7th Symposium on remediation in Jena
“Jenaer Sanierungskolloquium”

"Metal stress: biotic and abiotic factors"

22-23 September 2008

Friedrich Schiller University Jena
Wöllnitzer Str. 7, D-07749 Jena, Germany

Conference proceedings
Table of Contents

Conference Program
Welcome
Map of the meeting place
Acknowledgements for financial supports
Abstracts of the talks
Abstracts of the posters
List of participants
Notes
Conference Program

Monday, 22nd September 2008

09:00  Start of Symposium
Opening of the meeting & Introduction: Erika Kothe

Session 1 – Metal transport in acid mine drainage waters
Chair: Hans Bergmann
10:00  Mark G. Macklin, Aberystwyth, Wales, UK
       *A geomorphological approach to the management of rivers contaminated by metal mining*
10:30  Bert Allard, Örebro, Sweden
       *Metal releases from a historic sulphidic mine site (Ljusnarsberg, Sweden) - mobilization and attenuation processes*
11:00  Kilian Pollok, Bayreuth, Germany
       *Pyrhotite weathering and secondary mineral formation: A first survey on microstructural controls*
11:20  Martin Lonschinski, Jena, Germany
       *Heavy metals and radionuclides in groundwater and soil water on the remediated heap Gessenhalde*
11:40  Anja Grawunder, Jena, Germany
       *Rare earth elements in the soil-water-plant-system at the test site Gessenwiese*

12:00 Lunch

Session 2 – Precipitation and (bio) mineral formation
Chair: Georg Büchel
14:00  Giovanni De Giudici, Cagliari, Italy
       *Bioprecipitated hydrozincite in mine abandoned environment: investigation on the biological control*
14:30  Fernando Rull, Valladolid, Spain
       *Raman spectroscopic study of the chemical equilibrium and mineral precipitation processes in Rio Tinto, Spain*
15:00  Andreas Fritzschke, Jena, Germany
       *Changes in arsenic mobility as a result of stability changes of iron colloids after leaching from soils*
15:20  Juliane Hopf, Bayreuth, Germany
       *New phosphates and sulphates produced by a Streptomyces acidiscabies strain?*
15:40  Walid Salama, Jena, Germany
       *Origin of the Fe ooids and oncoids of the Lower Eocene ironstones, Bahariya Depression, Western Desert, Egypt*

16:00 Coffee break

Session 3 – Influence on microbial communication
Chair: Erika Kothe
16:30  Irene Lichtscheidl, Vienna, Austria
       *Copper mosses and their response towards heavy metal stress*
17:00  Anna-Rosa Sprocati, Rome, Italy
       *Harnessing natural degradation potentials: A challenge for bioremediation strategies*
17:30  Valerian Cioibota, Jena, Germany
       *Characterization of microorganisms by means of Raman spectroscopy*
17:50  Eva-Maria Burkhardt, Jena, Germany
       *Fe(III) reducing microbial communities and their influence on metal mobility*
18:10  Christian Lorenz, Jena, Germany
       *Geomicrobial transfer of heavy metals monitored by Rare Earth Elements*

19:00 Visit of the observatory and BBQ at the Institute of Microbiology
Tuesday, 23rd September

Session 4 – **Plant stress response**
Chair: Gerd Gleixner
9:00 Aurora Neagoe, Bucharest, Romania
   *Native vegetation in mining areas as source of contaminated hot spots remediation*
9:30 Katarzyna Turnau, Krakow, Poland
   *Practical use of plant-fungal interaction in phyto remediation of heavy metal rich industrial wastes*
10:00 Lars Zeggel, Jena, Germany
   *Microbe assisted phytoextraction on contaminated concrete*
10:20 Daniela Siegel, Jena, Germany
   *Analysis of fungal activity on black shale and graphite surfaces using vertical scanning interferometry*
10:40 Frank Schindler, Jena, Germany
   *Heavy metal resistance in Streptomyces*
11:00 Coffee break

Session 5 – **Mechanisms of interaction**
Chair: Kirsten Küsel
11:30 Thuro Arnold, Dresden-Rossendorf, Germany
   *Uranium in biofilms*
12:00 Virgil Iordache, Bucharest, Romania
   *Ecotoxicological mechanisms of microbial and plant resistance to metals at population and community level*
12:15 Ileana Cornelia Farcasanu, Bucharest, Romania
   *“Kamikaze” Saccharomyces cerevisiae mutant cells for heavy metal hyperaccumulation: A primary step to bioremediation by removal and recovery of heavy metals from contaminated environments*
12:30 Angela Meier, Jena, Germany
   *Experiments to the interaction of hematite in reservoir sandstones with organic compounds*
12:50 Evelyn Krawczyk-Bärsch, Dresden-Rossendorf, Germany
   *The response of biofilms to uranium impacts*
13:10 Felicia Gherghel, Jena, Germany
   *Heterogeneity of ectomycorrhizal community - heavy metals relationship*
13:30 Lunch

Session 6 – **Protection of water paths and the food chain**
Chair: Kai-Uwe Totsche
14:30 Kevin Hallberg, Bangor, Wales, UK
   *Biogeochemistry of AMD: key microorganisms and the roles they play in attenuation of mine pollution*
15:00 Hermann Bothe, Köln, Germany
   *Arbuscular mycorrhiza, heavy metal and salt tolerance*
15:30 Jana Sitte
   *Heavy metal tolerant sulfate-reducing prokaryotes in a gleyic creek soil at the former uranium mining district Ronneburg*
15:50 Kai-Uwe Totsche, Jena, Germany
   *Biogeochemical interfaces in soil – A DFG priority research program SPP 1315*
16:10 Denis Jianu, Bucharest, Romania
   *Integrated interpretation of geochemical and geophysical data for the estimation of metals’ stocks in contaminated areas*
16:30 Closing remarks: Hans Bergmann
End of the symposium
Welcome

to

7th Symposium on remediation in Jena "Jenaer Sanierungskolloquium"

focusing on

"Metal stress: biotic and abiotic factors"

The 7th Symposium on remediation is dealing with metals in the environment as ecotoxicological risk for organisms being influenced by biotic and abiotic factors. Among the biotic factors, especially in contaminated soil, the activities of microorganisms and the chemical components exuded by plant roots are major players. In terms of abiotic factors, the hydrogeological parameters, mineral formation and dissolution, or physico-chemical soil properties largely influence metal release and reactive transport.

In this year, participants from 8 countries will present their research in 6 sessions with 40 talks and poster presentations. Session 1 starts with metal transport in acid mine drainage waters, followed by precipitation as (bio)mineral formation (session 2). Session 3 focuses on influence on microbial communication, session 4 deals with plant stress response, and session 5 shows mechanisms of interaction. Finally, protection of water paths and the food chain will be discussed in session 6.

Bio-geo-interactions are in the focus of the research of the Friedrich Schiller University of Jena. PhD projects of the graduate school “Alteration and element mobility at the microbe-mineral interface” supported by the German Research Foundation (DFG), as well as research projects supported by the German Ministry of Education and Technology are presented in this symposium. All projects here are of the Jena School for Microbial Communication. This research focus of the Friedrich Schiller University is also reflected by the newly established BSc/MSc program Biogeosciences and the MSc Microbiology.

Hans Bergmann, Erika Kothe & Georg Büchsel
Map of the meeting place

http://www.jena.de/stplan/

x: Meeting place (Wöllnitzer Str. 7)

*: BBQ at the Institute of Microbiology (Neugasse 25)

symbol Sh: observatory "Universitätssternwarte" (Schillergäßchen 2/3)
Acknowledgements for financial supports
Abstracts of talks
A geomorphological approach to the management of rivers contaminated by metal mining

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As the result of current and historical metal mining, river channels and floodplains in many parts of the world have become contaminated by metal-rich waste in concentrations that may pose a hazard to human livelihoods and sustainable development. Environmental and human health impacts commonly arise because of the prolonged residence time of heavy metals in river sediments and alluvial soils and their bioaccumulative nature in plants and animals. This paper considers how an understanding of the processes of sediment-associated metal dispersion in rivers, and the space and timescales over which they operate, can be used in a practical way to help river basin managers more effectively control and remediate catchments affected by current and historical metal mining. A geomorphological approach to the management of rivers contaminated by metals is outlined and four emerging research themes are highlighted and critically reviewed. These are: (1) response and recovery of river systems following the failures of major tailings dams; (2) effects of flooding on river contamination and the sustainable use of floodplains; (3) new developments in isotopic fingerprinting, remote sensing and numerical modelling for identifying the sources of contaminant metals and for mapping the spatial distribution of contaminants in river channels and floodplains; and (4) current approaches to the remediation of river basins affected by mining, appraised in light of the European Union's Water Framework Directive (2000/60/EC). Future opportunities for geomorphologically-based assessments of mining-affected catchments are also identified.
Metal releases from a historic sulphidic mine site (Ljusnarsberg, Sweden) - mobilization and attenuation processes

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Mining started in the Bergslagen region, some 60 000 km² in South Central Sweden, more than a thousand years ago. In the beginning of the 20th century there were some 100 mines in operation, but the number is presently only 4. In 2004 the Bergskraft Bergslagen programme was created as a collaboration between local communities, companies, regional and national authorities and universities with the aim to stimulate exploration for new ore deposits and re-evaluation of old mines and previously neglected mineralisations in the Bergslagen region.

Another task for the programme is to elaborate and test strategies for remediation of the mine deposits and metal polluted areas in the region. Remains from mining operations can be seen at more than 8500 places, and there are at least some 500 deposits with sulphidic ore residues that would require remediation actions. The present project represents one of the Bergskraft Bergslagen activities towards the goal to assess a sustainable technique for remediation of historic sulphidic mine sites and mining waste deposits (see Allard and Herbert, 2008, as well as Höglund and Herbert, 2004).

Reactions in porous rock pieces typical of historic mine waste are: Sulphides (mainly pyrite and other iron sulphides) are oxidised by O₂. The iron is hydrolysed and precipitates eventually as Fe(III)- hydroxides. The sulphide oxidation can, however, take place without O₂, with Fe(III) serving as the electron acceptor. Present in the historic mine deposits and environments are generally substantial quantities of Fe(III). Thus, oxidative weathering would be expected even in the absence of air, although slowly at high pH when the iron would be present as a solid oxy-hydroxides.

The strategy selected for the present field demonstration of remediation of a historic mine site is:
- Injection and/or mixing the mine waste with components that may prevent the Fe(III)-driven sulphide oxidation
- Design of reactive barriers with capacity to immobilise the released metals in effluents
- Use of cheap additives and components - waste products if possible

Tests have been started (m³-scale in the field, as well as in laboratory scale) at the Ljusnarsberg mine field, Kopparberg:
1. Artificial precipitation is percolating through drums with mine waste and additives, either in homogeneous mixtures or in separate layers – 8 systems, started in 2007
2. Leachates or mine water is passing through a horizontal filter system with three compartments in sequence: pH-increase, aeration/iron precipitation and adsorption – 8 systems, started in 2008

Components used so far are:
Alkaline waste materials – lime kiln dust (from lime production), green liquor dreg (from pulp and paper production), steel slag (from steel production), bio-fuel fly-ash (from incineration plant)
Adsorbing agents – peat nuggets, limestone, apatite (phosphate), Leca pellets (illite)
Support – crushed brick

A steady increase in pH is observed in all systems, as well as a pronounced reduction of metal fluxes from the mine waste, but also appearance of ”new” metals from the additives (e.g. V). The systems have, however, not reached steady-state after more than 6 months. The field programme, with supporting laboratory tests, will continue for a minimum of three years. Detailed studies of oxidation of sulphides at high pH (above 10) in the absence of oxygen will be performed, in order to establish the role of Fe(III) as a possible electron acceptor.
Pyrrhotite weathering and secondary mineral formation: A first survey on microstructural controls

Kilian Pollok

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Sulfide dissolution and the formation of secondary phases play a fundamental role for the release and mobility of metals in acid mine drainage water. Pyrrhotite is, after pyrite, the most common iron sulfide in nature and is found in a wide range of magmatic and metamorphic rocks as well as in massive deposits. The oxidation rates of pyrrhotite are on the order of 20 to 100 times higher compared with pyrite. The variable Fe/S ratio (Fe$_{1-x}$S, with $x = 0 - 0.125$) allows for various stacking sequences and ordered superstructures. Furthermore, twinning, stacking faults, and dislocations are common in natural samples. Thus, the characterization of the structure and distribution of reactive surfaces requires methods of investigation that provide crystallographic, chemical, and textural data from the mineral-product interfaces with highest spatial resolution. This study focuses on the influence of the microstructure on the reacting front and the spatial relationship between the primary mineral and the secondary phases at micro- and nanometer scale. Pyrrhotite (from Dalnegorsk, Russia) is associated with pyrite and secondary goethite. Transmission electron microscopy (TEM) reveals the 4C modification as dominant superstructure as well as numerous stacking faults. Weathered rims have a thickness of about 20 µm and are compositionally heterogeneous. A first shell (about 15 µm) consists of fine-grained Fe-oxyhydroxide which is loosely bound to pyrrhotite. A second shell (less than 5 µm) is formed by massive secondary FeS$_2$ (probably marcasite). Where pore space is available, grains of goethite (5 to 20 µm in diameter) have been formed.

The results will be discussed on the background of interface-coupled dissolution-precipitation reactions and thermodynamic and kinetic considerations will be presented.
Heavy metals and radionuclides in groundwater and soil water on the remediated heap Gessenhalde

M. Lonschinski, D. Merten, G. Büchel

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The Gessenhalde was a leaching heap at the former Uranium mining area Ronneburg (Germany). During the active mining period (1971-1990) low ore substrate was leached by Acid Mine Drainage (AMD) and sulphuric acid (Beleites, 1992) to exploit the Uranium. The leachate infiltrated through the barrier soil and was retained on the glacial sediments underneath. The leaching process can be considered as the general source for the heavy metal contamination currently observed in groundwater. Processes like sorption/desorption or coprecipitation/remobilisation of heavy metals involving Fe-, Mn-, Al-oxides/hydroxides control the amount of heavy metals in the water phase today. The present study describes the residual contamination in the water phase in a post mining site that is said to be remediated according to the state of technology. The research is focussed on a part of the Gessenhalde of about 5.000 m², where a test site for planting trials was installed. High concentrations of Ni (up to 56 mg/l), Co (up to 20 mg/l), Zn (up to 20 mg/l), Rare Earth Elements -REE (up to 4 mg/L of Ce), Cu (up to 4 mg/l) and U (up to 3.5 mg/l) occur in the groundwater. The spatial distribution of the heavy metals is related to the different geological units acting as aquifers with different release potential of heavy metals. The sandy aquifer in the upstream area of the test site and the southern test site has a low release potential today, since the wash out seems to be complete. In contrast the central and northern part of the test site still have high release potentials leading to high amounts of heavy metals in the groundwater (Grawunder, 2008).

The concentrations of Ni, Co, Mn, Cd, Zn and the REE are correlating with the sulphate concentration (Pearson correlation coefficient > 0.75; level of significance < 0.01) in the water, indicating a relation of these elements to the AMD/sulphuric acid leaching. By contrast, elevated concentrations of Cu, Th and U in the groundwater are spatially related to small sites. These sites probably represent sources (not identified yet) with higher release potential for these elements. Studies of the temporal trends in the concentration of heavy metals indicate a general wash out of heavy metals from this area. Longer periods of dry weather conditions lead to increased concentrations of heavy metals, while rainy periods increase the wash out process dramatically. Studies on soil water indicate lower amounts of Ni, Co, Zn, Mn and SO₄²⁻ in relation to the groundwater, but concentrations of Cu (up to 21 mg/l), U (up to 13.7 mg/l) and Th (up to 70 µg/l) are partly significantly higher in the soil water. The high concentrations could be caused by low pH conditions (< 3.0).
Rare earth elements in the soil-water-plant-system at the test site Gessenwiese

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Rare earth elements (REE) comprise the elements from atomic number 54 to 71 (La to Lu). Because of their closely chemically coherent behaviour, REE can, after standardisation to Post Archean Australian Shale PAAS (Taylor & McLennan, 1985), be used to study processes. Here, REE are investigated in order to understand the processes in the water-soil-plant system.
To characterise the behaviour of REE in this system, studies were carried out with soil material, groundwater and plants of the test site Gessenwiese, situated on the basement area of the former uranium leaching heap Gessenhalde near Ronneburg, Thuringia, Germany. At this heap, Palaeozoic rocks with a low uranium mineralisation, which represent the source of the REE as well as other heavy metals, were dumped on a basement of Quaternary sediments and leached with acid mine drainage and sulphuric acid.
The method of sequential extractions is used to characterise the bonding of heavy metals to soil components (mobile, adsorbed, bound to Mn-/ Fe-oxides or organic matter) and with it the probability of their mobilisation.
The PAAS normalised patterns of Palaeozoic rocks, similar to those deposited on the former leaching heap, show MREE enrichments in the results of sequential extraction. Total REE concentrations of the Quaternary sediments result after standardisation to PAAS in straight lines, but regarding the results of sequential extraction, especially the mobile fraction shows comparable patterns to the groundwater: an enrichment of MREE as well as a positive Ce-anomaly. Sequential extraction indicates strongest Ce-anomalies in the fraction interpreted as Mn-oxide bound fraction. Furthermore, a Mn-Ce correlation was found in a hardpan layer by LA-ICP-MS. In general, Mn-oxides are well known to concentrate Ce through oxidation to immobile Ce^{4+}, while Fe-oxides enrich all REE in a similar order of magnitude. At the test site different Mn oxides were observed in the layers above the aquifers. Because of their different Eh-pH stability in acidic environments, a possible explanation is that the positive Ce anomaly is caused by dissolution of Mn-oxides and with it a stronger input of Ce into the ground-/soilwater.
The REE patterns of the mobile fraction can be followed up into the plants - shown on the example of Festuca rubra. Roots and blades of grass were digested and result in a fractionation of HREE with higher concentrations in the roots compared to the culms.

Bioprecipitated hydrozincite in mine abandoned environment: investigation on the biological control

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The occurrence of hydrozincite \([\text{Zn}_6(\text{CO}_3)_2\text{OH}_6]\) biomineralization at Naracauali creek (Sardinia, Italy) has been previously shown to be effective in the abatement of heavy metals in polluted stream waters (Podda et al. 2000). In this work, crystallinity and microscopic properties of hydrozincite samples from Naracauali and reference standards of hydrozincite were investigated by using X-ray diffraction, Infra Red spectroscopy, Nuclear Magnetic Resonance spectroscopy, Scanning Electron Microscopy and High Resolution Transmission Electron Microscopy. Overall, the experimental measurements indicate that crystallites of museum samples are larger than crystallites of synthetic and biomineral samples. \(^{13}\text{C}\) MAS and CPMAS NMR spectra show for all the sample investigated more than one peak, despite the carbon atoms have only one crystallographic position in the pristine hydrozincite structure. The additional peaks reflect the presence of lattice defects typical of nanocrystals as indicated by the HR-TEM images where high concentration of line, plane and surface defects can be observed especially in the nanocrystals both in the natural and synthesized samples. This work provides new insight on microscopic properties of biologically controlled hydrozincite that will be useful in predicting its reactivity and stability.
Raman spectroscopic study of the chemical equilibrium and mineral precipitation processes in Rio Tinto, Spain

Fernando Rull
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Rio Tinto mine waters contain extremely high concentrations of sulfuric acid and dissolved iron. In this paper Raman spectroscopy is used as the main tool to characterise the chemical equilibrium in acidic aqueous solutions as an approach to the study and characterisation of the equilibrium processes and sequence of mineral precipitation in Rio Tinto (SW Spain). Identification of the different chemical species and quantitative analysis of the chemical equilibrium in the system H₂O-H₂SO₄ and H₂O-H₂SO₄-FeSO₄ is studied using synthetic solutions. The activity coefficient product as deduced from Raman data is reported. Also diffusion coefficients for the different chemical species have been estimated from the evolution of the Raman spectra with distance and time in dedicated experiments in capillary and narrow glass tubes. Finally Raman spectra from Rio Tinto’s stream water obtained at the laboratory and in-situ on the river are reported together with the main mineral species detected. These results have proved that Raman spectroscopy is a powerful tool allowing to carry out an accurate quantitative analysis of concentrations of species present in the system iron(II)–sulfuric acid–water and their individual diffusion coefficients. Also these results show the capability of Raman spectroscopy to identify these species in the river acidic waters which in their turn represent an important step in developing appropriate models for the mineral precipitation processes.
Changes in arsenic mobility as a result of stability changes of iron colloids after leaching from soils

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Arsenic is known for its distinct affinity for sorption on Fe(III) hydroxides thus making the mobility of Fe hydroxides a crucial parameter for As mobility. After mobilisation and leaching from soils into oxic environments, Fe(II) is subject to re-oxidation which usually leads to sedimentation of Fe(III) hydroxides. However, we could prove a predictable and enduring presence of Fe hydroxide nanoparticles. Concurrently, As was found to be strongly associated with Fe, implying that increased Fe mobility due to smaller particle sizes considerably affects As mobility. We conducted a soil column experiment using material from the top horizon of a floodplain soil near the Mulde river (Saxony-Anhalt, Germany). The soil columns were operated under saturated flow conditions including flow interrupts up to several weeks. We especially focused on particle formation dynamics in the effluent by measuring particle size and Zeta potential ($U_E$). Fe was discharged completely as Fe(II) and oxidised rapidly after exposure to oxic conditions. However, we only observed sedimentation of Fe hydroxides after flow interrupts. This coincided with a maximum of particle size and $U_E \approx -10$ mV suggesting coagulation due to limited colloidal stability. During flow conditions, Fe colloids coagulated to a much lesser extent resulting in particle sizes $<100$ nm and strongly decreased sedimentation. However, $U_E$ only decreased to $\approx -18$ mV which is still within the range of limited interparticular stability. We suppose that the observed Fe colloid stability is a result of steric forces induced by organic coatings on the Fe colloids. These repulsive forces were only overcome by cation bridging that took place at elevated ionic strength present after flow interrupts. Therefore, increased As mobility by co-transport on mobile Fe colloids should be taken into account in natural aquatic environments where DOC can crucially enhance interparticulate stability.
New phosphates and sulphates produced by a *Streptomyces acidiscabies* strain?

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Multiple tolerances against heavy metals are known for some of the soil-dwelling *Streptomyces*. For example *Streptomyces acidiscabies* E13, isolated from a heavy metal contaminated former uranium mining site, is highly nickel tolerant (up to 10 mM). Under laboratory conditions this bacterium is capable of precipitating directly or indirectly a new biominal putatively named nickel struvite Ni(NH₄)₂(PO₄)·6H₂O [Haferburg et al. (2008)]. Microbial precipitation of such heavy metal containing compounds is considered to be a detoxification mechanism to prevent toxic effects of the heavy metal itself. Remediation processes could use this inducible formation of struvite for the decontamination of heavy metal polluted soils and waste waters.

In this study, new experiments have been performed to test the ability of *Streptomyces acidiscabies* E13 to form related biominerals with other heavy metals (Ba, Cd, Co, Cr, Cu, Mn, Zn). The bacteria were incubated in liquid growth media spiked with heavy metal chlorides. In the course of experiments small sample aliquots were studied with light microscopy. After 2 weeks of incubation at 28°C we observe the formation of polycrystalline spherical (about 50 μm in diameter) aggregates in experiments with barium, chromium and manganese cations.

The examination of the precipitated crystals was subsequently carried out using analytical TEM, SEM and microprobe techniques. A precipitation of phosphorous-containing barite could be observed in the experiment with solute barium. This finding is comparable with previous laboratory experiments by González-Munoz et al. (2003), observing the formation of barite in the presence of *Mycxoccoccus xanthus*. The incubation of *Streptomyces acidiscabies* E13 with chromium and manganese ions leads however to the formation of a specific magnesium-phosphate assimilable to the Mg₃(PO₄)·4H₂O compound synthesized by Kongshaug et al. (2001).

Further control experiments are under way to test whether these crystals would not form in absence of a biological component.

References


Origin of the Fe ooids and oncoïds of the Lower Eocene ironstones, 
Bahariya Depression, Western Desert, Egypt

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The Lower Eocene ironstones of the Bahariya Depression are composed mainly of autochthonous/para-autochthonous facies rich in Fe ooids and oncoïds and various ferruginized skeletal particles. The Fe ooids and oncoïds are identical in mineralogy and they are different in grain size, external morphology and internal microfabrics. The majority has discoidal and ellipsoidal forms, less frequently being spastolithic and spheroidal. Internally, they have skeletal or non-skeletal cores (nuclei) or they may lack cores. The ooidal cortical laminae are smooth to slightly wavy, while large oncoïds exhibit highly crenulated, knobby and club-shaped microstromatolitic cortical laminae. The Fe oncoïds may incorporate small ooids and nummulite tests within their cortices, forming hybrid grains. Large Fe oncoïds display multiphase encrustations with cross-cut cortical laminae.

The cortical laminae comprise alternating dark organic-rich green and brown laminae and organic-poor yellow and orange laminae. Under SEM, they have a random internal microfabric and they consist mainly of rounded grape-like aggregates of ferrihydrite together with acicular goethite. Amorphous silica and/or microcrystalline quartz laminae are also observed using petrographic microscopy, BSE and EDX. Authigenic plate-like patches of psilomelane are also observed within the dark green laminae.

The cortical laminae exhibit gradual contacts between each other and with the surrounding goethite and quartz matrix. They contain intercortical voids, associated with the organic-rich laminae.

The above internal microfabrics of both Fe ooids and oncoïds indicate intra-sedimentary autochthonous origin of these grains. The variable mineralogical composition of the cortical laminae reflects the role of early diagenesis and decomposition of organic matter in the mineral dissolution and segregation.

The autochthonous ooids and oncoïds are further reworked and concentrated by storm and/or tidal currents.
Copper mosses and their response towards heavy metal stress

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We investigate bryophytes from copper-rich soil in the Grossarl valley near Hüttenschlag in Salzburg (Hohe Tauern, Austria) and on prehistoric mining heaps on Hochkönig (Salzburg, Austria). The total copper content of the soil reaches up to 7,000 ppm, plant available copper is an order of magnitude less. The few higher plants growing there are specialists known also from other heavy metal habitats; some have distinct stress symptoms like nanism or overproduction of anthocyanins, leading to an intensive red colouration of the leaves.

Concerning bryophytes, we investigate 2 sorts of mosses, Mielichhoferia elongata and Pohlia drummondii, and the liverwort Scapania undulata. Their copper tolerance is of special interest with respect to interaction with soil microorganisms: while some liverworts were described to have symbiosis with different sorts of microorganisms, no interaction with mykorrhiza has been found for mosses so far.

Heavy metal content of plants and soils was analysed by flame atomic absorption spectroscopy (F-AAS) and inductively coupled plasma - mass spectrometry (ICP-MS). Copper values of up to 25,000 ppm in the green living plants suggest that copper is hyper-accumulated. Such enormous copper uptake appears even more striking because of the low plant availability of copper in the soil. Investigating single moss plants by X-ray microanalysis (EDAX) in the scanning electron microscope (SEM) showed the local distribution of copper within the plant; in living green parts, copper is distributed regularly or with a slight increase in the central region tip. In the dead bottom parts of the moss plants the concentration is highest. Heavy metals are bound mainly to the cell surface. Inconsistencies between EDAX and AAS suggest that only analysis of individual moss plants allows for reliable data.

Culture of the mosses on agar plates spiked with copper showed that also here copper is taken up, however, to a lesser extent. This indicates that mosses are capable of accumulating copper from the substrate in addition to collecting it over their whole surface.
Harnessing natural degradation potentials:  
A challenge for bioremediation strategies

Anna-Rosa Sprocati

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Microbial processes responsible for the biodegradation of organic contaminants and for the transformation of inorganic contaminants are the driving forces behind natural attenuation and detoxification.

Nevertheless the accumulation in the environment of highly toxic pollutants emphasises the fact that microorganisms, by them-self, are insufficient to protect the biosphere from anthropogenic pollution. They can be harnessed in enhanced bioremediation technologies.

Harnessing the natural bioremediation potentials is the current challenge to be addressed by the environmental research. This challenge must consider the main problems faced by bioremediation technology, i.e: i) most degradation potentials are ubiquitous but indigenous microbes are usually present in very small numbers, ii) the degradative metabolism towards specific pollutants often needs to be induced, iii) biodegradation is inhibited by the presence of heavy metals, iv) effective heavy metal capture and removal is not of easy solution. Possible ways to activate these natural potentials include changing physico-chemical parameters (pH, T, electron donors or acceptor etc) as well as a "niche adjustment", by the inoculation of competent microorganisms into these systems. Such biogeochemical management is realised through different bioremediation approaches, whereof the predominant techniques are biostimulation and bioaugmentation. Both these techniques have often originated controversy. Analysis of comparative works indicates that detailed site specific characterisation studies are needed prior to deciding on the proper bioremediation methods. Bioaugmentation offers a way to provide specific microbes in sufficient numbers to complete the biodegradation. On the other hand few issues in environmental biotechnology generated more controversy than the use of bioaugmentation. Dispite its apparent simplicity there have been many failures, described by exhaustive studies (Goldstein, 1985, Stephenson and Stephenson 1992, Bouchez 2000, Vogel and Walter 2001, Wagner-Döbler 2003). The interpretation of these results indicates that bioaugmentation studies generally suffer from a lack of ecological data concerning the fate and the activity of the inoculated microorganisms and their relationship with the indigenous microbial communities.

Based on the common belief that it is time to initiate more comprehensive approaches to find common rationales in bioremediation, this note will focus on bioaugmentation, discussing problems and factors associated with this technique, its benefits and failures, new strategies and new approaches suggested by different authors to enhance the efficacy of bioremediation processes (i.e: heirloom strains, in rhizo-directed strains selection, phytoaugmentation, cold adapted microorganisms, biofilm architecture, bacteria chemotaxis, tailor-made consortia, etc). Finally the strategy followed by the author’s group to select microbial formulas suitable for the treatment of co-contaminated matrices and the experimental work in progress will be illustrated.

Characterization of microorganisms by means of Raman spectroscopy

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An important direction of research in microbiology is to find a rapid method for reliable bacterial identification. The conventional microbiological techniques are time consuming. For a faster identification, one alternative is to use spectroscopic methods. Unlike other spectroscopic techniques, micro-Raman spectroscopy presents a few important advantages: it is a non-invasive method, requests limited sample preparation and one single bacterial cell is sufficient for identification. Therefore, no cultivation is required. In addition, information about the chemical composition of the investigated cell is obtained from the Raman spectra of the microorganisms. Since different factors influence the chemical composition of microorganisms, e.g. nutrients, age, oxygen and temperature, the effects of these parameters on Raman spectra have to be investigated.

The investigation of a facultative anaerobic bacterium is the optimal starting point for the study of oxygen influence on chemical composition of bacterial cells. The relatively easiness in cultivating *Acidiphilium cryptum* JF-5 in laboratory using various electron acceptors represented an important factor in choosing this strain.

The compositional variation of the bacterial cells due to different iron sources, oxygen and age was analyzed. A clear distinction could be made between spectra obtained from young culture and spectra from old culture. These changes in Raman spectra were due to the presence of polyhydroxybutirate (PHB) in cells. Using Support Vector Machine (SVM), we were able to distinguish between JF5 cells grown under oxic and anoxic conditions.
Fe(III) reducing microbial communities and their influence on metal mobility

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Near Ronneburg, Germany, uranium mining caused widespread contamination of soil and groundwater with heavy metals. Contaminants enriched in a creek soil downstream of the mining area. Metals peaked in the soil solution of an iron-rich groundwater influenced oxidized horizon (GolII). Iron minerals are important sorbents for metals. Geochemical data indicated the possibility for Fe(III) reduction in this horizon. Fe(III)-reducers can affect metal mobility directly or indirectly. The objectives of this study were i) to study the activity of Fe(III)-reducers and their effect on natural attenuation of heavy metals and ii) to identify the active Fe(III)-reducing microbial population. The abundance of Fe(III)-reducers, determined with a MPN approach, approximated $10^4$ cells g⁻¹ soil and was three orders of magnitude lower in the presence of heavy metals. Microbial Fe(II)-formation was low without additional carbon sources. In soil microcosms biostimulated with $^{13}$C-labeled ethanol or lactate Zn and Cd were immobilized, whereas Mn, Co, As, and U were mobilized during Fe(III)-reduction and the beginning of sulfate-reduction. Possibly Fe(III) reduction caused mobilisation of Co and U by dissolution of Fe(III) minerals and mobilisation of Mn and As by direct reduction. DNA stable isotope probing (SIP) was combined with terminal restriction fragment length polymorphism (TRFLP) analysis and cloning/sequencing methods to characterize the microbial community active during iron-reduction. The active Fe(III)-reducing population was dominated by Geobacter spp. in the $^{13}$C-ethanol treatment and by Geothrix spp., Pelosinus spp., Dechloromonas spp. and Pelobacter spp. in the $^{13}$C-lactate treatment.
Geomicrobial transfer of heavy metals monitored by Rare Earth Elements

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Fractionation of rare earth elements (REE), which means concentration changes within the element group of the lanthanides, is used as a monitoring tool for biogeochemical processes such as redox reactions, adsorption and desorption of metals in shaken microcosms. Four strains, among them three previously isolated from a former uranium mining site in Eastern Thuringia, Germany and a Cupriavidus metallidurans from the German Collection of Microorganisms and Cell Cultures (DSMZ), were inoculated to suspensions of contaminated soil including autochthonous soil microorganisms and complex media. Additionally, two approaches without inoculation and with either water or complex media as eluent instead of the complex media were used as a control. Metal transfer from the liquid to the solid phase was investigated by measuring the dissolved metal concentrations during 15 days of experimental time. During the experimental time, the colony forming units (cfu) demonstrated comparably decreasing cell numbers for all inoculated approaches (from $10^{10}$ to $10^7$ cfu) and the water elution (from $10^8$ to $10^5$ cfu), but increasing cfu numbers within the non-inoculated flasks (from $10^4$ to $10^7$ cfu). Microbial investigations showed the successive suppression of two of the inoculated strains (both of them previously isolated) by one autochthonous organism, which was probably better adapted to the used substrate. Measured pH values of complex media approaches strongly increased up to three units to pH 9.5 during the experimental time, whereas the water-soil-suspension showed constant values of pH 5. The high pH values for the complex media had the major influence on precipitation. Especially the dissolved fraction of the elements Al, Fe and Mn strongly decreased with time. Most of the inoculated flasks showed a loss of dissolved concentration down to values comparable to the eluent’s metal concentrations. But in the case of L. shinshuensis, C. metallidurans (DSMZ) and the non-inoculated approach, Al, Fe and Mn were much more separated from solution even below those values and a preferential loss of light (especially Ce and La) and middle REEs from solution was detected. The investigations showed strain-dependent results of various metal immobilization processes.
Native vegetation in mining areas
as source of contaminated hot spots remediation

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Designing the most appropriate phytoremediation method for a contaminated land raises interesting plant ecology questions. To what extent the contaminated hot spots are naturally colonised by plant species from the integrating landscape? Is it feasible to use only native species in the phytoremediation, and what should be the criteria for the selection of non-native species in the remediation? Answering the first question supposes the investigation of contaminated areas in different stages of secondary succession in comparison with the surrounding landscapes. The working hypothesis was that always the species pool of the integrating landscape includes early successional or euribiotic species (implicitly resistant to toxicity stress). We selected four catchments including four contaminated areas disturbed by different mechanisms (two mining dumps, a tailing dam, and an area with atmospheric deposition from a smelter). The list species common found on the contaminated lands are compared with the list of species found in the integrating catchment. Mechanisms of colonization are indirectly inferred in function of seed morphology, vegetative dispersal potential, type of fruit, phenology and life cycle (annual, perennial, shrub, and tree). The selection of the native species should include 1) inspection of euribiotic species pool for resistance to contamination stress and 2) inspection of early successional ecosystems from the landscape. In the rare cases when native species are not appropriate for use in remediation a key criteria in the selection of the non-native species is the low invasive potential. Care should be given also to the seed bank of the amendments used in the remediation process. In this context, one can identify a connection between biodiversity management and contaminated lands management. One of the objectives of biodiversity management in the landscapes integrating contaminated lands could be to ensure the renewal of successional seres and implicitly the pool of early successional species with potential use for phytoremediation.
Practical use of plant-fungal interaction in phytoremediation of heavy metal rich industrial wastes

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Post-flotation wastes rich in heavy metals are an example of substratum difficult for any biological reclamation. The slopes of the heap are not only toxic to plants, but also slide down and are extremely difficult to stabilize. Research on the mycorrhizal status of plants occurring on the Zn-Pb waste in Chrzanów has been carried out for the last 15 years. Several experiments were performed including the introduction of mycorrhizal inoculum followed by seeding various grasses, and also the introduction of mycorrhizal plants originating from xerothermic grasslands or using plants that appeared in waste area due to spread of seeds from the surrounding of the waste. Experimental plots were established in autumn 2003 and 2004 in Chrzanów (wastes of the ZG Trzebionka Mining Company). They were devoid of vegetation due to either accidental spills of the sedimental pulp or to chemical destruction of the surface layer. The plants together with the inoculum were introduced directly to the waste without using a soil cover, which is a very expensive practice and demands continuous care and the use of large volumes of water. The main aim was to select proper cultivars or plant species that are able to survive under these harsh conditions and will avoid the accumulation of toxic elements in shoots. As the nonmycorrhizal plants were mostly not able to survive under such conditions (field and laboratory data) the experiments were carried out focusing on mycorrhiza-assisted plants. In addition also the parasitic fungi were studied. The results showed that simple introduction of mycorrhizal inoculum was not enough in the case of metal-rich industrial wastes. Also the addition of hydrogel was not very helpful. As shown previously, selected plants originating from xerothermic grasslands (Turnau et al. 2008) could be used for phytostabilization. Certainly these plants are able to tolerate drought and high temperature. All the plants used in the experiment at Trzebionka belong to a group of pseudometalophytes that usually should show the metal exclusion strategy, comprising the avoidance of metal uptake and restriction of metal transport to the shoots. Almost all plant species had as high contents of Pb, Zn and As in shoots that are usually not considered as suitable for animal food. Such concentrations are, however, common in industrial areas. Still, we should try to select species that contain as little metals as possible. Among the studied species the most useful would be Molinia caerulea, Melica transsilvanica, Bromus innermis, Agropyron intermedium and Anthyllis vulneraria. These plants were also showing little differences in photosynthesis, while plants from the waste were compared to those growing on natural stands. Especially interesting for phytoremediation seems to be M. caerulea. This plant, however, was commonly infected by Claviceps purpurea that can create another type of health risk. Very high accumulation of potentially toxic metals was found in the case of Verbascum thapsus. The possibility to use it in phytoextraction will be checked in future.
Microbe assisted phytoextraction on contaminated concrete

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In terms of bioremediation most investigations have been done in more or less acidic environments, mainly influenced by acid mine drainage. These habitats show low pH and high concentrations of sulfate due to pyrite oxidation. The low pH increases the mobilities of several heavy metals and radionuclides.

However, there are also contaminated areas and materials with alkaline pH that are in need of being remediated. In alkaline environments, different element mobilities and different growth conditions for plants and microorganisms are present. Therefore, this project focused on the remediation of alkaline substrates.

Due to the decommissioning of nuclear facilities and the termination of the uranium mining activities in several countries vast amounts of heavy metal and radionuclide contaminated material is produced. The majority of this material consists of concrete. Moreover, large contaminated areas of mine heaps and topsoil of alkaline pH are left that allow only restricted use due to heavy metal and radionuclide contamination.

Existing chemical or physical separation methods are expensive and not without subsequent problems like production of acids and additional waste due to mechanical removal. Biological remediation strategies are an alternative to this common decontamination techniques.

To test the feasibility of a biological remediation a field experiment was set up which consists of 27 lysimeters with a volume of 1 m\textsuperscript{3} each. As substrates we used a waste rock material, concrete and a top soil as a control. To the substrates except the control compost were added to provide a basal fertilization. The lysimeters were treated with an arbuscular mycorrhizal fungus (Glomus intraradices) and/or with two soil borne bacteria of the genus Streptomyces to improve plant growth and alter mobility and uptake of elements. A third treatment was left untreated. The first two years different cultivars of sunflower and corn served as plants. In 2007 Sorghum sudanensis. was used.

The results showed a high bioavailability and plant uptake for the potential contaminants cesium and strontium compared to other heavy metals. Despite the high alkalinity and no further fertilization except the initial compost amendment in the substrates the plants showed proper growth over four vegetation periods. The inoculation with Glomus intraradices of the substrates lead to a higher mycorrhization rate. A higher cfu was detectable for the inoculated Streptomyces coelicolor after one vegetation period in the concrete material, but not in the waste rock material.

Biological removal of radionuclides can be suitable, but success depends on several circumstances like the conditions of the substrate, area, depth and level of contamination, microbial ecology, climate. Hence a biological remediation strategy has to be strongly adapted to the conditions given at the site. Additional inoculation with microorganisms can promote growth if nutrients are limited.
Analysis of fungal activity on black shale and graphite surfaces using vertical scanning interferometry

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The oxidative weathering of black shale organic matter (OM) plays an important role in diverse processes like atmospheric CO$_2$ balance and acid mine drainage. Fischer & Gaupp (2005) quantified the reactive portions of the black shale surface area during weathering. Here we want to test the influence of microorganisms on the degradation of black shale OM. In this study black shale as multi-component rock material and graphite as pure carbon source were used for incubation experiments with lignolytic fungi. *Schizophyllum commune*, a wood-rotting fungus, is able to excrete enzymes which are able to degrade (lignin like) carbon structures in organic rich rock materials. Amongst others, Fritsche et al (1999) and Wengel et al (2006) demonstrated the degradation of organic carbon due to fungal activity. Our investigations by vertical scanning interferometry microscopy quantified the rate of black shale OM and graphite degradation by white-rot fungus *Schizophyllum commune*. Due to microbial colonization the reacted rock surfaces got typical etch pits. The etch pits, with an average depth of 120-150nm, occur more frequently and with more continuous elongate morphology on the graphite surface.

References
Heavy metal resistance in *Streptomyces*

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A site in Eastern Thuringia near Ronneburg was investigated with regard to effects of acid mine drainage (AMD). Strains of actinobacteria isolated from the banks of a creek and from a former uranium mining site reveal high levels of resistance to the high heavy metal content. Within this Streptomyces, several strategies for coping with the contamination under high nickel concentrations were found. The adaptation to heavy metal rich environments resulted in microorganisms which show activities for biosorption, bioprecipitation, extracellular sequestration, transport mechanisms, and/or chelation. Specific adaptation strategies and high yields of (intra)cellular heavy metal retention could be seen and specific responses in actinobacteria were investigated.

Biomineralization is another specific way allowing the soil bacterium to withstand high metal concentrations. A new biomineral (Ni-struvite) was observed on colony surfaces of *Streptomyces acidiscabies* E13 and *Streptomyces mirabilis* P16B-1. The two strains of streptomycetes show mineral growth upon prolonged incubation of solid minimal or complex media containing NiCl₂. The capacity to induce biomineralization of a nickel containing mineral is postulated to constitute a resistance factor as an adaptation to their habitats.

The promotion of plant growth may be another effect of the adaptation to contaminated environments. Auxin and siderophore production allows the plants better sprout shoots and better root growth.

Such resistance mechanisms against heavy metals are the basis for the use of microorganisms in biotechnological applications, such as bioremediation and plant biomass conversion approaches.
Uranium in biofilms

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Biofilms show a complex architecture of heterogeneously distributed sessile bacteria embedded in extracellular polymeric substances (EPS), made of polysaccharides, proteins, lipoproteins and glycoproteins, which are interspersed by open water channels. Biofilms are made of 50-95% water and dissolved substances and are thus considered as hydrogels. Through these water channels nutrients and possibly toxic heavy metals from the surrounding bulk solution effectively infuse into the biofilms to the microorganisms and metabolites and exudates of the microorganisms are transported away. The attachment of microbial cells to surfaces during biofilm formation leads to major changes in metabolism, resistance, and survivability and therefore the retention of radionuclides by biofilms are probably different from the interactions with single cell suspensions of only one type of bacterial species.

In this study particulate uranium in a multi-species biofilm grown at the solid/liquid interface was visualized within the biofilm and spectroscopically identified by a combination of confocal laser scanning microscopy (CLSM) and laser-induced fluorescence spectroscopy as uranium(VI) and metastable uranium(V). The particles showed either a characteristic fluorescence spectrum in the wavelength range of 415-475 nm, indicative for uranium(V), or in the range of 480-560 nm, which is typical for uranium(VI). These uranium particles were attributed for uranium(VI) to biologically mediated precipitation and for uranium(V) to redox processes taking place within the biofilm. The detection of uranium(V) in a multispecies biofilm was interpreted as a short-lived intermediate of the uranium(VI) to uranium(IV) redox reaction. Its presence clearly documents that the uranium(VI) reduction is not a two electron step but that only one electron is involved.
Ecotoxicological mechanisms of resistance to metals at population and community level

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The measurements in bioremediation experiments for contaminated lands are usually directed to parameters characterizing the individual organisms, and neglects for operational reasons larger scale processes occurring at population and community level. Obtaining some insights into larger scale processes could be, however, a condition for realistic extrapolation of the experimental results in the field. The purpose of this presentation is to point several interesting issues from the literature published in this area. 1) The most intensively studied community processes, because of their small scale, in the context of bioremediation, are those occurring in the rhizosphere. The „in principle” knowledge is rich in this area, but the „ready for application” knowledge is rather scarce. I discuss the possible reasons for this situation. 2) Field based studies of population resistance mechanisms can be successfully supplemented by math model integrating the effects of metals on life history traits. In the case of mobile populations spatially explicit individual based landscape models are the most interesting. An example is provided. 3) Antifeedent effects can occur in the contaminated populations with significant effects on the trophic chain, and consequently on the secondary productivity 4) The increased tolerance of native communities in contaminated areas can be coupled with decreased tolerance to other type of stressor – which may be a key point when putting the bioremediation in a more integrating perspective. 5) Dispersal of organisms at landscape scale is a key mechanism supporting the homeostasis of primary productivity at that scale (see also discussion in the presentation of Neagoe et al. this symposium), but also of secondary productivity (for instance of fish in mining catchments). I suggest that integrating the management of contaminated lands with basin management should be followed as a next step with the integration with biodiversity management in the same areas.
“Kamikaze” *Saccharomyces cerevisiae* mutant cells for heavy metal hyperaccumulation: A primary step to bioremediation by removal and recovery of heavy metals from contaminated environments

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In recent years, environmental regulations compel industries to shift to cleaner production methods, asking for development of environmental friendly treatment technique for metal rich effluents. Under such circumstances, biotechnological approaches to clean contaminated environments received great deal of attention. Growing metal-resistant cells that accumulate heavy metals can ensure better removal through a combination of bioprecipitation, biosorption and continuous metabolic uptake of metals after physical adsorption. *Saccharomyces cerevisiae* cells can easily act as biosorbents, but isolation of mutants that are both hyperaccumulating and tolerant to heavy metals proved extremely difficult. We therefore turned our attention to mutants that are hypersensitive to heavy metals due to high intracellular accumulation. We present here the possibility to use such mutants to reduce the heavy metal content of synthetic effluents. As the heavy metal accumulation ultimately results in cell death, we refer to these mutants as “kamikaze” cells.
We investigate the interaction of petroleum compounds with hematite grain-coatings on mineral surfaces and their effects on porosity, permeability and pore structure of elastic reservoir rocks. The concept of this approach is that oxidation of petroleum compounds (n-alkanes) and simultaneous reduction (bleaching) of Fe$^{3+}$ minerals (hematite) of red bed sandstones can generate carbon dioxide and carboxylic acids (SHEBL & SURDAM 1996, SEEWALD 2003). The acids cause or promote further diagenetic reactions, e.g. dissolution of instable cements or clasts like carbonates and feldspars that lead to porosity enhancement and reservoir quality improvement.

Flow cell experiments were designed with red bed sandstones under elevated temperature (200 °C) and pressure (400 bar) conditions and different reactant fluids. The sandstone samples and fluids were characterised prior and after experiments. Short-term experiments started with deionised water. Mineral reactions are monitored by analysis of the ionic species in the post experimental fluids by ICP-MS/OES. Further experiments were accomplished with a long-chain n-alkane (C$_{16}$). Analyses of organic compounds were carried out by GC-MS. Experiments in an externally heated water pressure autoclave at Leibniz University Hannover showed a significant bleaching of the red bed sandstone samples. Comparisons of pure hematite surfaces in different resolutions by optical microscopy and Laser Scanning Microscopy (LSM) prior and after the experiments indicate changes which are caused by reactions with organic reactants.

Literature:

The response of biofilms to uranium impacts

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The effect of uranium added in ecologically relevant concentrations (1×10⁻⁵ M and 1×10⁻⁶ M) to stable multispecies biofilms was studied by electrochemical oxygen microsensors with tip diameters of 10 µm and by confocal laser fluorescence microscopy (CLSM). The microsensor profile measurements in the stable multispecies biofilms exposed to uranium showed that the oxygen concentration decreased faster with increasing biofilm depth compared to the uranium free biofilms. In the uranium containing biofilms, the oxygen consumption, calculated from the steady-state microprofiles, showed high consumption rates of up to 61.7 nmol cm⁻³ s⁻¹ in the top layer (0 - 70 µm) and much lower consumption rates in the lower zone of the biofilms. Staining experiments with 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) and 4,6-diamidino-2-phenylindole (DAPI) confirmed the high respiratory activities of the bacteria in the upper layer. Analysis of the amplified 16S rRNA gene fragments showed that the addition of uranium in ecologically relevant concentrations did not change the bacterial diversity in the stable multispecies biofilms and is therefore not responsible for the different oxygen profiles in the biofilms. The fast decrease in the oxygen concentrations in the biofilm profiles showed that the bacteria in the top region of the biofilms, i.e. the metabolically most active biofilm zone, battle the toxic effects of aqueous uranium with an increased respiratory activity. This increased respiratory activity results in O₂ depleted zones closer to the biofilm/air interface which may trigger uranium redox processes, since suitable redox partners, e.g. extracellular polymeric substance (EPS) and other organics (e.g. metabolites), are sufficiently available in the biofilm porewaters. Such redox reactions may lead to precipitation of uranium(IV) solids and consequently to a removal of uranium from the aqueous phase.
**Heterogeneity of ectomycorrhizal community - heavy metals relationship**

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At mining sites, acid mine drainage leads to heavy metal contamination. Fungi have been shown to be quite tolerant to heavy metals, which also applies to ectomycorrhizal (ECM) fungi living in mutual symbiosis with host plants. In order to understand the functional differences between fungal symbionts and the reasons for changes in ECM community structure the relationship between ECM diversity and exploration types of ECM were investigated. Three different Quercus sp. forests with high heterogeneity of soil parameters (heavy metals, essential nutrients and pH) and land use (mining, forest harvesting and revegetation) were examined. An experimental design was used in which different successional stages were investigated at tree, community and ecosystem level. The differences in ECM diversity from one ecosystem to another in space and time were explained by correlating them to the abiotic characteristics of the ecosystem or by attempting to build an ecosystem level succession theory. Our results show that the distribution and diversity of ECM was significantly correlated with clusters of metals. The correlation of long distance ECM type with heavy metals indicated that the toxicity of heavy metals may be ameliorated by the availability of P. The absence of the contact types of ECM could be used as sensitive biological indicators to detect changes in ecosystem. The ecosystem level hypotheses of this study could be generally validated.
Biogeochemistry of AMD: key microorganisms and the roles they play in attenuation of mine pollution

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The pollution of surface water by acidic, metal rich drainage (acid mine drainage or simply AMD) from abandoned mine sites is a widespread problem in former mining areas of the UK and many other countries. AMD forms from the oxidative dissolution of metal sulfide minerals, leading to the formation of acidic solutions that contain high concentrations of transition metals, and especially iron. This process occurs when the minerals are exposed to oxygen and water, and is greatly accelerated through the activity of iron- and sulfur-oxidizing, acidophilic microorganisms. It has been suggested that more than 600 km of rivers in the UK, and more than 3000 km in Europe, are degraded by AMD.

While it is understood that microorganisms can catalyze the formation of acid mine drainage, it is increasingly becoming understood that these same microorganisms can also decrease the solubility of the metals, thereby leading to natural attenuation of the AMD. In the Iberian Pyrite Belt, in Spain, it was observed that natural attenuation of iron was occurring in several streams draining abandoned mines. It was noted, however, that in others little or no attenuation was occurring. One such stream was filled with macroscopic gelatinous microbial growths (“acid streamers” or “mats”). The microbial communities of these streamers and mats, and of the water, were analyzed using a combined biomolecular and cultivation-based approach. In addition, activities of iron-oxidizing and iron-reducing microorganisms were determined.

Energy sources for the primary producers in this system included both sunlight and reduced inorganic chemicals (predominantly ferrous iron), which promoted complex interactions between microorganisms in the stream water and the gelatinous streamer growths. Microbial transformations were shown to impact the biogeochemical cycling of iron, sulfur and copper in the acidic stream, restricting the net oxidation of ferrous iron and thus the attenuation of iron by precipitation. The results of this study have led to the development of a model accounting for the biogeochemistry of iron and sulfur in the mine waters has been proposed, and has highlighted the significance of the acidophilic communities in regulating the geochemistry and natural attenuation of acidic, metal-rich waters is described.
Arbuscular mycorrhiza, heavy metal and salt tolerance

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Under diverse stress conditions, arbuscular mycorrhizal fungi (AMF) exploit water and mineral salts from soils more effectively than plant roots and transfer them to the host. In turn, the fungi receive up to 20 % of the organic carbon formed by the plants. Due to the colonization of the roots by AMF, plants become more resistant to mineral deficiency, drought, salt or heavy metal stress and attack by pathogens. As regard to heavy metal stress, an isolate of the AMF *Glomus intraradices* has been obtained from the zinc violet. This isolate effectively transfers heavy metal tolerance to diverse plants in diverse heavy metal soils, provided the fertilization of the soil is optimized. Elemental localization studies with maize (1) showed that AMF colonized roots contain less heavy metals than non-colonized controls. The heavy metals which unavoidably reach the inside of the roots are concentrated in the inner root parenchyma cells where most of the fungal structures inside the root reside. Northern analyses, Real-time PCR experiments and *in situ* hybridizations (2) showed that plant genes are differentially expressed upon heavy metal stress and mycorrhizal colonization. Among such genes, a specific metallothionein gene (*Lemt2*) product is assigned a specific role in the detoxification of heavy metals by tomato. The transcript levels of this root specific *Lemt2* are drastically upregulated upon heavy metal stress but downregulated upon AMF colonization of the plants. To find fungal counterparts which could be involved in the alleviation of heavy metal stress, a suppression subtractive hybridization library was constructed from fungal hyphae grown either in high or low concentrations of zinc. This approach did not provide any fungal counterpart of the plant genes being down regulated at the plant side. In contrast, several genes coding for enzymes potentially catalyzing the detoxification of reactive oxygen species were found. Thus the fungal cells in the symbiosis may primarily have to cope with the heavy metal-induced oxidative stress.

Many plants from salt marshes are also strongly colonized by AMF (3,4). Salt marshes are particularly rich in spores of *Glomus geosporum*. Roots of the salt aster are colonized both by *G. geosporum* and *G. intraradices* and of a group of *Glomus* sp. for with no morphological data available at present. Differential gene expression experiments with tomato showed that aquaporines but not Na+/H+ transporters respond to salt stress and AMF colonization (5). In nature, the degree of mycorrhizal colonization of halophytes correlates with drought and not so much with the salt concentration in the soils (6). AMF isolates which transfer heavy metal or salt resistance to plants could have an enormous potential for applications (7).

Heavy metal tolerant sulfate-reducing prokaryotes in a gleyic creek soil at the former uranium mining district Ronneburg

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Sulfate-reducing prokaryotes (SRPs) are known to directly or indirectly affect the mobility of metals by altering their valence state or immobilizing them via the formation of metal sulfides. In the former uranium mining district Ronneburg (eastern Thuringia, Germany) leaching of low grade black shale led to an acidic, sulfate and heavy metal enriched leakage water resulting in the contamination of the surrounding aquifers. Therefore, SRPs should tolerate increased metal concentrations in groundwater affected soils. The objectives of this study were (1) to determine sulfate-reducing activities in a gley soil and (2) to investigate the diversity and heavy metal tolerance of resident SRPs. Potential sulfate reduction rates measured with $^{35}$S tracer technique were restricted to water saturated reduced horizons and yielded activities up to 5 nmol cm$^{-3}$ day$^{-1}$ at in situ sulfate concentrations of approximately 3 mM. The horizon with the highest total sulfur (2%) and heavy metal concentrations was used for amplifying the functional gene marker $dsrAB$ (dissimilatory bisulfite reductase). Resident sulfate reducers were related to the Desulfobacteriales, Desulfovibrionales, Clostridiales and Syntrophobacteriales. When soil was incubated under anoxic conditions nickel and cobalt were immobilized, while uranium became surprisingly mobilized. Enrichment cultures in a modified Postgate-medium tolerated up to 0.5 mM cadmium, 1.0 mM copper, 2.0 mM zinc, 5.0 mM aluminum and 12 mM nickel. Thus, an active and highly diverse sulfate-reducing community was present in these horizons that appeared to be well adapted to the presence of heavy metals.
Biogeochemical interfaces in soil – A DFG priority research program SPP 1315

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Soil is the thin semi-permeable membrane of the Earth’s surface that develops as an interface between the biosphere, hydrosphere, atmosphere and lithosphere. It plays a central role as a transformer, buffer, accumulator and filter of matter and energy input. The biogeochemical and biophysical processes are the primary driving force for key ecosystem functions. The different organic, inorganic and biological components define a complex, hierarchically structured and extremely large biogeochemical interface, the physical, chemical, and biological heterogeneity of which is the source of a multitude of habitats and supports a vast biological and functional diversity. Chemicals entering soil might be transported to the vadose zone or even to the aquifer. However, the major fraction is retained and will be exposed to these interfaces. There, the interactions and reactions control bioavailability, in turn affecting biodegradation, metabolisation and effect. Until recently, soils opaque nature has limited our ability to investigate its interior architecture. Yet, the joint application of novel spectro-microscopic and -tomographic techniques allow explore the architecture of biogeochemical interfaces and will make available direct information on their structure, dynamics and functioning. The nationwide, inter-disciplinary priority programme “Biogeochemical Interfaces in Soil” aims at the systematic structural characterisation and functional exploration of biogeochemical interfaces in soils and their controlling role for the fate and effect of organic chemicals. This holds the promise to explain the fate and effect of organic chemicals within a general mechanistic framework and will launch the integration of this information into field-scale concepts and models.
Integrated interpretation of geochemical and geophysical data for the estimation of metals’ stocks in contaminated areas

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The design of management plans for the areas contaminated with metals requires knowledge about the stocks of metals in the abiotic and biotic compartments and about the mobility of these metals. Estimating the stocks of metals in geological and pedological compartments strictly by geochemical methods can be exceedingly expensive if the sampling is not based on previous knowledge about the system’s structure. Valuable information about the structure of the contaminated systems can be obtained by geophysical methods.

The geophysical investigation of waste dumps has made a huge progress in the last two decades, benefiting of a significant improving of the data acquisition, processing and interpretation techniques. Geophysical surveys, followed by surface and vertical geochemical sampling on identified anomalous areas seem to importantly fasten the data acquisition stage, and can be a solution for high quality results in the condition of relatively small investments in the data acquisition stage.

In this study we present an integrated investigation of metalliferous mining waste deposits using this method. The sites are located in catchments from the Zlatna area, Romania, where a portfolio of projects dealing with the integrated management of contaminated lands is implemented by a bio-geo-chemical consortium.
Abstracts of posters
Enhanced plant stress tolerance by mycorrhization and amine treatments

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Heavy metals (e.g. Cd, As, excess of Cu, Zn) in contaminated soil substrates from a mining site cause oxidative stress in plants (twofold increase of POD-activity, decrease of GSH) and lead to growth retardation. By AM-inoculation the plant growth and biomass formation increased up to 45 % in comparison to untreated soil substrates (lysimeter experiments with cereals for 10 weeks). The increase by AM (arbuscular mycorrhizal fungi)-treatment was comparable to the effect of a foliar spray with 2-aminoethanol (2-AE, 150 mg/m²). A soil inoculation with AM followed by an application of 2-AE resulted in a synergy effect (70 % increase in biomass). Because the metal uptake into the plants rose by AM and 2-AE-treatment as well, the enhanced plant growth indicated a higher metal tolerance.

In short-term experiments with cereals, clover and lettuce exogenous 2-AE counteracted “oxidative” stress responses, measured by enzyme activities and enzyme pattern of superoxide dismutases (SOD), peroxidases (POD), catalases (CAT) and the glutathione (GSH) metabolism as well. Exogenous 2-AE enhanced – as a short-term response (4 days after spraying) – the activities of SOD, POD, CAT or glutathione transferase and increased the content of GSH and phenolics (hydroxycinnamic acids) twofold and more. An AM inoculation caused in young plants – as a short-term response (e.g. 3 weeks after sowing) – similar effects as shown with 2-AE.

However after the long-term plant adaptation to AM and 2-AE treatments the improved plant growth was correlated with decreased SOD- and POD-activities (up to 50 % in comparison with untreated plants).

Soil amendments with compost enhanced enormously the POD activity in long term experiments (twofold or threefold). Thus the C_org content of soils could modify the oxidative stress response in plants.

The experimental consequences are discussed.
**Dynamics of Metal Mobilization during Fe(III)- and Sulfate-Reduction**

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About 40 years of uranium mining in the district of Ronneburg entailed a widespread contamination of groundwater with heavy metals. Contaminants accumulated in a groundwater influenced soil near the creek Gessenbach downstream of the mining sites. Heavy metal concentrations peaked in the soil solution of an iron-rich oxidized horizon (GoII) where microbial Fe(III)-reduction might be an on-going process. Fe(III)-reducers as well as sulfate-reducers can affect heavy metal mobility by direct or indirect mechanisms. The objectives of this study were i) to study the dynamics of metal mobilization during Fe(III)- and sulfate-reduction in this horizon and ii) to evaluate the heavy metal resistance of the resident Fe(III)-reducers. In anoxic soil microcosms biostimulated with ethanol or lactate Cu was immobilized but Mn, Ni, Co, As, and U were mobilized during Fe(III)-reduction. Mobilization of Mn and As might be due to direct reduction while mobilization of Ni, Co, and U were likely caused by desorption from reduced Fe(III) minerals. The subsequent immobilization of Ni, Co, and U paralleled sulfate-reduction probably due to the formation of Ni- and Co-sulfides and the direct reduction of U. However, especially the dynamics of Co and U mobilization varied between both carbon sources. Activity of Fe(III)-reducers could be observed in the presence of up to 0.8 µM Cu, 73 µM Ni, and 114 µM Zn. According to these metal tolerances activity of Fe(III) reducers should be possible under field conditions as measured by pore water analyses. However, analyses of soil solid phase revealed much higher metal concentrations in the bioavailable fractions. If these metals are mobilized in the pore water Fe(III) reduction might be inhibited.
Hydrological modelling in landscapes contaminated with metals: research program and preliminary results

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Estimating the surface and underground water fluxes connecting the contaminated lands with the rest of the landscape is a needed step towards the development of effective management plans for the contaminated landscapes and, at the same time, can support the testing of interesting basic research questions. Our objective is to compare the relative importance of surface and groundwater export of metals from contaminated lands in four different landscapes (two catchments in mountain regions, one catchment in a plain area, and an alluvial island in Danube floodplain) at various scale of investigation (from local landscape to subcatchment to large catchment). This research is performed in the frame of a portfolio of projects dealing with the integrated management of contaminated lands implemented by a bio-geo-chemical consortium. We present here the overall organisation of the research program and illustrate preliminary results with modelling of surface water fluxes in the alluvial island – Danube floodplain. For results illustrating groundwater modelling see the poster of Scrâdeanu et al. (this symposium).
Heavy-metal uptake by Chinese cabbage from metalliferous soil modified in its C\textsubscript{org} content

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Key words: Compost amendments, humic substances, leaching, organic N, plant uptake, soil attenuation, solubility, sorption

Availability of mineral N species in soil limits biomass production and the formation of N\textsubscript{org} in plants. Continuous N supply below the toxic threshold increases thus shoot dry wt production of Chinese cabbage 2 to 3 times and raises the content in N\textsubscript{org} up to the detrimental concentration of ≥ 4 %. The value of N\textsubscript{org} is representative for the concentration of (enzyme) metalloproteids. Concentrations of N\textsubscript{org} increase thus at the same rate as the sum of the biocatalytic, proteid-associated transition metals, Co, Cu, Mn, Ni, and Zn but not of Fe. Concentrations in Ca and Mg with their structural, metabolic, and enzyme regulatory functions are also elevated, whereas uptake of non-physiological elements increases only with the plant DW, but not in concentration. In the present study, Chinese cabbage was potted on uranium mine dump soil substrates. The original soil C\textsubscript{org} 2.5 % (w/w) was attenuated with builder’s sand (C\textsubscript{org} = 0) to substrates C\textsubscript{org} 0.5 and 1.55 %. It was also enriched with Fischer’s commercial compost (C\textsubscript{org} 21 %) to substrates C\textsubscript{org} 6.6 and 9.5 %. The soil was treated in this way to lower the concentrations of hazardous elements in the soil solution by simple attenuation or by sorption to organic matter and, thus, to restrict plant uptake and leaching of heavy metals. Due to the largely differing mineral-N resources from sand-attenuated to the compost-amended soil substrates, dry wt production (5.5 times) and N\textsubscript{org} content (5.0 times) of Chinese cabbage shoots varied considerably. In shoot tissues of plants from soil samples C\textsubscript{org} 0.5 to 2.5 %, concentrations of the proteid-associated transition metals and of Ca and Mg fell and rose near-linearly with N\textsubscript{org}. Uptake of non-essential elements was only reduced in the case of plants from soil C\textsubscript{org} 0.5 % with its low metal concentrations in solution. With the introduction of Fischer’s compost, the relation between the content in shoot N\textsubscript{org} and a proportionate uptake in the sum of the proteid-associated transition metals and in Ca and Mg was dramatically reversed, although these metals remained in solution and did not bind to the compost supplement. Shoot tissues of plants from soil samples C\textsubscript{org} 6.6/9.5 % with top concentrations in N\textsubscript{org} contained as little as 17/11 % from the quantity of the five transition metals which would theoretically be taken up from non-amended soil. Uptake of the excessively dissolved Ca and Mg was also repressed, whereas uptake of non-essential elements remained independent of soil C\textsubscript{org} and shoot N\textsubscript{org}. It is concluded that amendments of commercial compost could be disastrous for the uptake of (heavy) metals by phytoextractive crops and non-efficient in the prevention of leaching. Compost amendments may nevertheless greatly ameliorate heavy-metal stress to commercial crop plants. In addition, the results point to the potential role of microbial hormones on the metal uptake behaviour of plants which had been formed in compost during the industrial fermentation.
Microbial reduction of sulfate in the presence of heavy metals

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In the former uranium mining district Ronneburg, Thuringia (Germany), aquifers and groundwater influenced soils are still contaminated with heavy metals and radionuclides. Microorganisms, like sulfate-reducing prokaryotes (SRPs), are able to change the mobility of metals directly or indirectly by altering their valence state or via the formation of insoluble metal sulfides. This study aims (1) to elucidate if sulfate reduction is a dominating anaerobic microbial process in a groundwater affected soil at the bank of a contaminated stream, (2) to enumerate heavy metal-tolerant sulfate-reducing prokaryotes, (3) and to evaluate the effect of sulfate reduction on the mobility of heavy metals present in this soil. When soil of the groundwater influenced Gr I horizon was incubated under anoxic conditions, no increase of Fe(II) and Mn(II) was observed, whereas amended sulfate (10 mM) was rapidly consumed within 34 days of incubation. SRPs preferred acetate or propionate as carbon source. Small concentrations of arsenic (1 µM) and uranium (0.05 µM) were mobilized, while nickel and lead disappeared from solution. Numbers of cultures SRPs appeared to be higher in the more reduced deeper horizon. The addition of heavy metals in a concentration that was close to their in situ maximum concentration in the soil solution had only minor effects on the culturability of the SRPs in both horizons. Thus, the activity of heavy metal tolerant SRPs in both reduced horizons appeared to contribute to the retention of nickel and lead due to the potential formation of metal-sulfides.
Accumulation of arsenic and other heavy metals in rice

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The drinking water and food are main pathways of the heavy metals e.g. As, Cu, Pb, etc. in humans. Their intakes cause diseases i.e. skin cancer, vascular disorder, etc. The central India is seriously contaminated with the heavy metals i.e. Mn, Ni, Cu, As, Pb, etc. due to the huge exploitation of the minerals and coal. A wide variety of the rice species are cultivated in this part of the country. Ten different rice species (i.e. Culture, G. Gurmatia, IR-64, Kalinga, Luchai, Mahamaya, Masuri, Purnima, Safari, Shyamla) cultivated in ten contaminated agriculture fields (Ambagarh Chauki) in the rainy season have been selected for determination of the As, Cu and Pb contents to know their accumulation potentiality. The contents of As, Cu and Pb in the field water, soil, rice and husk were found to be ranged from 47 - 180, 78 - 131 and 7.0 - 19.0 µg L⁻¹; 8.8 – 252, 23.9 - 80.9 and 13.3 - 45.5 mg kg⁻¹; 0.018 – 0.446, 4.3 - 10.32 and 0.21 - 1.51 mg kg⁻¹; and 0.194 - 1.048, 5.3 - 16.3 and 0.56 - 6.28 mg kg⁻¹ with mean value of 108, 102 and 11.0 µg L⁻¹, 71, 49 and 25.0 mg kg⁻¹; 0.27, 6.6 and 0.64 mg kg⁻¹; and 0.50, 9.4 and 1.5 mg kg⁻¹, respectively. Amongst them, the highest biological absorption coefficient (BAC) i.e. plant to soil metal ratio, and the highest concentration factor (CF) i.e. plant to water metal ratio were observed for Cu (0.34) and for Pb (168), respectively. The effect of factors (i.e. metal contents in the water and soil, grain mass, cultivation period, plant height, yield, etc.) in the accumulation potentiality in 10 rice are discussed.
Microbial monitoring and population dynamics in an AMD affected field site

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Microbial communities in soil are a very important part of the ecosystem and play an enormous role in carbon, nitrogen and many other nutrient cycling. The composition of these living communities are dependent on soil characteristics, and also on natural or anthropogenic disturbances. Contamination with heavy metals is one of the strongest influencing factors. To understand the interdependencies between affecting conditions and to investigate the influence of heavy metals, the current environment at the former uranium mining site in Eastern Thuringia, Germany, was observed by determination of population dynamics, growth characteristics of single isolates including both cultivation-dependent and DNA-based fingerprinting methods to identify microorganisms.

On the investigated site differently treated plots (topsoil or compost) were installed to enhance soil fertility. The treatments brought new, unadapted microorganisms into this heavy metal polluted site which were confronted with high mobile amounts of heavy metals.

This adaptation force was addressed by monitoring the changing in number of microorganisms and composition of microbial communities in the topsoil or compost treated plots in comparison to an untreated control. Plating and strain isolation then was used to establish surface profiles at the heavy metal containing field site concerning microbial diversity.

To give an overview on the occurring microbial community, a 16SrDNA genebank was used. For a faster way to identify microorganisms a first Phylo-Chip was developed. This first array layout allows to distinguish between taxonomic classes. These data were verified by comparison to the cultivation-dependent methods.
A model of metal transport in the industrial area Pantelimon and a monitoring and analysis program for evaluation of plant stress response

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Pantelimon industrial area is the most polluted area from the Bucharest. Here are two major metallurgic plants (S.C. NEFERAL S.A. and S.C. ACUMULATORUL S.A.) that use lead for the storage batteries and recycling and processing lead from waste. Pollution with lead and other associated metals involve all media types: air emissions and deposition, soil and water contamination.

The model of metal transport is focused only on the contamination of soil and groundwater by leaching of metal from dump, transport through drain an infiltration through unsaturated zone in aquifer.

The fitting of the transport model is realized using 34 sampled investigated in the laboratory of Faculty of Geology from Ruhr University Bochum, Germany. Spatial distribution of models parameters are evaluated in two versions: by kriging, to minimize the dispersion by conditional simulation, to maximize the dispersion of contaminants.

The proposed monitoring program and methodology for evaluation the plants stress response in based on the detailed analysis of uncertainty distribution in the studied area. We propose a methodology based on entropy analysis and fictive point method. The analysis of plant response to the stress metal contamination needs a more detailed separation of spatial units in the Pantelimon area, taking in account the boundary of the ecosystems existing in the industrial area. The increase number of parameters will bee filtrated using the dispersional and factorial analysis.
Influence of soil copper pollution and *Fusarium culmorum* on the heavy metal content in wheat

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A greenhouse experiment in pots was carried out in order to assess the effect of combination of stress factors – copper pollution and *Fusarium culmorum* pathogen on the bioavailability of heavy metals in contaminated soils and their uptake from agricultural plants. Cu ions were introduced into the soil as a solution of CuCl₂·2H₂O in three concentrations: 150, 400 and 600 mg per kg. The soil was artificially inoculated with *Fusarium culmorum* pathogen. Wheat (*Triticum aestivum*) plants were grown at the phenophase “5-6 leaf”. Soil samples were analyzed to determine the content of elements: Pb, Fe, Zn, Cu, Cd, Mn.

An ICP technique was used for determination of total and mobile forms of heavy metals in the soil, before planting the seeds. Concentration of the same elements was measured also in the stems and roots of investigated plants, after the harvesting.

A higher concentration of all determined elements was established in the roots of wheat, compared to those in the stems. A substantial change of the heavy metal content in the infected and uninfected with *F. culmorum* plants was not determined.

**Key words:** Copper pollution, heavy metals, total and mobile forms of elements, pathogens, *Fusarium culmorum*, wheat
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