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Friedrich-Schiller-Universität Jena

11th Symposium on remediation in Jena
“Jenaer Sanierungskolloquium”

"Geobiotechnology: from lost areas to resources"

4th-5th October 2012

Friedrich Schiller University Jena
“Zur Rosen“, Johannisstraße 13, Jena, Germany

Conference proceedings

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Welcome
to
11th Symposium on remediation in Jena "Jenaer Sanierungskolloquium"
focusing on
"Geobiotechnology: from lost areas to resources"

The 11th symposium on remediation is featuring new developments arising from the field of bio-geo-interactions. These range from detailed sensoring and monitoring of metal contaminated sites leading to identification of processes at microbe-mineral interfaces to use metal contaminated sites for the production of renewable energy by application of bioaugmentation, including the formation and use of nanoparticles, or to the formation of new ore deposits and technology development for biomining. Especially the impact of bacteria and fungi on biogeochemical changes in the environment provide a rich source of processes which will allow reclamation of sites with metal contamination stemming from mining operations of sulfidic ores. These sites are characterized by acid mine drainage which, so far, has been viewed as a subject for geotechnological or, in best cases, natural attenuation applications. However, new technologies are appearing which promise economically feasible use of these “lost areas”.

International participants will present their research in 6 sessions with 28 talks and additional poster presentations. The research focus of bio-geo-interactions at the Friedrich Schiller University is reflected by several third party funded projects including the research training group “Alteration and element mobility at the microbe-mineral interface” supported by the German Research Foundation (DFG), the Excellence Graduate School “Jena School for Microbial Communication”, the International Max Planck Research School “Global biogeochemical cycles”, as well as research projects supported by the German Ministry of Education and Technology. PhD projects of these structured programs are presented in this symposium.

A guided tour by Prof. Dr. Hermann Bothe will be offered to the Bottendorfer Höhe (heavy metal plants) and to Artern (famous brine spring) on 3rd of October 2012.

We welcome you warmly to the 11th remediation symposium in Jena.

Georg Büchel & Erika Kothe

Conference Program

Thursday, 4th October 2012

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

Session 1 – **Nanoparticles: Potentials in Application**
Chair: Georg Büchel
10:00 Christian Dimkpa, Old Main Hill, U.S.A.
Metallic nanoparticles: Biotoxic? Yes, but it's not always a toxic story.
10:40 Giovanna Armiento, Rome, Italy
Nanotechnology applications for site remediation: potentialities and questions raised
11:20 Matthias Händel, Jena, Germany
Synthesis of Mn minerals at ambient temperature and pressure
11:40 Katarzyna Turnau, Krakow, Poland
Nanoparticles in rhizosphere

12:00 Lunch and Poster Session

Session 2 – **Bioleaching: From acid mine drainage to biomining**
Chair: Christian Dimkpa
14:00 Axel Schippers, Hannover, Germany
Biomining for metal recovery
14:40 Katja Nebelung, Steffi Formann, Jena, Germany
Heavy metal and radionuclide uptake of plants in the area of the former Gessen heap, Thuringia/Germany
15:00 Aurora Neagoe, Bucharest, Romania
The effects of inoculation with bacteria and mycorrhizal fungi on the development of Agrostis capillaris in multi-scale experiments with mine tailing substrate
15:20 Anja Grawunder, Jena, Germany
Element distribution in fruiting body compartments of Lactarius pubescens adapted to mining impacted conditions emphasizing rare earth elements
15:40 Annekatrin Voit, Jena, Germany
Chemotaxis of different environmental isolates to siderophores

16:00 Coffee break

Session 3 – **Phytoextraction versus phytostabilization: Renewable energy production**
Chair: Dirk Merten
16:30 Frank Hellwig, Jena, Germany
Adaptive traits in men made environment – plants on heavy metal soil in middle Germany
17:10 Anna Burger, Vienna, Austria
The response of the multiple hyperaccumulators Thlaspi caerulescens, Thlaspi goesingense Thlaspi cepaeifolium, Thlaspi minimum, Alyssum ovirense and excluder Plantago major towards radionuclide ^{238}U
17:30 Anika Kötschau, Jena, Germany
Phytoextraction of heavy metals and radionuclides by sunflowers (Helianthus annuus)
17:50 Daniel Mirgorodsky, Jena, Germany
Field scale phytoremediation experiments on a heavy metal and uranium contaminated site, and further utilization of the plant residues
18:10 Hermann Bothe, Köln, Germany
Violets of the section Melanium, their adaptation to heavy metal soils and their colonization by arbuscular mycorrhiza fungi

19:00 Visit of the "Herbarium Haussknecht" and BBQ at the Institute of Microbiology

Friday, 5th October

Session 4 – **Microbial impact: Biominerals and Bioweathering**
Chair: Matthias Gube
9:00 Giovanni de Giudici, Cagliari, Italy
Biomineralization and microscopic processes at the root-mineral interface
9:40 Eileen Schütze, Jena, Germany
Growth of Streptomyces mirabilis P16B1 in heavy metal contaminated soil and impact to Soil Organic Matter formation
10:00 Rene Phieler, Jena, Germany
Microbially assisted phytoremediation of the heavy metal contaminated site Gessenwiese
10:20 Tsilla Boisselet, France
Influence of selected endophytic microorganisms on plant growth and metal uptake on heavy metal contaminated soil
10:40 Tsing Bohu, Jena, Germany
Linking Mn(II)-oxidizing bacteria to natural attenuation at a former uranium mining site

11:00 Coffee break

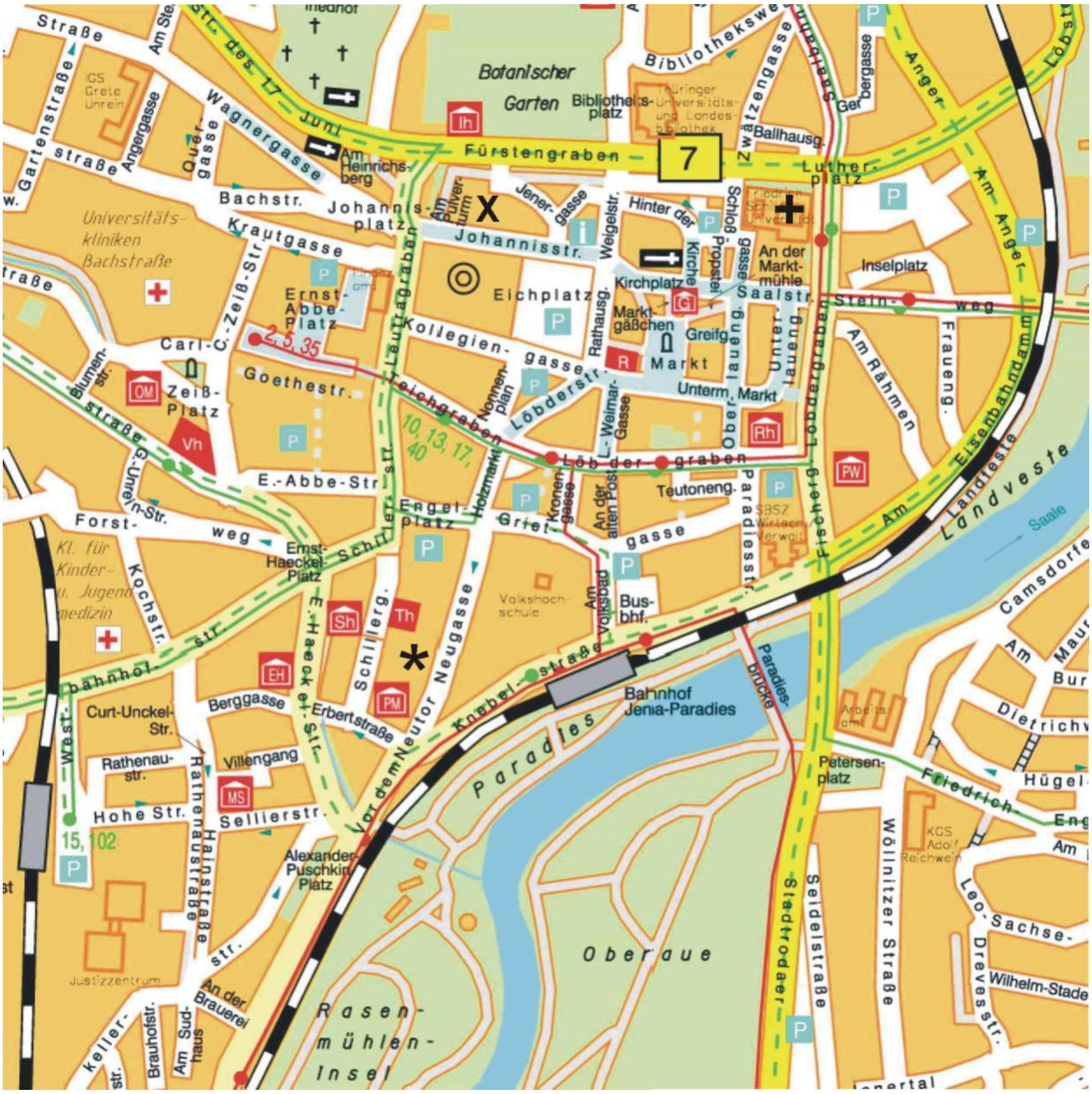
Session 5 – **Biosensing: Technologies for monitored natural attenuation**
Chair: Katrin Krause
11:30 Karl-Heinz Feller, Jena, Germany
Biosensing: Technologies for monitoring heavy metal concentrations
12:10 Steffi Rothhardt, Jena, Germany
Influence of Schizophyllum commune on organically coated sand grains
12:30 Giancarlo Renella, Florence, Italy
Harmonization of methods to assess the bioavailability of trace elements and to monitor the efficiency of various gentle remediation options in trace element contaminated soils
12:50 Francesca Langella, Jena, Germany
Plants growth promoting bacteria for the phytoremediation of heavy metal contaminated sites

13:10 Lunch and Poster Session

Session 6 – **Implications for geobiotechnologies**
Chair: Erika Kothe
15:00 Joern Geletneky, Jena, Germany
Development of remediation guidelines for contaminated sites based on the results of the EU-project UMBRELLA
15:20 Sneha Narvekar, Jena, Germany
Colloidal stability and mobility of nanoparticulate extracellular polymeric substance (EPS) coated iron oxides
15:40 Stefan Karlsson, Örebro, Sweden
Revegetating acidic mine waste using UMBRELLA guidelines - the second summer
16:00 Viktor Sjöberg, Örebro, Sweden
Revegetation of acidic mine waste – Impact of commercial bark compost
16:20 Maria Fabisch, Jena, Germany
Gallionella-like organisms as main iron oxidizers in metal-contaminated groundwater effluent

16:40 Concluding remarks
End of the symposium

Map of the meeting place



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

x: Meeting place “Zur Rosen“ (Johannisstraße 13)

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

+: “Herbarium Haussknecht” (Fürstengraben 1)

Busbhf: starting point of excursion on 3rd October 2012, 13:00
Guided tour to the Bottendorfer Höhe (heavy metal plants) and to Artern (famous brine spring)

The venue will be the historical building “Zur Rosen“ at Johannisstrasse 13 in the city centre of Jena.

Acknowledgements for financial supports

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umbrella
Using MicroBes for the REgulation of heavy metaL mobiLity at ecosystem and landscape scAle

Abstracts of the talks

Metallic nanoparticles: Biotoxic? Yes, but it's not always a toxic story.

Christian Dimkpa

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Projected to be worth $15 billion in 2015, the nanotechnology industry is associated with immense risks and benefits. The benefits stem from the inherent properties related to their small size, conferring them superior attributes over larger-sized particles of similar chemistry. Nanoparticles (NPs) have, thus, found applications in the manufacture of a wide variety of household, industrial and medical products. Of special biological benefit is their use for inactivating human pathogens, many of which are increasingly becoming resistant to the traditional suite of antibiotics. However, NPs are not selective for target organisms, so that beneficial microbes as well as other terrestrial and aquatic life forms are affected when NPs are deposited in the environment. Ag, CuO and ZnO are among the most applied NPs. This presentation focuses on the biological activity, transformation, and potential relevance of these metal-based NPs to agriculture and the environment. Findings show NPs exist in two states in different matrices: as stable NPs and as aggregates. Of no less significance is that they act as a point source for metal release, so that microbes and plants in contact with NPs are faced with both particle-specific and ion release-related consequences. However, at sub lethal levels, the NPs remodel bacterial and plant metabolisms, sometimes in positive ways, and the toxic effects may be mitigated by both bacterial and plant factors that interact at the NP surface. We further explore NP potentials to be harnessed for improving plant productivity and health, nutrients availability to plants from soils where uptake of essential metals is limited by solubility barriers, and use of plants to remediate NP-contaminated environments.

Nanotechnology applications for site remediation: potentialities and questions raised

Giovanna Armiento

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Sustainable remediation of contaminated soils represents a strategic target for European policies. In many cases conventional remediation and treatment technologies have shown only limited effectiveness in reducing the levels of pollutants; moreover current treatment technologies are often expensive and invasive. Remediation with nanoparticles (NPs) promises effective and possibly cheaper approaches than conventional methods and has the potential to treat large contaminated sites in-situ, eliminating the need for removal of contaminants. An undoubted advantage of using NPs is that surface modifications and small size make them reactive and mobile. The long-term feasibility of NPs-based remediation needs to be evaluated by appropriate research, as well as the interaction of NPs with geological and biological surroundings. This approach is very new, and more research is needed on the mobility of engineered NPs and their impact on the environment. While increased spreading capacity would allow more efficient remediation, it could also result in the migration of the NPs beyond the addressed contaminated area. Knowledge is limited on the fate and transport of NPs in the environment, their potentially toxicological effects, on the potential for bioaccumulation in environmentally-relevant species and effects on microbial communities. It is difficult to extrapolate toxicity data from bulk to nanoparticulate materials. The same properties (size, reactivity) that make NPs technologically interesting may at the same time place them in a novel category of potentially toxic substances. Consequently, questions of post-remediation persistence and potential human exposure to the particles are still open.

Synthesis of Mn minerals at ambient temperature and pressure

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Manganese minerals are widespread in soils, sediments, water and ores. Particularly, Mn oxides play important roles in many soil chemical processes, are effective sorbents for metal ions and can oxidize organic and inorganic contaminants. Manganese oxides mostly occur together with other minerals and are therefore difficult to separate. To study fundamental processes on reactivity and kinetics pure phases are needed. The objective of this work was to synthesize Mn minerals at ambient conditions and without the use of strong acids and bases. We designed four different experimental approaches based on $KMnO_4$ reduction. The reaction time varied between 1 and 550 days and the influence of the organic modificators acetate, citrate and lactate was checked. The reaction products were characterized mineralogically, morphologically and chemically.

After one day a poorly crystalline birnessite was the sole mineral phase that was formed in all reaction batches. Depending on the educts and on the modificator, several transformations of birnessite were observed over time. However, in some reaction batches birnessite remained stable. Birnessite that was formed in the presence of citrate partly transformed to rhodochrosite. Also lactate promotes the formation of rhodochrosite but the residual birnessite transformed to manganite or feitknechtite. The modificator acetate had no effect on mineral transformations. In two batches without modificator birnessite slowly dehydrated to cryptomelane.

Our pathways to synthesize Mn minerals are feasible on a large scale, can produce crystallites in different sizes and with a well-defined surface area. The products can serve as model minerals in studies on the biogeochemistry of Mn minerals, and may also have a potential for remediation of contaminated sites.

Nanoparticles in rhizosphere

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Nanoparticles (NPs) have unique physicochemical properties and a broad range of applications is proposed in medical treatments, various branches of industry, remediation etc. Yet the knowledge on the risks related to NPs impact on biological systems is limited. It is already known that, even below cytotoxicity threshold, NPs influence mitochondria and cytoskeleton functionality, ROS production, Ca signaling and membrane properties. The release of NPs might cause broad scale of dangerous changes in the environment. The pilot studies carried out on fungi showed that Ag and Ti nanoparticles are not always efficient against parasitic or saprobic fungi, on the contrary to efficient antibacterial effect. Not much attention has been paid that the presence of NPs leads to decrease of mycorrhiza propagule frequency and diversity what may be responsible for bringing serious changes in plant communities. The intensification of research in this area is clearly needed**.**

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Biomining for metal recovery

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Biomining is an increasingly applied biotechnological procedure for processing of ores in the mining industry (Biohydrometallurgy). Nowadays the production of copper from low-grade porphyry ores is the most important industrial application and a significant part of the world copper production already originates from heap or dump bioleaching. Conceptual differences exist between the industrial processes of bioleaching and biooxidation. Bioleaching on the one hand is a conversion of an insoluble worth metal in a soluble form by means of microorganisms. In biooxidation on the other hand predominantly gold is unlocked from refractory ores in large-scale stirred tank biooxidation arrangements for further processing steps. Besides copper and gold production, biomining is also used to produce cobalt, nickel, zinc and uranium. Dump and heap bioleaching and stirred tank bioleaching (or biooxidation) are nowadays the most important processes.

Up to now, biomining is merely used as a procedure in processing of sulfide ores and uranium ore. With regard to the bioleaching of metals that are used especially in the electronic industry, as well as of mine and industrial waste (e.g. mine tailings, ash etc.), promising laboratory methods and even pilot processes already exist. The newly developed Ferredox process (anaerobic bioleaching) enables the processing of silicate ores like laterites and oxide ores like manganese nodules, which is not possible in an industrial scale to date.

This contribution also estimates the amount of the world production in copper, gold and other metals by means of biomining and chemical leaching (bio- / hydrometallurgy) compared with the metal production by pyrometallurgical procedures. Data is mainly collected from data banks of BGR and public literature. In addition, an overview is given about the state of new developments in biomining, as well as perspectives for future field applications.

Literature:

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Heavy metal and radionuclide uptake of plants in the area of the former uranium mining pit Lichtenberg near Ronneburg, Thuringia/Germany

Katja Nebelung[1], Steffi Formann[2], D. Merten, K. Krause, M. Gube, G. Büchel[1], E. Kothe[2]

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The Gessen heap of the former open uranium mining pit Lichtenberg served as leaching heap where uranium was extracted with acids. The basis of the heap was sealed to the point of the subsoil with clay. Nevertheless parts of the heap solution seeped into the subsoil. The sub-project “Heavy metal and radionuclide uptake of plants in the area of the former Gessen heap” is funded by the Federal Ministry of Education and Research and is part of the project: “Radiation and Environment II”. This serves the purpose to investigate the connection between radiation, environment and human beings. The investigation of the area is focused on plants, because in general it is known that plants stabilize the soil through their roots and are able to take up and to store high amounts of heavy metals and radionuclides, and faced some obstacles as there was not much known about the distribution of uranium on the heap and the variety of plants (> 200 species of the heap) and its uranium uptake behaviour. Therefore a screening was performed to find characteristic sample sites and to learn more about the plants. Samples of two to three typical herbaceous plant species on every sample site (15x15m) with at least 5 individuals of each sampled species were taken. The leaves and stems of 36 plant species where used to measure the heavy metal and radionuclide concentration. All plants absorbed very less of the available uranium in the soil.

The Kanigsberg, in the south of the mining pit, was selected to study next to the herbaceous plants the mycorrhized shrubs and trees. In this study mycorrhized pioneer trees *Betula pendula*, *Picea abies* and *Pinus sylvestris* in symbiosis with two early stage fungi *Paxillus involutus* and *Pisolithus tinctorius* and the late stage fungus *Tricholoma vaccinum* where used to get more insides in the growing behavior under heavy metal and radionuclide load. In laboratory and pot experiments the beneficial work of the plant-fungi interaction and the ability of the plants to live with these confinements, xenobiotics, were studied and specified. Over the time it was obvious that the fungal contact with the plants enables the partner of this symbiosis to deal easier with high heavy metal and radionuclide amounts. They help each other by exploring and colonizing the soil and have the chance to live on some places inhospitable for the most organisms. In addition they clean up the polluted sites over the time. Cause through the binding of the pollutants on and in the cells they are thereby remoted from further contact with the soil, soil water, ground water and food chain of animals and humans.

Keywords: Uranium, mixed sample, plants, heavy metal, uptake, mycorrhiza, pioneer plants, symbiosis, early stage and late stage fungus

The effects of inoculation with bacteria and mycorrhizal fungi on the development of *Agrostis capillaris* in multi-scale experiments with mine tailing substrate

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In order to investigate the efficiency of the phytomediation techniques and to reduce the potential risks due to direct work at field scale, a multi-scale methodology has been applied. Investigations have been carried out using a native plant species *Agrostis capillaris* grown on a multiple metal polluted mine tailing substrate. Previous studies were performed in order to establish the suitable additives for improving the properties of the mine tailing substrate. Trying to find the best solution to manipulate the rhizosphere system, different plant-soil microorganism interactions were tested in the modified mine tailing substrate. Here will be presented two different experiments. The first consists of two experimental variants with and without bacterial inocula applied into a mine tailing substrate amended with compost. Each of the two experimental variants was replicated five times. The second experiment consists of three experimental variants: 1. with simple modified mine tailing substrate using a mix of top soil, zeolite and calcium carbonate 2. with the same modified mine tailing substrate inoculated with 1% mycorrhizal fungi and 3. with the same modified substrate and 7% mycorrhizal fungi. All three experimental variants were replicated five times at pot and lysimeter scales and 4 times at field scale. The simple modified and inoculated mine tailing substrates were characterized in both of the presented experiments by measuring pH, EC, CEC, LOI, TC, available nitrogen, available phosphorus, metals and metalloids (aqua regia and sequential extractions). For plant material biomass (excepting by field experiment), proteins, lipid peroxides, assimilating pigments, metals and metalloids were measured. The effects between scales for each inoculation type were compared using the ratio between values of parameters in inoculated and non-inoculated experimental variants. Moreover, the effects of the two inoculation types between scales have been compared using the ratio between the values of parameters in bacterial inocula and mycorrhizal fungi experimental variants. As a result of inoculation an increase of plant biomass was observed. In the case of experimental variant with bacterial inocula the biomass was higher as in the case of both mycorrhizal fungi variants. This result appeared as a consequence of the differences between modified mine tailing substrates. Relative effects of inoculation with fungi on biomass in relation to modified mine tailing substrates have been somewhat larger (50%) than those of inoculation with bacterial inocula (40%).

Keywords: metals, phytoremediation, bacterial inocula, mycorrhizal fungi.

Element distribution in fruiting body compartments of *Lactarius pubescens* adapted to mining impacted conditions emphasizing rare earth elements

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This study presents data on behaviour of elements emphasizing rare earth elements (REE) in the compartments of fungal fruiting bodies, which have been rarely studied. We focused on fruiting bodies of *Lactarius pubescens*, a common ectomycorrhizal fungus in the former Uranium mining area of Ronneburg/ Germany. REE (La to Lu) are a group of f-block metals with a unique coherent behaviour what allows them, after normalization to a suitable standard (here PAAS: Post Achaean Australian Shale; McLennan, 1989), to be used as process indicators also in bio-geo-processes.
Twelve individuals of *L. pubescens* were separated in the four compartments pileus, stalk, gills and pileipellis, which were digested with HNO_3 in microwave procedure. In their range, the contents do no exceed reference values of other wild growing mushrooms (e.g. Kalač, 2010). Based on the results, statistical analyses showed that macro-nutrients are mainly stored in the pilei and the gills, while potentially biotoxic elements including REE are rather stored in the pileipellis. Further, younger and older individuals seem to differ from this distribution, as proven by the PAAS-normalised REE patterns. Older fruiting bodies showed a similar REE pattern in both gills and pileipellis, contrasting to the younger ones. When compared to the bioavailable fraction in soil, light REE (La-Nd) are slightly stronger accumulated than the other REE, but the positive Ce-anomaly from soil is not transferred to the fruiting bodies. This indicates discriminating mechanisms for either uptake from soil or translocation from mycelium into fruiting bodies.

Literature:
Kalač, P. (2010): Trace element contents in European species of wild growing edible mushrooms: A review for the period 2000-2009. Food Chem. 122: 2-15
McLennan, S.M. (1989): Rare earth elements in sedimentary rocks: Influence of provenance and sedimentary processes. In: Lipin and McKay: Geochemistry and mineralogy of rare earth elements. Rev Min Geochem 21/1:169–200

Chemotaxis of different environmental isolates to siderophores

Annekatrin Voit, M. Greyer, T. Krauße, C. Oehler, M. Gube, E. Kothe

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Large soil contamination by mining activities poses a severe environmental problem. The former uranium mining site Wismut near Ronneburg, Germany, has been mined for more than 231.000 tons of uranium until remediation of the area started in the 1990's. Resulting from mining, the banks of creeks show highly concentrations of toxic heavy metals. Microorganisms are forced to adapt to heavy metal exposure, thus the area represents a store to screen for heavy metal resistant strains. One resistance mechanism of soil bacteria is excretion of chelating ligands like siderophores. These mobilise, transport and store iron(III) as well as other (heavy) metals in the environment. In association with that, a modulation of metal availability to other organisms takes place. Motile and growing organisms are able to perform chemotaxis in migrating or growing towards or away from a gradient to get into better living conditions.

In this study we used siderophore containing culture supernatants of three different heavy metal resistant *Streptomyces* strains to test for effects on other environmental isolates. Some of the tested strains could be shown to exhibit positive chemotaxis towards the siderophore containing medium. Fungal strains showed faster growth towards the siderophore-free side while there, changes in morphology to fruitbody producing stages was prominently seen, with more aerial mycelium developing. The tested *Streptomyces* strains showed significantly altered growth and a different pigment production. According to that, siderophores are indicated to play a role as signalling substance in nature.

Adaptive traits in men made environment – plants on heavy metal soil in middle Germany

Andreas Gerth, Frank Hellwig

Institute of Systematic Botany, Friedrich Schiller University Jena, Germany

Habitats with special edaphically conditions such as serpentine or salt sites and especially metal rich soils make great demands on plant growth and reproduction success.

Species adapted to metal soils have been investigated intensively in the last years and are still in the focus of different disciplines of science. Plant species have to evolve metal tolerance to establish stable populations on metal rich soils. These plants are then called metallophytes. There is evidence that the differentiation process takes 40 to 100 years or under high selection pressure even just 10 years. For this reason these species are especially well suited for studies about differentiation of ecotypes and adaptive traits.

In our research we studied the habitat properties on copper shale spoil heaps in the eastern foreland of the Harz Mountains. This is the habitat of *Minuartia verna* a typical metal soil plant and *Alyssum montanum*, a plant which is not yet known to be a typical plant for metal rich soils. The total mean concentration in soil and the sequential extraction showed high concentrations of lead, copper, zinc and other metals. So *Alyssum montanum* can be classified as a facultative metalophyte since it dwells on normal soils, too. Actual research is focused on differentiation processes and adaption in *Alyssum montanum* polyploid complex between populations from natural and normal metal supplied sites and populations from metal-rich soils.

The response of the multiple hyperaccumulators *Thlaspi caerulescens*, *Thlaspi goesingense* *Thlaspi cepaeifolium*, *Thlaspi minimum*, *Alyssum ovirense* and excluder *Plantago major* towards radionuclide ^{238}U

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8 plant species were cultivated hydroponically in medium containing uranium (U) in concentrations between 1 µM and 10 mM. The effects of U were tested by describing plant growth parameters such as biomass, length of roots, stems and leaves, the degree of chlorosis and necrosis, and the rate of photosynthesis. Uptake of U into roots, stems and leaves was analyzed from extracts by inductively coupled plasma mass spectrometry (ICP-MS). Localization of U in plant organs on a tissue-specific level was determined by energy-dispersive X-ray spectroscopy (EDX) in the scanning electron microscope (SEM).

The amount of accumulated U was strongly influenced by U concentration in the cultivation medium. Increased U contamination caused visible negative effects such as necrosis and chlorosis. Most sensitive was *Thlaspi goesingense* from Flatz, a non-contaminated habitat; it showed also the highest U uptake (17.200 mg U kg^{-1} in roots and 550 mg U kg^{-1} in leaves). Most tolerant up to 10 mM was *Plantago major*, which occurs also naturally on U containing soil such as Gessenwiese. On an average, roots contained most of the U, less U was transported further up to the leaves. Element analysis by EDX-SEM revealed, however, that U is not distributed evenly within leaves and roots, but that it is accumulated in the cortex of the root and in the central tissues of the leaves; in this mesophyll, U concentration may even surpass the amount of U in the roots, similar as it is observed in hyperaccumulating plants.

Phytoextraction of heavy metals and radionuclides by sunflowers (*Helianthus annuus*)

Anika Kötschau[1], J. W. Einax[2], G. Büchel[1], W. von Tümpling[3], D. Merten[1]

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Sunflowers (*Helianthus annuus*) are potential phytoextractors of heavy metals from soils. In phytoextraction contaminants are accumulated in the easily harvestable above-ground plant parts. To investigate the influence of bioavailability on phytoextraction, sunflower plants were grown over 24 weeks on the test field "Gessenwiese" on a former uranium mining site near Ronneburg (Thuringia, Germany) and at the lysimeter station Falkenberg (Saxony-Anhalt, Germany), in 2011. At the lysimeter station Falkenberg soil was used with similar grain size (sandy loam) like at the Gessenwiese, but it has a pH (H_2O) = 6.3 close to neutrality, whereas the soil pH at the Gessenwiese is about 4.4 (in H_2O). The soil at the test field Gessenwiese shows higher total contents for all investigated elements compared to Falkenberg. With exception of Cr and Zn all elements show higher bioavailability (according to Zeien and Brümmer) at the Gessenwiese than at Falkenberg. Every 4 to 5 weeks whole plant samples were taken at both sampling sites and dry weight as well as contents of metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Th, U and Zn) in roots, shoots and leaves were analyzed. After 24 weeks Cd, Co, Mn, and Ni were higher in above-ground tissues of sunflowers at the Gessenwiese than at Falkenberg, for the remaining elements a contrary behavior could be observed. Thus not only the general bioavailability seems so play a role in uptake and accumulation of the metals. Other influencing factors may be content of nutrients, stress and climate.

Field scale phytoremediation experiments on a heavy metal and uranium contaminated site, and further utilization of the plant residues

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Field scale investigation is applied to an area of the former uranium mining in East Thuringia, Germany. On this area, a low grade uranium ore leaching dump was situated, which was later removed during the remediation process of the area. Now, an underground slightly to moderately contaminated with heavy metals and radionuclides (HM/R) with restrictions of land use is remaining. In a joint project of the Friedrich Schiller University of Jena and the Technical University of Dresden concepts on remediation of HM/R-contaminated sites and for the subsequent utilization of the HM/R-loaded plant residues are developed (PHYTOREST).

Plant experiments with triticale, sunflower, Indian mustard and sorghum were performed revealing the influence of biological additives (mycorrhiza, HM-resistant streptomyces) and soil amendment strategies (increasing pH and organic matter, fertilizing) on biomass production and plant tolerance to heavy metals (research at the FSU Jena). The addition of mycorrhiza and streptomyces (MS) had a significant effect on biomass, and decreased slightly the bioavailable parts of HM/R (e.g. Ni, Sr, U) in soil in comparison to untreated polluted soil with all studied plants. In lysimeter experiments it was figured out, that the different soil amendment strategies, addition of MS and of calcareous top soil (MIX) resulted in a reduction of the concentration of contaminants in the seepage water, as well as of the seepage water rates and loads, thus decreasing the risk of groundwater contamination.

The contaminated biomass cannot be transferred in the food chain and should be energetically used by the production of biogas. This contributes to the minimization of wastes, to the production of energy and to material recycling (research at the TU Dresden). In fermentation experiments for the further utilization of heavy metal loaded triticale from the test field site, 89% and 91.5% yield of bioethanol was achieved in comparison with triticale from an uncontaminated field. These yields are in the optimal range for such a process, and they would be economical in practice.

Violets of the section Melanium, their adaptation to heavy metal soils and their colonization by arbuscular mycorrhiza fungi

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Violets of the section Melanium have the tendency to settle habitats with adverse soil conditions. Among these, the two zinc violets belong to the rarest, endemic plants of Central Europe. The yellow zinc violet, *Viola lutea* ssp. *calaminaria* occurs on Zn ditches and heaps in the Aachen - Liège area, where the blue form, *Viola lutea* ssp. *westfalica* can only be found in the Pb-heap at Blankenrode close to Paderborn. Both originate from the alpine *Viola lutea* as shown by analysis of their ITS1- 5.8S rDNA-ITS2 or 18S rDNA region (1) or by morphological criteria (2). The zinc violets are clear examples for heavy metal excluders (3,5) which is likely achieved by their colonization by arbuscular mycorrhizal fungi (=AMF) (4,5). An AMF isolate had been obtained from the yellow zinc violet which consistently conferred heavy metal tolerance to plants (3,4). In contrast to the zinc violets, heartsease (*Viola tricolor*) can occupy both heavy metal and non-polluted soils. Roots of heartsease that are distinctly colonized by AMF show lower heavy metal concentrations than the heavy metal soils in which they live (5). However, under extreme conditions in heavy metal soils, heartsease is not colonized (5,6). The use of AMF to protect endangered plants from extinction has been discussed (7). Future research will concentrate on Albanian violets that thrive on serpentine soils.

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Biomineralization and microscopic processes at the root-mineral interface

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Over the past decade, a growing body of literature is focused on the microscopic processes at the mineral-root interface. Such an effort is due to the role that biomineralization processes have on the Earth evolution. Besides fundamental science, knowledge of microscopic processes has relevant implications on geo-bio technologies such as phytoremediation, phytomining, and so on.

Velocity of mineral evolution at the mineral-root-interface is generally slow, several years of interaction are needed to achieve dissolution of primary minerals and formation of new minerals, and nanominerals are involved in the biomineralization processes. For this reasons, the challenge is understanding biomineralization processes at the nanometric scale. Synchrotron light facilities allow us to acquire invaluable information in terms of both chemistry and structure of materials. X-ray absorption spectroscopy (XAS) provides information in terms of the coordination environment of metals absorbed by plants, their atomic geometry, and the bioreduction of metals within phytoremediation systems. More recently, it has been applied to investigate metal interactions within biosystems by using microbeam facilities. In such a way, chemical and structural data can be acquired by scanning a microbeam in X-ray microscopy.

In this work, bio-mineralogical process relevant to geo-bio-technologies for remediation of mine areas will be out lined. Specifically, investigation strategy of mineral root interface and some experimental constraints observed during the former EU UMBRELLA project will be discussed.

Growth of *Streptomyces mirabilis* P16B1 in heavy metal contaminated soil and impact to Soil Organic Matter formation

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It has been shown that streptomycetes are a dominant group of bacteria in heavy metal contaminated soil and that growth of soil bacteria had positive effects on bioremediation, on bioavailability of metals in soil and on biogeochemical cycles.

The former uranium mining site Wismut in Eastern Thuringia, Germany, shows extreme environmental conditions such as scant nutrients, intense salt load and low pH, followed by high metal content. Such habitats only can be colonized by microbes which are adequately adapted. Actinobacteria isolated from this hostile environment show high resistances against a range of heavy metals like nickel, cobalt, cadmium or zinc. Growth of *Streptomyces mirabilis* P16B1 was investigated in mesocosms of contaminated soil from the especially nickel and zinc contaminated sample sites K7 (WISMUT area Ronneburg, Germany). As control uncontaminated soil PaO (paradise parc Jena, Germany) was used. Heavy metal sensitive *S. lividans* TK24 was used as control in both types of soil as well as dead biomass from both used strains. This experiment gave insight in growth and contribution to soil organic matter (SOM) formation of the strain, as well as its impact to heavy metal availability. Scanning electron microscopy and XRD analysis were used to detect the mycelium, spore production, as well as dead bacterial biomass and its attachment to soil particles as patchy fragments. The metal content of soil from the samples was determined by SE methods and MS. Superoxide-dismutase (SOD)-production of *S. mirabilis* P16B1 under natural conditions was detected via native PAGE and qualitative SOD-staining as well as quantitative Assay with extracted protein. Auxine production and siderophore production were measure via MS.

It could be shown that inoculation with the strain has an effect of SOM formation in soil, as well as heavy metal availability in mobile and specifically adsorbed fraction. Due to Fenton reaction and elevated concentration of heavy metals SOD expression could be seen as an important resistance factor of strains. Auxine and siderophore production by streptomycetes could be shown directly in soil. Thereby application of extremely heavy metal resistant strains from WISMUT area for microbial enhanced phytoremediation could be recommended.

Microbially assisted phytoremediation of the heavy metal contaminated site Gessenwiese

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Heavy metal contamination of soil as a result of, e.g., mining operations evokes worldwide concern. The use of selected metal-accumulating plants to clean up heavy metal contaminated sites represents a sustainable and inexpensive method. Microbially assisted bioremediation strategies such as phytostabilization and phytoextraction may be viewed as potentially useful methods for application in soil remediation. The test site Gessenwiese of the university at Jena is located at the former uranium-leaching heap site Gessenhalde near Ronneburg, Thuringia, Germany. A field experiment as well as pot experiments were carried out in order to assess the effect of microbial inoculation on the uptake of heavy metals by plants from contaminated soil. In order to investigate the effect of rhizosphere microbes on plant performance as well as heavy metal uptake, the soil was inoculated with mycorrhizal fungi and soil bacteria using two plant species, *Zea mays* and *Sorgum bicolor*. The current study indicated that biomass production can be enhanced by inoculation with multi resistant *Streptomycetes* isolated from the Gessewiese. The used microorganisms improved plant performance and the colonization of plant roots with mycorrhizal fungi and soil bacteria led to highly significant increase in plant growth.

Influence of selected endophytic microorganisms on plant growth and metal uptake on heavy metal contaminated soil

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The mobility of heavy metals plays a key role for their distribution in the soil complex dynamic system, and as a consequence for the prediction of their bioavailability and toxic effects on the biosphere. Plants and in soil living organisms are some of the many factors that can influence this parameter, by being an active part both of the hydrologic balance and the chemical interaction with metals.

In this study, we focus on the improvement of plant health and growth on heavy metal contaminated and nutrient depleted soil exploiting symbiotic endophytic bacteria. For this purpose, selected autochthonous microorganisms isolated from two plant species (*Trifolium pratense* and *Festuca rubra*) grown on the heavy metal contaminated soil of the former uranium mining site located in eastern Thuringia, Germany, were used. Further, consortia of these strains with complementary characteristics were also used as an inoculum. Plant health and growth, metal contents in soil and plants, and photosynthetic activity were analysed and compared to un-remediated soil. The pH and the bioavailable fraction of heavy metals including Rare Earth Elements (REE) in the soil was also determined

The results showed that inoculation of some bacterial strains improved plant growth on contaminated soil to a level comparable to that on non-contaminated soil. Further, root length increased due to the presence of specific microorganisms hence better stabilising the soil by the formation of a denser root system. Inoculation by consortia also led to very significant improvements of plant growth, suggesting a synergetic effect of the different strains. This was confirmed by chlorophyll fluorescence measurements which showed that, on a contaminated soil, inoculated plants were less stressed compared to non-inoculated plants. The mobile fraction of metals was lower with plants than for un-vegetated soil, indicating a stabilising effect of plants. Some bacteria could reduce the solubility of specific metals in the soil. Microbes and microbial consortia alone and in combination with their plant host, could influence the availability of some metals, with Al and REE behaving opposite to Mn, for which three inoculated strains caused a decrease of the soluble fraction compared to un-inoculated plants. Similarly, the uptake of metals by plant aerial parts depended on the plant species and the metal itself.

This shows that plants, especially in combination with symbiotic bacteria, can decrease locally the amounts of soluble metals in soil, increase the pH, influence the hydrologic budget and limit erosion, which are strategic properties for *in situ* phytoremediation of metal contaminated soils.

Linking Mn(II)-oxidizing bacteria to natural attenuation at a former uranium mining site

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Natural attenuation of heavy metals is occurring in Mn oxide rich layers at pH ~ 5 in the subsurface of former uranium mining district Ronneburg, Germany. It is unknown how these Mn-rich layers are formed. As microorganisms readily oxidize Mn(II) at pH 7, we sought to determine if Mn(II)-oxidizing bacteria were present and could precipitate Mn oxides under in situ conditions. Therefore, in this study, we (1) characterized microbial communities in the moderately acidic (pH 5) Mn-rich layer; (2) tested pH and heavy metal tolerance of isolated Mn(II)-oxidizing bacteria; and (3) identified biogenic Mn oxides. Bacterial 16S rRNA gene cloning and isolation showed the dominance of β-Proteobacteria and Actinobacteria in the Mn-rich layer, including known Mn(II)-oxidizing bacteria. Cell abundance was low (8.5×105 cells g soil-1 based on quantitative PCR) and Mn(II)-oxidizing bacteria represented 0.01% of the total bacterial community. Cultivation at acidic pH yielded several Mn(II)-oxidizing isolates related to members of the *Proteobacteria* and *Actinobacteria* phyla. The closest relative of the pH 5.5 isolate TB-2 was *Rhodoferax ferrireducens*, which represented 16% of total bacterial clones in the Mn-rich layer. Our Mn(II)-oxidizing isolates tolerated a wide range of heavy metal concentrations, ranging from 1 to 100 fold higher than in situ porewater concentrations (85 µM nickel, 0.44 µM cadmium, 23 µM zinc, and 0.8 µM copper). Transmission electron microscopy analysis techniques including imaging, energy-dispersive X-ray spectroscopy and selected area electron diffraction showed variable morphology of precipitates confirmed the presence of manganese oxides. Among these were highly crystalline Mn oxides of varying phase including groutite and birnessite formed by our isolates. The ability to grow at similar to *in situ* pH (5.0-6.0) and produce Mn oxides supports our isolates' environmental relevance. Since chemical oxidation is predicted to be slow at acidic pH, heavy metal tolerant Mn(II)-oxidizing bacteria may be involved in forming Mn oxides which are linked to natural attenuation in the former Ronneburg mining area.

Biosensing: Technologies for monitoring heavy metal concentrations

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Sensing of heavy metal concentrations in different media remains an important and challen-ging task. Beside elaborate analytical detection by atomic absorption spectrometry microbial biosensing with the help of immobilized cells or bacteria is a method of choice.

A microfluidic platform based on [1] is developed consisting of a cell-based in vitro assay as part of a lab-on-a-chip system for testing the physiological or rather the toxic effect of sub-stances towards human skin cells. The unique feature of the chip design is the combination of optical and electrochemical detection units, which will be used as an alternative and sensitive in vitro model with a high data throughput.

For the establishment of the in vitro assay the keratinocyte cell line HaCaT [2] was used. The cells were stable transfected with a plasmid, where a green fluorescent protein (GFP) and an additional promoter are involved. The decisive advantage is the fact that interaction with the analyte is converted into an optical signal (here the fluorescence intensity) which is direct proportional to the stress potential rather than to the analyte concentration. The system is by no means limited to the detection of stress potential but more generally applicable to inter-action sensing becoming an insight into molecular interactions:

- investigation of specificity and affinity of interaction sites,
- which functional groups are likely to interact with each other,
- which intra- and intermolecular interaction mechanisms are present, and
- how is the distribution of accessible and unaccessible interaction sites,

to announce only some of the special applications.

In case of heavy metal detection this general scheme is used by T. Charrier et al. to construct inducible bioluminescent strains via genetic engineering based on Escherichia coli bacteria [3]. Utilizing these sensors gave a range of 12 detected heavy metals including several cross-detections. Detection limits for each metal were often close to and sometimes lower than the European standards for water pollution. It has been proven that the sensor assay can be used to measure the heavy metal pollution of maize originating from heavy metal contaminated fields.

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Influence of *Schizophyllum commune* on organically coated sand grains

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Fungal alteration on organically coated sand particles, which show high retention ability towards heavy metals, was studied. Such sand particles can be applied as adsorbent to purify low heavy metal contaminated water. However, their stability has not been assessed yet especially in the oxic environment and, more specifically, in the presence of microorganisms. This is relevant for the evaluation whether coated sands could act as a reliable tool in remediation. To address this question we characterized the fungal alteration of organic coatings on sand grains using several techniques, including focused ion beam milling, scanning electron microscopy (SEM), scanning transmission X-ray microscopy (STXM) and vertical scanning interferometry (VSI). Sand grains coated with organics were analysed before and after incubation on agar medium in presence and absence of *Schizophyllum commune* to estimate changes in heavy metal retention. Formation of microbially mediated minerals and etch pits are induced by fungal colonization as shown by SEM and VSI. Using STXM we measured near-edge X-ray absorption fine structure spectra at the C and O K-edge to characterize the different organic compartments down to the 25 nm scale. We observed quantitative changes, which are presumably caused by the fungal alteration.

Our results suggest heterogeneous biodegradation of organic coatings on sand grains by spatially organized fungal exudation. An important implication can be the overall decrease in heavy metal retention potential of organically coated sand grains owing to the alteration processes by *S. commune*.

Harmonization of methods to assess the bioavailability of trace elements and to monitor the efficiency of various gentle remediation options in trace element contaminated soils

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Vast previous research on the potentials of plant-based gentle remediation options (GRO) for trace element contaminated soils (TECS) have demonstrated that both field scale demonstration projects and robust methods for evaluating the sustainability of GRO in practice, to convince policy makers and stakeholders about GRO feasibility. Although several GRO have been evaluated at field scale all over the World by various, there is still a need for identify suitable methods for option appraisal and carefully assess the initial and residual risks and the success of GRO.

Among the several methods to assess the risks associated with TECS through the quantification of the bioavailable and labile TE fractions, in Europe different countries still share different methodologies for the above mentioned aims.

Among the aims of the EU FP7 GREENLAND project, one of the main objectives was the evaluation and harmonization of methods for quantifying the bioavailable and bioaccessible TE fractions among several field trials across Europe, and select methods to be used as indicators for GRO success and as sustainability monitoring tools.

To achieve the aims, a list of most used tests was established based on available literature and the experience of the research partners, five chemical extraction methods to be tested were selected and evaluated in untreated soils and soils under GRO (e.g. phytoextraction, phytostabilisation).

The non conventional Diffusive Gradients in Thin-films (DGT) test was also performed on seven selected soils, to be compared with more conventional extractions and plant tests, to evaluate the bioavailable TE fractions.

Soils from untreated and treated plots were analysed in at least 3 replicates and statistical analyses and comparisons (ANOVA, t-test, regression analysis, multivariate data analysis) were made for selected elements such as Cd, Zn, Pb, and Cu.

Chemical extractions data were compared to well established plant toxicity tests (Plantox) and several soil biological and functional parameters such as microbial biomass, enzymes activities, nutrient mineralization capacity, to evaluate their predictive potentials towards adverse effects on soil microbial communities and soil functions during and after GRO implementation.

The preliminary results for Cd, Pb and Zn showed the following decreasing order of extraction strenght: $H_2O<NaNO_3<NH_4NO_3<EDTA<$aqua regia, with the exception of an acidic soil where no differences between the H_2O and saline extractants for Cd and Zn. Overall, the preliminary results of the chemical extractions showed correlations with some biological parameters such as plant growth, worm avoidance, growth and nematode reproduction.

Plants growth promoting bacteria for the phytoremediation of heavy metal contaminated sites

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Phytoremediation is a clean, cost-effective and non disruptive technology for the remediation of polluted areas like abandoned mine sites. Microbially aided phytoremediation offers in situ soil restoration and partial decontamination in case of phytoextraction, combined with maintenance of the biological activity and physical structure of the soil. Phytostabilization triggered by microbial inoculation, in contrast, is faster and allows for land-use, e.g. for the production of energy plants by lowering the metal mobility and hence both, uptake into plant biomass and wash-out with seepage water. Three former mining sites in Europe were selected for isolating plant growth promoting bacteria: the uranium mine in Ronneburg (Germany), the copper mine in Kopparberg (Sweden), and the tailing dam at Mica Valley in Zlatna (Romania). About 200 strains were isolated and screened for plant growth promotion traits as phosphate mobilization, nitrogen fixation, siderophore and phytohormone release and the resistance against the prevalent heavy metals was assessed, too. Three site specific consortia were derived to be augmented in greenhouse pot experiments together with six plant species adapted at the sites: *Helianthus annuus*, *Agrostis capillaris*, *Verbascum thapsus*, *Deschampsia flexuosa*, *Euphorbia pithyusa* and *Festuca rubra*. The effect of microbial inoculation on metal uptake into plant biomass or stabilization in the soil was investigated by assessing bioavailability of metals in soil, plant performance, and metal uptake in above ground biomass. The best performing plant-microbe consortium for the German soil (*Helianthus annuus* with the German consortium) was tested in a field trial to investigate metal mobility at large scale and in environmental conditions; an additional inoculation with the mycorrhizal fungus *Glomus intraradices* was investigated in combination of a soil amendment with compost. The study confirmed that sunflower was the best plant for a multi-metal contamination. The plant's performance could be microbially triggered to allow for different strategies: extraction in case of no augmentation and stabilization in case of addition of bacteria, mycorrhiza, or their combination.

Development of remediation guidelines for contaminated sites based on the results of the EU-project UMBRELLA

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The overall goal of the EU-financed project UMBRELLA (http://www.umbrella.uni-jena.de) is to use micro-organisms to develop cost-efficient and sustainable methods for soil remediation on heavy metal contaminated sites throughout Europe. UMBRELLA uses an integrated scientific approach, including different institutions from several European countries concerning microbiology, botany, and (hydro)geochemistry. All were centred on studying the microbial influence on metal biogeochemical cycles and their use in environmental protection.
A uniform definition of metal loads which necessitate action for remediation is still lacking. In particular in national soil and water guidelines, total contents rather than bio-available contents are the basis for decisions about remediation actions. From an ecological and eco-toxicological point of view, this is not overall supported by scientific data. The differences in geogenic metal contents in soils have led to problems with determination of relevant limits. Hence, the inclusion of governmental agencies as partner will allow to develop feasible guidelines that can be proposed to the EU to develop a combinatory guideline including both soil and water protection issues.
Thus, one outcome of UMBRELLA is a set of new phytoremediation techniques of metal contaminated soils. Based on these methods, bioremediation guidelines which focus both on soil and water protection, are developed. Those guidelines contain best practice rules for remediation in different scales based on methods and results of the project work-packages (microbiology, botany, (hydro)geochemistry) and individual laws and regulations of the European UMBRELLA partner countries.

Colloidal stability and mobility of nanoparticulate extracellular polymeric substance (EPS)coated iron oxides

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Solubility and transport of nutrients and pollutants is affected by the presence of colloidal and (organo) mineral compounds which may act as mobile geosorbents. In soils and aquifers, pure and organically modified Fe and Mn oxy-hydroxides are of particular importance as they are progressively used for environmental cleanup. Yet, colloidal stability, reactivity and thus the mobility of such colloids is governed by processes like flocculation/dispersion, filtration and straining, accumulation at the air-water interface, and sorptive interactions with the immobile solid surfaces. The dynamic interactions of the colloidal particles, organic coatings and the components of the natural environments can affect colloidal properties like size, surface charge and hence influence the colloidal stability.

In natural systems like soils, sediments and aquifers, Extracellular polymeric substances (EPS) are an important component of the soil organic matter pool. EPS are a complex mixture of biopolymers consisting primarily of polysaccharides and proteins, with variable amounts of lipids and nucleic acids. EPS and its components may form organomineral associates with soil borne iron oxide via adsorption and co-precipitate. Components of EPS are neutral moieties which can ionize as a function of pH, which can affect the colloidal properties of the iron oxides in the soil. These can also change the size and geometry of the particles and bring about a new arrangement of surface sites. The objective of our study is to fundamentally understand how the interactions with EPS affects the surface and colloidal properties of mobile natural geosorbents and their their reactivity and mobility.

Revegetating acidic mine waste using UMBRELLA guidelines - the second summer

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In 2011 the suitability of *Agrostis capillaris* for growth on historic acidic mine waste with the climatic conditions in central Sweden was evaluated with pot and field experiments. The UMBRELLA tool kit was followed, including additives to improve the water holding capacity (bark compost), nutritional status (mycorrhiza) and pH in the root zone (water works granules (wwg), i.e. $Ca/MgCO_3$) as well as wood chips (*Populus tremula*) in different combinations. The results showed that addition of bark compost was sufficient to maintain growth although the largest biomass was found when also mycorrhiza and wwg were present. The highest biomass was however rather low, some 15% of a reference with commercial compost. No differences (p 0.05) were found among the quality parameters (Electrical conductivity, pH, principal anions, dissolved organic carbon (DOC), inorganic carbon, principal and trace metals) in the water leaving the pots. It was therefore concluded that any geochemical changes induced by the plant growth were limited to the root zone or its immediate vicinity. The chemistry of the aqueous phase was controlled entirely by the release of DOC from the bark compost.

During the present growth season the sustainability of the treatments was evaluated by sowing A capillaris in the pots that were used in 2011 and then monitoring the same parameters. Until the end of August the systems without addition of wwg behaved almost identical with the previous season. Where the wwg had been added the plants began to wilt already in mid July. The wilting progressed most rapidly in the systems with only bark compost and wwg. Where living mycorrhiza had been added in 2011 the wilting process was slower and some 20% of the plants were alive at the end of August. Preliminary results of the composition of the drainage water show that the bark compost has ceased to release DOC (reduction with 98%) while other parameters are within the variation of the last season. So far there is no obvious reason for the negative impact of the wwg but a study is in progress. In the meanwhile, the wwg should be used with care.

Revegetation of acidic mine waste – Impact of commercial bark compost

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To minimize the mobilization of potentially toxic elements from historical acidic mine waste, establishment of a vegetative cover (e.g. grass) is often a successful method. To establish a vegetation cover pH and water holding capacity are often critical and must be increased to sufficient levels for the chosen plant species. One additive that may provide both properties is bark compost. It has been found that addition of 30 % (volume) commercial bark compost to historical acidic mine waste from the Ljusnarsbergs waste site increased the pH of the leachates with approximately 1 pH unit, from 3 to 4, at least for one growth season when precipitation gave an accumulated L/S of 5. To test whether this neutralizing capacity may extend over several seasons bark compost (diam 0.56 – 0.90 mm) was leached by sequential batch with 0.1 M NaCl at pH 3.0 and 6.1 in 24 hrs cycles. At L/S 10 to 26 the pH after 24 hrs contact time was in the range of 6.6 to 7.1, with no tendency for exhaustion of the capacity. Since the batch leaching did not contain any mine waste water constituents that could interfere, the ratio between absorbance at 254 nm and DOC (SUVA-index) was used for comparison. A fair correlation between L/S and SUVA was found (r^2 = 0.87) for the field experiment and sequential batch leaching at pH 3. At pH 6.1 the correlation was slightly lower (r^2 = 0.81) caused by the higher aromaticity of the DOC released at pH 6.1. This indicates that the sequential batch leaching data can be used to estimate the neutralizing capacity of the bark compost in contact with acidic mine waste. Since the annual precipitation in the field experiment reached an L/S of 5 the sequential batch leaching data indicates that the commercial bark compost would provide neutralizing capacity during at least 5 to 6 seasons.

Gallionella-like organisms as main iron oxidizers in metal-contaminated groundwater effluent

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In the former U mining area Ronneburg, Germany, groundwater effluent with high Fe(II) and metal content at pH 6.0 flows along a grassland into a small creek, forming iron-rich terraces at the creek bank. Microbially or chemically formed iron oxyhydroxides may adsorb/co-precipitate metals. We aimed to i) investigate microbial community structure with special focus on iron oxidizing (FeOB) and reducing (FeRB) bacteria, and ii) characterize iron oxides and heavy metals in waters and sediments. 16S rRNA gene cloning and sequencing with waters and sediments revealed high fractions of all clones having ≥97% sequence similarity to reported FeOB (32%) or FeRB (5%). The neutrophilic microaerobic FeOB *Gallionella* sp. ES 2 was the most dominant iron organism (up to 75% in one library). qPCR confirmed that a high proportion of the community was related to FeOB and FeRB (21-92% of bacterial 16S rRNA gene copies), clearly dominated by *Gallionella*-like organisms (up to 89%). ICP-MS analyses of filtered water fractions showed that Al, Cr, Cu, Pb, and U were mostly associated with particles (fraction >0.45 µm). The metals of most concern, Ni and Zn, stayed in the dissolved fraction, despite their potential for sorption/co-precipitation with iron oxyhydroxides at pH 6. However, sediments were enriched in heavy metals, for example Cd, Ni, or Zn. Raman spectroscopy of sediments revealed the dominance of schwertmannite at the terraces and ferrihydrite at the adjacent creek site. As *Gallionella* spp. represented such high fractions of the total microbial communities, they appear to contribute to the formation of iron oxyhydroxides influencing the uptake of metal contaminants into the sediment.

Abstracts of the posters
(in alphabetical order)

Mineralogy and microbiology of arsenic-rich waste dumps at Kutná Hora (Czech Republic)

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The chemical stability of waste materials from mining and mineral processing is an important study for investigation of impacts on the environment, especially ground water pollution and acid mine drainage (AMD). Subject of the current research investigation presented are highly weathered waste materials from the medieval dumps in the Kaňk deposit near the municipality of Kutná Hora (Czech Republic), with possible involvement of waste materials from Ashanti Belt (Ghana) and Rotguelden-Salzburger land (Austria).

The transformation of sulphide minerals generates predominantly Fe-rich weathering products which retain the pollutants. These secondary phases, are elusive, nanocrystalline, or amorphous, and will be studied extensively by mineralogical (TEM) and microbiological methods, with the aim of evaluating the role of microorganisms in the weathering of the primary sulphides and investigating the transformation and associated release of the pollutants.

Special attention will be given to the olive-green or yellow powdery masses resulting from the weathering of the mineral tetrahedrite [$(Cu,Fe,Ag,Zn,Hg)_{12}(Sb,As)_3S_{13}$], where extensive mineralogical studies have been or are being carried out (Majzlan et al., 2012). These minerals will be identified by Electron-microprobe, X-ray diffraction and Raman spectroscopy. The elements identified in both the primary sulphides and the weathered products will be categorised into mobile or immobile elements with the aim of understanding the partitioning of the elements into minerals and aqueous phases. Further mineralogical and geochemical work from this stage will be aimed at the identification of the microorganisms involved and a preliminary assessment of their role in the element mobilisation and immobilisation.

Literature:

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Nanosized iron oxides in microbial BTEX oxidation: a novel concept for groundwater remediation

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Microbial reduction of ferric iron is a major biogeochemical process in groundwater ecosystems and often associated with the degradation of organic contaminants, as bacteria couple iron reduction to the oxidation of organic molecules like e.g. BTEX. Yet the high crystallinity and low solubility of iron oxides limits reduction rates. However, environmental nanosized iron(oxy)hydroxide minerals seem to have an unequally enhanced reactivity potential compared to their bulk and highly crystalline parent materials of the same mineral.

Therefore, we examined the reactivity of nanosized, synthetic and environmental colloidal iron oxides in microbial iron reduction in static batch incubations and column experiments. Results Colloidal particles showed an enhancement of reaction rates of up to 2 orders of magnitude higher reaction rates than bulk phases, independent of the kind of mineral phase and or surface area. Furthermore, soil column experiments demonstrated the high persistence of nanosized iron oxides under simulated environmental conditions, opening the perspective for their technological application as electron acceptors in the remediation of BTEX and putatively PAH contaminated sites. First studies on the feasibility of this technology showed a 5x-fold enhancement of toluene oxidation after application of nanosized iron oxide particles to microbial incubations, as well as the possibility of custom-tailoring the subsurface mobility of these particles after injection into a contaminant plume.

Our results suggest that the injection of ferric iron nanoparticles as electron acceptors for microbial contaminant degradation in contaminated aquifers might develop into a novel bioremediation strategy by creating in situ reactive barriers.

Strata-specific bacterial diversity in aquifers of the Thuringian Basin/Germany

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The INFLUINS (Integrated fluid dynamics in sedimentary basins) project investigates coupled dynamics of near surface and deep flow patterns of fluids, transported materials and component substances in the Thuringian Basin. The extensive basin landscape is located in eastern Germany and belongs to the Triassic period of Bunter sandstone (Buntsandstein), shell limestone (Muschelkalk) and Keuper, which crop out at the surface. Older sediments and Permian (Zechstein) can be found at the edges of the basin. With microbial investigations, we are analyzing the bacterial diversity of ground- and mineral water at different locations to see whether there are special patterns in bacterial distributions originating from the different rock strata. This will facilitate understanding fluid movement in the Thuringian Basin. We determined bacterial diversity from water samples out of five natural springs and eight groundwater wells by cultivation and subsequent morphological, physiological and molecular identification. To elucidate differences to other rock strata, we compared these samples to two brine springs (4.62 M and 1.03 M salt content), located in Permian aquifers. First results show that the largest proportions were found to be members of Bacilli and γ- proteobacteria, including the genera Pseudomonas and Bacillus. Next steps will be a comparison of cultivation-dependent and cultivation-independent methods to gain further information on bacterial strains which where uncultivable or suppressed by other bacteria strains.

Revegetation of mine waste increases the selective pressure on microorganisms involved in occlusion of heavy metals

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Accumulation of plant residues is a prerequisite for the establishment of microbial heterotrophes and basis for the microbial formation of heavy metals (HM) chelants such as carboxylic and humic acids from lignocellulosic, and of amino acids from organic-N substrates. Chelants serve as shuttles in the occlusion of soluble HM in minerals and humin.

Microbial decay of plant residues under microaerobic conditions led to acid pH conditions and the formation and release of (volatile) alcohols, ketones, and esters of C_3 to C_7 alkanes with emphasis on butyrate derivatives but also on non-derivatized C_{12} to C_{19} alkanes. Under aerobic conditions, substrates alkalinized and exhaled, beside non-derivatized C_7 to C_{19} alkanes, numerous mono- and sesquiterpenes whose microbial formation was not bound to the obligate presence of grass residues as they were also formed on sucrose-amended soil. The combined volatiles formed in CO_2 controlled 1-L microcosms repressed the growth on malt extract agar of soil basidiomycetes such as *Agaricus bisporus*, *A. macrocarpus*, *A. macrosporoides*, *Marasmius oreades*, *Macrolepiota procera* (to 100%), *A. campestris*, *Lepista nuda* (to 60-88%), *Coprinus comatus*, *Stropharia rugoso-annulata* (to 11-14%), and of mitosporous Ascomycota/Basidiomycota such as *Aspergillus niger*, *Fusarium* sp., *Fusarium graminearum*, *Rhizoctonia solani* (to 3-92%). Growth of *Scytalidium lignicola* was stimulated (by 42%).

The volatiles (antibiotics) which penetrate the soil conveniently may thus impair establishment and competitive ability of efficient decay fungi and complicate the introduction of (engineered) remediative microbial isolates.

Bark compost for removal of Nickel in complex waste water

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The purpose of this study was to decrease the concentration of nickel in complex industrial waste water from a metal plating industry. According to the producer, the water contained 6 g/L nickel and a complexing agent similar to EDTA. The concentrations of Ni, Cu and Zn were lowered to 27 mg/L, 0.1 mg/L and 1.7 mg/L respectively, when the water was treated in a conventional fashion (pH adjustment, sulphide precipitation and flocculation). The outgoing water had a pH of 8.1 and an electrical conductivity of 9.1 mS/cm.

Additional precipitation with hydroxide decreased Ni, Cu and Zn to 23 mg/L, 0.06 mg/L and 0.11 mg/L respectively. Repeated sulphide precipitation then lowered the concentrations of Cu and Zn to < 0.02 mg/L and 0.12 mg/L respectively, but had no impact on nickel. The unknown complexing agent also prevented adsorption of nickel to hydrous ferric oxide phases.

In search for a cheap and readily available sorbent, saw dust (home made), peat (Econova Garden AB) and bark compost (Econova Garden AB) were tested. The evaluation was done by 20 hrs contact time at a L/S of 40 (undefined particle size) and moderate agitation. The nickel removal was 51 %, 74 % and 94 % for saw dust, peat and bark compost respectively. The corresponding removal of copper was 46 %, 94 % and 94 %, for zinc the removal was 79 %, 62 % and 91 %. Meanwhile the pH decreased to 7.5.

The high removal efficiency of the bark compost would in principle be controlled by its titrable surface groups in combination with a large surface area. For this particular batch the adsorption capacity ranged from 0.16 to 0.37 mEq/g for divalent cations such as Cu and Zn at pH 5.0-5.8. The bark compost buffered water (conductivity matrix 100-180 µS/cm) at pH 6.5 and had buffer capacity in the range 0.03 to 0.10 mEq/g after 20 hrs contact time. These conditions were not optimal for cation adsorption but rather satisfying since it allowed for anion adsorption.

Interactions of basidiomycetes with environment

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Fungi play an important role in biogeochemical cycles, act as decomposers and environmental indicators.

Therefore they interact with different compounds of the environment like wood, heavy metals and xenobiotics. Different enzymes and transporters are involved in these processes. We investigated laccases, aldehyde dehydrogenase *ald1* and multidrug and toxic compound extrusion (MATE) protein *mte1*. Expression analysis of two laccases and four laccase-like genes in *Schizophyllum commune* on basis of its substrate utilization and developmental stages were performed. Ald1 overexpressing mutants of *Tricholoma vaccinum* showed increased ethanol stress tolerance in comparison to wild type. By heterologous expression in *Saccharomyces cerevisiae*, different metals, xenobiotics like DNA-intercalating dyes and fungicides were identified as substrates for the MATE transporter.

To investigate the interaction with other microorganisms co-cultures of *S. commune* with six wood colonizing basidiomycetes and 14 bacterial strains were used. Differences in growth rate, specific interaction shapes, earlier fruiting body formation and pigment production along demarcation line were observed in *S. commune*-fungal interactions. The presence of *Bacillus subtilis*, *Providencia rettgeri*, and *Erwinia amylovora* provided a specific condition that triggered primordial and fruiting body formation in a monokaryotic strain of *S. commune*, which are normally formed still after mating interaction with compatible monokaryon. Analyses of amylase, cellulase, laccase, and lipase enzyme activities showed that only laccase plays a role in interactions with microorganisms.

Isolation and characterization of manganese-oxydizing bacteria (MOB) from a former uranium mining district, contaminated with heavy metals

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Heavy metal contamination in uranium mining sites is a common problem. The former uranium mining district in Eastern Thuringia, Germany, which was mined for four decades, was one of the most intensively mined and polluted sites. During mining the rocks consisting of low grade of uranium ores were leached leading to contamination with heavy metals forming secondary mineral enriched (SME) layers. The appearance of SME layers with manganese (Mn) and iron (Fe) oxides in the mining site provides a research platform on bioremediation and microbe-mineral interaction. This research focuses on the isolation and characterization of the manganese-oxidizing bacteria (MOB) from SME layers. Total digestion of the SME layer show high metal content of 18 mg/g, 55 mg/g and 73 mg/g soil of Mn, Al and Fe respectively. In contrast, the total carbon content of the SME layer is very low (0.28 g/kg soil) and pH is around 4.5. Culture dependent techniques revealed that total colony forming units (CFU) on modified K-medium from the SME layer is up to 107/g soil. The initial full sequencing results of the isolates represent four different manganese oxidizers which belong to the genus *Arthrobacter*, *Sphingomonas*, *Bacillus* and *Brevibacillus*. The manganese oxidizers were tested using Leucoberbelin blue, which reduces Mn oxides turning colonies to dark blue if manganese oxidation activity is present. Mn enrichment was further confirmed using LA-ICP-MS. Further perspectives will be to elucidate the microbial influence on the sorption of heavy metals to biogenic/synthetic Mn oxides.

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Signaling pathways are interconnected in *Aspergillus nidulans*

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The number of experimental data and publications indicating that signaling pathways are interconnected in the filamentous model fungus *Aspergillus nidulans* is increasing steadily. More recent observations from our laboratory demonstrated that cell wall integrity stress, oxidative stress and asexual sporulation signal transduction pathways are coupled and this complex signaling network may play a pivotal role in the regulation of several highly important physiological processes like autolysis. In this presentation, probable interplays between the elements of the stress sensing and signaling pathways (HOG mitogen activated protein kinase pathway, AtfA transcription factor, SrrA response regulator), the cell wall integrity stress signaling pathway (BckA-MkkA-MpkA mitogen activated protein kinase pathway, RlmA transcription factor) and the asexual development signaling pathway (FluG signaling, BrlA transcription factor) will be discussed. According to some observations from other laboratories, the FadA/FlbA heterotrimeric G protein vegetative growth signaling pathway, the TcsA/DevR sensor kinase/developmental regulator system and the Ca^{2+}/calmodulin system may also connected to the conidiation/stress signaling network shown above.

Formation and alteration of carbonates by microbial activity

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Calcification is a general phenomenon which appears in different environments such as soils and water. Various studies have shown examples for biotic and abiotic mineralization. Abiotic formation of carbonates is influenced by different factors such as temperature and changes in pH. Processes of biomineralization affected by the activity of organisms are known to occur through activity of gastropods or mussles with shells of carbonates, or by bacteria able to modify carbonates by creating an alkaline environment. The processes involved in increasing the pH are associated with the activity of enzymes such as urease and carbonic anhydrase. In this study, the microbial diversity in limestone was investigated, followed by experiments for the detection of biomineralization. The Thuringian Basin, which is located in the central part of Germany, belongs to the Triassic period and is surrounded by layers of Muschelkalk. The investigated sampling site was the quarry Bad Kösen that consists of the Middle Muschelkalk (Karstadt Formation) and the Lower Muschelkalk (Jena Formation). For the isolation of bacteria, rock samples were taken from the Lower Muschelkalk. The cultivation on different media demonstrated low cfu of 3*104 which is typical for rocks as extreme habitats. All in all, 40 strains could be isolated which were cultivated on calcium containing media to study their ability to form or degrade carbonates or to analyze urease activities.

Thermodynamic properties of antlerite, brochantite, and posnjakite

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Weathering of primary copper minerals (e.g., chalcopyrite, bornite) leads to the formation of secondary copper sulfates, phosphates, and arsenates. In this work, we focused on Cu sulfate-hydroxide minerals antlerite ($Cu_3SO_4(OH)_4$), brochantite ($Cu_4SO_4(OH)_6$) and posnjakite ($Cu_4SO_4(OH)_6 \cdot H_2O$). We synthesized these phases in the laboratory and measured their thermodynamic properties.
The synthesis procedure was a titration of a 0.1 M sodium hydroxide solution into a 0.001 M solution of copper sulfate. The temperature of the $CuSO_4$ solution ranged from 25 to 80 °C and the end-point pH was set between 6 and 11. By variations of temperature and the end-point pH, the precipitates contained brochantite, posnjakite, or tenorite (CuO). We were able to determine systematic variations of the nature of the precipitated product with temperature and pH. Antlerite was synthesized as described in [1]. All these samples were characterized by X-ray diffraction (XRD). Subsequently, the best samples were analyzed by scanning electron microscopy (SEM) for phase purity and prepared for calorimetric measurements.
The calorimetric measurements were carried out by an acid-solution calorimeter at T = 298 K in 5 N HCl as the solvent with the calibration compound KCl, using CuO, $CuSO_4 \cdot 5H_2O$, and H_2O as the reference compounds. For all measurements, appropriate thermochemical cycles were constructed. The formation enthalpies (ΔfH0) of antlerite, brochantite and posnjakite are -1733.6±5.3 kJ/mol, -2194.4±7.0 kJ/mol, and -2468.2±7.0 kJ/mol, respectively. Measurements of heat capacity are finished and standard entropies for each phase were calculated as 274.3±2.7 J/mol·K, 343.1±3.4 J/mol·K, and 390.9±3.9 J/mol·K, for antlerite, brochantite, and posnjakite, respectively. The standard Gibbs free energies of formation of antlerite, brochantite and posnajkite are -1454.7±5.4 kJ/mol, -1819.2±7.0 kJ/mol and -2031.3±7.1 kJ/mol, respectively. The ultimate goal of this study is a comprehensive model of Cu- and sulfate-rich fluids present in oxidation zones of ore deposits and tailings of Cu ores.

Literature:
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