptibility until after several weeks the microcosms reached new stable MS values, indicating that bioavailable Fe was a limiting factor for the formation of ferro(i)magnetic minerals.

Influence of gasoline addition

Compared to setups without carbon amendment addition of gasoline to the soils did not alter the direction of the MS change (increase vs. decrease) and the time needed until the MS values reached stable values were also similar for both treatments (Figures 1A-G, 2). However, regarding the MS change of the microcosms the gasoline addition had to some soils a stimulating (soils Wabu, Hclean), an inhibiting (soils Sbu, AW) or a similar (soils HG, Wabu, Hoil) effect as the addition of no carbon. This might explain why no correlation between χ and any soil parameter was observed for gasoline amended microbially active microcosms (Table 5). These results further show that the different soil microbial communities were differently well adapted to the degradation of gasoline.

Conclusion and environmental implications

In the present study we showed that the addition of hydrocarbons as well as the mobilization of organic carbon led to a change in MS of soils and that this change was mainly microbially mediated. Although the changes in MS were only around 4 to 11%, for most soil microcosms, the high sensitivity of the MS measurements allow to detect them significantly. MS measurements are able to detect even small changes in the ferro(i)magnetic soil mineral content as shown by a control experiment in which soil FB was mixed with different amounts of magnetite. An increase in the magnetite content by only 1 wt% of the total Fe content present in the soil-magnetite-mixture led to an increase of MS by 55%. Hence, an increase in κ by 10% corresponded to an increase in the magnetite content of 0.2 wt% of the total Fe content. Since in the microbially active soils of the present microcosm study also other ferro(i)magnetic Fe minerals than magnetite could have been formed and the shape and size of these minerals also determine MS of a sample (Mullins, 1977), the MS values of the microcosms can only be used semi-quantitatively. However, MS measurements are much more sensitive than Mössbauer spectroscopy and EXAFS measurements. Although MS measurements of microbially active microcosms with soil Sbu indicated an increase in ferro(i)magnetic minerals (e.g. magnetite, maghemite) such minerals were neither detected by Mössbauer spectroscopy nor by EXAFS.

Magnetic analyses of crude oil contaminated soil from a former oil field and of non-contaminated reference soil also revealed that contaminated soil had a higher MS than non-contaminated soil (Moti-Lal Rijal personal communication). The results of our microcosm experiments and the field study suggest that the measurement of the soil magnetic susceptibility is a suitable tool to localize hydrocarbon contaminations in the field. In comparison to conventional chemical analyses of soils in the lab, MS measurements have the advantage to be time and cost effecient. This method therefore allows the screening of large areas with high spatial resolution and thereby the identification of sampling points for further analytical analyses.

Except the bioavailable Fe content other soil properties did not seem to influence the change in MS, indicating that MS measurements can be applied to different soil types to localize hydrocarbon contamination. The mobilization of organic carbon led to a similar microbial (trans)formation of ferro(i)magnetic minerals than gasoline addition and might mask MS changes due to hydrocarbon input. Hence, future experiments have to reveal if hydrocarbon input into Fe-poor soils and in organic-rich soils with regular stagnant moisture also lead to measurable changes in MS. Furthermore, it can not be excluded that other soil properties than those considered in the present study (e.g. grain size distribution, water retention time in soils, porosity) also play an important role. Further investigations should therefore also include column experiments with undisturbed soil samples. Column experiments would also allow the simulation of rain fall events and thereby the formation of wet and anoxic conditions in the soils alternating with dry and oxic conditions as they can be found in natural soils.

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Supporting information

The supporting information contains results of a one-sample t-test determining the significance of changes in MS of sterile and microbially active microcosms (Table S1), results of a t-test for unpaired sample determining the significance of differences in MS changes of microbially active microcosms without carbon amendment and with gasoline amendment (Table S2) and the concentrations of dissolved organic acids and carbon in the pore water from microcosms of soils FB and AW (Figure S1).

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Table S1: Result of an one-sample t-test with a 95% confidence interval for microcosm setups with ≥3 parallels shown in Figure 1. The test value was 1 and represented no change in volume specific magnetic susceptibility (κ). As test variables the average of the last four to five volume specific magnetic susceptibility (κ) measurements of each bottle per setup was used. A microcosm setup differed significantly from 1, when the value zero was not covered by the confidence interval.

Sterility	Soil	Carbon addition	95% confidence interval		Sample significant different to 1
			lower	upper	
sterile	FB	no carbon	-0.0221	0.0207	no
		gasoline	0.0006	0.0106	yes
	AW	no carbon	-0.0240	-0.0073	yes
		gasoline	-0.0528	0.0356	no
	Hclean	no carbon	-0.0263	0.0080	no
		gasoline	-0.0139	0.0020	no
	Hoil	no carbon	-0.1027	-0.0044	yes
		gasoline	-0.0976	-0.0606	yes
	Sbu	no carbon	-0.0649	0.0236	no
		gasoline	-0.0532	0.0338	no
microbially active	FB	no carbon	-0.0768	-0.0577	yes
		gasoline	-0.1375	0.0438	no
	AW	no carbon	-0.7523	-0.4540	yes
		gasoline	-0.1353	-0.0830	yes
	Hclean	no carbon	0.0434	0.0619	yes
		gasoline	0.0749	0.1045	yes
	Hoil	no carbon	-0.0503	-0.0257	yes
		gasoline	-0.0612	-0.0376	yes
	Sbu	no carbon	0.0332	0.1930	yes
		gasoline	0.0572	0.0756	yes

Table S2: Result of a t-test for unpaired samples with a 95% confidence interval for microcosm setups with ≥3 parallels shown in Figure 1. The average extent of changes in volume specific magnetic susceptibility (κ) of microbially active microcosms without carbon addition and with gasoline was compared, as well as of microcosms with autoclaved soil Hoil. As test variables the average of the last four to five κ measurements of each bottle per setup was used. Microcosms without carbon and with gasoline differed significantly from each other, when the value zero was not covered by the confidence interval.

Soil	95% confidence interval lower	upper	significant differences between no carbon and gasoline setups
FB	-6.7765	10.8698	no
Hoil	-2.4519	0.1692	no
Hoil (autoclaved)	-5.5841	0.4737	no
Hclean	-5.1957	-2.2175	yes
Sbu	0.5818	8.7606	yes
AW	35.0660	63.7353	yes

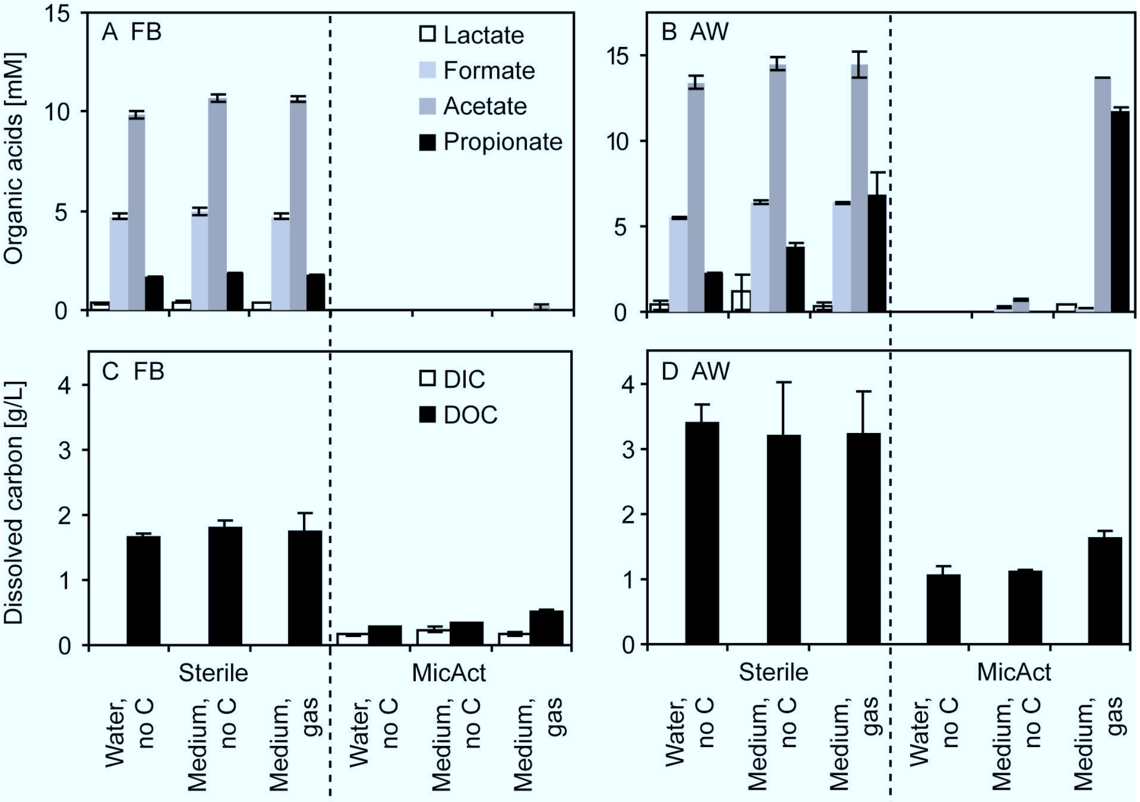

Figure S1: (A-B) Organic acids in the pore water samples after 85 weeks of incubation from sterile and microbially active (MicAct) microcosms of soils FB and AW without carbon amendment (no C) or with addition of 3.60 µL/g gasoline. As liquid either water or medium was added. (C-D) Dissolved inorganic (DIC) and organic carbon (DOC) in the same pore water as in (A). Results are means of duplicate measurements of one bottle per setup. Bars bracket the range of duplicates.

7

Conclusions and outlook

6.1 Conclusions

1. Generally, water saturation often causes dissolution of magnetic minerals in soils and sediments (Grimley and Vepraskas, 2000; Grimley and Arruda, 2007). However, we have for the first time observed that this is not the case in hydrocarbon contaminated groundwater, especially when the groundwater contains huge amounts of LNAPLs type contaminants and the water table continuously fluctuates due to remediation-driven mechanism. In this case, hydrocarbon contamination together with the fluctuating groundwater table caused an increase of magnetic susceptibility as well as of other concentration dependent magnetic parameters such as ARM and SIRM. Interestingly, the increase of magnetic parameters was lower at the site which contained lower contamination as well as lower frequency and magnitude of fluctuation.

A conceptual model for the increase of MS in the hydrocarbon contaminated groundwater table fluctuation zone is introduced. The increase of MS was interpreted as a new formation of magnetite grains and was related to dynamic redox zonation and bacterial activities. Though, the direct evidence of bacterial generated magnetic minerals and their morphological features were not identified. However, hydrocarbon degradable *Comamonas acidovorans* bacteria that was originally present at the site (Masak et al, 2003) could have played a significant role along with other iron oxidizers and reducers. Formation of magnetite during biodegradation of crude oil was also noticed in a previous study (McCabe et al., 1987). Therefore, the magnetic enhancement could have generated from the microbial degradation of hydrocarbons in various redox zones which have generated after hydrocarbons were spilled from the source zone into the groundwater. Some iron(III)-reducing microbes can use hydrocarbons as carbon source to reduce Fe(III) to Fe(II) and under certain conditions even magnetite is formed (Lovley et al., 1989; Lovley and Anderson, 2000). Therefore, the microbial degradation of hydrocarbons could be associated with the (trans)formation of magnetic iron minerals resulting differences in MS of hydrocarbon contaminated sediments in three different sections.

2. The magnetic signal of sediments, from the unsaturated zone of Hradcany site, which were formerly heavily contaminated and then remediated, showed different magnetic signal than in sediments of groundwater table fluctuation zones. These sediments were affected by remediation driven methods like soil vapor extraction and vacuum pumping implemented for the unsaturated zone, as well as air sparging and nutrient amendment (N, P and K) applied for the saturated zone for many years (Machackova et al., 2003). It is very important that the magnetic concentration signal was still high in samples from the remediated area that still contained a significant amount of hydrocarbon contamination.

3. At the former oil-field site Haenigsen in northern Germany organic contaminants such as mineral oil and diesel were present. Magnetic parameters were also significantly increased in hydrocarbon contaminated soil samples from the unsaturated zone but were significantly lower in water saturated and hydrocarbon contaminated sediment samples. Non-magnetic measurements such as maximum probable numbers (MPN) counts revealed that both iron reducers and oxidizers were present in water saturated and hydrocarbon contaminated sediment samples.

4. Natural processes are often very complicated because many natural and anthropogenic processes and pathways for the formation and transportation of magnetic iron minerals are coupling together. Therefore, laboratory investigations were performed to determine controlling factors for the change of magnetic properties due to the microbial (trans)formation of magnetic minerals by simulating hydrocarbon contamination in different soils.

The MS monitoring results clearly demonstrated that an increase of MS only appeared in non-sterile setups indicating that microorganisms played a crucial role to cause changes of magnetic signals of the soils and sediments. MS change (increase as well as decrease) was noticed immediately after beginning of incubation and reached saturation after around six weeks. However, there was no significant difference of MS between non-sterile samples with autochthonous microorganisms and non-sterile samples with added iron-metabolizing microorganisms. However, a similar MS increase was also noticed in microbially active setups without hydrocarbon contamination containing only added water. The reason for this is that the mobilization of soil carbon occurred after addition of water which could have enhanced microbial activities and caused changes of MS. Furthermore, the ferro(i)magnetic component has been increased more in microbially active setups than in sterile setups as obtained from hystereses measurements. This further supports previously mentioned argument that microbial processes were necessary for such an increase of the content of ferro(i)magnetic phases.

5. The role of the soil water content for changes of MS is quite versatile. For lower amounts of soil to added amount of water (ratio 3 g/ml), the water content has mobilization effect of soil carbon and other elements from soil matrix whereas an increase of added water amount above that limit, water has a dissolution effect for magnetic iron minerals. The former laboratory scenarios might be slightly linked with an increase of MS in Chinese loess corresponding with high rainfall and well drained condition whereas the latter one correspond with the decrease of MS in hydric soil.

6. In addition to soil batch experiments, magnetic properties of magnetite formed by *Shewanella oneidensis* MR-1 during the reduction of ferrihydrite was studied in the laboratory. MS can

provide a fast, cost-effective method that requires no sampling to follow changes of ferro(i)magnetic iron minerals that can form under different environmental conditions by various biogeochemical processes. This was well documented during the microbial transformation of ferrihydrite into magnetite. The result obtained from magnetic characterization of magnetic particles formed by *Shewanella oneidensis* MR-1 depicted that it produced a wide range of pure or partially pure magnetite particles within a superparmagnetic to single domain grain size range.

6.2 Outlook

6.2.1 Magnetic investigation of hydrocarbon contaminated soil and sediment

The use of MS as a proxy parameter to delineate polluted areas can be an attractive method because the method is fast, cost-effective, and sensitive to a low detection limit (Thompson and Oldfield, 1986; Walden et al., 1999; Maher and Thompson, 1999). However, a meaningful analysis of magnetic parameters, their interpretation as well as deriving conclusions from them requires additional knowledge about different processes and pathways of contaminants. Moreover, the measured integral signal has to be differentiated from the natural background signal for the determination of magnetic signals that are directly related with the contamination of soils and sediments. If natural processes and pathways are coupling together then it is a challenging task to separate the contributions of those processes from the bulk magnetic signal of a sample. Therefore, it is crucial to separate different types of pollution such as organic e.g. hydrocarbon pollution and inorganic e.g. heavy metal contamination and their contribution on the measured bulk magnetic signal. It would be an incorrect interpretation if the linkage between magnetic parameters and organic pollution are established when heavy metals are also present in a sample. In such scenario, the sole effect of organic pollution on magnetic signal can not be separated.

6.2.2 Influences of natural processes on magnetic properties

The study of organic pollution with magnetic methods is more complicated than in case of heavy metal pollution because the processes and pathways of change of iron iron minerals after hydrocarbon contamination are not as clear as in the case of heavy metal pollution. It is very important to understand the following factors such as magnetic signals of natural background material i.e. clean soils and sediments, the role of soil forming processes, hydrocarbon contamination, seasonal water table fluctuations, rainfall effects, yearly wet and dry cycles, periods and frequency of rainfall, water content, oxic and anoxic conditions and microbial and abiotic iron cycling. The effect of these factors on the changes of the magnetic signal and the effect of coupling of these factors need to be taken account. Additionally, variation of environmental geochemical conditions such as pH, presence of sulfate, carbonate, humic substances as well as iron bearing clay minerals, type and mechanism of release of contamination, aging of organic contaminants after their release into the environment, hydrogeological characteristics of natural materials and sorption capacities of iron oxides either to immobilize or release contaminants have to be understood. More importantly, the role of microorganisms for the formation and transformation of iron and/or magnetic iron minerals in hydrocarbon contaminated sites under different environmental conditions has to be examined. Therefore, it is important to understand microbial iron cycling in hydrocarbon contaminated sites and compare it with nearby sites having the same environmental conditions so that the effect of hydrocarbon contamination during microbial iron cycling can be clearly investigated. Ferro(i)magnetic minerals both in hydrocarbon contaminated sites and under natural field conditions can also be transformed abiotically due to variation of geochemical factors such as pH and organic acid leading to variations of MS without any direct contribution of microbial activity. Our laboratory experiments revealed that after addition of certain amount of water, MS increased in microbially active setups due to the mobilization of organic carbon from the soil matrix whereas a high concentration of humic acid impeded the formation of magnetite during ferrihydrite reduction with *Shewanella oneidensis* MR-1 in pure culture.

6.2.3 Sample characterization and methodological improvements for shallow sub-surface investigation

For the improvement of magnetic methods in terms of its sensitivity and depth of investigation for environmental purposes, especially for hydrocarbon pollution studies in both saturated and unsaturated conditions, analysis of geochemical parameters by sequential iron extraction, quantification of iron oxidizers and reducers by most probable number (MPN) method, Mössbauer spectroscopy at room as well as at low temperatures, scanning electron microscopy (SEM) and extended X-ray absorption fine structure (EXAFS) for mineralogical and morphological characterization of iron mineral grains can be combined together. Geochemical techniques, though expensive as well as more time consuming than grain-size dependent characterization by magnetic techniques, provide an additional confirmation for the characterization of magnetic minerals. Furthermore, this facilitates to better characterize newly formed iron mineral grains, the mechanism of their origin as well as their transportation pathways.

Not only mineral magnetic characterization techniques can be combined with chemical as well as microbial analytical techniques but also the depth of investigation capacity of magnetic methods for environmental investigation can be increased by integrating magnetic sensors such as SM400 with direct-push technique. This can be done by adapting the diameter of the pipe made up of non-magnetic material which exactly fits with the dimension of SM 400 instrument. This gives an opportunity to sample contaminated soils and sediments from more than a meter depth which is not the capacity of current sample corer which can retrieve material only from a half meter depth. This also provides constraints for the online measurement of MS with SM400 that is generally inserted into the hole that was formed after collecting the core sample. Hence, there is a better option for technical developments of instrumentation for the higher depth coverage and increasing the depth of investigation.

6.2.4 Suggestions for future research

For the future investigation of magnetic and geochemical parameters that can mainly play a role for the modification of the magnetic signal in soils and sediments, various types of laboratory experimental setups are proposed. Among them, well characterized iron minerals in combination with sediments/soils and with/without hydrocarbon contamination under variable geochemical conditions such as water content, drainage conditions and humic substances concentration are some options. Moreover, the role of seasonal water table variation and yearly wet and dry cycles for the change of MS can be setup simulating such conditions in the laboratory both with and without hydrocarbon contamination so that the role of hydrocarbon contamination is better understood. In such way, several column and tank experiments can be setup in a laboratory. However, simultaneous field investigations are also required. The ideal laboratory setups would be achieved by sampling field sites with uncontaminated material nearby a contaminated site, simulate contamination on it as observed at the nearby site and use this material in columns and tanks by varying a single factor at a time. This could give possible answers for the main controlling factors that are responsible for the change of magnetic parameters under such environmental conditions. By doing such investigations, it is likely that answers of many open questions which remain unanswered by this part of the research could be achieved from future experiments.

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