

20th Symposium on remediation in Jena "Jenaer Sanierungskolloquium"

Microbes matter – Environmental systems



September 29th-30th 2022

Friedrich Schiller University Jena Big Lecture Hall Erbertstr. 1, Jena, Germany

Conference proceedings

Table of Contents

	Page
Welcome	3
Conference Program	4
Map of the meeting place	6
Acknowledgements for financial supports	7
Abstracts of the talks	8
Session 1 - Microbe interactions	9
Session 2 - Colloids and biofilms	13
Session 3 - Plant interactions	18
Session 4 - Metals and speciation	21
Session 5 - Microenvironments	25
Session 6 - Remediation and circular economy	29
Poster contributions	33

Welcome

to 20th Symposium on remediation in Jena "Jenaer Sanierungskolloquium" focusing on Microbes matter – Environmental systems

We would like to welcome you to a hybrid format for our 20th symposium. The program will consist of six sessions highlighting environmental interactions with metals focusing on processes that include microbially as well as chemically driven reactions with metals spanning the geological, hydrogeochemical, mineralogical, materials sciences and microbiological aspects and implications to remediation and land use. Thus, basic scientific questions as well as applied sciences in the fields of biology and geosciences are presented. We hope the meeting will allow us to exchange ideas and provide a basis for future cooperations.

Thorsten Schäfer and Erika Kothe

Conference Program

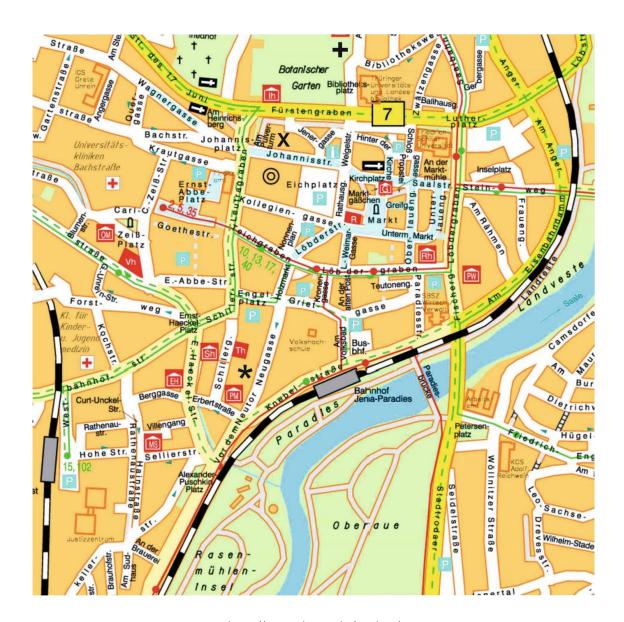
Thursday, September 29

09:15-09:30 09:30-11:00 09:30	Start: Big Lecture Hall Erbertstr. 1 (right at the Institute for Microbiology, Neugasse 25) Session 1: Microbe interactions Chair: Erika Kothe, FSU Miriam Rosenbaum, HKI: Droplet Microfluidics: Taking experimentation to the scale and
10:00	diversity of the microbial world Virgil Iordache, University of Bucharest, Romania: The influence of the order hydro-chemical events on the export of elements from floodplain sediments
10:20	Mario Krespach, HKI: Eavesdropping on microorganisms – arginine-derived polyketides as a means of microbial communication
10:40	Marie Harpke, FSU: Microbial adaptation to high salt combined with heavy metals
11:00	Coffee break (Seminarraum Neugasse 25)
11:30 - 13:20 11:30	Session 2: Colloids and biofilms Chair: Dirk Merten Elisabetta Dore, University of Cagliari, Italy: Historical mining sites: biomineral processes, environmental resilience and implications for risk analysis and management
12:00 12:20	Flavio Costa, FSU: Biominerals of the struvite family Sarah Nettemann, FSU: A multi-method approach to quantify and characterize the natural
12:40	colloidal inventory of AMD-influenced waters Steffen Hellmann, FSU: Single cell ICP-MS as new characterisation tool for biological cells: what can we learn about heavy metal uptake into/onto biological cells? online
13:00	Virginia H. Albarracín, CIME Tucumán, Argentina: Andean microbiomes: Bio-geo-interactions of novel multiresistant microbes
13:30-14:45	Lunch
14:45-16:15	Session 3: Plant interactions Chair: Katrin Krause
14:45	Philipp Franken, FH-Erfurt-FGK: Approaches for application of arbuscular mycorrhizal fungi in
14:45 15:15 15:35 15:55	
15:15 15:35	Philipp Franken, FH-Erfurt-FGK: Approaches for application of arbuscular mycorrhizal fungi in horticulture practise Hermann Bothe, Köln: Newer aspects of the biology of heavy metal plants Trang Vuong, FSU: Biotic interactions of a green alga in a nature-like microverse environment Katarzyna Turnau, Jagellonian University, Krakow, Poland: Symbiosis of Ni
15:15 15:35 15:55	Philipp Franken, FH-Erfurt-FGK: Approaches for application of arbuscular mycorrhizal fungi in horticulture practise Hermann Bothe, Köln: Newer aspects of the biology of heavy metal plants Trang Vuong, FSU: Biotic interactions of a green alga in a nature-like microverse environment Katarzyna Turnau, Jagellonian University, Krakow, Poland: Symbiosis of Ni hyperaccumulators online Coffee break Session 4: Metals and speciation Chair: Daniel Mirgorodsky Stefan Karlsson, University Örebro, Sweden: Wining and mining — Extraction of critical
15:15 15:35 15:55 16:15	Philipp Franken, FH-Erfurt-FGK: Approaches for application of arbuscular mycorrhizal fungi in horticulture practise Hermann Bothe, Köln: Newer aspects of the biology of heavy metal plants Trang Vuong, FSU: Biotic interactions of a green alga in a nature-like microverse environment Katarzyna Turnau, Jagellonian University, Krakow, Poland: Symbiosis of Ni hyperaccumulators online Coffee break Session 4: Metals and speciation Chair: Daniel Mirgorodsky Stefan Karlsson, University Örebro, Sweden: Wining and mining — Extraction of critical elements from steel slag with discarded wine online Antonio M. Newman-Portela, HZDR: Microbially induced reduction of uranium in contaminated
15:15 15:35 15:55 16:15 16:40-18:10 16:40	Philipp Franken, FH-Erfurt-FGK: Approaches for application of arbuscular mycorrhizal fungi in horticulture practise Hermann Bothe, Köln: Newer aspects of the biology of heavy metal plants Trang Vuong, FSU: Biotic interactions of a green alga in a nature-like microverse environment Katarzyna Turnau, Jagellonian University, Krakow, Poland: Symbiosis of Ni hyperaccumulators online Coffee break Session 4: Metals and speciation Chair: Daniel Mirgorodsky Stefan Karlsson, University Örebro, Sweden: Wining and mining — Extraction of critical elements from steel slag with discarded wine online Antonio M. Newman-Portela, HZDR: Microbially induced reduction of uranium in contaminated mine water for bioremediation purposes: A multidisciplinary approach study Aurora Neagoe, Bucharest, Romania: A pot experiment testing the effects of plant community structure and heavy metals contamination on the leaching of elements and functional traits of
15:15 15:35 15:55 16:15 16:40-18:10 16:40 17:10	Philipp Franken, FH-Erfurt-FGK: Approaches for application of arbuscular mycorrhizal fungi in horticulture practise Hermann Bothe, Köln: Newer aspects of the biology of heavy metal plants Trang Vuong, FSU: Biotic interactions of a green alga in a nature-like microverse environment Katarzyna Turnau, Jagellonian University, Krakow, Poland: Symbiosis of Ni hyperaccumulators online Coffee break Session 4: Metals and speciation Chair: Daniel Mirgorodsky Stefan Karlsson, University Örebro, Sweden: Wining and mining – Extraction of critical elements from steel slag with discarded wine online Antonio M. Newman-Portela, HZDR: Microbially induced reduction of uranium in contaminated mine water for bioremediation purposes: A multidisciplinary approach study Aurora Neagoe, Bucharest, Romania: A pot experiment testing the effects of plant community

Friday, September 30

09:30-11:00	Session 5: Microenvironments Chair: Kevin Lenk
09:30	Kumar Gaurav, FSU: Functional diversity of terrestrial and aquatic habitats of Planctomycetes
10:00	Katja Burow, FH Erfurt-FGK: The challenge of the targeted use of microbial consortia for an improved functionality of peat-free horticultural growing media
10:20	Anna Potysz, Wrozlaw, Poland: <i>Microbially accelerated weathering of metallurgical wastes: disposal scenario and treatment systems</i>
10:40	Oluwatosin Abdulsalam & Katrin Krause, FSU: Survival of Tricholoma vaccinum in ectomycorrhizospheric habitat: Facing the living and the non-living
11:00	Coffee break
11:30-13:00	Session 6: Remediation and circular economy Chair: Thorsten Schäfer
11:30-13:00 11:30	Session 6: Remediation and circular economy Chair: Thorsten Schäfer Mirko Köhler, WISMUT: Goals and strategies for monitoring of growth and soil development of covers and decontaminated sites
	Mirko Köhler, WISMUT: Goals and strategies for monitoring of growth and soil development
11:30	Mirko Köhler, WISMUT: Goals and strategies for monitoring of growth and soil development of covers and decontaminated sites Steffi Formann, DBFZ, Leipzig: Combined substantial and energetic use of biomass in closed-
11:30 12:00	Mirko Köhler, WISMUT: Goals and strategies for monitoring of growth and soil development of covers and decontaminated sites Steffi Formann, DBFZ, Leipzig: Combined substantial and energetic use of biomass in closed-loop systems of elements in the air-soil-organism interface online Lucian Staicu, University of Warsaw, Poland: Arsenic and selenium biomineralization:

Map of the meeting place



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

Coffee break / Lunch / BBQ at the Institute of Microbiology (Seminar room Neugasse 25)

^{*} Meeting place Big Lecture Hall Erbertstr. 1 (nearby Neugasse 25)



The venue will be the Big Lecture Hall Erbertstraße 1 (left) in the city centre of Jena, nearby the Institute of Microbiology at Neugasse 25 the location of Coffee break / Lunch / BBQ (right). (Photos: Dr. H. Pohl, https://www.zoologie.uni-jena.de/. M. Östreicher https://www.mikrobiologie.uni-jena.de).

Acknowledgements for financial supports













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

Abstracts of the talks

Droplet Microfluidics: Taking experimentation to the scale and diversity of the microbial world

Miriam A. Rosenbaum^{1,2}

¹Leibniz Institute for Natural Product Research and Infection Biology – Hans-Knöll-Institute, Jena, GERMANY

² Faculty of Biological Sciences, Friedrich Schiller University, Jena, GERMANY

Droplet microfluidics offer a unique opportunity to effectively and functionally explore complex microbial communities, even down to the single cell level. It allows for deeper sample analysis and ultra-high throughput screening. With the great need to combat the occurrence of antibiotic resistant pathogens, it is essential to screen for novel bioactive molecules.

We recently showed the great potential of droplet microfluidic cultivations to access a much broader and different part of the microbial community compared to classical strain isolation methods. In brief, we encapsulate and grow single cell inocula from different environmental sources in 200 pL size droplets. Our platform is integrated with optical fibers for multi-color fluorescence detection. Thus, we can multiplex the screening of different inhibition activities by adding multiple fluorescing microbial reporter strains to our pre-grown droplets. The applicability of our platform was first demonstrated by screening for gram-positive and gram-negative inhibiting molecules produced by known *Streptomyces* and verified by its application to unknown environmental inocula. We isolated new strains with antimicrobial activity. However, in this case, chemical analysis revealed already know antimicrobial compounds. But the results clearly showed the potential of this promising tool for exploring diverse microbial communities.

The influence of the order hydro-chemical events on the export of elements from floodplain sediments

<u>Virgil Iordache</u>¹, George Dincă^{1, 2}, Aurora Neagoe¹

¹University of Bucharest, ²Geological Institute of Romania

In mining areas floods of water contaminated by tailings or acid mine drainage occur in alternation with normal floods. The floodplain sediment of large river basins is contaminated with tailing materials of different ages, and also with nutrients from towns and villages. One could expect that the order of new reactions during floods is important for the fluxes of elements. In this context we tested the following hypothesis: the order of percolations with water having different pH and chemistry changes the export of elements by leaching from small columns of contaminated floodplains sediments. To test the hypothesis, we sampled 24 columns of sediments from two locations in the Aries floodplain, Romania. Two columns from each site were used to characterize the initial chemistry and mineralogy of the sediments, and 5 columns were percolated with water having different pH and chemistry. In the first variant the order of floods (six percolations for each type of water) was from neutral water, to technological basic water (sampled downstream an active tailing dam), to technological acid water (sampled upstream an active tailing dam). In the second variant the order was from acid technological water to basic technological water and to neutral water. We measured in the percolated water the pH, conductivity, heavy metals and total phosphorus, ammonium, nitrate, nitrite, and phosphate. After the 18 percolations we cut the columns longitudinally and made the chemical and mineralogical characterization of the sediment. We computed the total fluxes of elements exported from each sediment type in each order of hydro-chemical events, tested by bivariate ANOVA the hypothesis, and interpreted the results in function of fine structure of the exports of elements in time, and the differences between the initial and final chemistry and mineralogy of the sediments. In the end we discuss the results, and propose the structure of a more general program dealing with order hydrological events on biogeochemical processes.

Eavesdropping on microorganisms – arginine-derived polyketides as a means of microbial communication

Mario K. C. Krespach, Maria C. Stroe, Maira Rosin, Lukas M. Zehner, Johanna M. Beilmann, Anna J. Komor, Christian Hertweck, Axel A. Brakhage

Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany

Microorganisms produce numerous natural products (NPs) with important biological activities. For most of the time, these NPs were thought of as weapons to kill competitors. Now NPs are studied as means of communication rather than weapons. We discovered the complex tripartite interaction between the green alga Chlamydomonas reinhardtii, the bacterium Streptomyces iranensis, and the filamentous fungus Aspergillus nidulans and employ this tripartite interaction to study how microorganisms communicate (1). We found that S. iranensis communicates with C. reinhardtii as well as A. nidulans (2, 3). We discovered a distinct class of NPs that acts as signals: the arginine-derived polyketides. These NPs share a common, unusual biosynthetic origin, the amino acid arginine, which is further processed by a polyketide biosynthetic assembly line (4). C. reinhardtii as well as A. nidulans specifically react to these compounds. The green alga generates a novel aggregate form, that we named gloeocapsoids, which protects the alga from the toxic effect of these compounds, while A. nidulans starts to produce NPs itself (2, 5). Furthermore, we show that randomly isolated fungi from the soil can perceive compounds of this family and react with the production of NPs that may amplify the initial bacterial signal. In conclusion, argininederived polyketides form a family of compounds that is used as a communication molecule most likely all over the world that may shape the structure of microbial consortia.

- 1. M. K. C. Krespach *et al.*, Lichen-like association of *Chlamydomonas reinhardtii* and *Aspergillus nidulans* protects algal cells from bacteria. *ISME J.* 14, 2794-2805 (2020).
- 2. M. K. C. Krespach *et al.*, Bacterial marginolactones trigger formation of algal gloeocapsoids, protective aggregates on the verge of multicellularity. *Proc. Natl. Acad. Sci. U.S.A.* 118, e2100892118 (2021).
- 3. V. Schroeckh *et al.*, Intimate bacterial–fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans. Proc. Natl. Acad. Sci. U.S.A.* 106, 14558-14563 (2009).
- 4. H. Hong, T. Fill, P. F. Leadlay, A common origin for guanidinobutanoate starter units in antifungal natural products. *Angew. Chem. Int. Ed.* 52, 13096-13099 (2013).
- 5. M. K. C. Krespach, M. Stroe *et al.*, Ubiquitous bacterial polyketides induce cross-kingdom microbial interactions. *bioRxiv* 10.1101/2022.05.09.491136, 2022.2005.2009.491136 (2022).

Microbial adaptation to high salt combined with heavy metals

Marie Harpke¹, Sebastian Pietschmann¹, Flávio Silva Costa¹, Clara Gansert¹, Nico Ueberschaar², Thomas Krüger³, Olaf Kniemeyer³, Axel A. Brakhage^{1,3}, Sandor Nietzsche⁴, Falko Langenhorst ⁵, Erika Kothe¹

- ¹ Friedrich Schiller University Jena, Institute of Microbiology, Neugasse 25, 07743 Jena, Germany;
- ² Friedrich Schiller University Jena, Mass Spectrometry Platform, Humboldstr. 8, 07743 Jena, Germany;
- ³ Leibniz Institute for Natural Product Research and Infection Biology, Department of Molecular and Applied Microbiology, Adolf-Reichwein-St. 23, 07745 Jena, Germany;
- ⁴ Elektronenmikroskopisches Zentrum, Universitätsklinikum Jena, Ziegelmühlenweg 1, 07743 Jena, Germany;
- ⁵ Institute of Geosciences, Friedrich Schiller University Jena, Carl-Zeiss-Promenade 10, 07745 Jena, Germany

Adverse environmental conditions such as high salt and metal loads can lead to adapted microbial communities that may be screened for mechanisms involved in halophily and, in this case, metal tolerance. A former uranium mining and milling site in Seelingstädt, Germany, where 108.8 mio t of ore producing concentrate containing 86.273 t of uranium were processed, provides such a challenging habitat for microorganisms. Microbial communities from surface waters and sediment soils were analyzed and screened for halophilic, metallotolerant isolates. The contamination consisted mainly of high chloride and sulfate contents, accompanied by high metal loads with presence of cesium and strontium. Thus, isolates were scanned for tolerance towards 100 mM of either metal and the maximum soluble amount of sodium sulfate and chloride. Bacterial communities were dominated by Chloroflexi, Proteobacteria and Acidobacteriota; only at the highest contamination, Firmicutes and Desulfobacterota reached appreciable percentages in the DNA-based community analysis. The fungal community was dominated by Ascomycetes, followed by Mortirellomycota and Rozellomycota. The extreme conditions providing high stress were mirrored by low numbers of cultivable strains. 34 extremely halotolerant bacteria surviving 25 to 100 mM SrCl₂, CsCl and Cs₂SO₄ (23 Bacillus sp. and 4 other Bacillales, 5 Actinobacteria, 1 Gamma-Proteobacterium) as well as 3 halotolerant Aspergillus sp. were further analyzed. Mineral formation of strontium-apatite or cesium-struvite was observed, reducing bioavailability and thereby constituting the dominant metal and salt resistance strategy in this environment.

Historical mining sites: biomineral processes, environmental resilience and implications for risk analysis and management

Elisabetta Dore^{1*}, Giovanni De Giudici¹, Daniela Medas¹

¹University of Cagliari, Department of Chemical and Geological Sciences *elisabetta.dore@unica.it

Mine polluted areas account for ca. 0.1 per cent of the total land surface. Mine wastes are transported by rivers to the oceans resulting also there in chemical pollution. Mine pollution, thus, must be considered a global environmental problem. However, we still have poor knowledge on the global impact of chemical pollution and the response of our ecosystems to mine pollution perturbation.

Minerals, microbes, plants, water and air are all compartments of the ecosystems. Environmental resilience can be promoted by one of these compartments and it is generally sustained by the interaction among many of them. Moreover, state of the art knowledge on the effect of mineral reactions at larger scale allows one to recognize environmental drivers. Such a knowledge also can make a difference in resource and environmental management. This seminar will illustrate a case study in SW Sardinia (Italy) revealing a dynamic response of the environment to pollution, and its implications for risk analysis. In the study area (Iglesiente and Arburese mine districts), mine activities linked to Zn and Pb extraction from sulphides and calamine ores, left a seriously impacted environment due to widespread dumps of highly contaminated (Zn, Pb, Fe, Cd, etc.) mining residues. Authigenic metal sulphides occur due to sulphate reducing bacteria (SRB) under favourable conditions in the riverbed. When erosional processes do not prevail and thick muddy sediments lay in the hyporheic zone, biomineral processes occur also in plant systems (*Phragmites australis*, *Juncus acutus*) and are part of the resilient response of the environment to mine pollution. This seminar will highlight implications for management and circular economy.

Biominerals of the struvite family

Flávio Costa¹, Falko Langenhorst² and Erika Kothe¹

- ¹ Friedrich Schiller University Jena, Institute of Microbiology, Neugasse 25, 07743 Jena, Germany
- ² Institute of Geosciences, Friedrich Schiller University Jena, Carl-Zeiss-Promenade 10, 07745 Jena, Germany

Streptomyces mirabilis P16B-1 is a heavy metal resistant, filamentous Gram-positive bacterium that had been isolated from a former uranium mine. It carries a giant linear plasmid coding for heavy metal resistance genes. We aimed to investigate the role of this plasmid in the formation of biominerals, and investigate the incorporation of nickel into the mineral structure. Minerals produced by this strain were identified as struvite using Raman and TEM-EDX, or the nickel containing variant, Ni-struvite. The precipitation of Ni-struvite occurred in cultures containing nickel which reduced the overall nickel concentration in the supernatant. Therefore, biomineralization can work as a mechanism providing tolerance towards nickel. Despite the fact that the plasmid is not essential for the mineral formation, the expression of a nickel efflux transporter gene harbored on the plasmid increased the concentration of nickel ions in the cell vicinity, leading to the incorporation of nickel into struvite. Since the bacterium appears to have little control over the precipitation that occurs extracellularly, we tried a biomimetic synthesis of struvite using supernatant of cultures from S. mirabilis P16B-1. However, in our conditions, it was not possible to synthesize nickel containing struvite using the supernatant of P16B-1 culture, indicating some control by the bacterium in the incorporation of nickel into the mineral struvite. Overall, our experiments suggest that the precipitation of struvite and Ni-struvite are induced by S. mirabilis P16B-1 due to the interplay of metabolism and changed environmental physico-chemical conditions.

A multi-method approach to quantify and characterize the natural colloidal inventory of AMD-influenced waters

Sarah Nettemann¹, Linda Schönfeld¹, Daniel Mirgorodsky¹, Erika Kothe² and Thorsten Schäfer¹

¹Friedrich Schiller University Jena, Institute of Geosciences, Applied Geology, Burgweg 11, D-07747 Jena, Germany (*correspondence: sarah.nettemann@uni-jena.de) ²Friedrich Schiller University Jena, Institute of Microbiology, Microbial Communication, Neugasse 25, D-07743 Jena, Germany

Heavy metal (HM) and radionuclide (RN) transport in the environment is facilitated by natural inorganic and organic colloids as well as biocolloids such as bacteria [1]. This work determined the colloidal inventory and associated HM/RN in AMD-influenced pore- (PW) and groundwaters (GW) sampled over the past two years at the test field "Gessenwiese" [2] located in Eastern Germany. Due to uranium mining this area is moderately contaminated with HM and RN. For the quantification and characterization of colloids as well as associated HM/RN, a multi-method approach consisting of Liquid Chromatography - Organic Carbon Detection (LC-OCD), (Fluorescence-)Nanoparticle Tracking Analysis (Fluo-NTA), SEM, 1 kDa Ultrafiltration, and ICP-MS/-OES was implemented.

A median particle concentration of 1E+08 particles/mL with a median hydrodynamic diameter of 131 nm was determined using NTA. Particles in the GW are morphologically diverse and often form aggregates consisting of almost spherical particles with smooth surfaces, plate-like structures, or rods. High DOC concentrations of 2.1 mg/L to 485 mg/L and 0.8 mg/L to 5.4 mg/L, in PW and GW respectively, indicate an important role of organic colloids. Besides, first Fluo-NTA results using SYBR Green I for selective labeling of bacteria suggest that the mobile biocolloidal fraction accounts for up to 8% of the total colloidal inventory. Moreover, ultrafiltration revealed that a large percentage of the HM/RN are either truly dissolved or associated with organic humic-like compounds in the < 1 kDa fraction. A method to additionally quantify the association of HM/RN with specific organic fractions derived from LC-OCD is currently being developed. The presented work is part of the BMBF-funded project USER-II which aims to optimize on-site bioremediation strategies combined with bioenergy production.

- [1] Kretzschmar, R. and Schäfer, T. (2005). *Elements* 1, (4), 205-210.
- [2] Grawunder, A., et al. (2009). Chemie der Erde-Geochemistry 69, 5-19.

Single cell ICP-MS as new characterisation tool for biological cells: what can we learn about heavy metal uptake into/onto biological cells?

Steffen Hellmann^{1,2*}, Teba Gil-Díaz¹, Dirk Merten¹ and Thorsten Schäfer¹
¹Friedrich Schiller University Jena, Institute of Geosciences, Applied Geology
²Max Planck Institute for Biogeochemistry, Department of Biogeochemical Processes,
Molecular Biogeochemistry

*Correspondence: steffen.hellmann@uni-jena.de

How do bacteria survive in a heavy metal (HM) contaminated soil environment? In the past, methods such as Transmission Electron Microscopy with Energy Dispersive X-ray spectroscopy (TEM-EDX) or common Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) measurements following acid digestion were commonly used to characterise the HM's accumulated into/onto an overall mass of biological cells. However, these methods generally require high HM concentrations and are time-consuming. Single cell (sc)ICP-MS. a relatively new technique, can be used to directly characterise many single biological cells in aqueous suspensions quantifying their size (20-5,000 nm⁽¹⁾), number concentration and elemental composition without elaborate sample preparation. The main advantage of scICP-MS compared to classical ICP-MS is the concurrent quantification of both, ionic (dissolved) and cell elemental concentrations (i.e., no need for cell separation from the exposure media). In addition to the cell investigation, small particles such as biominerals can also be identified and simultaneously characterised. The state of the art of scICP-MS was recently published by Resano et al. 2022⁽²⁾, who pointed out that in the past three years the number of publications on scICP-MS per year have doubled. Authors mainly focussed on yeast, spherical and rod-shaped bacteria and even animal and human cells. They reported that current challenges are cell stability, adherence to surfaces and low transport efficiencies of, in particular, large cells, showing the importance of further scICP-MS improvement. In this work, we present several ideas to learn about HM uptake in biological cells. Since scICP-MS requires nearly spherical cells, particularly if the cell size is to be measured, we will focus on the roughly spherical spores of the filamentous bacteria Streptomyces, which are commonly used for bioremediation studies. This approach will lead to new insights in the direct quantification of single biological cells, providing at the same time, representative information about population dynamics regarding environmental contamination. We believe that this analytical approach is another important piece of the puzzle to unravel HM uptake mechanisms and resistances of biological cells.

- (1) Laborda, F.; Bolea, E.; Jiménez-Lamana, J. Single Particle Inductively Coupled Plasma Mass Spectrometry: A Powerful Tool for Nanoanalysis. *Anal. Chem.* 2014, *86* (5), 2270–2278. https://doi.org/10.1021/ac402980q.
- (2) Resano, M.; Aramendía, M.; García-Ruiz, E.; Bazo, A.; Bolea-Fernandez, E.; Vanhaecke, F. Living in a Transient World: ICP-MS Reinvented via Time-Resolved Analysis for Monitoring Single Events. *Chem. Sci.* 2022. https://doi.org/10.1039/d1sc05452j

Andean microbiomes: Bio-geo-interactions of novel multiresistant microbes

Albarracín V.H.

Laboratorio de Microbiología Ultraestructural y Molecular, Centro de Integral de Microscopia Electrónica (CIME), CONICET, FAZ-UNT, Tucumán, Argentina. E-mail: cime@conicet.gov.ar

In South-America, the Puna High Andes Region (3,000 to 6,000 m) displays a rich diversity of poly-extremophilic microbes distributed in salt flats, active volcanoes, hot-springs, fumaroles, and high-altitude Andean lakes. There, Andean microbiomes (AM) thrive under multiple hostile conditions: i.e. high arsenic and heavy-metals, hypersalinity, alkalinity and especially under the highest solar (total and UV) irradiation of the world. In spite of the multiple extreme conditions, AM's microbial communities composed mainly of Bacteria and Archaea are biodiverse in terms of species composition but also in the way they counteract the extreme conditions, i.e. forming associative networks such as biofilms, microbial mats and even a variety of microbialites, including modern stromatolites. Thus, the study of AM provides further understanding of the adaptation and function of proteins under multiple, extremely harsh conditions. In addition, the studies offer a high window to study ancient geobiochemical cycles in analogous stromatolites of their Precambrian counterparts. Likewise, AM offer a window to the future as the many biotechnological applications that are arising from studying the intricate and novel process to cope with extreme conditions, including toxic metals and metalloids. In this talk, we will present the biodiversity of multiresistant bacteria of the AM, with focus in actinobacteria. Likewise, the structural and molecular basis that allows these bacteria to adapt to multiple stress conditions will be dissected using omics and imaging approaches.

- Albarracín, V. H., Kurth, D., Ordoñez, O. F., Belfiore, C., Luccini, E., Salum, G. M., ... & Farías, M. E. (2015). High-up: a remote reservoir of microbial extremophiles in central Andean wetlands. Frontiers in microbiology, 6, 1404.
- 2. Albarracín, V. H., Gärtner, W., & Farias, M. E. (2016). Forged under the sun: life and art of extremophiles from Andean lakes. Photochemistry and photobiology, 92(1), 14-28.
- Alonso-Reyes, D. G., Galván, F. S., Portero, L. R., Alvarado, N. N., Farías, M. E., Vazquez, M. P., & Albarracín, V. H. (2021). Genomic insights into an andean multiresistant soil actinobacterium of biotechnological interest. World Journal of Microbiology and Biotechnology, 37(10), 1-16.

.

Approaches for application of arbuscular mycorrhizal fungi in horticulture practise

Alberico Bedini^{1,2}, Alicia Valera Alonso³, Louis Mercy², Carolin Schneider², Eva Lucic-Mercy², Philipp Franken^{1,3}

¹ Institute of Microbiology, Friedrich Schiller University Jena
 ² INOQ GmbH
 ³ Erfurt University of Applied Sciences, Erfurt Research Centre for Horticultural Crops

Contact: philipp.franken@uni-jena.de

Arbuscular mycorrhizal (AM) fungi possess a number of functions that are beneficial to the plant. They promote plant growth and increase plant defense and tolerance and the quality of plant products. Nutrient exchange is one of the central functions of the AM symbiosis. Phosphate is mainly provided by the fungus, which receives sugars and lipids from the plant. Many studies indicate that under certain conditions even the complete phosphate nutrition can be taken over. If plants are, however, sufficiently supplied with available phosphate, root colonization by the AM fungus is low and the symbiosis cannot perform its various functions.

Horticultural plant production systems usually contain sufficient available phosphate. Especially sustainable peat-free substrates supplemented with compost contain high amounts of plant-available phosphate and suffer rather from N deficiency. Therefore, phosphate-tolerant strains are needed, if producers want to take advantage from the other functions of the AM fungi symbiosis like protection against abiotic and biotic stresses. There are currently no such strains cannot among AM fungal products on the market, because isolation has been not successful. As alternative to new isolations, we propose the acclimatization of given AM fungal strains to high phosphate concentrations.

Root organ cultures have been established with the commercially available *Rhizophagus irregularis* strain QS 81. These cultures were propagated over five generation at high amounts of phosphate in the culture medium (low amounts have been used as control). The process resulted in putatively P-acclimatized (Acc+P) and non-acclimatized (Acc-P) strains. Under high Pi-concentrations, the Acc+P strain showed increased hyphal branching compared to the Acc-P strain, and in root organ cultures, the Acc+P strain developed more hyphae. Spore development was, however, low compared to the Acc-P strain irrespective of the Pi concentrations in the medium. After inoculation of plants in pot cultures, the Acc+P strain showed higher infection frequencies and increased biomasses and P uptake of shoots even at optimal Pi fertilization levels.

The results suggest that acclimatization can shift the ability of an AM fungus to develop at different conditions. This would allow a targeted AM fungal inoculum production. Further studies are directed to the other mycorrhizal functions in order to elucidate if the Acc+ strain is available to increase resistance and tolerance against biotic and abiotic stresses in horticultural substrates.

Newer aspects of the biology of heavy metal plants

Hermann Bothe

Botanical Institute, Biosciences, The University of Cologne, Zülpicherstr. 47b, D-50674 Cologne

Heavy metal plants (the metallophytes) can be differentiated into obligate and facultative species. Obligate heavy metal plants occur solely on heavy metal soils but not in Central Europe. Facultative species (also called pseudo-metallophytes) can live on heavy metal soils, but also on non-polluted habitats in far apart areas in most instances. Two species, both of the Brassiceae family, namely *Noccaea* (*Thlaspi*) caerulescens and *Arabidopsis* (*Cardaminopsis*) halleri are currently in the centre of molecular studies and are extensively discussed for phytoremediation purposes. Both species nowadays are amenable to genetic manipulations, thus to knock-out and overexpression of genes putatively involved in heavy metal tolerance. Both species are hyperaccumulators of heavy metals. They achieve this capability by duplications and cis-regulatory properties of genes coding for heavy metal transporting/excreting proteins.

The zinc violets employ arbuscular mycorrhizal fungi for heavy metal tolerance and have a rather restricted local distribution. The yellow form (*Viola lutea* ssp. *calaminaria*) is found on heavy metal soils in the Aachen-Lüttich (Liège) area, whereas the blue morph (*Viola lutea* ssp. *westfalica*) only lives at the Pb/Cu-ditch of Blankenrode near D-Paderborn. *Armeria maritima* ssp. *halleri* occurs on heavy metal soils but closely related subspecies occur in salt marshes or in the drier sandy areas of Northern Germany. *Armeria* employs modified stomata as glands to excrete evitably taken up excess of heavy metals or NaCl, respectively. The two members of the Caryophyllaceae, *Minuartia verna* and *Silene vulgaris* ssp. *humilis* have not thoroughly been investigated for their heavy tolerances. They apparently discard their leaves when overloaded with heavy metals and must be able to keep their leaf-forming meristems essentially free of these toxic elements.

Heavy metals often have an extremely patchy distributed in soils which are rather dry in most instances. Thus drought-tolerant plant species are often, but falsely, listed as metallophytes in the literature.

The subject has been reviewed by us (Bothe and Słomka, J. Plant Physiol. 219, 45-61, 2017). Newer aspects, published after this article, will be in the focus of the current presentation.

Biotic interactions of a green alga in a nature-like microverse environment

Trang Vuong¹, Harikumar Suma², Pierre Stallforth², Maria Mittag¹

¹Matthias Schleiden Institute of Genetics, Bioinformatics and Molecular Botany, Friedrich Schiller University Jena, Am Planetarium 1, 07743 Jena, Germany

²Department of Paleobiotechnology, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute (HKI), Beutenbergstraße 11a, 07745 Jena, Germany

The biflagellate unicellular green alga Chlamydomonas reinhardtii has been recently developed as a model for studying biotic interactions (Aiyar et al., 2017; Krespach et al., 2020; Hotter et al., 2021). C. reinhardtii was first isolated in a soil sample taken from a potato field near Amherst, Massachusetts, USA (Harris et al., 1989). In nature, it is usually found in moist soil. To study the growth of C. reinhardtii and its interaction with other microorganisms in a nature-like environment, 3-D structured components using glass beads have been introduced. Our results show that the growth of C. reinhardtii is enhanced in the presence of 3-D structured components compared to a control in pure liquid medium. The antagonistic interaction of the soil bacterium Pseudomonas protegens Pf-5 that deflagellates, blinds and lyses the algal cells (Aiyar et al., 2017; Hotter et al., 2021) was also studied in a 3-D environment. A FISH protocol was established to visualize bacterial and algal cells in 3-D. It shows that the bacteria also encircle the algal cells in 3-D as they do in liquid culture (Aiyar et al., 2017). Notably, C. reinhardtii recovers in 3-D environment after about 25 days, which is not the case in liquid culture under the same used conditions. Further soil bacteria, including such of potato fields, have been analyzed in co-cultures with C. reinhardtii. Some of them exhibit also a strong inhibitory effect on microalgal growth and will be presented.

References

Harris, E.H., et al., 1989, The Chlamydomonas Sourcebook.

Aiyar, P. et al., 2017, Antagonistic bacteria disrupt calcium homeostasis and immobilize algal cells. Nat. Commun, 8, 1756.

Krespach, M. K. C. et al., 2020, Lichen-like association of *Chlamydomonas reinhardtii* and *Aspergillus nidulans* protects algal cells from bacteria. ISME, 14, 2794–2805.

Hotter, V. et al., 2021, A polyyne toxin produced by an antagonistic bacterium blinds and lyses a Chlamydomonad alga. Proc. Natl. Acad. Sci., 118, 33.

Symbiosis of Ni hyperaccumulators

Katarzyna Turnau

Institute of Environmental Sciences of the Jagiellonian University, Kraków, Gronostajowa 7, Poland, Correspondence *E-mail:* katarzyna.turnau@uj.edu.pl

A wide range of microbes can be used in cost-efficient and sustainable techniques in phytoremediation, agromining and agriculture. Selection of the most efficient endophytic and mycorrhizal fungi assisted by symbiotic bacteria can be used not only to speed-up plant growth, but also to limit the use of fertilizers and pesticides and to defend plants from pathogens or harsh conditions. Research on microbes supporting plants in heavy metal rich areas have been carried out. Several plant species were mycorrhizal, however, even the same plant species under toxic condition had either only dark endophytes or they were cohabited by AMF, DSE and also other endophytes that seems to be of key importance under harsh conditions. Even so called "nonmycorrhizal plants" are also not devoid of fungi. Ni hyperaccumulative plants (such as Alyssum montanum, Noccaea caerulescens, N. goesingensis) are mostly associated by the DSE isolates that belong to the group of Dothideomycetes and Sordariomycetes. Some of them increased Ni accumulation at least twice in inoculated plants. A global gene expression analysis was performed using NGS (Next Generation Sequencing) and the genes of hyperaccumulative plants were identified, the expression of which was activated or inhibited in plants developing the Ni phenotype under the influence of symbiosis with endophyte. Increased gene expression of metal transporters has been demonstrated. The presentation will show several data resulting from long- and short-term studies carried out under local and international cooperation.

Wining and mining – Extraction of critical elements from steel slag with discarded wine

Stefan Karlsson¹, Viktor Sjöberg¹, Anders Herdenstam² and Henrik Scander²

¹School of Science and Technology, ²School of Hospitality, Culinary Arts and Meal Science, Örebro University

Roughly 25% of the annual wine import to Sweden is discarded by the whole sale companies because of quality issues. Since trade agreements prohibit consumption only a minute fraction is recycled, notably for extraction of ethanol. In an effort to find other use for the discarded wines their ability to extract critical elements from solid industrial refuse was systematically evaluated in a pilot project. Five red and five white wines were tested. They were selected according to their sensory profiles by master sommeliers in an effort to investigate any relationships between taste and complexing capacity. The solid refuse consisted of steel slag whose chemical properties had been characterized in detail and conventional batch leaching tests were performed.

The preliminary results show that the wines were highly efficient for metal extraction but the spectrum of elements as well as leaching capacities varied considerably between brands. So far, some of the red varieties mobilized metals rather indiscriminately while a higher specificity was found among the white. Depending on the brand of wine elements of environmental (Cd, Cr, Cu, Pb, Zn) or commercial concern (V, REE) were released at rather different amounts. Hence, in the future it might be possible to optimize both specificity and capacity for selected elements by careful mixing of different brands. This limited study does not provide any conclusions concerning the taste profile and leaching properties of the wines. Size exclusion chromatography of the metal complexes in the extracts indicates that the most active complexing agents is more related to the brand of the wine than what a trained sommelier experience through the senses. The chemical properties of the complexing agent is presently under way.

Microbially induced reduction of Uranium in contaminated mine water for bioremediation purposes: A multidisciplinary approach study

A.M. Newman-Portela^{1,2}, E. Krawczyk-Bärsch², M. Lopez-Fernandez¹, A. *Kassahun*³, A. Roßberg², K. Kvashnina⁴, E. Bazarkina⁴, J. Raff ², M.L. Merroun¹.

Department of Microbiology, Faculty of Science, University of Granada (Granada, Spain)
 Department of Biogeochemistry, Institute of Resource Ecology, Helmholtz-Zentrum
 Dresden-Rossendorf (Germany)
 WISMUT GmbH (Chemnitz, Germany)

⁴ European Synchrotron Radiation Facility (Grenoble, France)

Contact: antnewpor@ugr.es

The legacy of the former uranium (U) mining in Saxony and Thuringia (Germany) still shows uranium concentrations, e.g., in the mine water of some mines. The present study describes the biostimulation of the native U reducing microbial community in a U contaminated mine water as an efficient and eco-friendly strategy for *in situ* bioremediation to prospectively support or outperform chemical water treatments.

The microbial community was characterized by 16S and ITS1 rRNA gene analyses, showing a relative abundance of native microbial groups with the ability to alter the speciation and redox state of soluble U (e.g., Desulfovibrio, Gallionella, Penicillium and Aspergillus). Additionally, Inductively Coupled Plasma-Mass spectrometry (ICP-MS) and Ionic Chromatography (IC) were used to determine geochemical profile of the mine water, exhibiting a notable concentration of U (1.01mg/L), SO₄²⁻ (335mg/L), Fe (0.99mg/L) and Mn (1.44mg/L). Cryo-Time-Resolved Laser Fluorescence spectroscopy (cryo-TRLFS) and Parallel Factor Analysis (PARAFAC) determined the aqueous species Ca₂UO₂(CO₃)₃⁴- as the main U species in mine water. A set of anerobic microcosms, supplemented with glycerol (10mM) as electron donor to stimulate U reducing bacteria, were designed as basis of an in situ bioremediation strategy for uranium contaminated waters. A thermodynamic Eh-pH dominance diagram calculated using Geochemist's Workbench predicted the reduction of U(VI) and the formation of the solid U-ore (uraninite). At the end of the experiment, ICP-MS and lon-Chromatography analysis from the microcosms revealed a decrease of U (≈98%), Fe (≈91%) and SO₄²⁻ (≈88%). Furthermore, the black precipitate formed at the bottom of the microcosm was analyzed by High Energy Resolution Fluorescence Detected Near-edge Xray absorption fine structure (HERFD-XANES) and Extended X-ray absorption fine structure (EXAFS) identifying mainly U(IV) (≈80%) and U(V) (≈20%).

The results obtained revealed that microbial cycling processes have a significant impact on the complete enzymatic reduction of soluble U(VI) to U(IV) and U(V) by the addition of an electron donor in low U concentration contaminated mine water. Therefore, this methodology could be an efficient bioremediation approach for the management of U contaminated mine water, as well as low U contaminated mine water scenarios, through the biostimulation of its indigenous microbial community.

A pot experiment testing the effects of plant community structure and heavy metals contamination on the leaching of elements and functional traits of plants

Aurora Neagoe, Virgil Iordache, Gențiana Predan, Daniela Lazăr, Trițescu Bogdan

University of Bucharest, Romania

We performed a bivariate experiment in function of soil contamination and type of vegetation cover (one species, and two species of plants) in the frame of a research program dealing with the effects of heavy metals and temperature on the role of grassland plants in the production of several biogeochemical services (www.mettelflux.com). We tested the following hypotheses: H1. The export of N and P by leaching is lower in the case of twospecies pots than in the case of mono-species pots; H2. The morphometrical and mechanical treats of plants will be different in the case of two plant species variant compared to one species variant. H3. The heavy metals contamination will have a significant effect on the phenomena tested by H1 and H2. We used soils sampled from three contaminated sites: Paul Valley (VP), Copsa Mică (CM) and Metes (MT) each of them being taken from two areas: one with minimum and another with maximum content of HMs in the local range. An uncontaminated top soil was used for comparison. The design consisted in 7 experimental variants with 5 replicates each, seeded with one plant species (Agrostis capillaris) and with two plant species (A. capillaris and Melilotus albus), totaling 70 pots. This experiment was run for 3 months in a growth chamber at controlled optimal temperature, light (day / night cycles) and humidity. Pots were flooded 3 times after the plant developed, and the leachate was sampled and analyzed for phisico-chemical variables. Soil and vegetation were sampled after 3 months and analyzed for phisico-chemical analysis, and aboveground and below ground functional traits, respectively. We present here the effects of contamination degree/soil type and type of plant cover on the fluxes of elements exported by water, on their stoichiometry, and on the functional traits of plants relevant for the export of elements by runoff and leaching. We discuss the results in terms of plants interaction and the influence of functional traits on the export of elements by water, on the background of different contaminations with heavy metals. In the next phase the bivariate experiment will be repeated at a higher temperature, in order to have a three-variate experiment and test more complex hypotheses related to the effects of climate changes on the export of elements from grasslands by runoff and leaching.

Adsorption of uranium to low cost sorbents – AOD-slag and coniferous bark

<u>Viktor Sjöberg</u>¹, Kristina Åhlgren¹, Gunnar Ruist², Peder Eneroth³

¹Örebro University, Man-Technology-Environment Research Centre, Fakultetsgatan 1, 701 82 Örebro, Sweden

²Outokumpu Stainless AB, Koppardalsvägen 65, 774 41 Avesta, Sweden ³FlexiClean AB, Brandthovdagatan 16, 721 35 Västerås, Sweden

Remediation of contaminated waters is a worldwide issue. From a technical point of view is the possibilities regarding the preferred method endless but site specific conditions e.g. access to roads and especially electricity is often a limiting factor. With remote locations and limited infrastructure is the available technology often limited to passive methods such as gravity feed filter systems or reactive barriers. For such installations to be cost efficient is it often desirable to use cheap and readily available materials that has a high specificity for the contaminant at the site. Such materials can be bark from nearby wood industry or slag from nearby steel works.

Uranium is an element that occasionally has a high occurrence in the bedrock and high concentrations might be found in waters percolating through freshly crushed rock e.g. during construction of roads. Two materials, in Sweden readily available, with widely different characteristics were therefore tested for their ability to adsorb uranium, initially during controlled laboratory conditions. One of the materials was coniferous bark (pine and spruce) while the other was AOD-slags of different composition.

All the materials were evaluated using a relevant concentration range of uranium, 0.01 to 1 mg/L, and 24h equilibrium time. The sorbent concentration was set to 5 g/L and the sample volume to 40 mL. During those conditions the tested materials were able to adsorb between 60% and 90% of the available uranium. By using the SIPS-isotherm for the evaluation adsorption capacity it was found that the slags and the barks had adsorption capacities in the range of 125 μ g/g to 247 μ g/g and 187 to 308, respectively. Spruce bark was superior to pine bark and water cooled slag from the production of high temperature stainless steel was superior to the other tested slags.

	10738	10761	10771	bark	
٧	165	135	247	308	G
L	164	175	125	187	Т

Functional diversity of terrestrial and aquatic habitats *Planctomycetes*

Kumar Gaurav¹ & Christian Jogler¹

1, Department of Microbial Interactions, Institute of Microbiology, Friedrich Schiller University

The phylogeny of *Planctomycetes* was a subject of controversy since its discovery in the year 1924. These members were first recognised as eukaryotes due to their morphology resembling fungi. However, it was corrected and subsequently acknowledged as bacteria in the year 1972. The Planctomycetes along with other bacterial phyla like Verrucomicrobia and Chlamydiae form the PVC superphylum. Members of the phylum Planctomycetes are ubiquitous in distribution and are most commonly isolated from aquatic habitats and few from terrestrial habitats like Himalayas. Species of this phylum have unique physiology and cell morphology compared to other bacteria. The main biotechnological application of Planctomycetes is the removal of ammonium from waste water by anaerobic so-called anammox Planctomycetes. Presence of several biosynthetic gene clusters (BGCs) in the Planctomycetal genomes, make them untapped 'adroited producers' of bioactive small molecules that might comprise putative novel antimicrobial compounds with a potential therapeutic character such as novel antibiotics. Planctomycetes' role was observed for the polyhydroxyalkanoate production in dark fermented paperboard mills in the presence of acetate. Planctomycetes might also have an essential role in facilitating halotolerant organisms for the bauxite, arsenic and uranium remediation. Planctomycetes along with other microbes colonize and inhabit mine wastes, they tolerate high concentrations of metals and contribute to soil functioning and plant growth. Hence, through biotechnological approaches, their activities can be stimulated to remediate metal containing mine wastes.

The challenge of the targeted use of microbial consortia for an improved functionality of peat-free horticultural growing media

<u>Katja Burow</u>^{1*}, Julia Brandes¹, Sebastian Pietschmann², Julius Dawydow¹, Dirk Möcker¹, Erika Kothe², Philipp Franken^{1,2}

¹Erfurt University of Applied Sciences, Erfurt Research Centre for Horticultural Crops (FGK)

²Friedrich Schiller University Jena

*katja.burow@fh-erfurt.de

The development of sustainable products, of optimized processes and of methods to conserve natural resources finds currently its way into many areas of research. In horticultural sciences, the development and improvement of peat-free substrates becomes increasingly important. One field of research of the Erfurt Research Centre for Horticultural Crops (FGK) concerns the interactions between plant-beneficial microorganisms and plants. In frame of the current project, defined microbial consortia are selected to replace the functional properties of peat.

In a first step, the contribution of the microbiota derived from compost, from plants and from the greenhouse environment to microbial communities in substrates and plant roots was investigated, and analyzed whether the structure of these communities depended on the genotype and the developmental stage of the model plant petunia. During the culture period, plants were phenotyped, the physical and chemical properties of substrates were analyzed, substrate and root microbiomes were monitored and potential endophytic microorganisms were isolated, cultured and molecularly identified.

The results of the microbiome analysis indicated that Proteobacteria dominate the bacterial endophytic community and that representatives of Basidiomycota from compost prefer to colonize the roots of *P. exserta* during time. Comparing the different stages of plant development, the relative abundance of Verrucomicrobiota and Basidiomycota seems to be higher at later stages. Furthermore, an increase of Verrucomicrobiota and Sumerlaeota in substrates could be observed over time. Microorganisms from the greenhouse surrounding establish a complex microbial community in the substrates and in the roots, which differs substantially from the input from the compost.

Approximately 350 bacterial and fungal isolates are currently characterized with respect to their physiological and biochemical properties to assemble a functional plant beneficial microbial community. Out of this strain collection a bacterial consortium consisting of *Pseudomonas alvandae*, *Paraburkholderia strydomiana*, *Rhizobium gei* and *Paenibacillus cineris* were used to investigate their plant-growth-promoting (PGP) potential on *Petunia hybrida* cv. ,Mitchell' in a sterile/unsterile peat-free substrate. For the improvement of physical and chemical properties, the white rot fungus *Schizophyllum commune* was added to the system. First results indicated PGP effects in peat-free substrate by the chosen bacterial consortium and the fungal biomass, and due to the sterilization process. Additional studies are intended to further improve the design of the microbial consortia, to test the benefits of fungal mycelium additions and to evaluate the effect of sterilization.

ċ

Microbially accelerated weathering of metallurgical wastes: disposal scenario and treatment systems

Anna Potysz

¹Department of Experimental Petrology, Institute of Geological Sciences, University of Wrocław, Pl. M. Borna 9 50-204 Wrocław, POLAND; e-mail: anna.potysz@uwr.edu.pl

Metallurgical industry by its very nature produces large amounts of metal-bearing wastes including slags, mattes, and ashes. These wastes are of environmental concern due to potential release of contaminants if managed improperly. Of particular interest is the fate of metallurgical wastes landfilled long ago without appropriate supervision concerning metal migration (Ettler and Vítková, 2021). Thus, biogeochemical processes affecting dissolution rates of metallurgical wastes have received relevant interest in recent years (Kierczak et al., 2021; Potysz et al., 2015). On the other hand, the search for alternative management options become crucial for the metallurgical industry due to the finite reserves of natural metal resources (Potysz and van Hullebusch, 2021). In this regard, experimental simulation of bioweathering processes ongoing in the field and development of recovery systems have been evaluated in the frame of this study.

Pseudomonas fluorescens (DSM 50091) is a siderophore producing microorganism. Bacterium was incubated in Fe-poor growth medium expecting that enhanced siderophore production may be observed. Consequently, potential impact on dissolution of Fe-bearing phases incorporated in metallurgical wastes could also be expected. The incubation of polymetallic wastes (metallurgical slag and matte) for 119 days in a semi-open pass flow through reactor mode was carried out under the following conditions: (i) water, (ii) sterile growth medium and (iii) medium with bacterium Pseudomonas fluorescens. In addition, comparison of extraction mediated by Pseudomonas fluorescens and Acidithiobacillus thiooxidans was made in batch experiments during 28 days.

Generally, after 119 days of the experiment the following extraction yields were achieved under abiotic weathering with water and sterile medium: up to 6% of Cu, up to 0.4% of Zn, and up to 2.6% of Pb. The presence of bacterium increased the release of contaminants reaching up to 9.4% of Cu, 0.5% of Zn, and up to 4% of Pb. What is important, microbial biomass revealed an important contribution in elements capturing as proven by the total content of metal incorporated in mineralized biomass: up to 16.8% of Cu, up to 1.60% of Zn and up to 11.2% of Pb. Metal immobilization by secondary phases was predicted by applying a geochemical model which demonstrated the presence of various Cu-bearing and Zn-bearing precipitates that were specific to the waste incubated and varied between the exposure conditions.

This study demonstrated the accelerated solubility of elements from studied metallurgical wastes. Preferential release of metallic elements was observed with respect to the major elements indicating that application of *Pseudomonas fluorescens* in the selective recovery systems is a promising treatment route. The analysis of elements captured by the biomass showed that immobilization mechanisms related to activity of this bacterium at the disposal site may be crucial for development of remediation techniques. Comparison of efficiency of *Pseudomonas fluorescens* with that of *Acidithiobacillus thiooxidans* showed a good recovery performance (up to 56%) of the latter strain; thus combined approach involving two-stage recovery process is promising for treatment of such wastes.

Ettler, V., Vítková, M., 2021. CHAPTER 5. Slag Leaching Properties and Release of Contaminants. https://doi.org/10.1039/9781839164576-00151

Kierczak, J., Pietranik, A., Piatak, N.M., 2021. CHAPTER 4. Weathering of Slags. https://doi.org/10.1039/9781839164576-00125 Potysz, A., van Hullebusch, E.D., 2021. Secondary metal recovery from slags, in: Piatak, N.M., Ettler, V. (Eds.), Metallurgical Slags: Environmental Geochemistry and Resource Potential, Chemistry in the Environment. Royal Society of Chemistry, London, pp. 268–301. Potysz, A., van Hullebusch, E.D., Kierczak, J., Grybos, M., Lens, P.N.L., Guibaud, G., 2015. Copper metallurgical slags - current knowledge and fate: A review. Crit. Rev. Environ. Sci. Technol. 45. https://doi.org/10.1080/10643389.2015.1046769

Survival of *Tricholoma vaccinum* in ectomycorrhizospheric habitat: Facing the living and the non-living

Oluwatosin Abdulsalam¹, Katrin Krause¹, Hasan Rahman Khattak¹, Katharina Wagner¹, Wilhelm Boland², Erika Kothe¹

1 Friedrich Schiller University, Institute of Microbiology, Jena, Germany 2 Max Planck Institute for Chemical Ecology, Jena, Germany

Ectomycorrhiza-forming fungi like Tricholoma vaccinum have been shown to be very important in the balance of the forest ecosystem. Their roles in supporting plants through nutrient allocation and/or through wading off disease-causing pathogens and their role in making better soils has been well documented. These fungi have shown their ability to survive challenging environment such as sites contaminated with xenobiotics with evidence of a robust evolutionarily distinct relationship with many terrestrial plants. To ascertain stress in plant host – mycorrhiza fungi interaction, simple co-culture experiments were carried out to measure biomass change as a function of fitness in the interacting partners. In these studies, we suggest the roles of specific genes predicted to code for effector proteins, that are important in the interaction of the fungi (*T. vaccinum*) with its plant host (*Picea abies*). We also show the expression of different GST genes in T. vaccinum while interacting with biotic and abiotic stressors. RNASeq experiments were then carried out to analyse transcriptomic changes in T. vaccinum while interacting with abiotic stressors like metalcontaining seepage water and while interacting with biotic partners like the plant host. Attention is being driven towards the use of ectomycorrhiza fungi in the treatment of contaminated waste lands, as it helps in the remediation of sites while supporting the growth of host plants on the site, allowing for complete recovery of soil health. Therefore our continued contribution to the understanding of how these super resources functions will make them a great tool for reforestation.

Goals and strategies for monitoring of growth and soil development of covers and decontaminated sites

Ulf Barnekow, Mirko Köhler

Wismut GmbH

Over the past three decades, Wismut GmbH has rehabilitated the legacy of uranium ore mining in Saxony and Thuringia on behalf of the federal government. Numerous waste rock piles, operating areas and a residual open pit mine have been put to new use after comprehensive remediation and revegetation. What has been achieved now poses new challenges for the company in dealing with the areas that have been made usable again. It is important to preserve what has been achieved in a responsible manner. In particular, afforestation must be developed into resilient forests under the climatic changes of recent years. This is all the more important because the woody plants and their influence on the soil water balance are an essential functional component of the cover systems.

Based on these backgrounds and tasks, Wismut GmbH is open to further cooperation with the Friedrich Schiller University. In addition to support for the continued operation of test fields in the "Seidemannsche Schlucht" near Ronneburg in Thuringia, joint research activities are planned. Specifically, extensive studies on cover and soil, vegetation, microbiology, water balance and climate are planned for the waste rock dump Beerwalde.

Wismut's research priorities include the development of innovative biomonitoring methods to describe and evaluate the development of vegetation. The data and results are to support Wismut under the influence of climatic changes in ensuring the long-term functions of the tailings cover and the established forest for securing the radioactive contaminated site.

Combined substantial and energetic use of biomass in closed-loop systems of elements in the air-soil-organism interface

Steffi Formann*, Thomas Schliermann, Clement Owusu Prempeh, Ingo Hartmann

Department of Thermochemical Conversion, DBFZ - Deutsches Biomasseforschungszentrum gGmbH, Torgauer Straße 116, 04347 Leipzig, Germany *Correspondence: steffi.formann@dbfz.de

The growth and activity of organisms enable diverse environmental functions like regulatory (e.g. purification of air and water, climate control), supporting (enable e.g. primary production, soil formation, nutrient cycles) and providing services (production of e.g. food, biomass, raw materials, energy sources). In particular, the biomass production deliver thereby high potentials by association to the interface ambient air, soil, and other organisms. By the help of natural uptake from the soil for physiological supply of cells, diverse elements are transferred into the biomass of organisms. Elemental availability in the soil is geogen or anthropogenic induced and directly connected to soil-living organisms. Via dust formation by human activities and thermochemical conversion/combustion processes are both, biomass and soil, immediately related to the ambient air. Pollutant emissions in turn can lead to poor soil properties and excessive use of toxic metals, e.g. in industrial manufacturing and application, can also negatively affect soils and therin living organisms and biomass.

Therefore our research focuses on energetic use of elemental-enriched biomass combined with recovery of conversion products for technical or industrial applications, elemental use in circuitry and regenerative heat production with regard to emission control [1]. In order to prepare them for material use in environmental engineering processes, elemental-enriched biogenic byproducts are generated during the material utilization, coupled with a heat controlled production. In previous investigations, a production process for the generation of amorphous and porous silicon dioxide (SiO₂) from silicon rich biogenic cereal residues was developed [2]. The environmental and economic viability of the process is given in particular by the fact that heat - controlled production can be combined with the material utilization of the incineration products [3]. As case study the recovered biogenic SiO₂ finds application in flue gas cleaning and ambient air purification. This research work represents an applicable example of combining sustainable utilization of the resource soil and agricultural residues with the generation of energy inclusive the recovery of value elements for further valueadded processes and applications. The recyclable materials recovered in this way are valuable resources and should moreover increasingly be used in the sense of recycling. Elemental biomass accumulation with vision for potential commercial applications will furthermore bring in progress the bioeconomy aspects to combine renewable energy processes with the recovery substantial of raw materials.

^[1] Schofield K (2020) Combustion Emission – Formation, Reaction, and Removal of Trace Metals in Combustion Products. Academic Press; 1. Edition.

^[2] Schliermann T, Hartmann I, Dizaji HB, Zeng T, Schneider D, Wassersleben S, Enke D, Jobst T, Lange A, Roelofs F, Fellner A, Schneider P (2018) High Quality Biogenic Silica from Combined Energetic and Material Utilization of Agricultural Residues. Conference: 7th International Symposium on Energy from Biomass and Waste at: Venice, Italy, ISBN 978-8-86-265013-7.

^[3] Bachmaier H, Kuptz D, Hartmann H, Heimler F (2021) Stoffliche Nutzung von Biomasseaschen als Baustein der Bioökonomie. Berichte aus dem TFZ 72, Straubing..

Arsenic and selenium biomineralization: biominerals to bioremediation

Staicu LC¹, Wojtowicz PJ¹, Molnár Z², Ruiz-Agudo E³, Gallego JLR⁴, Baragaño D⁴, and Pósfai M²

¹Departmet of Bacterial Genetics, Faculty of Biology, University of Warsaw, Poland. *staicu@biol.uw.edu.pl || https://staiculab.com/

²Research Institute of Biomolecular and Chemical Engineering, University of Pannonia, Veszprém, Hungary

³Department of Mineralogy and Petrology, University of Granada, Spain
 ⁴Environmental Biogeochemistry & Raw Materials Group and INDUROT, University of Oviedo, Mieres, Spain

Shewanella sp. O23S (Shewanella baltica; isolated from a former gold mine in SW Poland) is a versatile anaerobic respirer [1] and a potential candidate for the bioremediation of polymetallic industrial streams [2]. The strain can use selenium (Se) and arsenic (As) as terminal electron acceptors in anaerobic respiration. In this process, selenate (SeO₄²⁻) and selenite (SeO₃²⁻) are reduced to extracellular red Se⁰ particles, while arsenate (AsO4³⁻) is reduced to arsenite (AsO₃³⁻) [1,2]. In the presence of H₂S released from cysteine metabolism extracellular As-S biominerals are formed. After seven days of incubation with only either Se or As oxyanions, kinetic analysis indicated the following reduction yields: SeO₃²⁻ (90%), AsO₄³⁻ (60%), and SeO₄²⁻ (<10%). The mix of SeO₃²⁻ with AsO₄³⁻ led to a decrease in As removal to 30%, while Se reduction yield was unaffected (88%). Interestingly, SeO₄²⁻ incubated with AsO₄³⁻ boosted Se removal (71%). These results indicate a complex metabolic relation between As and Se oxyanions leading to either inhibition or stimulation outcomes. When As and Se oxyanions were mixed, both As-S and Se⁰ biominerals were synthesized. All biominerals formed were extracellular, amorphous and presented a negative surface charge in the -24 to -38 mV range [1].

In a separate study, the incubation of *Shewanella* sp. O23S in a polymetallic real industrial effluent (retentate from a full-scale reverse osmosis plant) for 14 days under anoxic conditions [3]. The effluent was amended with yeast extract (source of micronutrients), lactate (carbon source and electron donor) and cysteine (reducing agent and source of H_2S [4]), and the incubation was performed in batch ode. After 14 days of incubation the following removal yields were recorded: As (8%), Co (11%), Mo (3%), Se (62%), Sb (30%) and Zn (40%) [3]. The addition of 1 mM of cysteine increased the performance as follows: As (27%), Co (80%), Mo (78%), Se (88%), Sb (83%), and Zn (90%). The contribution of cysteine as a source of H_2S to enhancing the removal yield was confirmed by its addition after 7 days of incubations initially lacking it [3]. This study showed that real metal-laden industrial effluents can be treated to medium-to-high efficiency using a biological system (naturally sourced inocula) and inexpensive reagents (yeast extract, lactate and cysteine).

References:

- [1] Staicu et al. (2022) Environ Pollut 306:119451 https://doi.org/10.1016/j.envpol.2022.119451
- [2] Uhrynowski et al. (2019) Int J Mol Sci 20(5):1018 https://doi.org/10.3390/ijms20051018
- [3] Staicu et al. (2021) Lett Appl Microbiol https://doi.org/10.1111/lam.13578
- [4] Staicu and Barton (2021) J Inorg Biochem 222:111509 https://doi.org/10.1016/j.jinorgbio.2021.111509 Austria

Estimation of biomass productivity using microdrone laser scanning in a Bioremediation experiment at field scale

Mirgorodsky, D.¹, Nettemann, S.¹, Riefenstahl, M.¹, Pietschmann, D.², Fürst, D.², Lenk, K.², Ziethe, J.², Kothe, E.², Schäfer, T.¹

Tree height is a key parameter to accurately quantify above ground biomass (AGB) of trees. Measuring biophysical and structural parameters of trees within a forest to determine biomass and quantify carbon storage is often difficult and labor intensive. Approaches that integrate laser scanning with field-based measurements may improve estimation of tree heights.

Field scale investigations were undertaken in areas of moderate HM/R contaminated substrates at testsites near Ronneburg, to investigate bioremediation strategies (USER-II-project, PTKA, FKZ 15S9417).

Here, main foci lie on production of woody biomass with fast growing, metal tolerant plants (*Betula pendula, Sorbus aucuparia, Alnus, Pinus, Salix*) for the production of renewable energy in a short-rotation-coppice (SRC). In this context, carbonatic soil material (rendzina) and microbial amendments (VA-mycorrhiza, actinobacteria *Streptomyces mirabilis P16-B1*) should improve biomass productivity, provides a positive effect on biodiversity and erosion protection and should design sustainable landscapes by reducing bioavailability of contaminants.

Furthermore, quantification of biomass productivity, integration of drone laser scanning data with field-based measurements of heights and AGB, and HM/R-transfer within the soil-plant system are main scopes of this project. Here, plant growth could be monitored and determined with a laser scanning system installed on a microdrone on each test areas over the year period 2016-2019 and 2019-2021 within two vegetation periods. This confirms the advantage of *Alnus* over *Betula pendula* (birch) and *Salix* (willow) in terms of height growth and biomass productivity on soil-amended plots which could be verified by in-situ data.

¹ Friedrich-Schiller-University Jena, Institute of Geosciences, Burgweg 11, 07749 Jena, Germany

² Friedrich-Schiller-University Jena, Institute of Microbiology, Neugasse 25, 07743 Jena, Germany

Poster contributions

Tuber aestivum: Microbiome & cultivation

<u>Marla Josephine Jeckstiess</u>, Katrin Krause, Erika Kothe Friedrich Schiller University, Institute of Microbiology, Jena, Germany

Interactions in contaminated environment: Investigation of Schizophyllum commune in Chernobyl soil

<u>Lea Traxler</u>, Erika Kothe Friedrich Schiller University, Institute of Microbiology, Jena, Germany