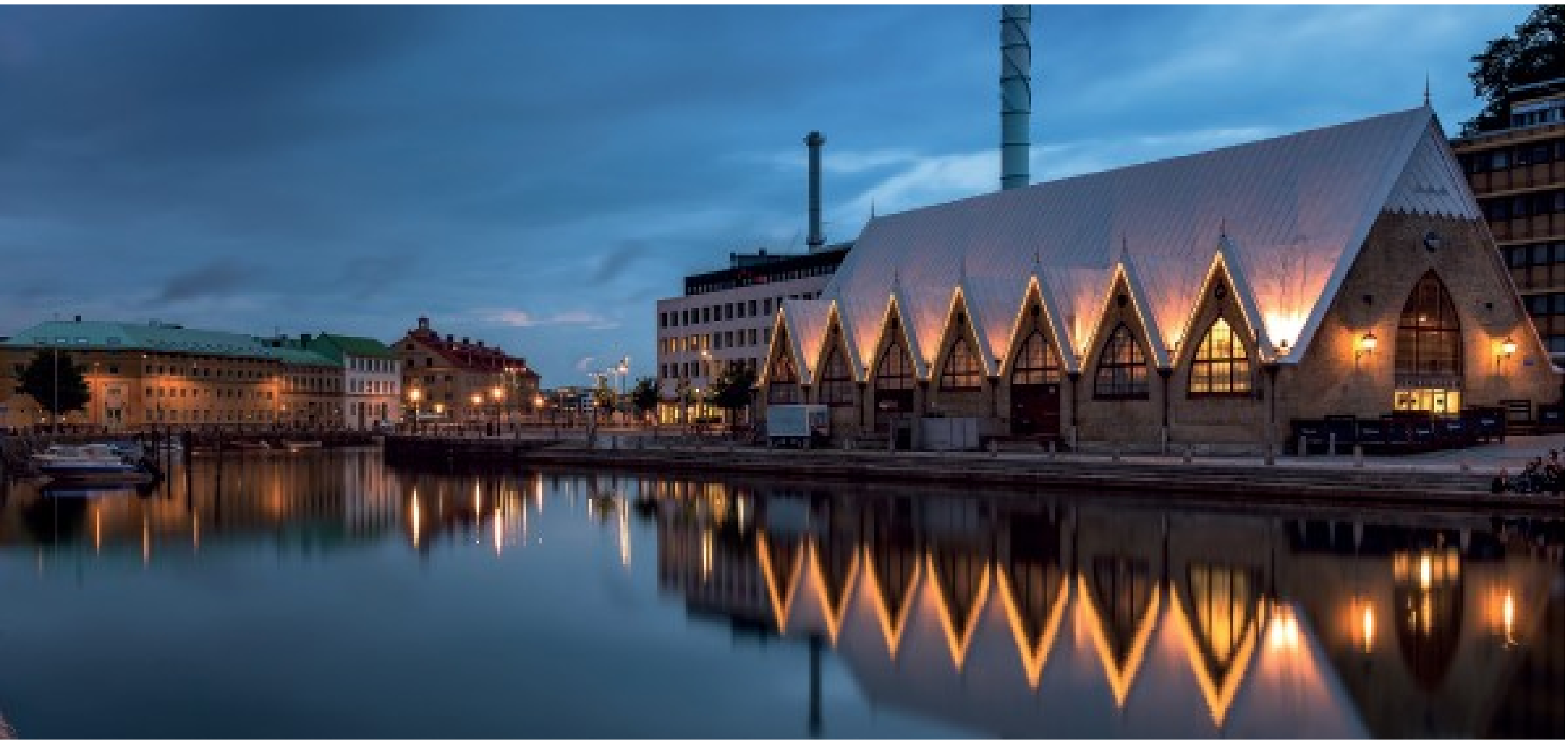

EcotoxicoMic 2024

4th International Conference on Microbial Ecotoxicology

12-14 November 2024

Gothenburg, Sweden

BOOK OF ABSTRACTS

last updated 22 November 2024

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Welcome to EcotoxicoMic 2024

Dear colleagues,

On behalf of the Organizing and the Scientific Committees, I am pleased to invite you to participate in EcotoxicoMic 2024. EcotoxicoMic was established in the last decade as a leading bi-annual international conference in microbial ecotoxicology. After the first three editions, organized in 2017 in Lyon (France), in 2020 in virtual format, and in 2022 in Montpellier (France), the 4th EcotoxicoMic conference in 2024 is held in Gothenburg (Sweden).

The overall aim of the conference is to provide an up-to-date state-of-the-art in the field of **microbial ecotoxicology** and pinpoint emerging issues and areas of research where future efforts are needed, considering all types of **microorganisms including prokaryotes and microeukaryotes**.

EcotoxicoMic 2024 will bring together scientists from various disciplines (microbial ecotoxicology, environmental microbiology, environmental chemistry, antimicrobial resistance, risk assessment, and big data), in a highly interactive environment.

The scientific programme covers presentations from invited speakers and presentations selected from submitted abstracts. There will be ample opportunities to present posters.

The scope of the topics that **EcotoxicoMic 2024** addresses includes four regular sessions:

- Impact of contaminants on microbial diversity and function
- Microbial roles in contaminant fate and bioremediation
- Microorganisms as a tool for environmental risk assessment
- Antimicrobials resistance in the environment

And two special sessions:

- Microbiome-aware ecotoxicology of animals
- Occurrence, properties, fate, and microbial ecotoxicity of natural toxins

We are pleased to welcome you in Gothenburg for EcotoxicoMic 2024!

Yours sincerely,

Assoc. Prof. Natàlia Corcoll, Conference Chair
Senior lecturer, Dept of Biological and Environmental Sciences

University of Gothenburg, Sweden

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Dr. Fabrice Martin-Laurent
INRAE, France

Organizers special sessions

Dr. Louis Carles
INRAE, France

Prof. Sébastien Duperron
MNHN, France

Dr. Philipp Hess
Ifremer

Assoc. Prof. Carmen Palacios
University of Perpignan, France

Prof. Thomas Ostenfeld Larsen
Technical University of Denmark, Denmark

FEMS MICROBIOLOGY ECOLOGY Editor-in-Chief: Max M. Häggblom
OXFORD UNIVERSITY PRESS

Call for Papers: Thematic Issue on Microbial Ecotoxicology of Contaminants of Emerging Concern

Guest Editors:
Natàlia Corcoll (University of Gothenburg, Sweden), **Kristian K. Brandt** (University of Copenhagen, Denmark), **Sébastien Duperron** (National Museum of Natural History, France), **Mechthild Schmitt-Jansen** (Helmholtz Centre for Environmental Research, Germany), **Stéphane Vuilleumier** (University of Strasbourg, France)

FEMS Microbiology Ecology invites submissions to a thematic issue on “Microbial Ecotoxicology of Contaminants of Emerging Concern”. The thematic issue will be associated with the 4th International Conference on Microbial Ecotoxicology - EcotoxicoMic 2024, 12 -14 November 2024, Gothenburg, Sweden. Please visit the conference website for more information: https://ecotoxicomic24.sciencesconf.org

Microbial ecotoxicology seeks to assess the toxicokinetics (fate) and toxicodynamics (effects) of interactions between contaminants and both prokaryotic and microeukaryotic microorganisms. Contaminants of Emerging Concern (CECs) represent recently identified environmental contaminants which may pose significant threats to humans and ecosystems or have recently been shown to be hazardous. CECs include synthetic compounds (*e.g.* pharmaceuticals, personal care products, per- and polyfluoroalkyl substances, phthalates, bisphenols, antibiotics, nanoparticles, microplastics) and natural toxins (*e.g.* cyanotoxins) or biological agents (*e.g.* antibiotic-resistant microorganisms, antibiotic resistant genes, viruses or biomolecular contaminants). There is an urgent need to assess and characterize microbe-CEC interactions and associated environmental and human health risks.

The scope of this thematic issue includes but is not limited to the following topics:

- Impacts of CECs on microbial diversity and function
- Microbial transformation of CECs and bioremediation
- Methods for studying microbe-CEC interactions; *e.g.* association of classical ecotoxicology endpoints such as growth, biomass, species composition or community functions with omics approaches such as metagenomics, transcriptomics or metabolomics
- Microorganisms as tools for environmental risk assessment of CECs
- Antimicrobial resistance in the environment
- Microbiome-aware ecotoxicology

FEMS Microbiology Ecology will consider original research articles, reviews, and perspectives that address these topics for publication in the context of this thematic issue.

SUBMISSION TARGET DATE: 31 May 2025

Authors should specify in their cover letter that their paper is submitted in the frame of the ‘Thematic issue on Microbial Ecotoxicology of Contaminants of Emerging Concern”. For instructions on submitting a manuscript, please consult FEMS Microbiology Ecology journal instructions at https://academic.oup.com/femsec. All manuscripts will undergo regular review by members of the Editorial Board and other appropriate experts.

Accepted manuscripts will be published in regular issues of the journal upon acceptance, and the Thematic Issue will be compiled and made available online upon completion.

Venue

The conference takes place at **Lindholmen Conference Centre** which is located in the Linholmen Science Park.

Address: Lindholmspiren 5, 417 56 Göteborg, Sweden

Conference centre website: https://chalmerskonferens.se/en/konferens/lindholmen-conference-centre/

Events @ EcotoxicoMic 2024

YR EcotoxicoMic evening in a Pub

A **Young Researchers (YR) evening** with a **Quizz** will be held on **Tuesday,** 12th November at 19.00 at **Haket** Pub.

A drink will be covered by the EcotoxicoMic Network. Participants will be responsible for covering the expenses of the rest of the drinks/dinner.

Address: Första Långgatan 32; Website:http://www.haketpub.se

Conference dinner

The **conference dinner** will be held on **Wednesday** evening, 13th November at 18:15 at **L's resto** in the Lindholmen conference centre. Dinner tickets are required for this event.

Programme overview

	Tuesday, 12 November		Wednesday, 13 November		Thursday, 14 November
11:00	*Registration opens*	08:00	*Registration opens*	08:00	*Registration opens*
13:00	Welcome	08:35	Keynote: **Stéphane Pesce**	08:35	Keynote: **Thomas Backhaus**
13:15	Plenary talk: **Fang Wang**	09:05	Impact of contamination on microbial diversity and function	09:05	Microorganisms as a tool for environmental risk assessment
14:20	Keynote: **Monika Dolejska**	10:05	COFFEE BREAK	10:05	COFFEE BREAK
14:55	COFFEE BREAK	10:30	Impact of contamination on microbial diversity and function	10:30	Microorganisms as a tool for environmental risk assessment
15:15	Antimicrobials Resistance in the Environment	11:45	LUNCH	11:30	Poster Session
16:45	Opening Ceremony & Welcome cocktail	12:45	Poster Session	12:25	LUNCH
17:15	Poster Session	13:45	Keynote: **Elena Gorokhova**	13:15	Keynote: **Hans Peter Heinrich Arp**
18:00		14:15	Microbiome-aware ecotoxicology of animals	13:45	Microbial roles in contaminant fate and bioremediation
18:30 … 22:00	Student evening	15:00	COFFEE BREAK	15:00	COFFEE BREAK
		15:30	Keynote: **Hans Christian Bruun Hansen**	15:25	Poster corner
		16:10	Occurrence, properties, fate, and microbial ecotoxicity of natural toxins	15:55	Microbial roles in contaminant fate and bioremediation
		17:00	Poster corner	17:15	Closing ceremony
		17:30		17:45	
		18:15 … 22:00	Conference dinner		

Programme overview

Regular sessions

Antimicrobials resistance in the environment

Chairs: Anne Farewell and Johan Bengtsson-Palme

Keynote: Monika Dolejska, University of Veterinary Sciences Brno, Czech Republic

National and international authorities are now recognizing that the environmental dimension of antimicrobial resistance must be considered when developing strategies to combat this public health menace. Antimicrobial chemicals enter the environment indirectly through human and animal waste streams and manufacturing effluents, and directly through their use in aquaculture and as pesticides in plant-based food production systems. Since these chemicals are designed to kill microorganisms (viruses, bacteria, fungi, parasites) they have potent antimicrobial activity.

Thus, there are concerns about their impacts on microorganisms undertaking key ecosystem services. Furthermore, microorganisms in anthropogenically impacted environments may adapt by evolving or acquiring genes that confer antimicrobial resistance, increasing the reservoir of resistance genes that ultimately can be transmitted to microorganisms of human health concern. Presentations concerning any aspects of environmental development and transfer of antimicrobial resistance or antimicrobial impacts on environmental microorganisms and their functions are welcome.

Keywords: antibiotic; fungicide; antiviral, impacts, fate

Impact of contaminants on microbial diversity and function

Chairs: Chloé Bonnineau, Natàlia Corcoll, Aurélie Cébron, and Pedro Inostroza

Keynote: Stéphane Pesce, INRAE, France

Microorganisms participate in all major biogeochemical cycles, and their diversity represents a keystone for ecosystem functioning and resilience. The adaptability of microorganisms is unequaled among living organisms. Nevertheless, similar to other groups of organisms, such as invertebrates and fish, microorganisms face increasing threats from multiple environmental stressors, particularly human-derived pollution. This session invites research on the impact of contaminants and other environmental stressors on the taxonomic and functional diversity of the microbial world, including microbial responses at the individual, population, and community levels. These investigations may involve the use of 'omics' and ‘metaomics’ tools, including genomics, transcriptomics, proteomics, and metabolomics. Laboratory and field studies dealing with microbes from soil, freshwater, marine, and human-made environments are welcome.

Keywords: diversity; functions; bioindicators; omics

Programme overview

Regular sessions

Microorganisms as a tool for environmental risk assessment

Chairs: Thomas Backhaus, Kristian Brandt, and Fabrice Martin-Laurent

Keynote: Thomas Backhaus, RWTH University Aachen, Germany

Microorganisms are key players of earth life: they drive important ecosystem processes and contribute substantially to global biogeochemical cycles. They exhibit a broad range of sensitivity to many toxicants and are known to be natural early warning systems that detect acute and long-term effects produced by toxic pollutants.

Until now chemical analysis remains essential to evidence contamination, however, biological indicators can provide valuable complementary information on both the impact and fate of contaminants. Microorganisms are good candidates as a tool for environmental risk assessment (ERA) since they can be found in all types of environments (water, soil…) and have a large range of sensitivity to a wide range of chemical pollutants. Innovative methods and tools for environmental risk assessment based on microorganisms have been developed in order to i/predict hazard and assess risk before the release on the market of a new active compound (a priori ERA) as well as ii/ assess, the ecotoxicological impacts of chemical residues in the environment (a posteriori ERA). Despite the recognized importance of microbial communities in supporting a range of functions and ecosystem services, microorganisms are barely considered in both a priori and a posteriori environmental risk assessment and they are typically not yet implemented in the current regulations or legislations. This session will focus on the use of microbial bioindicators/biomarkers/bioassays as well as microbial biosensors to assess the environmental risk of contaminants in various ecosystems. In particular, communications on the interest of monitoring in response to pollutant exposure the diversity and function of microbial communities using omic approaches for ERA in various ecosystems are welcome. In this session, we also intend to discuss the possible strategies to promote the use of microorganisms (at different levels of biological organization) in both a priori and a posteriori environmental risk assessment.

Keywords: bioindicators/biomarkers, biosensors, risk assessment, environmental managers, biomonitoring

Programme overview

Regular sessions

Microbial roles in contaminant fate and bioremediation

Chairs: Lise Barthelmebs, Jennifer Hellal, and Dimitros Karpouzas

Keynote: Hans Peter Heinrich Arp, Norwegian University of Science and Technology & Norwegian Geotechnical Institute, Norway

Microorganisms have developed a variety of metabolic adaptations and resistance mechanisms to cope with the presence of toxic elements in their environment. Microbial activity thus has a considerable impact on environmental contaminants and strongly contributes to their fate. Many microbially-driven processes may contribute to contaminant degradation, detoxification, or immobilization (e.g. sequestration, precipitation) and thus to pollution mitigation. Conversely, some others may enhance the toxicity of contaminants and their dissemination in the biosphere.

Evaluation and understanding of these processes is still challenging, in the field and the laboratory. Thus, prediction of the fate of contaminants in ecosystems remains difficult. The bioavailability and mobility of chemical contaminants in the environment, as key factors of microbial transformation of chemicals, require particular attention today. Moreover, linking available evidence of contaminant biotransformation to key microbial players involved in these processes remains mostly indirect, especially in complex ecosystems. Finally, understanding the environmental factors governing microbial activity is essential to developing efficient bioremediation strategies. In this session, we welcome original reports on all aspects of these challenging research areas, especially those involving interdisciplinary approaches at the interface of chemistry, physics, and microbiology, including modeling studies.

Keywords: bioremediation, biodegradation, biotransformation, bioavailability, immobilization, dissipation, ecological engineering

Programme overview

Special sessions

Microbiome-aware ecotoxicology of animals

Chairs: Sébastien Duperron and Carmen Palacios

Keynote: Elena Gorokhova, Stockholm University, Sweden

The holobiont concept, accounting for hosts and their associated organisms, offers a new holistic perspective in Life Sciences. Because the microbiota can react to and interact with contaminants like metals, pesticides, plastics or pharmaceuticals, the relevance and impact of animal-associated microbiomes to ecotoxicology is now well-established, and necessary for understanding the relationships between organisms and their environments. We expect communications that will show how disruptions caused by contaminants can affect, or be mitigated, by microbiota associated with wildlife, modulating the health and adaptability of holobionts (either in nature or in the lab). Our sessions will inform how a "microbiome-aware ecotoxicology" can open new avenues into more effective strategies for the conservation of species and/or environments, recognizing the intricate dynamics between environmental stressors and the delicate balance of microbiomes and their hosts in populations.

Keywords: Holobiont, animal wildlife, environmental stressors, adaptation, conservation.

Occurrence, properties, fate & microbial ecotoxicology of natural toxins

Chairs: Louis Carles, Philipp Hess and Thomas Ostenfeld Larsen

Keynote: Hans Christian Bruun Hansen, University of Copenhagen, Denmark

Microbial ecotoxicology has developed considerably in recent years. At the interface between microbial ecology, toxicology, and biogeochemistry, this research area unravels the interactions between organic pollutants and microorganisms at different levels of organization to better understand the impact of man-made chemicals. However, anthropogenic activities are not only releasing chemicals into the environment but are also constantly modifying landscapes and impacting ecosystem functioning, leading to the development of species that produce natural toxins. This is particularly true for instance in agricultural areas with the proliferation of phytopathogenic fungi producing mycotoxins, and in aquatic environments with blooms of cyanobacteria producing cyanotoxins.

Despite their potential impacts on microbial communities, natural toxins are rarely included in microbial ecotoxicology studies. The aim of this special session is therefore to present the current state of knowledge on the interactions between microorganisms and natural toxins and to draw potential future directions for this research area.

In this session, we welcome communications on all aspects of occurrence, properties, and fate of natural toxins, as well as on their toxicity towards microorganisms at different organization levels and from different environments (air, water bodies, soil, animal, and plant microbiota). We also encourage submissions of communications on the capacity of microorganisms to biotransform natural toxins.

Keywords: secondary metabolites, phycotoxins, cyanotoxins, mycotoxins

Scientific programme

Tuesday, 12 November

11:00	Registration opens
13:00 – 13:15	Welcome - **Natàlia Corcoll**, University of Gothenburg
13:15 – 14:15	Plenary talk: Emerging Contaminants: A One Health Perspective, by **Fang Wang,** Chinese Academy of Sciences, China
14:20	**Antimicrobial Resistance in the Environment** Chairs: **Johan Bengtsson-Palme** & **Anne Farewell**
14:25 – 14:55	Keynote: Antibiotic resistance in wildlife, by **Monika Dolejska**, University of Veterinary Sciences Brno; University Hospital Brno, Czech Republic
14:55 – 15:15	COFFEE BREAK
15:15 – 15:30	Occurrence of antibiotic resistance in the Göta Älv and associated wastewater treatment plants. ***D. Gómez-Martínez***, *J. Sorel Ngou, V. Ugolini, FY Lai, E. Kristiansson, N. Corcoll*
15:30 – 15:45	Computational Assessment of Antibiotic Transformation Product Activity: Molecular Dynamics Simulations and Free Energy Calculations. ***P. Löffler***, *H. Henschel, T. Nyman, FY Lai*
15:45 – 16:00	Are on-site sewage facilities contributing to the spread of antimicrobial resistance (AMR)? – A Swedish case study. ***V. Ugolini,*** *H. Flores Quintana, J. Subirats Medina, P. Löffler, L. Ahrens, E. Ulinder, P. Rapp, C. Dunge, FY Lai*
16:00 – 16:15	Disturbance Intensity and Sub-Inhibitory Ciprofloxacin Concentrations Shape Diversity, Assembly Mechanisms, and Antibiotic Resistance in Aquatic Bacterial Communities. ***MKS Jensen***, *SB Lassen, D. Ning, BW. Strobel, JQ. Su, KK. Brandt*
16:15 – 16:30	Monitoring of antimicrobial resistance in the environment: what? why? and how? ***J. Bengtsson-Palme***,
16:30 – 16:45	Effect of Emerging pollutants at environmentally relevant concentrations on the transference of antibiotic-resistant genes. ***A. Rey-Sogo***, *N. Goñi, V. Costa, H. Budzinski, I. Alkorta*
16:45 – 17:15	**Opening ceremony & welcome cocktail** Chairs: **Natàlia Corcoll** & **Ingela Dahllöf**, University of Gothenburg **Margareta Broang**, Deputy Lord Mayor, City of Gothenburg
17:15 – 18:00	**Poster session** – Antimicrobial Resistance in the Environment
19:00	Young Researchers (YR) EcotoxicoMic evening Quizz in Haket Pub

Scientific programme

Wednesday, 13 November

08:00	Registration
08:30	**Impact of contaminants on microbial diversity and function** Chairs: **Chloé Bonnineau, Aurélie Cébron, Natàlia Corcoll** & **Pedro Inostroza**
08:35 – 09:05	Keynote: Influence of coalescence on microbial responses to chemical exposure: towards a better consideration of the environmental continuum in microbial ecotoxicology, by **Stéphane Pesce**, INRAE, France
09:05 – 09:20	Coastal chemical mixtures affect microzooplankton abundance and diversity, contrary to results from risk assessment. ***I. Dahllöf****, C. Jönander, J. Egardt*
09:20 – 09:35	Drying-rewetting stress on total and nitrifying communities in contaminated soils increase negative effects of contaminants. ***CM. Jones****, L. Müller, MTO. Hidalgo, L. Berkelund, A. Enell, M. Larsson, A. Taylor, M. Viketoft, J. Weiss , AK. Dahlberg, K. Wiberg, D. Berggren-Kleja, S. Hallin*
09:35 – 09:50	Microbial Existence under Chemical Persistence: Analysing the PFAS Impact. ***M. Sims****, C. Selva, A. Juhasz, L. Yu, J. Schwerdt, V. Bulone*
09:50 – 10:05	Impact of PFOS, PFOA, PFHxS and PFBS addition in 4 contrasted soils: impact on microbial communities and soil N/C cycles, PFAS dynamics. ***M. Crampon****, MP. Norini, F. Battaglia-Brunet, D. Relic, M. Ljesevic, M. Charron, H. Tris, J. Begoña, A. Juan Muñoz, V. Pere Colomer, V. Beskoski*
10:05 – 10:30	COFFEE BREAK
10:30 – 10:45	Microbial ecotoxicology of conventional and bio-based polymers in soil. ***S. Granderath-Miegel****, KS. Bitter, LM. Blank*
10:45 – 11:00	Influence of plastic substrata on the plastisphere's tolerance to freshwater pollutants assessed by Pollution-Induced Community Tolerance (PICT) ***C. Touchet****, S. Pesce, C. Bonnineau, H. Rogue, P. Laurency, J. Morel, F. Mermillod-Blondin, B. Motte, B. Volat, C. Rosy, E. Lyautey, S. Krause, L. Simon*
11:00 – 11:15	Multi-compartment impact of micropollutants and particularly antibiotics on bacterial communities using environmental DNA at river basin-level. ***P. Inostroza,*** *LJ. Gerdhard, L. Feilon, Z. Xiaowei, B. Werner, T. Backhaus*
11:15 – 11:30	Effect of pesticides on soil microbial communities: Scenario with variable predator-prey interactions. ***ME. Pérez-Villanueva****, C. Malandain, S. Jurburg, N. Ghanem, A. Chatzinotas*
11:30 – 11:45	Ecological warnings: pesticides biodegradation could be a problem! ***VL. Lozano****, NH. Pizarro*
11:45 – 12:45	LUNCH

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Scientific programme

Wednesday, 13 November

12:45 – 13:40	**Poster sessions:** Microbiome-aware ecotoxicology of animals Impact of contaminants on microbial diversity and function Occurrence, properties, fate, microbial ecotoxicity of natural toxins
13:40	**Microbiome-aware ecotoxicology of animals** Chairs: **Sébastien Duperron** & **Carmen Palacios**
13:45 – 14:15	Keynote: Microbiome of Ecotoxicity Assays, by **Elena Gorokhova**, Stockholm University, Sweden
14:15 – 14:30	Exposure to copper-based nanopesticide shape the gut microbiota and induce physiological alterations in model organisms: Insight from the Nanopestis project. ***E. Lauris****, E. Casale Eva, T. Moura, F. Chapeau, Y. Malaisé, F. Candaudap, L. Chevallier, C. Cartier Christel, E. Gauthier, F. Mouchet, E. Pinelli, E. Houdeau, L. Gauthier, B. Lamas*
14:30 – 14:45	Invasive and native lake fish species display different response of their gut microbiota and metabolite compositions along a eutrophication gradient. ***S. Duperron****, A Navarro, P. Foucault, C. Duval, N. Loiseau, M. Troussellier, M. Quiquand, B. Marie*
14:45 – 15:00	Assessing eco-exposome impact along juvenile fish development in artificial port reefs through population kinetics, metabolome and microbiome studies. ***C. Palacios****, I. Bonnard, D. Aubert, T. Rauturier, C. Sotin, M. Desmalades, D. Raviglione, S. Kunesch, M. Henry, B. Charrière, N. Lucchini, G. Saragoni, P. Lenfant*
15:00 – 15:30	COFFEE BREAK
15:30	**Occurrence, properties, fate & microbial ecotoxicity of natural toxins** Chairs: **Louis Carles, Philipp Hess** & **Thomas Osternfeld Larsen**
15:35 – 16:05	Keynote: Natural toxins from plants: which are of concern ? By Hans **Christian Bruun Hansen**, University of Copenhagen, Denmark
16:10 – 16:25	Effects of temperature and salinity on the growth and toxin production of the bloom-forming cyanobacteria *Nodularia spumigena*. ***D. Martins****, N. Corcoll, AB. Barbosa, A. Tronholm*
16:25 – 16:40	Insights into the chemistry of the microalgal toxins from the 2019 *Chrysochromulina leadbeateri* Blooms in Northern Norwegian fjords. X. Wang, M. *Fon, Mathias, A. Andersen, A. Solhaug, R. Ingebrigtsen, I. Samdal, S. Uhlig, C. Miles, B. Edvardsen,* ***TO. Larsen***
16:40 – 16:55	Sensitivity of wheat-associated bacteria to mycotoxins produced by *Fusarium* spp. ***L. Carles****,C. Nicollet-Malicot*
17:00 – 17:30	Poster corner – Microbial ecotoxicology of pesticides
18:15 – 22:00	Conference dinner – L's Resto, Lindholmen Conference Center

Scientific programme

Thursday, 14 November

08:00	Registration
8:30	**Microorganisms as a tool for environmental risk assessment** Chairs: **Thomas Backhaus, Kristian Brandt** & **Fabrice Martin-Laurent**
08:35 – 09:05	Keynote: Microbial Ecotoxicology: A Key to Environmental Protection in the Spirit of the Non-Toxic Environment and the Sustainable Development Goals, by **Thomas Backhaus**, RWTH-Aachen University, Germany
09:05 – 09:20	Prediction biases in Quantitative Structure-Activity Relationship (QSAR) models. ***P. Svedberg***, *PA. Inostroza, M. Gustavsson, E. Kristiansson, T. Backhaus*
09:20 – 09:35	Electrochemical Aptasensors in Action: Rapid Detection of Potential Environmental Threats. ***ML. Pham***, *S. Maghsoomi, M. Brandl*
09:35 – 09:50	Single species *in vitro* bioassays, using soil nitrifiers as bioindicators, as a first-tier tool for assessing the toxicity of pesticides on soil microorganisms. ***E. Bachtsevani***, *M. Kolovou, ES. Papadopoulou, DG. Karpouzas, C. Hazard, NW Graeme*
09:50 – 10:05	A systematic approach for a holistic ecotoxicological assessment strategy of polymers from the laboratory to field scale. ***M. Winter***, *G. Broll, B. Philipp, CA. Diaz Navarrete, K. Schlich*
10:05 – 10:30	COFFEE BREAK
10:30 – 10:45	Contaminant bioaccumulation and diatom community in biofilms are relevant bioindicators of coastal Mediterranean seawater quality. *A. Barre, M. Bouchoucha, V. Vaccher, N. Briant, M. Briand, A. Ortalo-Magné, P. Boissery,* ***JF. Briand***
10:45 – 11:00	Assessing the risks of agricultural run-off to microphytobenthic communities in small streams. ***M. Schmitt-Jansen***, *F. Larras, K. Eberhard, S. Lips*
11:00 – 11:15	Characterizing the potential effects of a complex textile industry contamination on structure, function and nutritional quality of phototrophic biofilms: the case study of the Cleurie River. ***C. Bonnineau***, *S. Chéron, M. Jean, L. Ughetto, N. Creusot, M. Eon, N. Mazzella, A. Tourret, S. Morin, S. Devin, V. Felten, M. Laviale*
11:15 – 11:30	Cobalt effects on the diversity, functions, and co-occurrence patterns of prokaryotic and eukaryotic communities in growing river biofilms. ***S. Gourgues***, *M. Goñi-Urriza, M. Milhe-Poutingon, Y. Corsellis, BN. Gurieff, C. Gelber, S. Le Faucheur*
11:30– 12:25	**Poster sessions** Microorganisms as a tool for environmental risk assessment Microbial roles in contaminant fate and bioremediation
12:25 – 13:15	LUNCH

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Scientific programme

Thursday, 14 November

13:15	**Microbial roles in contaminant fate and bioremediation** Chairs: **Lise Barthelmebs, Jennifer Hellal** & **Dimitros Karpouzas**
13:20 – 13:40	Keynote: A persistent problem: identifying and confronting persistent, mobile and toxic (PMT) substances, by **Hans Peter Heinrich Arp**, Norwegian University of Science and Technology & Norwegian Geotechnical Institute, Norway
13:45 – 14:00	Impact of Seasonal Pesticide Exposure on Periphyton Structure and Nutritional Quality in Agricultural Streams. ***A. Koch***, *DCP. Lau, O. Jonsson, M. Gönczi, N. Corcoll*
14:00 – 14:15	Effect of hydrological variability on the capacity of biofilms to dissipate pesticides. M. Luzet, L. *Bertrans-Tubau, Y. Menard, I. Batisson, N. Creusot, N. Mazzella, D. Millan-Navarro, A. Moreira, S. Morin, AM Romaní,* ***J. Artigas***
14:15 – 14:30	Surfactants in glyphosate-based herbicides have a concentration-dependent impact on glyphosate biodegraders. ***C. Lesuis***, *K. Thompson, F. Matthee, S. Kleindienst*
14:30 – 14:45	Carrier material for bacterial biofilms formation: production of biocomposites to inoculate and treat pesticides contaminated soils via bioaugmentation. *F. Meite Fatima, C. Joulian, A. Seron, K. Michel, H. Tris, A. Spor, M. Devers, C. Soulier, J. Paume, M. Motelica-Heino, C. Le Milbeau, N. Surdyk,* ***C. Michel***,
14:45 – 15:00	Exploring microbial PFAS degradation in soils from two AFFF contaminated sites. ***N. Messinger***, *L. Ahrens, S. Bertilsson, D. Berggren Kleja, F. Fagerlund*
15:00 – 15:25	COFFEE BREAK
15:25 – 15:55	Poster Corner - Microbial biodegradation of fluorinated & chlorinated compounds
15:55 – 16:10	Exploring the degradation pathway and metabolites toxicity of a promising Chlorpyrifos-degrading strain, *Priestia aryabhattai* 1-3I, isolated from a local phosphogypsum landfill. ***A. Hanano***, *M. Shaban, Nour Moursel, MH Obeid.*
16:10 – 16:25	Biodegradation of several organic UV filters by an in-house synthetic consortium. ***SK. Fagervold***,*C. Rohée, P. Lebaron*
16:25 – 16:40	Microbial role in the fate of "biodegradable" plastics in the marine environment. *L. Philip, K. Lebaron, I. Calvès, M. Hingant, M. Pujo-Pay, A. ter Halle, V. Barbe, B. Eyheraguibel, AL. Meistertzheim,* ***JF. Ghiglione***
16:40 – 16:55	Assessment of hydrocarbon degradation, microbiome diversity and enrichment of diesel degraders from oil contaminated soil for bioremediation purposes. *J. Qiao, A.Vauloup, J. Elsmoortel, E. Depoorter, P. Vandamme,* ***A. Cebron***
16:55 – 17:10	Microbial leaching of metals: a nature based solution for recovering critical metals from mine waste. *E. Laroche, L. Spadini, L. Oxarango, P. Morais, R. Branco, A. Crouzet,* ***J. Martins***
17:15 – 17:45	Closing & Awards ceremony

Poster corners

Wednesday, 13 November 17:00 – 17:30

Microbial ecotoxicology of pesticides

Chair: **Dimitrios Karpouzas**

- Evaluating the potential impact of pesticides on soil microbial communities: Integrating *in vitro* single species tests of soil nitrifiers with in soil tests. ***M. Kolovou***, *E. Papadopoulou, DG. Karpouzas* −ThP51
- Effect of herbicide exposure on ammonia and nitrite oxidizing microorganisms and their subsequent resistance to drought. ***LJ. Müller***, *A. Saghaï, CM Jones, S. Hallin* −ThP53
- Understanding Variability in Arbuscular Mycorrhizal Fungi Responses to Pesticides: Impact of Fungicide Application Methods and Dosages on AMF sensitivity. ***A. Manukyan***, *H. Zumsande, M. Triffterer, E. Hilz, TM. Marx, K. Papadopoulou, C. Schneider, L. Mercy, U. Menke* −ThP56
- *In vitro* evaluation of endpoint values (EC50) of nineteen pesticides on the arbuscular mycorrhizal fungus Rhizophagus intraradices. ***M. Roshanfekrrad***, *K. Zhang, C. Schneider, M. Calonne-Salmon, S. Declerck* - ThP47

Thursday, 14 November 15:30 – 16:00

Microbial biodegradation of fluorinated & chlorinated compounds

Chair: **Lise Barthelmebs**

- Bacteria *versus* PFAS: let the defluorination begin! ***E. Bocconetti***, *R. Khodr, C. Badel, C. Husser, M. Ryckelynck, S. Vuilleumier* −ThP79
- Breaking news: Carbon-fluoride bond cleavage by gut microbes. ***SI. Probst***, *FD. Felder, V. Poltorak, R. Mewalal, IK. Blaby, SL. Robinson* −ThP69
- Screening for PFAS co-metabolizing bacteria. ***A. Forouzandeh***, *A. Nan Vernon, M. Hansen, TK. Nielsen* −ThP66
- Biodegradation of PCE and 1,2-DCP by a natural groundwater bacterial community: kinetics, isotopic fractionation and evolution of the microbial community. ***D. Barrollier***, *M. Crampon, C. Malandain, M. Blessing, M. Cochennec, S. Colombano, D. Cazaux, P. Höhener* − ThP77

Communication abstracts

Talk

Opening plenary talk

Prof. Fang Wang, *Institute of Soil Science, Chinese Academy of Sciences, China*

Dr. Fang Wang is a Professor at the Institute of Soil Science, Chinese Academy of Sciences, and University of Chinese Academy of Sciences. She has worked at Munich Technical University, RWTH Aachen University, Helmholtz Munich Center, and Jülich Research Center in Germany and was a Visiting Professor at Michigan State University. Her research work focuses on soil pollution and remediation, especially for organic chemicals and emerging contaminants. She is the author of 200 journal articles in The Innovation, Environ Sci Technol, Water Research, and Soil Biol Biochem, etc. Dr. Wang has been awarded the Alexander von Humboldt Fellow for Experienced Researcher from Germany; Shennong Young Talent from the Ministry of Agriculture and Rural Affairs of China; Distinguished Young Scholar from the Natural Science Foundation of Jiangsu Province of China; and a Young Talent of Lu Jia-Xi Award from Chinese Academy of Sciences. She serves as Co-Editor-in-Chief of Environmental Technology & Innovation, Associate Editor of Science of the Total Environment, Academic Editor of The Innovation, Editor of Sustainable Horizons, and editorial board member of The Innovation Geoscience. She serves on the Steering committee of UN FAO International Network on Soil Pollution and the standing committee of the International Union of Soil Science. She is the Chair of the International Collaboration Committee of Soil Science Society of China (SSSC), Co-Chair of the working group of Collaboration between SSSC and Soil Science Society of America, and a member of the International Panel on Chemical Pollution.

Keynote conference - Emerging Contaminants: A One Health Perspective

Keynote

Assoc. Prof. Monika Dolejska, *University of Veterinary Sciences Brno; University Hospital Brno, Czech Republic*

She is an expert on molecular epidemiology and comparative genomics of antibiotic resistant Gram-negative bacteria with a special focus on clinically important resistance mechanisms to cephalosporins, carbapenems and colistin transferrable by plasmids. Using cutting-edge molecular techniques her studies recognized novel reservoirs and potential vehicles of multi-drug resistant bacteria in the environment and wildlife, especially in wild birds and waste waters. She described the role of particular wildlife species in context to their behaviour and ecology in the carriage and transmission of antimicrobial resistance. She utilizes the collaboration with clinicians, veterinarians and environmental biologists, emphasizing the importance of One Health concept when studying antibiotic resistance. Recently, she focuses on the co-evolution of pathogenic Escherichia coli strains and their plasmids with the aim to reveal the features staying behind the successful dissemination of high-risk clones.

Keynote conference - Antibiotic resistance in wildlife

Occurrence of antibiotic resistance in the Göta Älv and associated wastewater treatment plants.

<u>Gómez-Martínez, Daniela</u>[1]*; Sorel Ngou, Judith*[1] *; Ugolini, Valentina*[2]*; Lai, Foon Yin*[2]*; Kristiansson, Erik*[3]*; Corcoll, Natàlia*[1]
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Effluent water from municipal wastewater treatment plants (WWTPs) and other sewage systems have been proven to be emission sources of antibiotic resistance genes (ARG) and antimicrobial drugs into natural environments. However, the extent of this event and the capacity of ARG to persist in the environment, especially in large rivers, remains unclear. This study aims to characterize and quantify the bacterial resistome in sewage sludge and effluent water of four WWTPs and in sediment bacteria in seven sites along the Göta Älv river, using quantitative-polymerase chain reaction (qPCR) and shotgun metagenomics. Originating in Lake Vänern, it flows 93 km through six municipalities, including Gothenburg, the country's second largest city. Out of 30 screened antimicrobial drugs, ciprofloxacin, sulfamethoxazole and clarithromycin were detected above their Predicted No Effect Concentrations (PNECs) for resistance selection in the effluent samples. We identified all target ARGs (Sul1 and SadA; tetA and tetX; ermB and mphA; qnrS; and BlaCTX-M) that confer resistance to sulfonamides, tetracyclines, macrolides, quinolones, and penicillins and cephalosporins antibiotic classes, respectively, in the sewage sludge, the effluent water and the bacterial sediment samples. Each WWTP exhibited unique ARG profiles, which might be explained by the different users (*i.e.* hospitals, households). We identified an increasing gradient of ARG abundance in bacterial sediment from the river source (Lake Vänern) to the mouth (Gothenburg estuary), suggesting that WWTPs may contribute to the increasingly presence of ARG in the river's sediment communities. The more abundant genes were mphA and ermB, which confer resistance to macrolides, and sul1, which confer resistance to sulfonamides. Results from the shotgun metagenomics will allow us to profile the full list of ARGs, mobile genetic elements (MGE), bacterial taxa present sediment bacteria on the Göta Älv and to correlate their abundance profile to the abundance profile in the WWTPs.

Keywords: ARG, bacteria, sediment, river, antibiotics

Computational Assessment of Antibiotic Transformation Product Activity: Molecular Dynamics Simulations and Free Energy Calculations.

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The global proliferation of antimicrobial resistance (AMR) poses a significant threat to human, animal, and the environmental health within the concept of One Health. Current evaluations of AMR and associated environmental risks predominantly focus on antimicrobial parent compounds, often neglecting their transformation products (TPs). Being generated through organismal excretion and/or transformed during wastewater treatments, TPs frequently emerge through subtle alterations in their molecular structure, often resulting in increased stability and persistence, ultimately infiltrating surface water ecosystems. Given the structural similarity between TPs and their parent compounds, the potential interaction of TPs with the target protein of their parent compounds remains a critical concern for the emergence of AMR genes in the environment. This study used the open-source molecular dynamics software, GROMACS, together with PyAutoFEP for free energy calculations to assess the binding affinity differences to the respective target protein between Tps and their parent compounds. We explored several sulfonamide TPs, as well as TPs from trimethoprim. Many TPs exhibited predicted binding affinities within the similar range as the parent compound, with some showing increased affinity, suggesting potential activity. This research highlights the importance of expanding our approach for AMR assessment by recognizing the significance of antimicrobial transformation products.

Keywords: Antimicrobial Resistance, Metabolite, Medicinal Chemistry, Molecular Docking

Are on-site sewage facilities contributing to the spread of antimicrobial resistance (AMR)? – A Swedish case study.

Ugolini Valentina[1], *Flores Quintana Harold*[1], *Subirats Medina Jessica*[2], *Löffler Paul*[1], *Ahrens Lutz*[1], *Ulinder Elin*[4], *Rapp Pia*[3], *Dunge Catarina*[3], *Lai Foon Yin*[1]

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Wastewater is a major reservoir of contaminants, both chemicals and antibiotic resistance genes (ARGs), that can lead to antimicrobial resistance (AMR). On-site sewage facilities (OSSFs) account for 15% of the wastewater treatment in Sweden, and are widely overlooked sources of spreading AMR. OSSF effluent can be discharged to groundwater, often used for drinking water production. This study aims to investigate the spread of antimicrobial contaminants from an OSSF to the groundwater, and the related risks for AMR development. Wastewater and groundwater samples were collected in four different months, and analyzed for antimicrobial chemicals, sewage markers and AMR-related genetic contaminants (i.e., ARGs, mobile genetic elements and integrons) using liquid chromatography coupled with tandem mass spectrometry and also high throughput qPCR. A nearby wastewater treatment plant (WWTP) and two drinking water treatment plants in the same area were also sampled for chemical analysis. Antimicrobial chemicals were less frequently found in the OSSF compared to the nearby WWTP, which could be due to less usage in the small population and its disconnection from hospitals' or manufacturing industries' sewage. Antimicrobial removal in OSSF was similar to the WWTP, but some chemicals, such as trimethoprim and ciprofloxacin, were removed less effectively in OSSF. Fluconazole showed negative removal in both systems, and occurred with the highest detection frequency in all water types. Most genes were considerably removed in the OSSF, although some (*e.g.*, IncP_oriT, sul1, intI1) were found at higher abundance after treatment. In downstream groundwater, the abundance of the tested ARG classes (e.g., sulfonamide, multidrugs) was significantly higher than upstream groundwater. Occurrence of antimicrobial contaminants was subjected to seasonal variation. Risk for resistance selection was determined. Our findings suggest that OSSFs contribute to AMR dissemination, highlighting the importance of monitoring these systems as potential AMR sources in the future.

Keywords: pharmaceuticals, antibiotics, groundwater, risk assessment, antibiotic resistance genes

Disturbance Intensity and Sub-Inhibitory Ciprofloxacin Concentrations Shape Diversity, Assembly Mechanisms, and Antibiotic Resistance in Aquatic Bacterial Communities.

Mia Kristine Staal Jensen[a,b], *Simon Bo Lassen*[a], *Daliang Ning*[c,d], *Bjarne W. Strobel*[a], *Jian-Qiang Su*[e], *Kristian Koefoed Brandt*[a,b]

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Environmental pollution with antibiotics can promote the spread of antibiotic-resistant bacteria and resistance genes (ARGs), even at low concentrations, but little is known about the impacts of toxicant-induced disturbance intensity on the relative importance of different bacterial community assembly processes. In a replicated (n = 6) lake water microcosm experiment, we investigated, how the antibiotic ciprofloxacin, at both sub-inhibitory and inhibitory concentrations, affects the diversity, assembly mechanisms, and antibiotic resistome of aquatic bacterial communities. Bacterial productivity (growth), community composition and the antibiotic resistome were studied by [3H]leucine incorporation, 16S rRNA gene amplicon sequencing, and high-throughput qPCR ARG chip, respectively. The relative importance of different bacterial community assembly processes is being investigated using iCAMP (Infer Community Assembly Mechanisms by Phylogenetic-bin-based null model analysis).

Bacterial growth, community composition and diversity were affected by ciprofloxacin in a dose-dependent manner. Significant reductions in species diversity were observed at ciprofloxacin concentrations of 15.6 µg/L and higher. Additionally, ciprofloxacin altered the bacterial community composition and the abundance and diversity of ARGs and mobile genetic elements (MGEs). Interestingly, contrary to expectations, the relative abundance of fluoroquinolone ARGs did not increase with higher ciprofloxacin concentrations, and the relative abundance of total ARGs actually decreased. However, specific ARGs associated with resistance to multidrug determinants and β-lactams increased in relative abundance even at 1.95 µg/L. At 62.5 µg/L, MGEs, including insertional encoding genes, which were highly correlated with Limnohabitans, increased significantly in relative abundance.

Our ongoing iCAMP analysis will provide deeper insights into how environmental disturbance intensity, such as varying concentrations of ciprofloxacin, can modulate the relative contributions of community assembly processes. In this abtract the presenting author is a PhD student.

Keywords: Antimicrobial Resistance (AMR), Fluoroquinolones, Community Assembly Processes, Sub-Inhibitory Concentrations.

Monitoring of antimicrobial resistance in the environment: what? why? and how?

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Understanding antimicrobial resistance (AMR) dynamics requires monitoring of resistant bacteria and resistance genes in all relevant settings. AMR monitoring has been implemented in clinical and veterinary settings, but comprehensive monitoring of AMR in the environment is almost completely lacking, aside from the use of sewage to understand AMR in the general population. Yet, the environmental dimension of AMR is critical for understanding the dissemination routes and selection of resistant microorganisms, as well as the human health risks related to environmental AMR. In this presentation, the most important outcomes of the EMBARK project (http://antimicrobialresistance.eu) will be presented. This project has established the 'normal' background levels of environmental AMR, defined high-risk environments for transmission, and developed methods to detect resistance genes that are not already circulating among pathogens. We have also compared different methods for monitoring environmental AMR, including selective culturing, qPCR and shotgun metagenomics. We have also identified priority targets for AMR monitoring. Finally, we propose a monitoring scheme that will be applicable in a modular fashion depending on the available resources. This framework will be useful for regulatory as well as research purposes, and is flexible to future technological advances.

Keywords: antibiotic resistance, surveillance, environmental monitoring, methodology

Effect of Emerging pollutants at environmentally relevant concentrations on the transference of antibiotic-resistant genes.

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Today, the rate at which new substances are released into the environment exceeds our capacity toassess and monitor them. This gap between production and assessment poses a significant threat, asthe impact of these substances on biological systems is underestimated. There is a growing concernrelated to the presence of these contaminants in the environment and the possible selectivepressure on microbial communities that could enhance the transference of Antibiotic-ResistantGenes (ARG) to cope with the stress. It is widely accepted that antimicrobials such as antibiotics anddisinfectants enhance horizontal gene transfer of ARGs. New studies, point out other EmergingPollutants (EPs) without apparent antimicrobial activity to be exerting this same effect.Consequently, the window of substances that could be enhancing antibiotic resistance disseminationshould be expanded and carefully assessed.The aim of this work was to establish a standard protocol to characterize the effect of EPs fromdifferent classes on the transfer of ARGs by namely conjugation. To this end, a semi-HTP platformwas employed to establish a controlled system for the study of the transference of a conjugativeplasmid between Escherichia coli strains. Two representative compounds from each class (i.e.antibiotics, disinfectants, pharmaceuticals, pesticides, personal care products and industrialcompounds) were selected and evaluated at environmentally relevant concentrations. In parallel, westudied the underlying molecular mechanisms (i.e. membrane permeability and SOS response) tounravel their implication in the enhanced plasmid transfer under the exposure to these pollutants.Furthermore, we repeated the study using water from two WWTP effluents, known as hotspots ofARGs, as inductors of conjugation to gain insight into the effect that the mixture of contaminantscould exert.

Keywords: Antibiotic Resistance, Conjugation, Emerging Pollutants

Keynote

Dr. Stéphane Pesce, *French National Institute for Agriculture, Food and Environment (INRAE), France*

Dr. Stéphane Pesce is Senior Researcher (Director of Research) and Group leader for the research group on Aquatic Microbial Ecotoxicology, at INRAE. His group studies the interactions between benthic microbial communities and the chemical contaminants to which they are exposed in aquatic ecosystems (trace elements, pesticides, pharmaceuticals, etc.). The research carried out combines microcosm experiments and in situ surveys to provide knowledge and develop innovative tools and approaches to 1/describe how micropollutants affect the vulnerability and resilience of benthic microbial biodiversity and associated ecological functions in a context of multiple stresses, and 2/ evaluate the ecological quality of contaminated aquatic ecosystems through the study of natural periphytic and sediment microbial communities. Stéphane Pesce is one of the co-founders of the EcotoxicoMic network, created in France in 2013.

Keynote conference - <u>Influence of coalescence on microbial responses to chemical exposure: towards a better consideration of the environmental continuum in microbial ecotoxicology</u>

Coastal chemical mixtures affect microzooplankton abundance and diversity, contrary to results from risk assessment

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Marine surface waters contain complex mixtures of chemicals that can adversely affect marine organisms such as zooplankton. The microzooplankton community consists of many protists, but there is limited data on the toxicities of single chemicals to species in this organism group, and even less on mixtures. A general limitation in chemical risk assessment is indeed the lack of ecotoxicity data for both individual chemicals and mixtures, and here we use two different methodologies to fill this gap. We tested the toxicity of three chemical mixtures of polar organic compounds from marine surface water to microzooplankton communities, using both a component-based approach and a whole-mixture approach. Using the component-based approach, we estimated cumulative toxic units for each mixture based on their single compound concentrations, together with observed hazard data for zooplankton. When data was missing, we supplemented with ECOSAR generated QSAR daphnid LC50s. This component-based approached suggested that none of the mixtures should be toxic to zooplankton at the respective measured environmental concentrations. The second methodology, whole-mixture approach, gave contrasting results. We found that mixture exposure reduced the number of ciliates and dinoflagellates, as well as the overall microzooplankton diversity at the respective measured environmental concentrations, and with indications of effects even below measured environmental concentrations. Our findings suggest that the contaminant mixtures present in Swedish marine surface water have the potential to adversely affect organisms with a central role in the marine pelagic food-web, which could have negative implications for water quality in the area.

Keywords: Mixtures, marine microzooplankton, component-based and whole-mixture approach.

Drying-rewetting stress on total and nitrifying communities in contaminated soils increase negative effects of contaminants

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Abstract : Microbial communities are primary drivers of biogeochemical processes that constitute vital soil functions, and thus are indicators of the status of soil functioning in response to anthropogenic disturbances. However, the response of microbial communities to compounded disturbances is of increasing interest given the multiple threats to soils. We hypothesized that microbial communities under continuous exposure to soil contaminants will be more affected when also confronted with a second stressor than undisturbed communities. To test this, we established outdoor soil mesocosms to assess the impact of separate Cu and polycyclic aromatic hydrocarbon (PAH) contamination gradients on total bacterial and nitrifying microbial communities in two soils with contrasting properties over a 16-month period. We then subjected contaminated and uncontaminated soils to an additional drying-rewetting stress in laboratory microcosms to test whether pollution-induced shifts in microbial communities altered their resistance and resilience in response to an unrelated disturbance, in this case osmotic stress caused by drying and rewetting. We found that Cu and PAH contamination decreased the diversity and changed the structure of total microbial communities, and that specific nitrifier guilds responded differently to each contaminant, with detectable negative effects of both Cu and PAH on ammonia oxidizing archaea and Nitrospira-type nitrite oxidizers and nitrification activity. The structure and diversity of total microbial communities in contaminated soils were less stable in response to drying-rewetting stress, although the legacy of Cu contamination was more pronounced. In contrast, nitrifier community abundances and activity responded differently to drying-rewetting stress across both soil and contamination types. These results underscore the context-dependent nature of microbial community responses to stressors as well the increased impact of climate change on microorganism in contaminated soils.

Keywords: soil contamination, multiple stress, drought, nitrification, microbial community

Microbial Existence under Chemical Persistence: Analysing the PFAS Impact

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Per- and poly-fluoroalkyl substances (PFAS) are a large family of synthetic chemicals with mounting evidence of adverse health effects in humans and animals. PFAS are present in many consumer and industrial products, including aqueous film-forming foams (AFFF) used to supress liquid fuel fires. Extensive use of AFFF has resulted in contamination at airports, defence bases, and numerous firefighting training areas worldwide. Due to their volatility, and solubility in water, many PFAS travel long distances from their original site, which has led to PFAS contamination in groundwater, sea spray, agricultural land, rainwater, and even household dust. Despite their environmental ubiquity, the impact of PFAS on microorganisms, especially fungi, remains relatively unknown. We aim to understand the impact of AFFF on microbial diversity and richness, as well as the effects of four common PFAS (PFOS, PFOA, PFHxS, and 6:2 FTS) on morphology, growth, and hyphal width in individual fungal species.

To assess diversity within the microbial community, we cultivated and identified fungi and bacteria growing in AFFF-impacted soils. Preliminary results suggest a high prevalence of fungal micro- organisms that are typically encountered in plant and human hosts. This discovery warrants further investigation of these contaminated soils using metagenomics to understand the impact of AFFF on microbial complexity (fungi, bacteria, and archaea) and richness over time (0-400 days).

Studies on individual fungal species found PFOA and 6:2 FTS reduced growth and altered hyphal thickness in Phialemonium spp. and Neocosmospora spp., respectively. Sulphonic acids such as PFOS and PFHxS induced the formation of ‘bubble-like’ structures in hyphae of Neocosmospora spp. These aberrant phenotypes will be investigated further using transmission electron microscopy and transcriptomics to determine the affected pathways.

Together, this research will increase our understanding of the impact of widespread PFAS contamination on microorganisms, providing essential insight into PFAS-induced micro-biosphere disruption to inform remediation strategies.

Keywords: PFAS, AFFF, fungi, microscopy, metagenomics

Impact of PFOS, PFOA, PFHxS and PFBS addition in 4 contrasted soils: impact on microbial communities and soil N/C cycles, PFAS dynamics

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Per- and polyfluoroalkyl substances (PFAS) have emerged as a significant class of pollutants due totheir broad environmental and health implications. PFAS have been used in a range of industrial andconsumer applications since the 1940s, leading to their widespread presence in the environment,including among others, water sources, soil, and even the human body. Increasing scientific andregulatory attention is being paid to the occurrence and fate of PFAS in drinking water, wastewater,and the environment in general. Particularly, PFAS-contaminated soil has become increasinglyreported in many parts of the world, and is now being recognized as a serious environmental hazard.When these chemicals are present in soil, they can infiltrate the groundwater, potentially leading todrinking water and food contamination. Consequently, there is a pressing need to develop, optimize,and implement remediation technologies specifically tailored to PFAS-contaminated soils. Thepresent study aims to present experiments on 4 different soils with different physico chemicalproperties, artificially contaminated by a mixture of 4 PFAS (PFOS, PFOA, PFHxS and PFBS) at aconcentration of 10 mg.kg-1each.

Each condition was set up with 3 replicates and 3 controls (without PFAS) per soil. The impact ofPFAS addition was monitored on carbon cycle (CO2 release trapped in NaOH), nitrogen cycle (Nextraction by K2SO4 0,5M (analysis by ion chromatography) and enzymatic activities. The abundanceof 16S rRNA and 18S rRNA genes was monitored during 70 days, and the evolution of bacterial,archaeal and fungal biodiversity over time was monitored by metabarcoding of the 16S rRNA andITS2 genes. The PFAS concentrations were also quantified at T0 and at the end of the microcosmsexperiment, highlighting minor changes. Results highlighted contrasted impact of PFAS addition onthe 4 studied soils, with an impact of physico chemical properties and contrasted impact on microbialcommunities.

Keywords: PFAS, soils, microbial ecotoxicology

Microbial ecotoxicology of conventional and bio-based polymers in soil

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Various applications in construction, agriculture or forestry deliberatelyintroduce plastics into the soil environment, such as geotextiles, mulch films, orantifeedants. To avoid long-term plastic residues in the environment, alternativeproducts made from bio-based materials are being developed. Microbialecotoxicology is a key discipline for validating the effects of chemicals on the variousfunctions microorganisms perform in ecosystems. In this work, the potential impact ofthe above-mentioned polymers on soil microorganisms and soil nutrient cycles areexamined, comparing conventional products with bio-based ones.

This study aims to investigate potential impacts of the polymers on soil functions,potential differences between the effects of conventional and bio-based products,and microbial activity directly on the polymer surface compared to the surroundingsoil.

In order to assess this, field soil was incubated in microcosms with 0.4 % (w/w) ofeach polymer for 16 weeks at room temperature. At week 1, 2, 4, 8 and 16, flaskswere removed for sampling: First, soil microbial biomass was estimated via soilrespiration by measuring the emitted CO2 via GC-TCD. The pH of the soilsurrounding the polymers was measured, as was the activity of the soil enzymes β-D-glucosidase, cellobiosidase and acid phosphatase. Possible divergent activities onthe polymer surface were investigated by directly measuring enzymatic activities onthe polymer surface at final sampling.

Our results provide a comparison between the effects of conventional and bio-basedpolymers on soil functions in terms of pH and enzymatic activities. The emergence ofa so-called plastisphere on non-biodegradable polymers as a newly evolved micro-ecosystem is researched, accompanied by the abundance of polymer-degradingenzymes and differences in microbial activity between polymer surface and surrounding soil.

As the regulatory risk assessment of polymers is under development in theEuropean Union, our results represent a further step towards the sustainable use ofpolymers.

Keywords: Geotextiles; Polymers; Soil; Risk Assessment

Influence of plastic substrata on the plastisphere's tolerance to freshwater pollutants assessed by Pollution-Induced Community Tolerance (PICT)

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The growing pollution of all ecosystems by plastics is a major concern for both environmental andhuman health. Most of plastic waste accumulates in the environment and represents new substratesfor microbial communities that can develop on them as biofilms to form the ‘Plastisphere’. Despite agrowing scientific interest for these new microbial ecosystems there is still a lack of knowledge ofplastics effects on microbial communities and by extent on ecosystem functioning, especially infreshwaters. Previous studies showed effects of plastics on the composition of microbial communitiesand changes on their metabolic activities, notably regarding to the xenobiotic degradation pathways.A hypothesis to explain these changes could be the selection of xenobiotic-tolerant microorganismson plastics linked to the wide variety of chemical compounds present in these materials. To test thishypothesis, a Pollution Induced Community Tolerance (PICT) approach has been conducted onmicrobial communities developed naturally in a freshwater pond on four of the most produced plasticmaterials (PE - polyethylene, PP -polypropylene, PVC – polyvinylchloride, PS - polystyrene) and a glasscontrol material. The microbial communities were exposed to growing concentrations of fourfrequently found pollutants: a trace metal (copper), an herbicide (terbuthylazine), a non-steroidal anti-inflammatory drug (diclofenac) and a beta-blocker (atenolol). Communities’ tolerance to thesecontaminants was evaluated by measuring photosynthetic and beta-glucosidase activities after a four-hour acute exposure. Tolerance levels were then quantified by the EC50 values. Results showed aninfluence of the substrate on heterotrophic activities of biofilms both before and after exposure tocopper. Contrary to our hypothesis, microbial communities on plastics were more sensitive to copperthan the glass-associated ones, except for the PVC substrate. In contrast, the activity of phototrophiccommunities, as well as their capacity to tolerate the tested pollutants, were not influenced by thesubstrate.

Keywords: microbial ecotoxicology, adaptation, toxic stress, functional ecology, PICT (‘pollutioninduced community tolerance’)

Multi-compartment impact of micropollutants and particularly antibiotics on bacterial communities using environmental DNA at river basin-level

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Bacterial communities respond to environmental conditions with diverse structural and functional changes depending on their compartment (water, biofilm or sediment), type of environmental stress, and type of pollution to which they are exposed. In this study, we combined amplicon sequencing of bacterial 16S rRNA genes from water, biofilm, and sediment samples collected in the anthropogenically impacted River Aconcagua basin (Central Chile, South America), in order to evaluate whether micropollutants alter bacterial community structure and functioning based on the type and degree of chemical pollution. Furthermore, we evaluated the potential of bacterial communities from differently polluted sites to degrade contaminants. Our results show a lower diversity at sites impacted by agriculture and urban areas, featuring high loads of micropollution with pesticides, pharmaceuticals and personal care products as well as industrial chemicals. Nutrients, antibiotic stress, and micropollutant loads explain most of the variability in the sediment and biofilm bacterial community, showing a significant increase of bacterial groups known for their capabilities to degrade various organic pollutants, such as Nitrospira and also selecting for taxa known for antibiotic resistance such as Exiguobacterium and Planomicrobium. Moreover, potential ecological functions linked to the biodegradation of toxic chemicals at the basin level revealed significant reductions in ecosystem-related services in sites affected by agriculture and wastewater treatment plant (WWTP) discharges across all investigated environmental compartments. Finally, we suggest transitioning from simple concentration-based assessments of environmental pollution to more meaningful toxic pressure values in order to comprehensively evaluate the role of micropollutants at the ecological (biodiversity) level.

Keywords: bacterial communities, microbial ecotoxicology, trace-organic pollutants, antibiotic pressure

Effect of pesticides on soil microbial communities: Scenario with variable predator-prey interactions

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The adverse effects of pesticides on the environment have been a major concern for years. Nevertheless, traditional ecotoxicological assessments focus on macro-organisms and little is known about the effects of these compounds on soil microbial communities and microbial predator-prey interactions. Non-target microorganisms in the soil food web such as protists (micropredators) may be affected by pesticides, with potential cascading consequences for soil microbial diversity, composition, and functions. We assessed the effect of two pesticides at their recommended application doses on soil bacterial communities exposed to variable protist diversity. Quintuplicate microcosms were assembled in gamma-sterilized soils with increasing predator diversity: i. bacteria only (i.e. bacterial extract from non-sterilized soil), ii. bacteria + selected flagellate bacterivorous protists and iii. bacteria + protists with a higher diversity of traits. Samples were taken on days 0, 7 and 21 after pesticide addition. The abundance and composition of the bacterial community on each microcosm were assessed by qPCR and Illumina 16S rRNA gene sequencing. Our results showed that the soil bacterial community composition changed gradually according to protist diversity. Dominant bacteria were more prone to decrease in abundance due to protist presence. Pesticides had a lower influence on the differentiation of the bacterial community than the protist diversity. Nevertheless, we demonstrated that the effects of pesticides on the soil bacterial community are highly dependent on the protist diversity. These results unravel key aspects of the microbial response to pesticide exposure in soil, paving the path for understanding their effect on complex communities with predator-prey interactions.

Keywords: pesticides, trophic interactions, protists, bacteria, soil

Ecological warnings: pesticides biodegradation could be a problem!

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Historically, we have explored the biodegradation processes of environmental contaminants from a remediation perspective. The case of DDT, with its high persistence and bioaccumulation risks, has been pivotal in our vision of environmental risk assessment. However, does low environmental persistence due to rapid biodegradation always reduce environmental risk?

Recently, we leveraged the impact of glyphosate, the most widely used pesticide globally, on aquatic ecosystems to challenge this notion. A large series of ecological-scale studies spanning controlled laboratory conditions, outdoor mesocosms utilizing consolidated microbial communities, and field determinations; reveal that glyphosate's biodegradation pathway is the primary driver of its ecosystem impact. Glyphosate introduces phosphorus into aquatic systems, leading to eutrophication and, via a differential biodegradation process involving pico-cyanobacteria, alters the microbial and functional ecosystem structure. The combination of differential biodegradation and phosphorus input impacts the freshwater ecosystem, being evident in the long term within Argentina's shallow-lakes in agricultural landscapes as a turbid-organic status.

The example of glyphosate demonstrates that biodegradation is a selective process. It impacts microbial structure, leading to a significant ecological transformation from clear to organic-turbid systems. Organic contaminant biodegradation, such as pesticides, is an ecologically impactful process, requiring a broader ecological perspective. Incorporating a deeper ecological understanding is crucial for microbial ecotoxicology. The risk assessment of pesticides should integral ecological analyses including both: toxicity and degradation process.

Keywords: glyphosate; biodegradation; ecological impacts

Keynote

Prof. Elena Gorokhova, *Dept of Environmental Science, Stockholm University, Sweden*

Her research program focuses on the stress and adaptations in aquatic organisms and on the development of methods to measure it and understand the ecological and evolutionary mechanisms involved. We combine elements of molecular ecology, physiology, and informatics with long-term monitoring in the Baltic Sea to understand the wide range of environmental stressors in the environment. Through ecotoxicology and modeling, we also aim to understand the adverse impacts on the environmental status and develop indicators to measure these impacts.

Keynote conference - Microbiome of Ecotoxicity Assays

Exposure to copper-based nanopesticide shape the gut microbiota and induce physiological alterations in model organisms: Insight from the Nanopestis project

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Repeated application of Copper (Cu)-based pesticides in agriculture is the main source of Cu pollution in agricultural soils, contributing to contamination of aquatic ecosystems and human exposure. Given its environmental and health impacts, the European Commission (EC) recommended to reduce Cu use. Nanopesticides based on Cu nanoparticles constitute an alternative to conventional pesticides and are already used in conventional and organic agriculture outside Europe. Their nanometric size facilitates systemic passage for greater biocidal effects than conventional forms leading toto reduce Cu application rates. Thus, these "nano" formulations are likely to soon reach the European market given the need to reduce the use of Cu. Through a one health approach, the aim of the project is to assess the nanospecific risk to agrosystems and aquatic ecosystems, as well as to animal and human health linked to the dissemination of Cu nanoparticles. A multi-model approach was applied, using representative biological models for aquatic ecosystems and humans (e.g. chironomids and mice respectively). The impact of exposure to a commercial Cu(OH2)-based nanopesticides was evaluated on organisms physiology and microbiota, in comparison to a non- nanosized formulation. In chironomids, exposures to commercial formulations are associated to differential microbiota remodeling during larval stages both leading to alteration of adult traits, through a decreased adult emergence rate which can impair population dynamics. In mice, in vivo and in vitro experiments were performed to monitor the effects on the gut barrier homeostasis, including gut microbiota, and on colitis sensitivity using a microbiota-dependent colitis model. Data shown that long-term exposure to nanoformulation through the diet aggravates colitis in a sex-dependent manner, possibly due to microbiota structural shift toward pro-inflammatory dysbiosis profile. These data indicate that biocidal properties of Cu and nano-Cu are able to alter the gut microbiota structure of organisms from different biota, with deleterious effects on population dynamic and disease severity.

Keywords: Microbiota, nanopesticide, nanotoxicology

Invasive and native lake fish species display different response of their gut microbiota and metabolite compositions along a eutrophication gradient

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Because they occupy higher trophic levels, fish are important markers of freshwater ecosystem health, and cyanobacterial and algal blooms associated with eutrophication are the most common stress to which they are exposed. Meanwhile recent lab studies confirm that exposure to cyanobacteria has the potential to modify gut microbiota composition and holobionts functions, confirmative field studies are still lacking. In this study, specimens from the native perch (Perca fluviatilis) and the invasive pumpkinseed (Lepomis gibbosus) were collected from six lakes along a eutrophication gradient (mesotrophic to hypereutrophic) located in ~70km radius around Paris, France. Fish gut-associated bacterial community compositions were investigated by 16S rRNA gene sequencing, and variations in holobionts metabolism were evaluated using non-targeted metabolomics. Results indicate that gut communities from the two species were distinct and varied according to eutrophication level. Gut community as well as metabolite compositions were more variable among lakes in the perch compared to pumpkinseed, with the former more influenced by chlorophyll levels (reflecting the influence of phototrophs abundance). At higher chlorophyll levels, the gut of perch sheltered a higher proportion of bacterial lineages originating from water compared to that of the pumpkinseed, suggesting a less resistant microbiota. This study thus confirms that high chlorophyll levels typically associated with higher eutrophication have a major impact on fish gut community and holobiont metabolites compositions in natural populations. However, the response is species-specific, with invasive L. gibbosus microbiota and metabolism being stable over a broader range of conditions compared to native P. fluviatilis. Ability to tolerate and acclimatize to a broader range of environmental conditions could represent a competitive advantage in the context of increasing bloom frequencies and intensities worldwide. Increased resistance of gut microbiota and functions could thus be one yet-underestimated asset allowing some invasive species to outcompete native ones.

Keywords: Symbiosis, invasive species, resistance

Assessing eco-exposome impact along juvenile fish development in artificial port reefs through population kinetics, metabolome and microbiome studies

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Ports have been subject to ecological restorations to compensate for the decline in fishery resources by the installation of artificial habitats used as nurseries by coastal fishes, which are recruited at the larval stage in coastal areas and migrate offshore only when adults. But harbour activities disturb water quality through chemical and biological pollutants released from antifouling paints and runoff. To study the impact of the harbour eco-exposome along coastal fish development, a holistic interdisciplinary approach was used. We performed estimations of mortality through monthly individual fish counts. We performed metabolomics, microbiote resistance genes quantification and estimated trace metals elements (TME) concentrations in a coastal fish recruited from February to July. We also monitored TME in the water column through automatic samplers equipped with membranes.

Fish counts results corroborated the positive contribution of harbours to recruitment, being here almost 5 times higher than neighbouring natural areas. Even though differences in metabolome signatures between sites became pronounced from spring, copper resistance signatures were equally present in the microbiome of fishes and in the water column along their development in both, port and natural zones. These results are not consistent with a higher significant concentration of copper and most other metals in fishes from the port zone, excepted zinc and cadmium. Antimicrobials resistances were however present in both areas, but much more abundant in the port zone only in free-living bacteria. The water exposome resulted in all TME being more abundant in the port than in the natural area. Our results demonstrate that port artificial reefs can provide a solution to fisheries decline, but further complementary studies of both the exposome and the health status of fish growing in harbours must be performed to better conclude on their real benefits.

Keywords: Coastal fishes, *Diplodus vulgaris*, metabolome and microbiome signatures, ports as nurseries, metal exposome

Keynote

Prof. Hans Christian Bruun Hansen, *University of Copenhagen, Denmark*

The overall theme of the research group (4 professors, 2 lab techs, 7 PhD students, 2 post-docs) is soil and water biogeochemistry with focus on pollutant fate processes at the molecular level. This insight feeds into research and innovation on sustainable pollutant remediation technologies, resource recycling, risk assessment, soil and water cleaning, and modelling. Since 2017 research on plant-produced toxins/biologicals (NaToxAq), biochar-catalysed pollutant degradation and remediation, iron oxide redox dynamics and ecosystem functions, phosphorus release and capture, organic pollutant sorption and advanced oxidation/reduction, and heavy metal immobilization have made up more than 80 % of our research activities. Our research on natural toxins taps in with research on new crops as part of the foodshift paradigm as well as exploration and fate of plant derived biopesticides such as saponins. The research group teaches 7 courses in soil, water, and environmental chemistry from BSc to MSc level.

Keynote conference - <u>Natural toxins from plants: which are of concern ?</u>

Effects of temperature and salinity on the growth and toxin production of the bloom-forming cyanobacteria *Nodularia spumigena*

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Cyanobacterial harmful algal blooms (cyanoHABs) are increasing globally in response to climate change and anthropogenic eutrophication, and often produce potent toxins hazardous to human and ecosystem health. Given the diversity and regional variability of cyanoHABs, information on how this diverse group of microorganisms will respond to current and future climate scenarios is still limited. The Baltic Sea and the interconnected regional brackish and freshwater systems have been plagued by extensive cyanobacterial blooms in recent years. Among the cyanobacterial species forming these blooms, *Nodularia spumigena* is particularly notorious for producing the hepatotoxin nodularin, among others natural toxins. Here, we tested the effects of temperature (18°C, 25°C, and 29°C) and salinity (2 PSU and 8 PSU) on the physiological and metabolic responses of *N. spumigena* KAC13 in laboratory, specifically on cell growth and toxin production of known and uncharacterized secondary metabolites. Results indicated that *N. spumigena* can grow across a wide range of salinity and temperature, with salinity having a more significant impact on its physiological and metabolic responses. Optimal growth was observed at 25°C and 8 PSU, common conditions in the Baltic Sea during summer blooms, whereas the lowest growth occurred at 29°C and 2 PSU. LCMS results indicated that while the production of the nodularin R was consistent across all treatments, the production of other toxins varied, including anabaenopeptin 841, spumigin D, and two uncharacterized compounds. Remarkably, anabaenopeptin NZ825 was only produced at 2 PSU at all temperature treatments. These findings suggest that *N. spumigena* may prioritize growth over toxin production under favorable conditions, implying that future blooms in the Baltic Sea could result in higher biomasses without a proportional increase in toxin release. This highlights the need to adapt monitoring strategies to effectively manage the ecological and public health risks associated with cyanoHABs in the Baltic Sea.

Keywords: climate change, cyanoHABs, cyanotoxins, *Nodularia spumigena*, secondary metabolites

Insights into the chemistry of the microalgal toxins from the 2019 *Chrysochromulina leadbeateri* Blooms in Northern Norwegian fjords

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During the summer of 2019 numerous fjords in the northwestern part of Norway experienced a huge harmful algal bloom (HAB) by the microalgae *Chrysochromulina leadbeateri*, which lead to massive fish kills of more than 14.000 tonnes of farmed salmons. Motivated by the fact that no algal toxins have previously been described from *C. leadbeateri*, we set to investigate the chemical nature and toxicity of secondary metabolites of strains isolated from the 2019 bloom, as well as one older strain isolated during a smaller Norwegian bloom in 1991. Initial HPLC-DAD-MS/MS based metabolomics analysis of the crude methanolic extracts and supernatant of cultivated the strains (10 L) showed that their profiles of small organic molecules where very similar, indicating that similar similar toxin(s) have been the causative agents of the two HAB events. Next, dried organic extracts of the three cultures were separated based on low-pressure C18 based chromatography into 5 crude fractions, that were tested for toxicity in an Rtgill-W1 cell line. Only one fraction turned out to be highly toxic in a 0,1% concentration, why this fraction was further fractionated into 15 fractions using semi-preparative chromatography again leading to only one highly toxic fraction. Notably, again only one fraction turned out to be highly active in the Rtgill-W1 cell line, narrowing the major likely ichtyotoxin, detected as the mono charged ion [M+H]+ at m/z 1399.8306, down to a single compound with the formula C67H127ClO27, as well as several minor analouges. We note that the elemental composition, including 4 double bond equivalents, is highly similar to those of sterolysins such as karlotoxins that are known ichtyotoxins from several Karlodinium species. Intrigingly, the same suite of karlotoxin-like molecules could also be detected from the bloom sample further supporting that these compounds are indeed the causative ichtyotoxins.

Keywords: harmful algal blooms, ichtyotoxins, fish farming, sterolysins

Sensitivity of wheat-associated bacteria to mycotoxins produced by *Fusarium spp*.

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Fusarium head blight (FHB) is a damaging disease of cereal crops worldwide caused by a complex of pathogenic *Fusarium* species. This disease causes reductions not only in grain yield but also in grain quality due to the production of mycotoxins by the pathogen during the infection. The studies about mycotoxin toxicity mostly focused on animals, foods, humans and plants despite the widespread distribution of these compounds in different compartments of the agroecosystem (crops, water, manure, sewage sludge) as well as in freshwater. Only a few studies reported the toxicity of mycotoxins to other biological models in aquatic environments and nothing is known regarding the toxicity of mycotoxins to the wheat microbiota. In this context, the main objective of this study is to characterize the sensitivity of wheat-associated bacteria to mycotoxins produced by *Fusarium spp*. A selection of bacteria described as being associated to wheat will be exposed in vitro to deoxynivalenol (DON), zearalenone (ZEA) and to enniatins (ENNs), three groups of mycotoxins that are frequently detected on European wheat. These bacteria are expected to be exposed to mycotoxins in the context of FHB, are representative of different plant compartments (rhizosphere, phyllosphere, spicosphere, etc.) and are representative of the phylogenetic and functional diversity of wheat-associated bacteria. The toxicity values obtained in our study will help building toxicity models for the plant microbiota and the expected consequences for the host. The results of our study will also help to further characterize the interactions between bacterial candidates (*e.g.* the most sensitive and tolerant strains to mycotoxins), the plant and Fusarium by using transcriptomics and/or metabolomics. We also see our study as the first step of the construction of a mycotoxin toxicity database that will contain all toxicity data for wheat-associated bacteria and that will be extended to other compartments of the environment.

Keywords: plant microbiota; secondary metabolites; bioassays; microbial toxicity

Keynote

Prof. Thomas Backhaus, *RWTH-Aachen University, Germany*

He is the chair of Ecotoxicology and Environmental Risk Management. Thomas Backhaus's main research interest is the toxicology and ecotoxicology of chemical mixtures and its regulatory assessment. In this context, he has been working with several regulatory authorities in Europe and elsewhere. Additional research interests include the environmental assessment of emerging pollutants (e.g. pharmaceuticals, veterinary drugs, biocides, nanoparticles) and the substitution of hazardous chemicals. He is currently co-leading the risk characterization work of the OECD project on combined exposures and OSPARs intersessional communication group on cumulative stressors.

Keynote conference - Microbial Ecotoxicology: A Key to Environmental Protection in the Spirit of the Non-Toxic Environment and the Sustainable Development Goals

Prediction biases in Quantitative Structure-Activity Relationship (QSAR) models

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The total number of chemicals on the world market continues to increase, and toxicity testing is struggling to keep up. An important element of chemical risk assessment is trustworthy estimations of toxicity to green algae, daphnids and fish. In silico tools, such as ecotoxicological quantitative structure-activity relationship (QSAR) models, are being used to fill data gaps when empirical data is missing, but to what extent can we trust these predictions? Three freely available ecotoxicity QSAR platforms (ECOSAR, Vega and T.E.S.T.) have been tested against empirical data from the United States Environmental Protection Agency (USEPA) ECOTOX database, to determine model predictivity for both acute and chronic toxicity. The empirical database was filtered based on OECD guidelines, and for every chemical with data for at least one endpoint, QSAR predictions were generated. In cases where multiple models from within each platform were available, the geometric mean was used to summarize predictions, and, similarly, when multiple empirical data was available, the geometric mean was used to summarize the empirical measurement. Predictivity for each platform was assessed with coverage (percent successful predictions across the list of chemicals), median absolute error and large deviations (percent predictions deviating from empirical data by more than a factor 10). Additionally, CLP-classification thresholds were used to examine predictivity for regulatory toxicity ranges, to find out if models are more or less accurate for different classes. Results indicate some differences in platform predictivity among the tested endpoints, and, more importantly, a problematic trend when stratifying with CLP-classes – toxicity of the most toxic compounds is underestimated. These issues will be explored, using microalgae as an example.

Keywords: modelling, QSAR, computational ecotoxicology, chemical risk assessment

Electrochemical Aptasensors in Action: Rapid Detection of Potential Environmental Threats

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Rapid and accurate detection of waterborne contaminants is increasingly essential for safeguarding public health and environmental sustainability. Traditional detection methods often involve time-consuming sample preparation and expensive instrumentation, rendering them impractical for real-time monitoring in field settings. To address these challenges, we developed electrochemical aptasensors as a promising solution due to their high sensitivity, selectivity, and rapid response capabilities, allowing for the detection of contaminants at trace levels within minutes. Our approach leverages tailored aptamers that undergo conformational changes upon interaction with whole cells of target bacteria. We developed an electrochemical aptasensor utilizing a bespoke aptamer for the swift detection of Aphanizomenon sp. ULC602, a filamentous cyanobacterium found in freshwater. This aptasensor demonstrated exceptional sensitivity and specificity, achieving rapid detection with a detection limit of OD750 ~0.3. Additionally, we created another aptasensor for the swift diagnosis of Pseudomonas aeruginosa, with a detection limit of 8 CFU/ml. Our findings highlight the potential of electrochemical aptasensors as efficient tools for real-time monitoring of waterborne contaminants, ensuring timely and effective responses to potential public health threats.

Keywords: Aptamer, Biosensor, Cyanobacteria, Pseudomonas aeruginosa, Electrochemical

Single species in vitro bioassays, using soil nitrifiers as bioindicators, as a first-tier tool for assessing the toxicity of pesticides on soil microorganisms

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Single species tests using representative organisms of different trophic levels constitute a cornerstone of aquatic and terrestrial ecotoxicology and a major tool for assessing the ecotoxicity of pesticides. However, no corresponding assays are available for soil microorganisms due to the lack of identified microbial indicators, and standardized in vitro assays. Recent studies have indicated that ammonia-oxidizing microbes (AOM), which perform the first and rate-limiting step of nitrification, are ideal microbial indicators of the effects of abiotic stressors on the soil microbial community due to their key functional role, their sensitivity to xenobiotic compounds, and the availability of established tools to measure their activity and abundance. We aimed to develop and standardize a single species in vitro testing system as a Tier I ecotoxicity assay for soil nitrifiers recognized as potential indicators of pesticide toxicity on soil microorganisms. Specifically, relative toxicity thresholds (EC50) were determined via monitoring nitrite production or consumption in liquid cultures by ammonia-oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA), and nitrite- oxidizing bacteria (NOB), representing globally distributed lineages found in soil. All tested pesticides affected at least one nitrifier, with different strains exhibiting contrasting levels of sensitivity. For AOB, pesticides were most toxic for Nitrosomonas ureae and Nitrosospira briensis, while Nitrosotalea sinensis was the most sensitive AOA strain. NOB strains were less affected by pesticides than their ammonia-oxidizing counterparts, with Ca. Nitrobacter laanbroekii being the most sensitive. Fungicides and insecticides were more toxic to AOM than NOB, whereas herbicides had a wide range of toxicity on all tested groups of nitrifiers. Our study introduces a new testing tool for the assessment of the ecotoxicity of pesticides on soil microorganisms that could be readily implemented as a standard tier I type assay in environmental risk analysis.

Keywords: ammonia-oxidizing microorganisms, nitrite-oxidizing bacteria, risk assessment, singe species in vitro tests, pesticides

ACKNOWLEDGEMENTS This work is part of the projects:

- “ARISTO -The European Industry – Academia Network for RevIsing and Advancing the Assessment of the Soil Microbial TOxicity of Pesticides, an MSCA-ITN-EID-H2020 project which is funded by the European Commission under the Grant Agreement No.956496 and support E. Bachtsevani.
- “ReASSESS–is funded by the Hellenic Foundation for Research and Innovation (HFRI) and the General Secretariat for Research and Innovation (GSRI) and support M. Kolovou.

A systematic approach for a holistic ecotoxicological assessment strategy of polymers from the laboratory to field scale

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To enhance crop growing conditions and a secure yield, commercial agricultural practice resorts to e.g. seed coatings or mulch films. Those products often contain polymers favoured by their physico- chemical properties. However, the substance group is not regulated except for intentionally added microplastics, which were recently banned from the European market. Evaluation concepts for polymers, e.g. PRR-Concept (Polymers Requiring Registration), do not comprehensively include ecotoxicity as indicator for environmental concern. As a result, the environmental behaviour of polymers remains unknown. The study aims to develop a systematic ecotoxicological hazard assessment including terrestrial and aquatic toxicity screening tests for (bio)polymers. As main driver of terrestrial ecosystem services our focus was on effects on functional and molecular biological levels as indicator for polymer driven ecotoxicity on soil microorganisms. To determine the impact on soil function, sensitivity of nitrogen transformation test (OECD 216), usually used in environmental risk assessments, is compared to the alternative endpoints potential nitrification (ISO 15685) and extracellular enzymatic activities (ISO 20130), combined with molecular biological analysis using ARISA-PCR and qPCR (targets: amoA, amoB). By this, we aim to connect soil function with microbial diversity and specific gene expression potential. The tests will be performed with different types of polymers, i.e. biopolymers (e.g. chitosan), synthetically modified polymers (e.g. carboxymethylcellulose), bioplastics (polylactic acid) and microplastics (polyethylene, polycaprolactam). To evaluate ecotoxicological relevance of lab data, lysimeter studies are conducted under environmental conditions to gather corresponding data. First results are available for modified polymers and biopolymers. Comparing test derived NOECs (No Observed Effect Concentration), nitrogen transformation and enzymatic activity inhibition appears to be substance specific inhibited. Potential ammonia oxidation was adversely affected by most substances tested at lower test concentrations. Molecular biological testing and first lysimeter tests are currently carried out. An overview of the results will be presented at the conference.

Keywords: screening; polymers; functional ecotoxicity; molecular biological analysis

Contaminant bioaccumulation and diatom community in biofilms are relevant bioindicators of coastal Mediterranean seawater quality

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The bioaccumulation potential of contaminants from the marine environment in biofilms was investigated and compared with caged mussels for a wide range of both organic and metallic contaminants and considering a large geographical area. Marine biofilms were sampled after three months of sub-surface immersion at 49 locations along the 1800 km of the French Mediterranean coasts. 37 chemical elements (including Cr, Mn, Co, Ni, Cu, Zn, As, Cd, Hg and Pb) and 57 organic compounds (i.e., 18 polycyclic aromatic hydrocarbons (PAHs), 8 dioxin-like and 6 non-dioxin-like polychlorinated biphenyls (PCBs) and 25 organochlorine pesticides (OCPs)) were quantified, showing different multi-contaminated profiles in the different sites along the coast. Moreover, a remarkable correlation between concentrations in both biological matrices was observed for PAHs and PCBs, whereas OCP and metal bioaccumulation varied depending on each compound.

Corresponding biofilm diatom communities determined by a metabarcoding approach (rbcL sequences affiliated using the diatbarcode database) were primarily shaped by major environmental parameters, ie temperature and salinity, leading to the definition of three ecoregions. Inside these, dbRDA showed that cocktails of pollutants clearly shaped diatom communities and selected tolerant taxa. These results highlighted the potential of biofilms as relevant bioindicators of the marine multicontamination.

Assessing the risks of agricultural run-off to microphytobenthic communities in small streams

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Small streams and shallow ditches are wide spread components of agricultural landscapes and frequently exposed to agricultural mixtures of fertilizers and pesticides. Despite their importance as habitat for a variety of organisms and key ecosystem functions like primary production or the retention and transformation of pollutants, they are not included in regular monitoring programs like the water framework directive. There is a lack of data on toxic effects of agricultural stressors, both, from an effect-based perspective as well as from field surveys of microbial communities.

This contribution outlines a 'multiple-line of evidence approach' that combines different assessment strategies for phytotoxicity applied in a German-wide 'small-stream monitoring program'. Effect- based monitoring was performed using a diagnostic high-throughput microalgal assay that assessed the phytotoxic potential of >100 SPE-samples taken during rain events and showed good correlations with toxic units of site-specific pesticide profiles. Benthic diatom assemblages from 65 sampling sites were characterized using a metabarcoding approach revealing a total of 4008 ASVs (identified as 208 species). Multivariate statistics based on ASVs distinguished clusters for sites characterized by high toxicity or nutrients. We conclude (a) that agricultural run-off poses a high risk to autotrophic microbial communities in small streams and (b) that diatom communities in agricultural landscapes reflect the effects of pesticide and/or nutrient exposure. The multiple-line of evidence approach will be discussed in terms of the applicability for monitoring to assess impacts from agricultural run-off on small streams.

Keywords: effect-based monitoring, metabarcoding, multiple agricultural stressors

Characterizing the potential effects of a complex textile industry contamination on structure, function and nutritional quality of phototrophic biofilms: the case study of the Cleurie River.

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The Cleurie River (France) is a forested headwater stream contaminated by industrial activities (textiles). The cocktail of contaminants is characterized by high concentrations of glyphosate and AMPA as well as a high load of dissolved organic matter dominated by optical brighteners. This type of complex contamination, close to environmental quality standards, chronic and episodic, is usually poorly reflected by the bioindicators currently implemented within the European Water Framework Directive. This apparent contradiction feeds an environmental controversy involving end-users of the river, stakeholders and industrialists,

In this context, we investigated the effects of such contamination on phototrophic biofilms, a pivotal ecological player in this ecosystem. The objectives were (1) to evaluate the structure and function of phototrophic biofilms in situ, along the pollution gradient, (2) to establish causal relationship between the main contaminants of La Cleurie and biofilms structure and function in controlled experiment and (3) to estimate indirect effects of this contamination by evaluating its impact on the quality of the biofilm as a food resource for consumers.

To do so, biofilms were collected during a seasonal in-situ monitoring in the Cleurie river and several controlled lab-experiments. Glyphosate was found to have little effects on photosynthesis or heterotrophic activities of biofilms after 4 weeks of exposure to 0.1 to 150 µg L -1, nevertheless the microbial metabolome was modified in response to exposure. Further experiments highlighted the strong decrease in green algae cell density in phototrophic biofilms exposed to environmental concentrations of an optical brightener for 4 weeks. Exposure to those contaminants also led to changes in lipid profiles of biofilms potentially altering its nutritional quality. Therefore, those biofilms were provided as a food resource to a model consumer (Gammarus fossarum) whose life history traits were estimated (survival, growth) to estimate indirect effects of those contaminants on the food chain.

These results will be further used within the framework of a broader interdisciplinary approach aiming at studying the circulation of scientific knowledge between the different actors (researchers, end-users, stakeholders…) involved in this controversy.

Keywords : pesticides, glyphosate, periphyton, trophic chain

Cobalt effects on the diversity, functions, and co-occurrence patterns of prokaryotic and eukaryotic communities in growing river biofilms.

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Cobalt (Co) is a key metal in the energy transition, which has led to an increase of its extraction and use over recent decades. Nevertheless, its impact on aquatic ecosystems is poorly understood. Biofilms are important biological components and indicators for river health. The present study thus examined Co impacts on the structure, interactions and functioning of prokaryotic and eukaryotic communities in growing biofilms.

Artificial substrata were placed in mesocosms (TotalEnergies facilities, Lacq, France) without (control) and with Co addition (0.1, 0.5 and 1 µM). These substrata were then colonized by biofilms for 28 days. After the exposure period, a further 35 days without Co contamination was applied to simulate a recovery period. Water and biofilm were collected every 7 days during Co exposure. Water quality was analyzed, and biofilms were examined for Co bioaccumulation, structure and functions.

Cobalt bioaccumulation in growing biofilms was correlated with ambient Co concentrations. Exposure at 0.5 and 1 µM Co impacted beta-diversity of both prokaryotic and microeukaryotic communities from the earliest stages of biofilms colonization. Proportions of major prokaryotic and microeukaryotic taxa varied, highlighting sensitive and resistant taxa in both populations. Functional predictions revealed that Co exposure also influenced processes of biofilm formation (primary production and cellular processes). Finally, co-occurrence analyses indicated a reorganization of networks structure and dynamics in response to Co contamination. A loss of connectivity within microbial communities from exposed biofilms was highlighted and associated with a decreasing number and diversity of keystone OTUs. However, networks were denser with a concentration of interactions around the last central nodes, which were essential in the stress response and continued ecological function of the biofilms. Our results highlighted a new approach to assess Co impacts on biofilms considering co-occurrence patterns between microorganisms, in addition to analyses of taxonomic or functional profiles.

Keywords: bioindication; microbial ecotoxicology; metal; metabarcoding; microbial networks

Keynote

Prof. Hans Peter Heinrich Arp, *Norwegian University of Science and Technology & Norwegian Geotechnical Institute, Norway*

His research interests intersect fundamental and applied aspects related to pollutants in the environment. On the fundamental level, he is interested in how different types of pollutants, such as microplastics, PFAS, PAHs, POPs and metals behave in the environment. On the applied level, he uses this fundamental knowledge to design solutions within the context of emerging technologies and policy mechanisms, such as REACH and the circular economy, to create a zero pollution society.

Keynote conference - **A persistent problem: identifying and confronting persistent, mobile and toxic (PMT) substances,**

Impact of Seasonal Pesticide Exposure on Periphyton Structure and Nutritional Quality in Agricultural Streams

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Periphyton in agricultural streams are exposed to a wide array of pesticides throughout growing season of crops, potentially impacting their structure and nutritional quality as a food source for aquatic consumers. This study aimed to assess the temporal variations of pesticide concentration accumulated in periphyton as well as algal assemblages and food quality of the periphyton in response to pesticide exposure over the course of one growing season. Field experiments were conducted in two agricultural streams and one reference stream in southern Sweden (Skåne) in summer 2022. Artificial substrata for periphyton colonization (tiles) were deployed in each stream and let grow on site for three months. Water and periphyton samples were taken once a month. Analysis included residue of 109 pesticides, in surface water and in periphyton, 37 fatty acids as food quality indicator, ash-free dry mass, and 11 photosynthetic pigments as proxy of algal assemblages in periphyton.

Pesticide residues were detected in a mixture of 31 pesticides in periphyton, with a mean maximum concentration of 899 ng/g dry weight pyroxsulam (adjusted for the organic content of the periphyton). Mean herbicide concentrations were higher than those of fungicides and insecticides. Significant temporal and spatial variations in pesticide concentrations and occurrences were observed, indicating dynamic changes in pesticide exposure and accumulation in periphyton throughout the study period. Distinct pigment profiles were observed among different sites and over time. Further statistical analysis will explore correlations between pesticide accumulation in periphyton, periphyton metrics, and fatty acid profiles to better link the periphyton responses to pesticide pollution. These findings highlight the complex interplay between pesticide exposure and response of periphyton in aquatic ecosystems.

Keywords: Periphyton, pesticide exposure, fatty acids, algal assemblages

Effect of hydrological variability on the capacity of biofilms to dissipate pesticides

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The DECODRY project focuses on the effect of hydrological variability in rivers, as a consequence of climate change, anthropogenic activities and/or natural processes, and its repercussion on the ecosystem functions provided by aquatic biofilms. More specifically, the project examined the effects of hydrological variability history on the ability of biofilms to mitigate pesticide pollution in rivers. With this aim, a series of experiments using stream flumes enabled the re-creation of biofilms having undergone different histories of droughts and floods varying in frequency and duration, that were further tested for their capacity to dissipate a cocktail composed by herbicide (terbuthylazine, glyphosate, AMPA), fungicide (tebuconazole) and insecticide (imidaclopride) molecules. Hydrological variability effects on the structure (biomass, thickness, EPS content) and activity (respiration, community-level physiological profile) of biofilms were analyzed and correlated to the capacity to dissipate and bioaccumulate pesticides. The highest pesticide dissipation rates were measured in biofilms having experienced short and frequent hydrological disturbances (either drought or flood events), showing high microbial areal densities despite of their low thickness, compared to those having experienced more spaced events of longer duration or no flow variations (i. e. controls). In both drought and flood experiments, a higher bioaccumulation of hydrophobic (tebuconazole and terbuthylazine) than hydrophilic (glyphosate, imidaclopride) molecules were observed. Pesticide dissipation and bioaccumulation differences between biofilms having experienced different hydrological histories were confirmed both in open-flow (using stream flumes) and batch (using incubation flasks) experimental approaches. The results from DECODRY are relevant to understand how hydrological variability can modify the self- depuration capacity of rivers for pesticides, and for implementing strategies to optimize this natural process.

Keywords: pesticide cocktail, dissipation, bioaccumulation, biofilm, droughts, floods

Surfactants in glyphosate-based herbicides have a concentration-dependent impact on glyphosate biodegraders.

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Glyphosate-based herbicides (GBHs) are among the most used agricultural herbicides world-wide and continue to be so despite increasing evidence for their toxicity. Glyphosate itself is considered biodegradable in soil environments and both the degradation of pure glyphosate and its effect on the soil microbiome are well-characterized. However, studies on GBHs that tease apart the effects of their individual components, such as surfactants, on glyphosate degradation and microbial community structure are lacking. Here, we show that one surfactant commonly found in GBHs, pelargonic acid (PA), has a concentration-dependent effect on the growth and glyphosate degradation of individual glyphosate-degrading strains. At concentrations of 10-50 mg L-1, PA was found to promote growth of Ochrobactrum pituitosum and Achromobacter insolitus in media containing glyphosate as a sole source of phosphate. Under the same conditions, higher PA concentrations (100-1000 mg L-1) resulted in retardation or complete inhibition of growth. In aerobic soil microcosms exposed to either pure glyphosate or a GBH at concentrations corresponding to the recommended field application rate (i.e., 15 mg/kg) or a local hotspot (i.e., 150 mg/kg), near complete degradation of glyphosate and production of its degradation by-product AMPA occurred within 14 days. Notably, the initial rate of glyphosate degradation was slightly faster when provided in the form of a GBH. These findings underline the importance of considering the combined as well as individual effects of GBH components when assessing their frequent use in agriculture. Furthermore, since PA is not only added into GBHs but is also the main ingredient of the currently used organic alternatives to GBHs, it is especially important to take its concentration-dependent effects into account during application and when discussing its environmental applications.

Keywords: glyphosate, glyphosate-based herbicides, pelargonic acid, biodegradation

Carrier material for bacterial biofilms formation: production of biocomposites to inoculate and treat pesticides contaminated soils via bioaugmentation

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Soil inoculation with active microorganisms is a key step for the success of bioaugmentation for on- site restoration of contaminated soils. Introducing the desired microorganisms as biofilm can favor their activity and survival once in soil. A key parameter for biofilm growth is the choice of the carrier material. In the frame of pesticides-contaminated agricultural soils bioremediation using bioaugmentation, the activity, viability and biodiversity of a bacterial MCPA- and 2,4-D-degrading consortium grown on various carrier materials was studied. For this, three natural materials (zeolite, pozzolana, oyster shell) as raw or modified (surface covering with Ca/fe-Layered Double Hydroxides (LDH)) carrier materials were used to support the development of the degrading consortium. Such an LDH composition was chosen as, if validated as non-toxic to bacteria, it will allow the introduction of nutrients into its structure to improve the bioaugmentation process. All carrier materials, modified or not, allowed the growth of a pesticide-degrading biofilm whose biodiversity mainly depended on the carrier material rather than on surface modification, even if the presence of LDH on zeolite increased (Pseudomonas) or decreased (Azohydromonas, Sphingopyxis) the proportion of some bacterial strains. LDH was non-toxic at the concentration (10 g L-1) used for carrier material surface modification, and lead to MCPA adsorption. All biocomposites, i.e. biofilm grown on a carrier material, had a MCPA- and 2,4-D-degrading activity, whatever the carrier material or LDH presence/absence. Pesticide biodegradation was rapid (DT50 < 1.5 days with initial [MCPA]=5 mg L-1) and mineralization (radiorespirometry experiments with labelled 2,4-D) was observed. Several MCPA-degrading strains (Pseudomonas, Rhodococcus, Cupriavidus, Variovorax, Ralstonia, Aminobacter) were isolated from the zeolite-biocomposite. In conclusion, biocomposites made from selected carrier materials and consortia with the desired biodiversity and degradation activity could improve soil bioaugmentation approach. Modification of the surface of the carrier material could be a mean to further improve this biotechnology.

Keywords: Bioaugmentation, pesticide biodegradation, biofilm, carrier material, surface modification

Exploring microbial PFAS degradation in soils from two AFFF contaminated sites

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Per- and polyfluoroalkyl substances (PFAS) are anthropogenic persistent organic pollutants that have adverse health and environmental effects. Although PFAS are commonly referred to as “forever chemicals”, there is growing evidence for microbial degradation of PFAS in laboratory settings, indicating that this approach has the potential for development into a cost-efficient in-situ remediation technique. The aim of this research project is to improve the knowledge on biodegradation of PFAS. This includes identifying (i) the microbes involved in the process, (ii) the degradation pathways, and (iii) the degradation products formed. For this, soil-cores were drilled into PFAS contamination plumes at two former firefighting training sites, and samples collected at different depth of the core. Depending on the sample position relative to the groundwater level, enrichment cultures were set up either in two different aerobic media, Tryptic soy broth and Raymond medium, or in two anaerobic media, Basal media and A6 medium. A total of 230 enrichment cultures were spiked with perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), 6:2 fluorotelomer sulfonic acid (6:2FTSA) and perfluorooctane sulfonaminde (FOSA), whereafter PFAS degradation was screened by measuring the fluoride concentration using an Ion Selective Electrode (ISE). After 3 months, aliquots of the 60 cultures showing an increased fluoride concentration were transferred into new vials containing the same media but with either 20 ppm 6:2 FTSA and FOSA or 20 ppm of PFOS and PFOA. An increase in fluoride was observed in several cultures. Next steps involve PFAS analysis to confirm degradation, DNA sequencing and enrichment or isolation of the most successful strains.

Keywords: PFAS, bioremediation, biodegradation, enrichment cultures

Exploring the degradation pathway and metabolites toxicity of a promising Chlorpyrifos-degrading strain, *Priestia aryabhattai* 1-3I, isolated from a local phosphogypsum landfill

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Chlorpyrifos (CP), an organophosphorus pesticide extensively employed in the agricultural sector, poses substantial risks to the environmental biosystems and human health when overused. In particular, microbial remediation has emerged as a focal point in research for addressing CP-induced environmental contamination. The current study emphasizes the potential of the microbial community within phosphogypsum landfills to identify promising microorganisms involved in CP biodegradation. In this study, we isolated 26 bacterial strains from CP-enriched phosphogypsum sample contaminated with 100 mg Kg-1 CP and subsequently identified them through 16S rRNA sequencing. Among these isolates, *Priestia aryabhattai* 1-3I displayed remarkable proficiency in utilizing CP as a sole carbon source. Furthermore, P. aryabhattai 1-3I was found to harbor an oph-encoding gene, a crucial component in the CP degradation pathway, with a highly conserved 694 bp region shared by at least 24 homologous oph bacterial genes. The CP-degrading ability of *P. aryabhattai* 1-3I was assessed both in liquid medium and soil samples, achieving degradation rates of 95% and 60%, respectively, starting from an initial concentration of 100 mg L-1 CP after 4 weeks. This pronounced CP-degrading activity correlated with a rapid and significant increase in oph transcripts and was accompanied by a notable rise in the accumulation of a major protein band with a molecular weight of 39 kDa, consistent with the molecular weight of previously characterized oph proteins. Of particular interest, the toxicity of CP degradation products resulting from both CP-bacterial biodegradation experiments exhibited minimal effects on neural cells, as indicated by acetylcholinesterase (AChE) activity and cell viability, underscoring the safety of the CP degradation pathway. Our study underscores the exceptional biological capabilities of the phosphogypsum microbial community and provides a promising example of harnessing their potential for CP biodegradation.

Keywords: Chlorpyrifos, phosphogypsum, *Priestia aryabhattai* 1-3I, biodegradation and acetylcholinesterase (AChE) activity

Biodegradation of several organic UV filters by an in-house synthetic consortium

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Chemical UV filters are widely used in a wide variety of personal care products (PCPs), especially sunscreen creams. There are currently 28 different organic UV filters allowed in cosmetic products (EU 2021). These compounds have varying chemical properties and only few of them have been shown to be readily biodegradable. Some organic UV filters have, however, been shown to degrade in microcosm experiments with wastewater treatment plant. Interestingly, degrading enrichment cultures by multiple serial transfers have proven an efficient strategy for the isolation of specific bacterial strains capable of degrading several organic UV filters. This resulted in a synthetic consortium of 28 bacterial strains capable of degrading 5 different UV filters. 19 of the strains could degrade at least one UV filter alone. This in-house synthetic consortium was more efficient in UV filter degradation than several commercial consortia. Nevertheless, many organic UV filters were not degraded by any of the microbial consortia. This recalcitrance is most probably due to their size and lack of bioavailability.

Keywords: bacterial consortium; UV-filters; biodegradation

Microbial role in the fate of “biodegradable” plastics in the marine environment

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The “biodegradable” polymers have been proposed as one of the solutions to face plastic pollution. Nevertheless, biodegradability standard tests have been widely criticized by scientists for their lack of representativeness of natural conditions. Here, we provide feedbacks from years of experience on the influence of experimental conditions on tests carried out for the marine environment, with particular emphasis on the choice of microbial inoculum (concentration and origin) and test medium (seawater or synthetic medium). Experimental conditions were miniaturized to enable a multiplication of the modalities and parameters analyzed. Biodegradability tests were carried out for various biodegradable and conventional polymers by measuring bacterial diversity (16S rRNA sequencing), activity (O2 consumption, CO2 production and heterotrophic bacterial activity) and the presence of oligomers (1H NMR) for several months. Our results support the use of mature biofilms grown in natural seawater as test inoculum, rather than the use of free bacteria in seawater. In addition, we encourage the use of a synthetic medium enriched with nutrients rather than natural seawater, particularly for oligotrophic conditions (offshore seawater). Finally, the analysis of microbial diversity underlines the limits of the representativeness of these tests, which reflect potential biodegradability and whose applications should be relativized according to variations in the conditions of the natural environment.

Keywords: Plastic pollution, contaminant fate, biodegradability, biofilm

Assessment of hydrocarbon degradation, microbiome diversity and enrichment of diesel degraders from oil contaminated soil for bioremediation purposes

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A large number of industrial sites are contaminated by hydrocarbons, mainly due to negligence leading to the release of petroleum or oil to surface soils. As these hydrocarbons are toxic to humans and the environment, there is an urgent need for developing remediation solutions. Generally, when soils contaminated with excessive levels of total hydrocarbons, the excavation and conventional physical or chemical treatment are applied, which often disrupt the environment. We are therefore seeking to increase the use of bioremediation processes that represent environmentally friendly and sustainable approaches for environmental clean-up, however, they are still rarely used in situ. The European MIBIREM project (https://www.mibirem.eu/) aims to characterize soil microbiomes that are effective in degrading three typical types of contaminants, i.e. petroleum hydrocarbons, cyanide and hexachlorocyclohexane for in situ bio-augmentation for remediation of soil pollution. The present study focuses on the petroleum hydrocarbon use case. The objective of our study is first to identify soil microbiome exhibiting a strong hydrocarbons degradation capacity, next enrich the potential microbiome, identify the associated taxa, and finally isolate them.

Here we leveraged a contaminated soil collected from Ploufragan, France for a three-month degradation test, during which the microbial activity (mineralization), biodegradation of hydrocarbons and bacterial diversity were monitored. In parallel, two complementary approaches (liquid medium-based and soil BacTraps-based) were implemented to enrich the bacteria capable of degrading hydrocarbons by screening them using diesel oil as sole carbon source. The diversity of these microbiomes was characterized by 16S rDNA metabarcoding. In addition, known functional genes (alkB and CYP 153A P450) that encode the enzymes involved in alkane biodegradation were quantified through quantitative PCR. Finally, high-throughput isolation was carried out on the most promising samples to isolate the functionally active strains for further taxonomic and genomic characterization.

Keywords: petroleum hydrocarbon, microbiome, diversity, enrichment, isolates

Microbial leaching of metals: a nature based solution for recovering critical metals from mine waste

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Exploiting primary resources is becoming more and more crucial due to the constant growth in global demand for raw materials, resulting in the production of large quantities of waste from mining activities. Innovative approaches consider mining waste as secondary raw materials in a circular economy concept. As part of the REVIVING project, which aims to apply bioleaching processes to waste from a tungsten mine in Portugal in a bioengineering process, the aim is to transpose the small-scale optimized acid mine drainage biological process to medium and large scales via manipulation of the waste's indigenous microbiome. We tested the application of isolated bacterial strains in 20g batches, as well as nutrient biostimulation of the waste's indigenous microbiome (eg ferro-oxidant and sulfo- oxidant micro-organisms), and their bioaugmentation, through culture in a specific medium. Unconvincing, the bacterial monoculture approach was abandoned, whereas the 2 last approaches were very efficient for bioleaching metals of interest (Cu, Mn, Mg, Zn, W and Mo). For the sake of simplicity and efficiency, and because of important cells' filtration through the waste, biostimulation was selected for the process upscaling (x100) in 2Kg waste columns subject to controlled operating conditions (liquid and air flow) with a monitoring including water saturation, pH, O2, geochemistry, microbial community abundance and structure. Our tests validated the efficiency of the metal bioleaching process, compared with abiotic conditions. A second scale-up (x100) of the process was efficiently initiated in a 200Kg pilot set-up at the Portuguese-mine, and is still in progress after 4- months of operation using the same biostimulation process and monitoring. A biophysical-chemical process is currently being developed for the recovery of leached critical metals. The whole process should meet circular economy objectives by valorizing mining waste both through the secondary production of critical metals and of metal-free mineral material for potential civil engineering applications.

Keywords: bioleaching, critical metals, bacteria, mining wastes, Eco-engineering, column, field pilot.

Communication abstracts

Posters

QMRA model to assess the human exposure to ESBL E. coli from poultry production through different pathways

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Antimicrobial resistance (AMR) is a significant public health threat, exacerbated by environmental pathways that facilitate the spread of resistant pathogens such as Extended-spectrum beta-lactamase (ESBL) producing E. coli from agricultural activities. Within the framework of the ENVIRE project, our research focuses on the complex dynamics of ESBL-producing E. coli transmission through environmental vectors originating from poultry manure. Manure from poultry production is an exceptional nutrient source for plants, having high mineral content. However, the manure may also be a source of contamination, and if originating from flocks positive for resistant bacteria, such as ESBL E. coli, it could potentially spread pathogens or resistance genes.

Utilizing a Quantitative Microbial Risk Assessment (QMRA) model approach, we aim to identify and quantify the pathways that could lead to human exposure to these bacteria, including surface water, soil, and groundwater. A special focus will be placed on water contamination, exploring how manure-contaminated stream water may lead to human exposure, for example, through recreational swimming or the use of contaminated water for agricultural purposes.

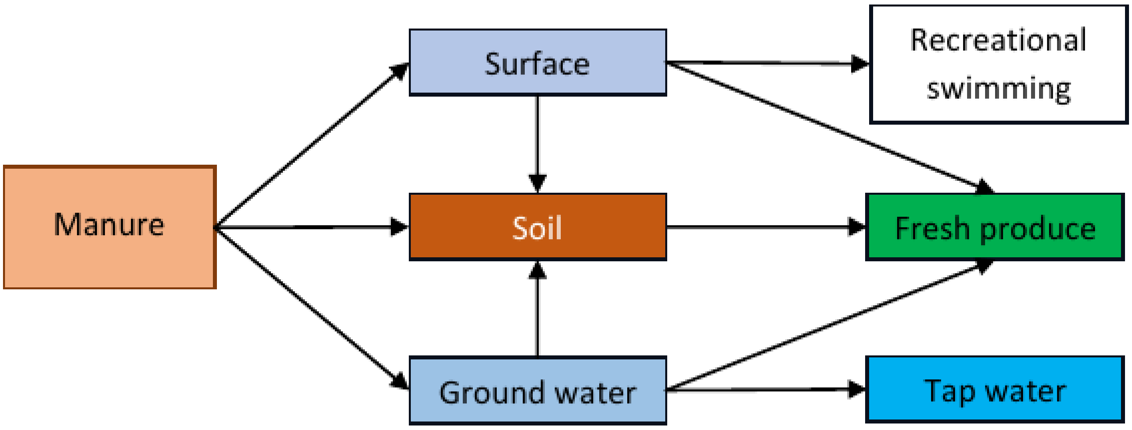

Figure 1. Transmission pathways

Our model not only prioritizes these pathways (see Figure 1.) based on their risk contribution but also explores low-frequency, high-risk scenarios to assess the impact of less common but potentially more severe exposure events. The findings aim to fill critical knowledge gaps in the environmental transmission of AMR, serving as a foundation for further research and guiding targeted interventions. This research is pivotal for informing targeted interventions and regulatory measures to mitigate the spread of AMR in the environment, ultimately reducing public health risks. Future work will refine pathway assessments and explore the effectiveness of specific mitigation strategies to minimize human exposure to resistant E. coli from poultry production.

Funding: This work is part of the JPIAMR-ACTION and funded by the French National Research Agency (Support Code: ANR-21-AAMR-0003-03) and the German Federal Ministry of Research and Education (Support Code: 01KI2202A).

Keywords: Antimicrobial resistance, poultry production, environmental AMR, water contamination, human exposure

Assessment of heavy metals resistance bacteria isolated from fishes with different habitats: case of Sardina pilchardus and Mullus barbatus collected from Bouharoune region, Algeria

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Pollution and anthropogenic inputs lead to maintenance and dissemination of bacterial resistance to antimicrobial agents in different compartments such as sediment and biota.The aim of this study was the evaluation of heavy metals resistance in the total flora of fishes from different ecological habitats : a pelagic fish, Sardina pilchardus (S) and demersal fish, Mullus barbatus (M). The results showed low to medium heavy metals resistance rates for the total flora: Pb (S: 7.89, 19, 0.45% and M: 0.36, 0.46, 2.3%), Cu (S: 0.34, 36, 0.3% and M : 0.63, 1.5, 2.9%), Zn (S: 2.89, 1, 0.22% and M: 5.83, 2.6, 3.6%), a very low levels for Cd (S: 0.026, 1, 0% and M: 0, 0.035, 0%), Hg (S: 0.033, 0, 5.2% and M: 0, 1, 0%) and total absence of resistance to Chromium in the total flora of the two fish. The statistical analysis of these results revealed a non-significant difference ($p>0.05$) between the results obtained for the two fish and showed that the habitat does not influence the state of resistance to heavy metals. Twenty five strains were identified, they have been assigned to the following species: Serratia odorifera (n=9), Pseudomonas aeruginosa (n=4), Pseudomonas luteola (n=3), Raoultella ornithinoytica(n=2), Staphylococcus aureus (n=2), Vibrio vulnificus (n=1), Leclercia adecarboxylata (n=1), Citrobacter koseri (n=1), Proteus mirabilis (n=1), Kluyvera spp (n=1). Analysis of heavy metal resistance profiles revealed resistance of 3 to 5 metals for the majority of strains as well as resistance associated with the main families of antibiotics (Betalactams, quinolones,aminosides and rifampicin). We can conclude that these resistant bacteria can cause significant ecological and health risks, especially since most of them have an associated resistance to antibiotics and can reach humans via the food chain.

Keywords: heavy metals-resistance, antibiotics-resistance, bacteria, Sardina pilchardus, Mullus Barbatus.

WaterParc - Wastewater treatment with an oxidizing agent: efficiency on Pathogens and antimicrobial resistance

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Pathogens and antimicrobial resistance (AMR) in wastewater and effluents of wastewater treatment plants (WWTP) represent a global health and environmental challenge that demands urgent attention. AMR has emerged as a leading cause of global mortality. According to a World Health Organization (WHO) report, antimicrobial-resistant pathogens are the primary health threat, causing over 700,000 annual deaths. Alarming projections anticipate 10 million deaths by 2050. Given the pressing consequences of AMR, numerous disinfection processes have been explored in WWTP to mitigate the release of ARB and ARGs in surface waters, including chlorination, peracetic acid, and UV disinfection, ozonation, and membrane bioreactors. However, in real-scale WWTPs, the reduction in AMR is generally limited, typically achieving 0 to 3-log reduction in ARGs. In some cases, an increase in the availability of genetic material, including plasmids and ARGs, has even been noted in treated water. The continued discharge of untreated or inadequately treated wastewater containing AMR and AMR-acquired pathogens contributes to the spread of infections and the proliferation of antimicrobial resistance, posing significant risks to human health and ecosystem integrity. There is therefore an urgent need to improve treatment strategies and implement comprehensive monitoring programs in order to preserve water quality and mitigate the risks associated with wastewater discharges.

The WaterParc project aims to tackle these challenges by assessing the effectiveness of a novel disinfection method utilizing performic acid (PFA, HCO3H). This emerging technology has garnered increasing attention in wastewater disinfection due to its potent oxidizing properties, cost- effectiveness and greater environmental compatibility. Our findings demonstrate higher PFA- concentration and longer contact time result in increased efficiency in controlling microbiomes and antimicrobial resistance genes. These results underscore PFA's effectiveness in reducing microbial and antimicrobial resistance gene levels, even with minimal concentrations and short contact times, thus demonstrating its suitability for widespread application in WWTPs.

Keywords: antimicrobial resistance (AMR), disinfection, performic acid

The Air Plasmidome: A Discreet Route for pathogens and the Transmission of Antibiotic Resistance Genes?

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Since the COVID-19 pandemic started, preventing airborne microorganism transmission has become everyone's concern. Viruses, bacteria, fungal spores … are airborne biological particles referring as bioaerosols. Through bacteria, a panel of genes (e.g., resistance or metabolic genes) might be found in bioaerosols and disseminated. However, genes found on plasmids are potentially more exposed to the risk of transmissibility than those harbored by chromosomes due to horizontal gene transfer (HGT). From 2422 metagenomes, among the microorganisms identified as present in air, bacteria, mainly Proteobacteria, were the most abundant with pathogens representing between 0.2 to 35.3% of the bacterial community per country; the highest relative proportions of pathogens were observed in the United States and Europe. We identified 674,495 representative plasmids. A few of them were present in plasmid databases excepted 16, some associated with pathogenic bacteria. Numerous genes such as mob, rep or mpf involved in HGT were detected. Antibiotic resistance genes represented <1% of predicted plasmid genes and were mainly efflux pump genes. The US and China were the most prevalent locations for these genes. A recent analysis presents strong evidence that increasing levels of air pollution are associated with increased risk of antibiotic resistance. The plasmid similarity was significantly linked to the location and to the microbial community ($P < 0.001$). Based on CRISPR detection, we determined that plasmids of bacteria living in built environments and soils were overrepresented.

Keywords: plasmidome, antibiotic resistance gene, metagenome, bioaerosol, resistome

The Impact of Pesticides on the Transmission of Antibiotic Resistance

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The widespread application of pesticides is a critical component of modern farming practices. While the direct effects of these chemicals on human health are extensively studied, their potential role in facilitating or decreasing the spread of antibiotic resistance remains poorly understood. Prior findings within our group (Palm & Fransson et al., 2022) indicate that copper, one of the most commonly used pesticides in organic farming, notably decreases the conjugation efficiency of an IncF plasmid in E.coli. We now wish to evaluate the impact of various pesticides on the transfer rates of antibiotic resistance plasmids. We investigated the effect of different chemical classes of pesticides on bacterial growth, including glyphosate (organophosphorus), dicamba (benzoic acid), 2,4-D (synthetic auxin), atrazine (triazine) and fenvalerate (synthetic pyrethroid). We found these compounds to have little to no effect on the growth of our reference E. coli strain. We are conducting quantitative mating assays to assess the potential differing impacts on conjugation efficiency of these pesticides. Results from these initial studies will guide further research into the mechanisms by which pesticides affect bacterial conjugation. Ultimately, we hope to contribute to a deeper understanding of the impact of agricultural practices on the spread of antibiotic resistance, and conjugation as a whole.

References: Palm M, Fransson A, Hultén J, et al. The Effect of Heavy Metals on Conjugation Efficiency of an F-Plasmid in Escherichia coli. Antibiotics (Basel). 2022;11(8):1123. Published 2022 Aug 19. doi:10.3390/antibiotics11081123

Keywords: pesticides, conjugation, E.coli

Coastal Cocktail: Pollution & Resistance Rise, the case of Toulon Bay

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The various sources of anthropogenic contamination in coastal areas impact water quality and pose a threat to biodiversity, ecosystem functioning and, potentially, the spread of pathogens. Increased multi-contamination generates selective pressure on biota, often correlated with the enrichment of metal resistance genes (MRG) and antibiotic resistance genes (ARG) - part of the resistome - in the local microbiome. Within biofilms, the polymeric matrix produced by the diverse community of microorganisms adhering to surfaces confers them an ability to tolerate environmental variations, and possibly bioaccumulate metals and antibiotics. In a study at the Toulon Bay, we tested whether the pollution gradient leads to an increase in the relative abundance of resistome-related genes in bacterial communities from marine biofilms. DNA was extracted from biofilms at three sites and shotgun metagenomic sequencing enabled targeted analysis for the description of MRG and ARG present (in comparison to the BacMet and SARG databases, respectively) on top of taxonomic analysis. The classification of the unassembled reads with MetaPhlAn indicated Flavobacteriia as the most abundant class at all sites averaging 42% of total abundance; and Chao1 index indicated a higher α-diversity at the least and most contaminated sites. No taxonomic diversity gradient was observed along the contamination in the bay as shown previously by metabarcoding. For metals, genes predicted to encode resistance to mercury and copper were the most abundant; while for antibiotics, most ARGs were classified as in the aminoglycoside family. Furthermore, around 2% of contigs for all sites (N50 of 961 bp) were identified as belonging to MRGs and ARGs. However, contigs matching both databases were mostly found for the Lazaret site, where a possible presence of cross-resistance mechanisms for metals and antibiotics resistance should be investigated.

Keywords: marine biofilms, metagenomics, resistomes, coastal pollution

Effect of Copper on Bacterial Conjugation and Antibiotic Resistance

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Antibiotic resistance is a growing concern in healthcare due to the increasing ability of bacteria to withstand treatments that were once effective. This resistance spreads rapidly through bacterial populations, often facilitated by a process called conjugation, where genetic material, including resistance genes, is transferred between bacteria. Inhibition of conjugation has been suggested as an approach to reduce the spread of antibiotic resistance. Further it’s known that heavy metals can co- select for antibiotic resistance but the effects of heavy metals themselves on conjugation has been in debate. In this work we investigate the effects of five heavy metals (Arsenic, Cadmium, Copper, Manganese, and Zinc) on the transfer of the conjugative F-plasmid in Escherichia coli. Copper had the biggest effect with a reduction of the transfer of the F-plasmid by approximately 100-fold. It was found that addition of copper did not affect transcription of the major PY promoter which regulates transcription of the major tra operon, including the conjugative machinery. It was also found that copper needed to be present during the mating event to have a severe impact on the transfer efficiency. Using copper chelators, we tried to determine if extracellular or intracellular copper could be causing the observed decrease in conjugation, and we now speculate that copper interact with the outer membrane to disrupt transfer of the F-plasmid. Further, we have examined a large number of natural E. coli strains to determine overlap between copper and antibiotic resistance and have performed genome-wide association studies to identify genes which are important for these resistances. We will next be looking at their association with conjugative elements.

Keywords: antibiotic resistance, heavy metals, copper, bacterial conjugation

Comparison of biogeochemical pollutant fluxes between combined sewer overflows and along spatial and temporal gradients in a Mediterranean river: how indicators could help monitor urban environments

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During precipitation events, soil surfaces are washed, river sediments are remobilized, and urban systems are drained. In urban areas, these "first flushes" carry materials collected by the sewage network to wastewater treatment plants. Although there is evidence that first flushes are major contributors of contaminants to surface waters, it was only during a thorough study of physical-bio- geochemical parameters that we were able to unambiguously attribute to combined sewer overflows (CSO) the highest levels of biogeochemical pollution mixtures ever recorded in a typically Mediterranean coastal river, constantly monitored otherwise. To demonstrate the repeatability of these results, we monitored during 5-years 24/24 hours during several days and sampled at strategical moments five CSOs. We calculated river fluxes of pollutants, seldom reported in the literature, of nutrients, metallic trace elements, pharmaceutical and pesticide present molecules among 330 monitored, faecal bacteria, calculated through cultural methods, all major families of antibiotic resistant genes, estimated using quantitative PCR. We also studied bacterial and archaeal through metabarcoding and performed an analysis of pathogen relative abundances.

During CSOs, fluxes of all biogeochemical pollutants encountered exceeded by several orders of magnitude the average levels observed in this same study at the river-to-sea continuum, during precipitations and during drought times. We argue CSOs contribution to pollution released into surface waters is underestimated, particularly in Mediterranean weather regions, as they occur more often than elsewhere. Several biogeochemical pollution indicators were derived from our study, which could be integrated into in situ detection tools to help decide which CSOs contribute most to pollution and should be closed to mitigate their threat to freshwater and coastal ecosystems and human health.

Keywords: Urban sewer systems monitoring, wastewater exposome, antibiotic resistance, pathogens, biogeochemical indicators

Exploring the Plastisphere as an arena of Microbial Evolution for New Catabolic and Antibiotic Resistance Traits

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+ equal contribution

Soils serve as a major reservoir for organic pollutants and plastics, whether released intentionally or unintentionally during agricultural practices. Plastics provide a micro-surface that can be effectively colonized by soil microorganisms, forming a new niche known as the plastisphere. Due to their hydrophobic nature, plastics are also expected to interact with pesticides and other organic pollutants present in agricultural soils. We hypothesize that the plastisphere acts as a hotspot where pesticides and antibiotics are adsorbed and directly interact with soil microbiota, leading to the rapid evolution of novel catabolic traits or antibiotic resistance genes (ARGs) compared to the bulk soil. To test this hypothesis, a soil microcosm experiment was conducted using two different types of plastics: a conventional plastic (LDPE) and a biodegradable plastic (PBAT).Plastic macro-fragments were implanted in the soil which was repeatedly treated, at monthly intervals, with biodegradable pesticides (iprodione, oxamyl) and antibiotics (sulfamethoxazole, tetracycline) with different hydrophobicity. At the first, third, and fifth cycle of organic pollutant application, bulk soil and plastisphere samples were collected. The former were used for measuring the degradation of the organic compounds to verify or not the establishment of an acclimated microbiota towards they enhanced biodegradation. In addition, plastisphere and bulk soil samples were used for DNA extraction and downstream (a) amplicon sequencing (b) q-PCR analysis for cehA (oxamyl-degrading gene), ipaH (iprodione-degrading gene), sul (sulfamethoxazole ARG) and tetA (tetracycline ARG) to verify the evolution of catabolic traits. The results on the dissipation of organic pollutants indicated an acceleration of iprodione dissipation in the LDPE-amended soil compared to a retardation in the PBAT-amended soil. Furthermore, there was a slight enhancement in dissipation of sulfamethoxazole, particularly in the LDPE- amended soil, though no significant difference was observed between the two types of plastics. qPCR analyses is in progress and the results will be presented at the conference.

Acknowledgements: This work is performed in the frame of the project MINAGRIS (Grant Agreement number: 101000407) funded by the European Union’s Horizon 2020 Programme for research & innovation.

Keywords: Plastisphere, ARGs, biodegradation, microbial evolution

Investigating the Role of Plastics in Transmitting Antimicrobial Resistance and Pathogens from Wastewater to the Marine Environment

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Antimicrobial resistance (AMR) is a major global health threat and recently, spread through environmental routes have been recognized. Plastics have been suggested to act as a hotspot for the development and spread of AMR. Plastics and other anthropogenic pollutants are discharged into the marine environment through waste streams such as wastewater. However, there is limited knowledge concerning the occurrence of pathogens and antibiotic resistant bacteria (ARB) in urban wastewater (WW) and in marine environments into which they are discharged. Moreover, the role of plastics in spreading pathogens, antibiotic-resistant bacteria (ARB), and antibiotic-resistance genes (ARG) in the marine environment is not well understood.

In the PlastiSpread project, a combination of lab- and field biofilm model systems enables investigation of plastic-associated biofilms and their potential to act as vector for transmission of AMR and pathogens from wastewater throughout the marine environment. The influence of WW composition and polymer type on the bacterial community composition, including the occurrence of pathogens, ARBs and ARGs will be studied. Biofilm will be formed on plastic materials (PS, PE, PVC) and natural materials (wood, glass). The laboratory experiments will be performed in Norway and Greece, which differ in antibiotic consumption rates. Transfer of resistance genes from wastewater bacteria to susceptible biofilm bacteria, and the effect of selective pressures from antibiotics and potentially toxic metals on this process, will also be investigated. In the field model system, materials will be placed at sites close to wastewater outfalls and compared to a non-exposed reference site. Marine bivalve molluscs will be used as indicators for exploring effects on marine organisms present at the same sites.

The knowledge obtained in the PlastiSpread project will have a long-term impact on WW legislation and treatment plant design. The project will increase our understanding of marine plastics as a vector for pathogens and AMR in the marine environment.

Keywords: antimicrobial resistance, pathogens, wastewater, marine environment

Environmental resistome of the Baltic Sea benthic ecosystem

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Antimicrobial resistance is a major global health concern, and the environment plays a crucial role in its emergence and dissemination across different sectors. Understanding the relationships between environmental factors, microbial communities, and their resistance mechanisms will provide crucial information on the dynamics of environmental resistomes in high- risk areas. In this study, we used the Baltic Sea as a model ecosystem to characterize and assess the relationships between benthic microbial communities, antimicrobial resistance genes (ARGs), their associated functional genes, and the influence of various physicochemical factors to improve our knowledge of the resistance mechanisms in complex aquatic ecosystems. We analyzed the metagenomes of benthic sediments collected from 59 monitoring stations across a 1,150 km distance of the Baltic Sea. The environmental resistome of the Baltic Sea benthic ecosystem consisted of ARGs conferring resistance to 26 antibiotic classes. We observed spatial variation in its resistance profile, with higher ARG diversity in the northern regions and a decline in the dead zones and the southern areas. Multidrug and glycopeptide resistance were the most abundant ARG types across all regions, with genes conferring multidrug resistance significantly more abundant in the Bothnian Bay and glycopeptide resistance in the Southern Baltic region. The combined effects of salinity and temperature gradients, alongside nutrient availability, created a complex environmental landscape that shaped the diversity and distribution of ARGs. Furthermore, we found that the structure of benthic microbial communities had strong direct effects on the composition of mobile genetic elements and intermediate direct effects on ARG composition. Our study provided critical information for establishing effective strategies for assessing the emergence and dissemination of antimicrobial resistance in the environment. Understanding how environmental factors and microbial communities modulate environmental resistomes will help predict the impact of future environmental changes on resistance mechanisms in high-risk areas.

Keywords: Antimicrobial Resistance, Baltic Sea, Benthic Ecosystem, Microbiome, Resistome.

Monitoring AMR in Aquatic Animals and the Environment: Developing Methodology for AMR and Antibiotic Residue Screening.

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Antibiotics are extensively used in both human and veterinary medicine. A high percentage of antibiotics are often excreted unaltered or as active metabolites. Wastewater treatment plants (WWTPs), though effective in removing many pollutants, are not specifically designed to eliminate antibiotics. Environmental contamination of antibiotic-resistant genes and residues due to discharge of raw and treated sewage from both centralised and decentralised sewage systems degrades the ecological health of waterbodies and contributes to the spread of antimicrobial resistance (AMR). Despite WWTPs being recognised as key contributors to the presence of antibiotics and antimicrobial resistance (AMR) genes in the environment, many countries lack regulations specifying antibiotic concentration limits in WWTP discharges. This gap in regulation is concerning as the presence of antimicrobial compounds in aquatic environments may exacerbate the spread of AMR, posing risks to human, animal, and ecosystem health.

In response to this threat, Cefas through its UK FAO Reference Centre for AMR, has developed sampling methodologies to utilise water and fish tissue samples for the effective isolation of antibiotic-resistant bacteria for phenotypic and genotypic AMR analysis from both freshwater and marine environments (Light E et al 2023).

This poster showcases the use of bacteria isolated from water and aquatic animals tissue samples for environmental monitoring of AMR using 2 case studies. The development of these methodologies/approaches enhances current understanding of AMR dynamics in aquatic environments, supporting ‘One Health’ AMR surveillance and environmental health protection efforts.

Reference: Edel Light , Craig Baker-Austin , Roderick M. Card , David Ryder ,Mickael Teixeira Alves , Hanan A. Al- Sarawi , Khalil Hasan Abdulla , Henrik Stahl ,Aliya Al-Ghabshi , Majed F. Alghoribi , Hanan H. Balkhy , Andrew Joseph , Alexandra Hughes ,Will J.F. Le Quesne , David W. Verner-Jeffreys , Brett P. Lyons , Establishing a marine monitoring programme to assess antibiotic resistance: a case study from the Gulf Cooperation Council (GCC)region, Environmental Advances (2022), doi: https://doi.org/10.1016/j.envadv.2022.

Keywords: Antibiotics, Wastewater, One Health, Bacteria, Survaillance.

Alterations in the aquatic microbial communities and toxicological effects due to ketoprofen and fluoxetine and their mixture at microcosm scale

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Pharmaceutical compounds are considered as contaminants of emerging concern (Khan et al., 2022) and their occurrence has been described in diverse environmental compartments including surface and groundwater, soil, sediments and biota (Aus der Beek et al., 2016). In general, the effects of these compounds on non-target organisms are still largely unexplored. In particular, this work aimed at determining the effect of sublethal concentrations of fluoxetine (antidepressant) and ketoprofen (analgesic) individually (0.050 mg/L) and their mixture (0.025 mg/L each) on aquatic communities at a microcosm scale for a period of 14 d. Model organisms, i.e., Daphnia magna, Lemna sp., and Raphidocelis subcapitata were added to the microcosm to assess effects on specific populations; while changes in eukaryotic and prokaryotic communities were determined according to data obtained from the sequencing of 16S/18S rRNA genes. Also, several physicochemical parameters were monitored to estimate functional alterations in the ecosystem. Effects due to pharmaceutical exposure were mostly observed by the end of the assay, by day 14. The microcosms containing fluoxetine (either alone or in combination with ketoprofen) exerted major alterations on physicochemical and biological parameters, compared to the less prominent disturbances observed in the microcosm containing only ketoprofen. Alterations in the N-cycle are likely to occur in the fluoxetine-containing microcosm according to the profiles in N-species; similarly, a decrease in the brood rate in D. magna was determined in every condition, while inhibition in the growth of Lemna sp. was observed in the systems containing individual pharmaceuticals. Alterations in the structure of micro-eukaryotic communities was determined in the fluoxetine-containing systems, whereas the mixture of pharmaceuticals seemed to exert a major effect in the structure of bacterial communities. The disturbances detected in the microcosm equilibrium provide valuable data to describe the risk these compounds pose to non-target communities in aquatic ecosystems.

Keywords: pharmaceuticals; toxicity; mixture of pollutants; microbial communities; eukaryotic communities

References:

Aus der Beek, T., Weber, F. A., Bergmann, A., Hickmann, S., Ebert, I., Hein, A., Küster, A. (2016). Pharmaceuticals in the environment—Global occurrences and perspectives. Environmental Toxicology and Chemistry. 35, 823-835.

Khan, S., Naushad, M., Govarthanan, M., Iqbal, J., Alfadul, S. M. (2022). Emerging contaminants of high concern for the environment: Current trends and future research. Environmental Research. 207, 112609.

Exploring Long-term Metal Pollution Effects on Freshwater Biofilm Eukaryotic Microbial Composition by Metabarcoding: A Novel Approach Beyond Traditional Biodiversity Assessment

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Environmental monitoring worldwide is governed by specific legislation establishing protocols for conducting ecotoxicological assays to determine contaminant thresholds and biodiversity assessment. However, conventional ex-situ assays often employ single pollutants on specific organism populations (e.g. Daphnia magna) failing to represent the diverse community of a natural environment and their interrelationships, or account for the combined effects of co-occurring metallic pollutants. Additionally, microscopic taxonomic identification, set by biodiversity assessment`s legislation, presents several limitations such as the susceptibility to taxonomic misidentification, overlooking impacts on entire aquatic communities, neglecting trophic and functional interactions crucial for ecosystem health.

To address these limitations, this study conducted water quality and biodiversity assessments on aquatic biofilms - focusing on eukaryotic composition - upstream and downstream of metal hotspots in Flanders (Belgium), historically contaminated by multiple metals from industry activity. Applying advanced high-throughput biodiversity approach, this study marks a departure from conventional monitoring strategies towards community-level perspectives enabled by environmental DNA metabarcoding techniques.

In addition to the conventional water quality assessments, which includes dissolved metals (As, Cd, Co, Cr, Cu, Mn, Pb, Zn by ICP-OES), total/dissolved organic carbon (TOC-L analyzer; Shimadzu), and nutrients (Cl-, SO42-, PO43-, NH4+ , NO3-, NO2- - Spectroquant kits; spectrophotometric analysis) contents, this research explored the structure and the diversity of eukaryotic microbial communities in aquatic biofilms from up and downstream the industrial effluent release using 18S rRNA gene metabarcoding (Illumina MiSeq) data.

By examining the relationship between species abundance and environmental stressors (e.g. nutrients and metals), the study aims to identify eukaryotic metal-resistant and metal-sensitive indicators, shedding light on contaminant impacts on species relationships and ecosystem functions.

Overall, this study demonstrates the efficacy of incorporating advanced molecular techniques into environmental monitoring efforts, providing a more comprehensive understanding of ecosystem health and resilience in the face of anthropogenic pressures.

Keywords: Biodiversity assessment; Ecosystem health; Anthropogenic pressure; High-throughput sequencing; Metal bioindicator.

Impacts of microplastics and synthetic compounds on soil bacterial diversity and function

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The fate and impact of microplastics, macroplastic disintegration products, have been documented in aquatic environments but very little research has been carried out in soils. As part of the MINAGRIS European project, which brings together twenty partners from thirteen European countries, we are measuring the effect of microplastics on telluric bacterial communities, major players in the ecosystem services provided by soils, particularly in relation to the nitrogen cycle. We hypothesize that microplastics could promote the development of biofilms and thus, create a new niche for these communities, impacting not only their abundance and diversity, but also their response to other pollutants in a multi-stress context.

To test this hypothesis, soil microcosms were treated, or not (control), with three types of microplastics at different doses in combination, or not (control), with synthetic compounds used in livestock farming (albendazole, an antiparasitic) and agriculture (pyraclostrobin, a fungicide) or a mixture of the two. Three months later, nitrate and ammonium concentrations and nitrification potential were measured. At the same time, soil DNA was extracted to measure the abundance of the bacterial community by q-PCR and its composition and diversity by Illumina sequencing of the 16S rRNA amplicons.

The effects measured on nitrate concentration and nitrification potential depend on the type of plastic considered. Abundance and diversity data analyses are on-going and will be presented during the conference. Ultimately, the obtained results should allow us to assess the ecotoxicological impact of microplastics on soil microorganisms and supported ecological functions to propose recommendations to reduce microplastic impact in agriculture.

Keywords: microplastics; soil bacterial community; nitrogen cycle; multi-stress

Soil Bacterial communities' turnover with the immergence of Bio-indicator genera correlated with physiochemical changes as a result of treated wastewater irrigation: case study of Tunisian agricultural soil.

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TWW has been used in northern Africa to irrigate fodder crops, but there haven't been enough studies done to show correlations between physiochemical properties of the soil changes', the composition and structure of bacterial communities, and the soil resistome over the long and short term TWW irrigation. Our results showed an acidification of soils correlated with TWW irrigation years, in addition to a significant increase of salinity, soil organic matter, concentrations of heavy metals, and major soil ions. Pollution-induced bacterial community tolerance (PICT) was investigated for Cu, Zn, Pb, and tetracycline with H3-Leucine incorporation. Our results showed a significantly higher tolerance index ($P<0.05$) for long-term TWW irrigated bacterial communities compared to control and short-term TWW irrigated communities. To highlight the impact of this irrigation on the bacterial communities' structure and composition, 16S rRNA gene amplicon sequencing was performed on the same samples as used for PICT detection. Biosensor analysis, using genetically modified Bacteria strains highly sensitive to bioavailable Cu and Zn present, was carried out on different samples showing a significant increase ($P< 0.05$) in the mentioned heavy metals in long-term TWW irrigated samples compared to control and short-term TWW irrigated samples. Statistical analysis based on phyla and genera relative abundance, alpha and beta-diversity analysis, and environmental factors correlation were performed. A significant difference in alpha diversity indexes such as Chao1 and ACE was observed. Surprisingly, short-term TWW irrigated bacterial communities had the higher biodiversity index ($P< 0.05$) with a better bacterial richness implication. A turnover in bacterial communities' composition was proved especially at genus level with a dominant appearance and development of Bio-indicator genera such as Defluviococcus, Pseudarthrobacter, Nocardioides, and Gaiella in long-term TWW irrigation compared to the rest of treatment, which lead us to consider them as TWW contamination bio-indicator.

Keywords: Bacterial communities, TWW irrigation, Heavy metals, resistome, PICT, Biosensor, and Bio-indicator genera.

Changes of bacteriome structure in the organs of Pacific oysters *Crassostrea gigas* from differently polluted sites

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Every organism can be considered as an holobiont made up by the association of a host and a complex microbial community of bacteria, archaea, virus and small eukaryotes, commonly referred to as microbiota. Microbiota can perform specific functions and thus participates in the enantiostasis of the organism. Like other invertebrates, oysters harbour a rich microbial community. Previous studies revealed a distinct microbiome specific to oyster tissues (hemolymph, gills, mantle and intestine) that is also different from the surrounding seawaters microbial. Multiple factors, including environmental changes, diet, infection, and probiotic use, have been found to influence the composition of oyster microbiome at certain life stages and in different tissue types. Coastal ecosystems are exposed to pollution produced by anthropogenic activities (industrial activities, harbours, transport, coastal facilities or recreational activities). Amongst environmental pollutants, trace metals are known to have toxic effects on aquatic organisms, even at environmental concentrations. Due to their filter feeding life style, oyster bioaccumulates metals from both abiotic (habitat) and biotic (food) sources. In line with the "One Health" global strategy, more and more studies are focusing on characterising the exposome of living organisms to better understand the toxic effects of chemical and physical agents on all living organisms, especially on populations and communities within defined ecosystems.

In this field study, we investigated the potential impact of trace metal pollution on the bacteriome associated with different oysters tissues sampled from six sites of the French coastline.

Keywords : Microbiome, exposome, ecotoxicology, metabarcoding, oysters

Ecotoxicology of a biocontrol bacterial strain against *Sclerotinia* stem rot in rapeseed

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Sclerotinia stem rot in rapeseed is caused by a phytopathogenic fungus, namely Sclerotinia sclerotiorum. It poses a significant threat to crop yields and economic losses. Traditionally, chemical fungicides have been used to combat this pathogen, but their environmental risks have raised concerns, leading to the development of more sustainable biocontrol solutions. In this study, we focus on evaluating the unintended effects resulting from the inoculation of one strain of biocontrol agents against Sclerotinia stem rot in three different soils presenting contrasted physicochemical properties.

We analysed the persistence kinetics of the biocontrol strain in soil microcosms using qPCR quantification with strain-specific primers and selective plate counts of a rifampicin-resistant variant. After a rapid decline in the first week, the strain persisted for six months in both low (6x10 3 bact/g of soil) and high dose (1x108 bact/g of soil).

The effects of the biocontrol agent on soil bacterial and fungal communities were measured by sequencing 16S and ITS rRNA gene amplicons as compared to those caused by a chemical fungicide or a commercial biocontrol agent. While fungal alpha diversity remained unaffected by exposure to the biocontrol strain, fungal beta-diversity was altered at short term, however resilience was observed in two of the soils by two months. The commercial biocontrol showed no significant effects on fungal alpha and beta diversities, while the chemical fungicide, tebuconazole, altered fungal beta- diversity in two soils after two months of exposure, without resilience of the fungal communities.

Further analysis of bacterial communities will provide more insights into the ecotoxicological impacts of the biocontrol strain. The ecotoxicological assessment of the biological agent will soon be scaled- up in assay conducted on rapeseed planted soils at both greenhouse and field levels. This comprehensive investigation will enable us to assess the unintended effects of these novel rapeseed stem rot biocontrol products on soil microbial communities.

Keywords: ecotoxicology, biocontrol bacteria, microbial communities, specific soil detection

Evaluating Hppd Activity And Pyomelanin Production In Soil Non-Target Bacteria Exposed To Beta-Triketone Herbicides

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Pyomelanin, a red/brown pigment produced by certain bacteria, plays a crucial role in physiological functions, such as resistance against various stresses. Pyomelanin results from the accumulation, oxidation and polymerization of homogentisic acid (HGA), an intermediate product of the L-tyrosine catabolic pathway, present in different microorganisms, where 4-hydroxyphenylpyruvate dioxygenase (HPPD) is involved in the conversion of 4-hydroxyphenylpyruvate (HPP) into HGA. HPPD enzyme is known to be the molecular target of Beta-triketones herbicides, mainly used on maize cultures. Thus, when applied to crops, Beta triketones could potentially impact non-target soil microorganisms, through HPPD inhibition and disruption of the L-tyrosine pathway and pyomelanin production.

In this work 28 HPPD bacterial soil strains were isolated and identified, representing 12 species. The addition, in agar medium, of two Beta triketones, tembotrione (TBT) or mesotrione (MST), revealed distinct levels of HPPD susceptibility among the strains, corroborated by a comprehensive colorimetric assay using resting cells. MST and TBT EC50 for HPPD inhibition of Stenotrophomonas chelatiphaga 3F10-1, Lysobacter antibioticus 13G10, Xanthomonas transluscens 1B1, Pseudomonas vancouverensis 3C7 and Stenotrophomonas rhizophila 5H8-2 strain values ranged from 0.13 ± 0.01 to 0.61 ± 0.13 mg/L, corresponding to agronomically relevant doses. Among these isolates, some bacterial strains showed greater tolerance to HPPD inhibition, with EC50 values ranging from 1.1 ± 0.19 to 14.21 ± 0.8 mg/L for the moderately tolerant strains Pseudomonas laurylsulfatphila LF8 and Pseudomonas bijieensis 5H8-1 and reaching up to 433.63 ± 24.99 mg/L for the strain Pseudomonas rheidholzensis 1A11. HPPD inhibition by TBT or MST did not have a significant impact on growth of the most sensitive strains. Multiple sequence alignment of HPPD sequences across the different strains revealed conserved key residues crucial for enzymatic activity. Interestingly, sensitive HPPDs demonstrated a higher degree of similarity among themselves compared to tolerant ones. This distinction could potentially be used to develop specific hppd primers targeting sensitive bacterial populations in soil, to be used as a biomarker of Beta-triketone exposure.

Keywords: Pyomelanin - 4-hydroxyphenylpyruvate dioxygenase - Soil bacteria - β-Triketone herbicides - Non target microorganisms

Assessing ecotoxicology in Mediterranean wetlands: Insights from microbial bioindicators

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The Mediterranean wetlands, renowned for their biodiversity and crucial role in supporting numerous species, are facing increasing pollution resulting from human activities such as intensive agriculture, urbanization, and coastal tourism. This pollution poses a significant threat to the ecological health of these fragile ecosystems and compromises their ability to provide essential ecosystem services. To enhance their preservation, a better understanding of their functioning is imperative. Microorganisms exhibit fast growth rates and are highly sensitive to environmental change. This suggest they have potential to be efficient bioindicators to assess and monitor the status of the ecosystems within they occur. Here, biogeographic study at dry and wet seasons, covering six distinct ecosystems under diverse anthropogenic pressure, is presented. We hypothesize that the level, type of pollution and local environmental conditions will drive microbial composition and that the spatiotemporal dynamic of microbial communities respond to global changes. In order to identify bioindicators of pollution a comprehensive approach was adopted analysing prokaryotic and eukaryotic compartments based on environmental DNA. Pollutant-tolerant or indifferent taxa increase while sensitive taxa decrease. The correlation of these data with physical-chemical factors allowed to determine the parameters shaping microbial communities. This information is crucial to better understand the impact of pollutant on these ecosystems and so assessing their ecological status.

Keywords: eDNA, ecological status, pollutants, microbial indicators, microbial ecotoxicology

Exposure to bilge water impact the growth rate of the marine diatom Nitzschia sp.

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The bilge is the lowest point in marine vessels, and water collected here originates from several different on-board areas, primarily condense water from the engine room. Bilge water contains residues of fuel, oils, metals, detergents, degreasers etc.

Bilge water can be discharged if it meets the IMO regulation that oil content is below 15 ppm. To achieve that, the bilge water is treated in an oily water separator. This however, may not make it environmentally safe to discharge. It has been shown in analysis of treated bilge water that although the total content of organic substances was below 15 ppm, individual PAH:s were above their predicted no effect levels. Metals and surfactants also remained after treatment.

The amount of produced bilge water depends on a vessel's engine capacity, and for passenger ships and RoPax vessels the daily production can be between 70-10900 L. Considering the number of vessels of the global fleet trafficking our seas there is therefore potential for a negative impact on the marine environment.

In our study, we will show results from exposure of the diatom *Nitzschia* sp. to bilge water from six different vessels to assess toxicity to a common primary producer in the Baltic Sea, and compare difference in toxicity between bilge waters with known content. As effects of bilge water is not yet well studied and results from studies vary depending on the ship, it's not established if it is specific compounds or the mixture that is driving the toxicity.

By comparing effects of bilge waters with known contents we can start narrowing down potential suspects that cause harm to marine organisms and aid in getting regulations in place to protect the marine environment.

Keywords: bilge water, oil content, Nitzschia, marine diatom

Effectiveness of commercial bacterial consortium of plant-biostimulant in soil affected by copper oxide nanomaterial and copper sulfide contamination.

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Plant-biostimulants based on beneficial bacteria (mPB) are used to boost crop productivity by increasing nutrient use efficiency in soil, which represents an environmentally friendly alternative to conventional fertilizers. These natural-based products increase the ability of plants to grow under abiotic stress conditions. However, the compatibility of these mPBs with either conventional or novel nano-based agrochemicals is not fully understood. To address this knowledge gap, our study aimed to study the viability of using commercial bacterial plant-biostimulants containing Azospirillum brasilense and Pseudomonas fluorescens in soils previously exposed to copper-based pesticides. For this, two distinct phases were evaluated. In Phase-I, the inhibition of mPB activity was tested using microdilution assays, testing three doses of mPB (including the recommended dose: 3 x 106; 6 x 106; 1 x 107 cell/ml), and a wide range of concentrations of copper: 0 to 50 mg/L. Phase-II was done to validate the most relevant compatible concentration of these products in soil. A pot experiment was carried out using natural soil exposed to copper formulations in the presence of Medicago sativa and inoculated with mPB. After days 14 and 60, parameters related to plant growth (shoot/root biomass) and soil properties (enzymatic activity, basal respiration) were measured. As a result for Phase-I, bacterial- based biostimulants were susceptible to copper-formulations in a dose-dependent manner, in which the highest inhibition rate was observed in the highest concentrations of pesticide. Phase-II revealed that the mPB (regardless of copper presence) increased the soil microbial activity over time, suggesting a positive effect on the soil properties. This study emphasizes the potential use of this bacterial plant- biostimulant in conventional agricultural systems. Thus, this study emphasizes the potential use of this bacterial plant-biostimulant in conventional agriculture. Furthermore, this data can also be used as a baseline for an in-depth investigation of the functional recovery of degraded soils.

Keywords: rhizosphere, promoting-growth bacteria, agrochemicals, nanomaterials, sustainable agriculture.

To grow or not to grow? The growth inhibition endpoint in the context of phytoplankton life cycle and behavior

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Growth inhibition is undoubtedly the most investigated endpoint in phytoplankton ecotoxicology, providing important information on the impact of contaminants on phytoplankton biomass production. However, this endpoint does not allow to extrapolate long-term impact of pollutants on phytoplankton dynamics. Cells that do not grow are not necessarily dead cells or unable to return to an active growth state once the pressure associated with the presence of the pollutant is released. The no-growth state occurs naturally in the life cycle of phytoplankton in presence of biotic and abiotic stresses that are present aquatic environments. In addition, most of the phytoplankton species have the ability to move within the water column and eventually settle in benthic environments where they can enter a resting phase thus generating a seed bank, a reservoir of propagules that can contribute to the assemblage and biodiversity of future phytoplanktonic communities.

The behaviour of phytoplankton in the presence of contaminants has rarely been investigated. In the event of contaminant exposure, are phytoplankton capable of moving into the water column and entering a resting phase? Are resting cells capable of re-entering a growth phase after a long period (months) of rest?

The aim of this work was to investigate the possible impact of contaminants on the behavior and resting phases of a model species of marine phytoplankton, the diatom Phaedactylum tricornutum. Cells were exposed to diclofenac (DCF) and ethynyl estradiol (EE2) under standard laboratory conditions (72 hours incubation under constant light and agitation). They were then transferred to a column where had the “freedom” to move along a vertical light gradient toward areas of very low light intensity. Their growth, viability, mitochondrial activity and ability to accumulate reserve lipids were investigated after two months of incubation, along with the ability of the viable cells to re-enter the active growth phase.

The impact of DCF on cells occurred mainly in the initial active growth phase (72h standard incubation) than in the later non-growth phase (two months of semi-dark incubation). During this second phase, the presence of DCF appeared to promote cell sedimentation and this resulted in an survival rate comparable to the control despite the prolonged exposure to the contaminant. In contrast, EE2 appeared to influence cell growth in the first phase and also survival in the second phase, possibly altering the ability of cells to make the transition into the non-growth phase. Prolonged exposure to EE2 resulted in a significant (3-fold) reduction in survival rate compared to the control.

Keywords: Diatoms, growth inhibition, behavior, resting cells, organic contaminants

Assessment of agricultural soil for heavy metal pollution from dumpsite leachates

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Disposal of solid waste is a major environmental problem faced by Nigerian cities and towns. The most common forms of waste disposal in the country are dumping in open spaces, uncontrolled burning and disposal in surface water bodies. Solid wastes are generated by almost every activity of man and the amount varies by source, season, geography and time. Co-disposal of industrial, municipal, residential and medical trash in unsanitary dumpsites is common in Nigerian communities and this indiscriminate disposal of refuse increases salts and trace metals in the soil and water bodies. When leachates run through dumpsites, they carry salts and organic and inorganic compounds. Dumpsite leachate is generated as a consequence of water percolation through the solid wastes, oxidation and corrosion of the wastes. This research was carried out to assess dumpsite leachates and their impacts on surrounding agricultural lands within the vicinity of the Umunze metropolis of Anambra state, Nigeria. Leachate samples were collected from two active dumpsites and analyzed for physiochemical and heavy metal parameters according to internationally accepted procedures. The results show that dumpsite 2 has lower values for nitrate (21.1mg/l), sulphate (92.0mg/l), Total hardness (665mg/l) and nitrite (12mg/l) than dumpsite 1 with values (28mg/l), (108mg/l), (720mg/l) and(16mg/l), and higher mean values for conductivity (184700πS/cm), salinity (100psu) and chloride (14.97mg/l) than dumpsite 1 with values (7.70,41.401mg/l, 52,800 mg/l, 58.2psu and 14.99mg/l) respectively. The pH, sulphate, and chloride values for both dumpsites are within the WHO permissible limit whereas the total hardness, conductivity, nitrate, and turbidity values are higher than the permissible limit. The essential metals present are all within limit, hence less toxic to the environment. The heavy metal concentrations fall within range when compared to Standard except that of Arsenic which can lead to arsenic contamination and accumulation by plants and vegetables grown on the land.

Evaluating impact of chemicals on marine phytoplankton communities using flow cytometry

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Phytoplankton communities provide key services to marine ecosystems. Chemical pollution is recognised as one of the five drivers for biodiversity loss and ecosystem change. Currently, the assessment of contaminant impacts on marine phytoplankton is primarily confined to standard toxicity testing often utilizing the model diatom Skeletonema spp.. However, our understanding of the broader effects of chemicals on phytoplankton communities remains limited. This work aims to address this knowledge gap by exploring the use of natural phytoplankton communities and with focus on the picophytoplankton component. Chlorine was selected as an example contaminant which is commonly used as antifouling agent in marine and estuarine environments. Different techniques were used to better understanding changes in the community. Thus, the more standard biomass was evaluated with chlorophyll a; diversity with functional diversity obtained with flowcytometry analysis and complemented with pigment composition obtained with HPLC analysis; finally, photosynthetic activity was evaluated with PAM fluorometry. Environmental relevant concentrations of chlorine had no impact on total biomass. Nevertheless, changes in size structure and functional diversity were quantified using flow cytometry with a reduction in smaller cells, particularly eukaryote picophytoplankton. Effects of chlorination discharges are likely to be localised close to the effluents only. Nevertheless, impact on coastal food webs and biogeochemical cycles should be further evaluated. Flow cytometry was the most sensitive method in detecting effects to the phytoplankton community and provided important additional information over more standard ecotoxicology methods. This approach is fast and low cost compared to other methods and is a promising addition to standard ecotoxicological approaches with numerous applications to microbial communities.

Keywords: flowcytometry, phytoplankton, picophytoplankton

Investigating the effects of urease inhibitors and nitrification inhibitors on the soil microbiome

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Stabilized nitrogen fertilizers contain urease inhibitors (UI) or nitrification inhibitors (NI). These enzyme inhibitors are added to slow down nutrient transformations and to reduce emissions of ammonia, nitrous oxide, and nitrate leaching. However, little is known about whether UIs and NIs have unintended effects on the soil microbiome. We investigate soil parameters (pH, NH4+, NO3-), enzyme activities (dehydrogenase, urease, nitrification, arginine ammonification, glucosidase, acid phosphatase, arylsulfatase), and the community structure (DNA sequencing and dPCR) after application of different fertilization treatments in a lab incubation study. Three different types of agricultural soils (sandy, silty, and clayey) are compared. At the time of abstract submission, the first results showed that the UI N-(2-nitrophenyl) phosphoric triamide reduced urease activity by more than 50% but also affected the non-target process of arginine ammonification, where urease enzymes are involved. Thus, the UI not only inhibited ammonium production from urea but also from other organic substrates. However, this might occur without affecting other than the target enzymes on a molecular level. The NI 3,4-dimethylpyrazole phosphate decreased the nitrate content and the nitrification rate (target effect). The C-, P-, and S-cycle related enzyme activities were not affected by the inhibitors. The preliminary results showed that UIs and NIs have their intended effect. However, the reduced arginine ammonification rate after UI application indicated that other than the targeted soil processes might be affected. We will further investigate this in the currently ongoing project and check for unintended side effects on functional microbial groups and the microbial community structure in different soils.

Keywords: Nitrogen; Fertilization; Enzyme activity; Microbial community; Inhibitors

Metabolomic insight in species sensitivity differences within periphytic communities

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In the face of global changes, periphyton serves as an important model for assessing the disruption of ecosystem functions in aquatic environments. However, there remains limited understanding of how species within these communities vary in their sensitivity to chemical stress, a factor crucial to their structure and function. This study aims to address this knowledge gap at the biochemical level by comparing the molecular phenotypes of a cyanobacterium, a green alga, a diatom, and their co-culture under chemical stress. To achieve this, the metabolomes of these three species and their co-culture were analyzed before and after a seven-day exposure to atrazine, S-metolachlor, and their mixture at three concentrations (10, 100, and 1000 µg/L) using UPLC-HRMS-based untargeted metabolomics. The comparison of metabolic profiles revealed that, before exposure, the metabolism of the diatom and green alga was more similar to each other than to that of the cyanobacterium, while the co-culture's metabolism was closer to that of the diatom. Additionally, atrazine, S-metolachlor, and their mixture affected the metabolome of each species differently, indicating potential variations in toxicity pathways. Further metabolite class annotation confirms the marked species differences in terms of number and up/down regulation of annotated metabolites. Finally, molecular networking, aiming to provide a comprehensive picture of the chemical landscape of the three species prior the exposure, highlighted that the abundance of some annotated metabolite differed strongly between the species. Overall, this study will contribute to a deeper understanding of species-specific sensitivities to chemical stress in periphyton.

Bacterial-fungal community patterns associated to the discharge of an urban wastewater affluent in the Swedish West Coast (Askeröfjorden – Stenungsund)

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The continuum of the Swedish West coast, particularly its fjord system, represents an opportunity to explore the impact of emerging contaminants on marine microbial assemblies. Emerging contaminants derived from wastewater treatment plants, agricultural runoff and urban runoff could jeopardize the ecological status of coastal areas. The Hakefjord and Havstensfjord are part of a fjord system on the Swedish West Coast surrounding the city of Stenungsund. Stenungsund harbours a variety of industry branches but is chiefly known for being the centre of the Swedish petrochemical industry. Here, we uncover the biodiversity status of bacterial and fungal communities across a coastal transect impacted by a marine outflow from a WWTP and runoffs from the agricultural areas and the industrial hub in Stenungsund. Additionally, two freshwater samples were collected to compare them to the marine samples. Our metabarcoding results suggest that bacterial (hypervariable region V3-V4 16S rDNA gene) and fungal communities (internal transcribed spacer ITS2) are richer and more diverse in freshwater samples than in the marine environment. However, we observed a clear decrease bacterial and fungal diversity in samples associated to the WWTP marine outflow. Indeed, the bacterial and fungal genotypes detected in the WWTP marine outflow showed a large degree of dissimilarity compared to the freshwater and marine samples, and only the fungal community showed some level of similarity to the nearest marine area. Multivariate analyses suggested that ammonium, phosphate, pH as well as the antibiotics and fungicides, could be potential drivers affecting the structure of the studied communities. Furthermore, we identified genotypes of taxa that are significantly correlated to the measured parameters. Our results provide an initial snapshot of how bacterial-fungal assemblages respond to WWTP discharges in the marine environment.

Keywords: Environmental microbiome, Metabarcoding, Micropollutants

Soil microbial community fragmentation reveals indirect effects of fungicide exposure mediated by biotic interactions between microorganisms

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Fungicides are used worldwide to improve crop yields, but they can affect non-target soil microorganisms which are essential for ecosystem functioning. Microorganisms form complex communities characterized by a myriad of interspecies interactions, yet it remains unclear to what extent non-target microorganisms are indirectly affected by fungicides through biotic interactions with sensitive taxa. To quantify such indirect effects, we fragmented a soil microbial community by filtration to alter biotic interactions and compared the effect of the fungicide hymexazol between fractions in soil microcosms. We postulated that OTUs which are indirectly affected would exhibit a different response to the fungicide across the fragmented communities. We found that hymexazol primarily affected bacterial and fungal communities through indirect effects, which were responsible for more than 75% of the shifts in relative abundance of the dominant microbial OTUs after exposure to an agronomic dose of hymexazol. However, these indirect effects decreased for the bacterial community when hymexazol doses increased. Our results also suggest that N-cycling processes such as ammonia oxidation can be impacted indirectly by fungicide application. This work sheds light on the indirect impact of fungicide exposure on soil microorganisms through biotic interactions, which underscores the need for higher-tier risk assessment.

Keywords: Interactions, networks, fungicide, soil communities

Experimental assessment of the chronic exposure thresholds to pharmaceuticals that can impact sediment microbial community diversity, activities and antibiotic tolerance.

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There is a growing concern about the global pollution of rivers by pharmaceuticals and their effects on aquatic life. Several studies have shown the potential impacts of these pollutants on microbial communities, including biofilms living in surface sediments where some pharmaceuticals can accumulate. However, there is still a lack of knowledge concerning the chronic exposure thresholds to pharmaceuticals, both in terms of concentration and duration, which induce structural and functional changes in sediment biofilms. In this context, our objectives were to evaluate the temporal evolution of the chronic effects of increasing concentrations of three pharmaceutical model substances– sulfamethazine, diclofenac, ofloxacin – on sediment microbial community activities, structure, diversity, and antibiotic tolerance.

We conducted a 47-day laboratory experiment using 18 laboratory channels containing natural river sediments spiked or not with one of the model pharmaceuticals. For each substance, five concentrations were tested, using a logarithmic design, starting with environmentally relevant concentrations (nominal concentrations from 0.14 to 10,000 µg/kg). Effective concentrations were measured at days 0 and 47 using liquid chromatography coupled with mass spectrometry. Pharmaceutical effects on the sediment microbial communities were assessed at days 0, 19, 33, and 47: five heterotrophic microbial activities were measured, community tolerance (and cross-tolerance) to sulfamethazine and ofloxacin was evaluated using a PICT approach (Pollution Induced Community Tolerance), and community structure and diversity were explored using a metabarcoding approach on the 16S rRNA gene.

The observed effects varied according to the substance, duration and level of exposure and the type of effect considered. Overall, the most marked effects were observed with the antibiotic ofloxacin. At the highest concentrations, this substance led to the inhibition, of several microbial activities and affected the structure and diversity of exposed communities. It also induced an increase in tolerance to the antibiotic sulfamethazine, suggesting the possible existence of a co-tolerance phenomenon.

Keywords: freshwater; biofilm; microcosms; PICT; metabarcoding

The greenhouse gas emitting potential of densely urbanized tropical estuary sediments inferred from metabarcoding microbial community analysis.

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The microbial community from impacted coastal sediments reflects on their functional dynamics the effects of pollutants exposition. Thus, accessing microbial communities' disruptions at an early stage is crucial for designing mitigation strategies. The Sepetiba Bay (SB, Rio de Janeiro – Brazil) presents long-term contamination by metals, and the current contamination scenario is mostly influenced by the urban expansion (domestic effluents) that contributes to eutrophication and pollutant loads to the bay, mainly impacting the SB internal sector stations (eastern portion). While external sector stations (western portion) are less polluted, mostly influenced by tidal currents. Using prokaryotic community composition data (16S rRNA metabarcoding) and functional prediction analysis (Tax4Fun2), this research aims to decipher the main energy metabolisms (emitters / consumers of greenhouse gases - GHGs) selected by metal pollution. Although not a substitute for the metagenomic analysis, the functional prediction profiles from 16S rRNA amplicon sequencing offer an overview of the main functional trends performed by the microbial community. Nitrogen (N2) fixation, methanogenesis, nitrate (NO3) reduction, and denitrification are resistant to the SB metallic pollution, prevailing in the internal sector stations (Mann Whitney's U test; $p < 0.05$). The enrichment of predicted genes linked to anaerobic metabolisms (e.g. denitrification) in the most polluted stations express the natural attenuation of contaminants, warning to the emission nitrous oxide. In addition, N2 fixation is pointed as the energy metabolism indicator typical of highly polluted sediments. Our study also revealed a strong correlation among methanogenesis, N2 fixation, and NO3 reduction, in which Spirochaetia, Deltaproteobacteria, and Thermodesulfovibrionia are possibly involved in these metabolisms coupling. Adversely, the external sector sediments act as GHGs consumer as metabolisms such as methane oxidation are favored. However, excessive pollutants in coastal ecosystems are closely linked to climate imbalances, driving global consequences; this highlights the need to strengthen microbial ecotoxicology monitoring.

Keywords: One health; Environmental imbalances; Ecological interactions; Metallic pollutants; Microbial ecotoxicology.

Deciphering the effects of polyethylene and additive cocktail on microbial communities in an agricultural soil

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The adverse effects of plastic particles on soil microorganisms have been a subject of growing interest in recent years. Indeed, the presence of microplastics (MP) can be detrimental to soil microbial communities whose role is essential to the maintenance of essential functions such as biogeochemical cycles [1]. However, whether most studies have focused on the influence of polymer type, size or shape on soil microbial community, they did not consider the presence of chemical additives contained in plastics, the latter being able to negatively impact soil microorganisms.

We compared the effects of different sizes of pristine polyethylene (PE) microparticles with those of PE containing a cocktail of additives on microbial communities involved in N and C microbial activities in an agricultural soil. Two blends of PE with two concentrations (1X and 10X) of additives (antioxidant and light stabilizers, slip agents, lubricants…) were prepared and grinded to obtain 3 MP sizes (<100µm, 200-300µm, mix: 1-300µm). Soil microcosms were exposed for 15 and 60 days at 20 °C in the dark to 0.01, 0.1 and 1% of each MP size with and without additives. Substrate-induced respiration (SIR), nitrifying and denitrifying enzyme activity (NEA, DEA) were measured to account for the effects of MP size and additives. The results show that PE has negative effects on SIR and DEA after 15 days. These deleterious effects are still visible at 60 days only for SIR. NEA increased after 60 days for MP<100µm and 200-300µm. Additives for sizes 200-300µm and mix sizes induce a toxicity on SIR after 15 days no longer observable after 60 days. For DEA, 10X additives induced contrasted effects according to the MP size. The additives seem to have no effect on NEA.

This study shows the toxicity of PE and the contrasting effects of additives depending on the microbial activities and the concentration of MP and additives.

Keywords: Microplastic, Soil, Microbial Community, Activities, Additives

Bacterial players and biomarker genes in the degradation of chloromethane in the marine environment

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Chloromethane (CH3Cl) is a toxic gas of mainly natural origin involved in the destruction of atmospheric ozone. This compound is a source of carbon and energy for certain methylotrophic bacteria capable of using compounds without carbon-carbon bonds for their growth. The ocean is one of the environments with the highest production of CH3Cl on our planet (around 600,000 tonnes per year). To date, only one CH3Cl degradation pathway has been described, the cmu (chloromethane utilisation) pathway. Another degradation pathway recently identified in a marine bacterium is currently being characterised in the laboratory. Enrichment cultures were established with marine sediments from a protected site (Six-Fours-Gaou, Var) and from a polluted site (Berre l'Etang, Bouches-du-Rhône) and at two CH3Cl concentrations (1 mM and 10 mM). Diversity analyses performed by sequencing of 16S rRNA gene amplicons showed the increasing dominance of the phylum Pseudomonodota in these cultures. The dynamics of bacterial community composition differed depending on the sediment and the concentration of CH3Cl used for enrichment. CH3Cl-degrading bacterial strains were also isolated and their pathways for using CH3Cl are currently being characterised. New taxa associated with CH3Cl degradation were identified, indicating that the ability to degrade CH3Cl in the bacterial world is probably more widespread than previously thought. These initial results contribute to a better knowledge of bacterial players of CH3Cl degradation, as a prerequisite for more detailed studies of the biogeochemical cycle of CH3Cl in the marine environment.

Keywords: Chloromethane, bacterial diversity, degradation, marine sediment

Using Metabarcoding in Mesocosms and Biofilm Microbiomes for the Environmental Assessment of Technologies to Mitigate Mining Impact

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Mine waste (MW) is a relevant environmental issue in the EU and worldwide, and it is usually considered a major source of impact on human health and the environment. However, recovering valuable critical raw materials (CRMs) from MS could entail an economic benefit and reduce the dependence from import in EU. The European project RAWMINA aims to recover CRMs from MW by a combination of bioleaching and membrane technologies. However, there is not a standardized method to assess whether these technologies suppose a significant reduction of the environmental impacts of MW, particularly on freshwater ecosystems. We have developed an easy and cost-effective mesocosms approach based on the analysis of freshwater biofilms as ecological indicators to assess the effectiveness of these technologies in reducing the ecological impact of mining effluents.

In these experiments we aimed to understand whether MW treated by bioleaching suppose future risk for freshwater ecosystems. Experiments were developed in artificial streams, previously colonized by natural biofilms. Two different biofilm communities obtained in a natural stream, upstream and downstream the input of the effluent of an abandoned mine, were exposed for 2 weeks to: i) bioleachates of raw MW with high concentration of metals and pH ≈ 2; ii) bioleachates of treated MW and iii) o clean water as control condition (6 treatments x 3 replicates).

The biofilm responses were evaluated as density of main photosynthetic groups, photosynthetic activity, and microbial diversity (analyzed using Novaseq sequencing of the 16s rRNA region). The results demonstrated a high mortality rate as well as a decrease in diversity in the channels directly affected by bioleachates from raw MW. However, the control biofilms and those exposed to the bioleacheates of previously treated MW showed higher diversity, survival rates, and more similar microbial communities indicating the potential of the bioleaching treatment to reduce the pollution risk associated to MW.

Keywords: Mine waste, mitigation, aquatic biofilm, metabarcoding, mesocosms

Dynamics of sub-Sahelian soil microbiome in response to 7 years of repeated application of organic waste products

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Soil microorganisms ensure vital ecosystem processes for agricultural production, such as maintenance and regeneration of soil fertility, regulation of pests, and resistance of plants to environmental stresses. Organic fertilization is increasingly recognized as one of the most favorable practices for soil microbial communities. However, organic waste products (OWP) might also represent a source of contaminants, including heavy metals that can accumulate in the soil. It is therefore essential to evaluate the consequences of a repeated use of these OWP on soil microbial communities. Soils were sampled from a market gardening field experiment in Dakar region (Senegal), where sewage sludge, poultry litter and cow manure digestates were applied three times a year for 7 years. These OWP presented diverse heavy metal content, sewage sludge being the highest. A dynamic sampling of 13 points was performed in 2022, to compare the short term (days), medium term (cultural season) and long term (years) effects of OWP application. We used DNA metabarcoding (16S and ITS target) and PLFA to investigate the evolution of the bacterial and fungal communities. The cumulative effect of OWP application explained the highest proportion of the total variation in soil bacterial and fungal communities (75 and 30%, respectively), followed by the time since last application (4 and 12 %) and the season (<1 and 3%). A significant increase of microbial biomass (~40 %) and richness (~75 and ~33 % for bacteria and fungi, respectively) was observed in all OWP treatments, compared to the mineral control. Changes in microbial communities were positively impacted by the increase in soil pH and organic carbon content in OWP treated soils, and negatively by the zinc content. Our study highlights that the benefits on soil microorganisms of replacing mineral fertilizers by OPW outweigh the risks due to heavy metal content after 7years of repeated inputs.

Keywords: Organic and inorganic fertilization, microbial communities, high throughput sequencing

Assessing bacterial community responses in four chemically stressed small rivers in Kenya using environmental DNA

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Microbial communities, in particular bacterial assemblages, are key players in the provision of essential ecosystem services such as biogeochemical cycles as well as the degradation of chemical pollutants. The responses of bacterial communities to changing environmental conditions are manifold and include structural and functional alterations depending on the environmental stressors and toxic chemicals (e.g. pharmaceuticals, personal care products, pesticides, and industrial chemicals) they are exposed to. In this study, environmental DNA (eDNA) was extracted from surface water samples collected from four small rivers in the Lake Victoria South Basin (western Kenya) to : i) evaluate how alpha- and beta-diversity vary across land-use types, ii) identify environmental variables influencing community structure, iii) assess the qualitative and quantitative impacts of antimicrobial stress on bacterial communities, and iv) evaluate bacterial functional changes related to the degradation of organic chemicals. Our findings suggest that, compared to alpha diversity, bacterial community composition is a more sensitive indicator of the impact of chemical pollution from different land-use types. Nutrients and chemical pollution stress explained the dissimilarities in bacterial communities across small, urbanized, and agricultural rivers. Furthermore, an assessment of potential ecological functions unveiled a season-specific decline in bacterial degradation potential of toxic chemicals in all four rivers. Finally, our results indicate that the potential impact in bacterial communities in small Kenyan rivers, running through areas with low urbanization or subsistence agriculture is comparable to that of rivers exposed to high urbanization, intensive agricultural, or mixed land-use in other parts of the world.

Keywords: microbial ecotoxicology, micropollutants, eDNA, land use, nutrients

Evaluation of antibiotic and copper mixture effects on a soil plant-microbiome system

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The use of manure and biosolids in agriculture as organic amendments can be a source of emerging contaminants, such as antibiotics (ABs) and heavy metals, e.g. copper (Cu) [1]. For this reason, agricultural soils can be considered as an environmental sinks for ABs and Cu, and their accumulation in plants and eventual transfer through food chains to animals and humans needs to be investigated [2].

The objective of the present work was to study the effect of three antibiotics (sulfamethoxazole, chlortetracycline and ciprofloxacin, 7 mg/kg each) and Cu (10 mg/Kg) on the growth and development of lettuce plants (Lactuca sativa, var. Crispa) and soil and plant-associated microbial communities, in presence/absence of compost (1%) as organic amendment. Soil microbial communities were evaluated in terms of abundance (DAPI counts), and enzymatic activities (dehydrogenase, phosphatase, β glucosidase). Antibiotic resistance gene abundances were also quantified. Microbial community structures were determined through NGS and bioinformatic analyses. Plant growth and physiology were assessed as biomass, number of leaves, leaf area, root elongation, chlorophyll content and root exudates. Antibiotic and copper amounts were determined in soil and plant leaves through HPLC/MS and ICP-OES analyses.

Preliminary plant results showed the highest values for biomass, number of leaves, leaf area, root elongation and chlorophyll content in the condition with compost (Soil+compost), followed by Control Soil, Soil+Cu and Soil+ABs+Cu+compost. The presence of ABs and Cu appeared to cause detrimental effects on lettuce development. For all conditions an increase in soil microbial abundance and activities was found at the end of the experiment (46 days), compared to the initial time, except for β glucosidase, which showed no significant difference. In presence of compost, microbial abundance and activities were particularly favoured.

Further analyses are in progress to determine ABs and Cu in soil and plant.

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Keywords: Soil microbial communities, Lettuce, Antibiotics, Copper

Bioaccumulation of metals in the marine plastisphere and trophic transfert

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The plastisphere is defined as all the microbial communities that colonise the surface of plastics, forming a biofilm, a ubiquitous and specific way of life on the surface of polymers. It has been shown that the presence of this organic layer around microplastics (MPs) promotes the bioconcentration of metals. However, the impact of environmental parameters, in particular nutrients, on the shaping of the biofilm community and their consequence on the level of metal load remains to be understood. In addition, this bioconcentration of contaminants raises the question of the impact of ingesting or grazing MPs colonised by contaminated biofilms. To study these two issues, we generated plastispheres on polyethylene MPs under controlled conditions in mesocosms with natural seawater from the bay of Toulon. The diversity and composition of the prokaryotic and eukaryotic communities in the biofilms and the corresponding metal load were determined. These MPs were then used as pasture for the grazing crustacean Artemia salina. After a week, the artemia were recovered and their metal load was determined. Differential metal bioaccumulation in the biofilms as a function of nutrient conditions and the presence of metals in the artemia through grazing of the contaminated biofilm were demonstrated, amplifying the impact of PMs in marine ecosystems.

Combination of metagenomics and meta-metabolomics to unravel the relationship between taxonomic and functional biodiversity in freshwater periphyton

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Facing chemical contamination of aquatic ecosystems, it is crucial to better understand how fluctuation in structural and functional microbial biodiversity due to environmental conditions may modulate the sensitivity of microbiomes to chemical stress, as these communities play a significant role in ecosystem function and services. To address this challenge, the combination of metabolomics and metagenomics is particularly relevant as it allows for the assessment of microbial activity and taxonomic and genic diversity, respectively. The aim of this study is to characterize the relationship between the fluctuation of microbial activity (i.e. meta-metabolome) and the structural biodiversity (diversity of species and genes) due to changing environmental conditions over the course of a year. Periphytons were colonized monthly on glass slides in a hypereutrophic pilot pond. After quenching and freeze-drying, samples were split for both omics. The meta-metabolome was extracted using biphasic extraction and analysed through LC-HRMS-based untargeted metabolomics. Data processing and annotation were performed using Mzmine4, GNPS tools and Sirius6. Shotgun whole genome sequencing was carried out on an Illumina NextSeq 2000 platform. Taxonomic assignation was performed with Kraken2 by using the NCBI RefSeq V208 nt, while relative abundance was determined at the genus level using Bracken. Both datasets were analysed in QIIME2 to determine alpha and beta-diversity. The results showed significant shift in the richness and evenness of both the meta-metabolome and the microbiome but also a significant correlation between both richness. Ordination and PERMANOVA based on (un)weighted UniFrac distances or Robust Aitchison PCA highlighted significant phylogenetic and meta-metabolome discrepancies in periphyton between months, respectively. Further correlation network (mmvec) highlighted the specific relationship between some taxa and some metabolite class at months with peculiar climatic conditions. Altogether, these results will further support better understanding of potential functional and structural impairment of microbiomes by the chemical stress in the global changes context.

Keywords: freshwater periphyton, microbiome-metabolome association study, longitudinal study, metagenomics, untargeted meta-metabolomics

Impact of a multi-metal contamination on the taxonomic and functional trait composition of bacterial communities in watercourses

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Metal contamination of watercourses modifies the taxonomic organization of local communities. These changes can lead to loss of functional diversity, potentially limited by functional redundancy. Bacteria, the first link in the trophic chains, play a major role in biogeochemical cycles and could be good bioindicators of environmental changes. We hypothesized that metal contamination could decrease the abundance of bacterial communities and modify their taxonomic composition and functional trait profiles. To test these hypotheses, samples from the water column of 40 rivers in north-eastern France with contrasted metal contamination levels, were collected during spring and autumn 2023. The abundance of bacteria was estimated through 16S rDNA qPCR and bacterial diversity was assessed using high throughput sequencing (Illumina). Bacterial trait inference was done using an actualized version of the BactoTraits database (Cébron et al. 2021), including 29 morphological and physiological traits (e.g. oxygen tolerance, halophily, pH and temperature preferences, spore formation, motility, cell length and width, Gram stain and GC content). Canonical Correspondence Analyses (CCAs) were used to relate taxonomic and trait-based changes in bacterial communities to physico-chemical characteristics of water, including metal concentrations. 22.978 OTUs were identified. Preliminary analyses revealed no significant effect of the level of metal contamination on the bacteria abundance and alpha-diversity. The level of contamination had also little impact on the taxonomic structure of bacterial communities, but specific metals (Mo, Al, Cd, Co and Mn) did. From a functional point of view, Gram positive bacteria and bacteria with a high GC content tended to be favored in metal contaminated water. This could be linked to a compromise in their energy allocation. Other traits are currently being explored and could contribute to a better understanding of the selection of traits in metal-contaminated environments. This will enable us to propose additional hypotheses on the underlying mechanisms.

Keywords: bacteria – metal gradient – freshwater – diversity – functional traits

The MicrobMonitor holobiont project: aquaculture ecosystem microbiome status as a link between the environment and fish health

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In nature, there is a link between the health status of all organisms in ecosystems through shared microbes, known as the concept One Health. Microbial diversity is a key factor regulating the health status of the host: eubiosis/dysbiosis condition of the associated microbiota strongly influences health and disease status of the host in a specific ecosystem. Given that in mind we have started a multidisciplinary One Health research holobiont project MicrobMonitor with a primary mission to tackle the subject of a “healthy” microbiome and to give answers to human, animal and environmental health problems. The project includes sampling in a closed-loop system freshwater aquaculture basin Ribnjak 1961 (Sišćani, Croatia) rearing the common carp (Cyprinus carpio). The project will integrate microbiome research, in which changes in bacterial assemblages in one aquaculture pond (poultry manure, water, sediment and fish tissue) in the hatchery/nursery system during the first 8 months of carp breeding year. Additionally, the climate change heatwave impact on the aquaculture ecosystem dynamic in regarding to microbiota-host-environment interactions will be investigated during summer period. Methodologically the MicrobMonitor holobiont project will include metagenomic NGS and network analysis to analyze alternations in microbiome community structure and function. The MicrobMonitor holobiont project intends to provide us with the answers to an important scientific question: What is a connection force between the health status of a host and the environment in a closed-loop aquaculture ecosystem through shared microbiomes, known as the concept of One Health?

Keywords: holobiont project MicrobMonitor; One Health; aquaculture ecosystem; microbiome; common carp

Acesulfame K as an Emerging Contaminant: Effects on Gut Microbiome and Neurotransmitter Dynamics in *Daphnia magna*

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The gut microbiome plays a crucial role in an organism's overall health, influencing various physiological processes, including metabolism, immune function, and development. Emerging evidence suggests that alterations in the gut microbiome can affect the production and regulation of neurotransmitters in the host organism, potentially impacting behavior and physiological responses. Acesulfame K (ACE) is an artificial sweetener widely used in food products and pharmaceuticals. Due to its chemical stability and low removal efficiency in wastewater treatment plants, ACE is increasingly found in aquatic environments, raising concerns as an emerging contaminant. Recent studies have shown that exposure to ACE can lead to gut dysbiosis in humans and rodents. Moreover, ACE exposure has been associated with changes in behavior and neurotransmitter levels in daphnids and zebrafish, suggesting a potential neurotoxic effect. To investigate the impacts of ACE on the gut microbiome and neurotransmitter regulation, we use the model organism *Daphnia magna*.

The aim of this project is to investigate (I) whether ACE exposure induces dysbiosis in *Daphnia*, (II) the effects of ACE on neurotransmitter gene expression in the host, and (III) the potential link between microbiome alterations and neurotransmitter expression. A chronic experiment was performed using Daphnia magna exposed to two environmentally relevant concentrations of ACE (1 µg/L and 100 µg/L) and a control. At different time points throughout the exposure, samples were collected for gut microbiome analysis via 16S rRNA sequencing and neurotransmitter gene expression analysis. A structural equation model was employed to explore potential relationships between microbiome changes and neurotransmitter expression. The outcomes of this project will be used to link ACE induced microbiome alterations to neurotransmitter regulation in *Daphnia magna*.

Keywords: *Daphnia magna*, Microbiome, Dysbiosis, Neurotransmitter, Artificial Sweeteners

Long-term effects of the chemical warfare agent Adamsite on the life history traits and microbiota of *Danio rerio*

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Several hundred thousand tons of chemical warfare agents (CWAs) were disposed of at sea during and after major conflicts of the 20th century, leading to widespread environmental contamination. Among these agents, Adamsite (DM [10-chloro-5,10-dihydrophenazarsinine]) is recognized as one of the most toxic and persistent. Yet, comprehensive ecotoxicological data on its long-term effects are still limited. This study aimed to fill that knowledge gap by investigating the chronic effects of Adamsite exposure on aquatic organisms, focusing specifically on its impact on the model fish species, *Danio rerio* (zebrafish).

In accordance with the OECD test no. 215 guidelines, a 28-day controlled exposure experiment was conducted to assess the effects of Adamsite on key life history parameters, including body length, body mass, and growth rate. A major focus of the study was to analyze changes in gut microbiota composition using sequencing of the V3-V4 region of the 16S rRNA gene. Gut microbiota plays a critical role in the overall health, immune function, and metabolic processes of the host, and is highly sensitive to environmental pollutants. In addition, tissue samples were collected to determine the bioaccumulation potential of Adamsite in the muscles of fish.

The findings of this study provide valuable data on the potential ecological risks posed by Adamsite and contribute to a better understanding of the long-term environmental consequences of CWAs. Notably, significant alterations in microbiota composition were observed in the exposed fish, suggesting that even trace concentrations of Adamsite can disrupt the delicate balance of microbial communities, which may have cascading effects on host physiology and disease resistance. This underscores the importance of microbiota as a sensitive bioindicator of environmental stress in ecotoxicological studies.

The research was financed by grant no. 2020/37/N/NZ8/04099 (National Science Centre, Poland).

Keywords: Sea-dumped munitions; Zebrafish; DM; Long-term toxicity; Intestinal microbiota

Evaluation of the impact of biological nitrification inhibitors in different experimental scales

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The application of biological nitrification inhibitors (BNIs) is a promising tool for reducing nitrification, the microbial conversion of ammonium-based fertilisers to nitrate, and mitigating the fluxes of N loss through leaching and production of the greenhouse gas nitrous oxide. While synthetic nitrification inhibitors (SNIs) have been widely used, they often have a narrow effect in a restricted range of soils or on a restricted selection of target organisms, and concerns exist regarding their sustainability and cost. While biological nitrogen inhibitors (BNIs) represent an attractive alternative to SNIs, their efficacy and impact on non-target organisms must be evaluated. Agricultural soils “Halastra” and “Ecully” from cultivated areas in Greece and France respectively, were used to establish microcosms and 5 BNIs 1.9 decanediol, MHPP, Zeanone, its analogue MQN as well as Sakuranetin, and tested at a range of application rates, measuring ammonium and nitrate concentrations up to 35 days after incubation. Despite previous studies demonstrating efficacy using other assay types, all compounds had limited efficacy in reducing nitrification rates compared to the application of established SNIs such as Ethoxyquin and DMPP individually or as a mixture. Dissipation analyses indicate that BNIs Zeanone, MQN and Sakuranetin were rapidly transformed in soil and may explain why limited activity was observed at the rates added. Current work is comparing this soil microcosm approach with other widely used assays involving potential nitrification assays (soil slurries) or individual strains grown in pure culture to determine whether assay choice impacts evaluation of BNI efficacy and whether some ex situ approaches have limited relevance in predicting *in situ* activity.

Keywords: biological nitrification inhibitors, experimental complexity

Biotechnological evaluation of chitinases of Shewanella basaltis strain isolated from the Mediterranean Sea: application as bioisecticide and Fungicide against legume pests

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Since antiquity, chemical pesticides were the most used means for the fight against the increase in damage caused by pests in agricultural crops. But growing concerns of man in relation to the reliability of these means and adverse effects of these pesticides for the environment led him to design more efficient and healthier ways to protect crops and seeds. Today, and this new approach, bio pesticides are in the process of taking over the place in the markets for pesticides and their use became more widespread. These new ways, were more effective and have attracted, thus more interest and investment. Bio pesticides are mainly from bacteria. They have been the subject of biological study. Chitinases have stimulated interest in various biotechnology applications due to their ability to degrade chitin in the cell walls of fungi and insects, which leads to the use as antifungal agents and insecticides. The research in this study are part of this axis and aim to screen the chitinolytic strains and select a candidate bacterium for the production of chitinases low cost using marine biomass as the sole carbon source and nitrogen. Among the seven strains grown on rich medium colloidal chitin, a labeled "SM 50" showed a degree of hydrolysis of chitin 94%. The morphological study of the strain allowed describing as a coccobacillus mobile Gram negative. The biochemical and phylogenetic identification revealed a percent similarity of 98% with Shewanella basaltis. To enhance bacterial growth and the production of chitinases, the physico-chemical parameters were optimized. The results have mounted a very significant production of chitinases pH 8, at a temperature of 30 ° C and a salinity of 30 mg / l. The insecticidal activity tests chitinases against weevil wheat and fungicide against Fusarium oxysporium responsible for fusarium wheat was made. The results revealed a broad spectrum of antifungal and insecticidal activity (zones of inhibition) (mortality) in function of the doses tested.

Keywords: chitinanases; Shewanella basaltis; bioinsecticide; biofongicide

Cytotoxic and behavioral effects of exposure to secondary metabolites produced by *Trichodesmium* blooms on the RTgill-W1 fish cell line and fish larvae

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Blooms of toxic cyanobacteria are increasing worldwide and are expected to be accelerated by global warming. These complex environmental events can cause, among others, severe ecosystem degradation, wildlife mortality, threat to nations' drinking water supplies, and human health issues. The methodological advances and the integration of biochemical and genetic tools have allowed us to expand our knowledge on cyanobacterial diversity and the biosynthesis of potent toxic compounds. Yet, the identity of bloom-forming species and the toxins they produce remain poorly understood. Despite numerous toxins have been described for the marine cyanobacterium *Trichodesmium*, only a few studies on its toxic effects are available. Here, we assessed the cytotoxicity and behavioral effects of the crude extract of Trichodesmium blooms. Cell viability assays in-vitro using the cell line RTgill-W1 from gill tissue of the rainbow trout showed a significant decrease in cell viability when exposed to fractions VLC1-3 of the crude extract after 72 hours at a concentration of 10 µg/ml, and when exposed to fraction VLC3 after 48h at concentrations as low as 0.01 µg/ml. Further fractionation of the crude extract was used to narrow down the fractions that showed more bioactivity. A significant decrease in cell viability was observed when RTgill-W1 cultures were exposed to fraction HPLC5 and HPLC7 after 24 and 48h, to concentrations of 10 µg/ml and 1 µg/ml. In-vivo behavioral assays with sand goby appeared to show an effect of exposure to fraction VLC3 on locomotion, but results were not conclusive. Further behavioral assays with zebrafish larvae did not show a significant effect on locomotion changes when exposed to fraction VLC3. Our findings provide evidence of the potential risk (cytotoxicity) of *Trichodesmium* blooms to the environment and human health, and call for thorough analyses of these blooms and further evaluations of the potential toxic effects.

Keywords: behavioral assay, cyanotoxins, cytotoxicity, secondary metabolites, *Trichodesmium*

In vitro evaluation of endpoint values (EC50) of nineteen pesticides on the arbuscular mycorrhizal fungus *Rhizophagus intraradices*

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Pesticides as the key agricultural input essential in crops protection to control weeds and diseases. Unfortunately, they can severely affect non-target microorganisms as arbuscular mycorrhizal (AM) *fungi*. Based on the core objectives outlined by the European Commission, it is essential to evaluate the effects of pesticides active ingredients (a.i.) before their release on non-target microorganisms prior to market authorization, using a standard, easy-to-use and reproducible method.

To this end, a fast-track *in vitro* cultivation system was employed, utilizing 12-wells microplates containing individual spores of the AM fungus *R. intraradices*, which were exposed to increasing concentrations of pesticides a.i., specifically at 0.01, 0.1, 1, 10, and 20 times the recommended field concentration (FC). This allowed the calculation of endpoint values (EC50), as defined by Papadopoulou et al. (2020), corresponding to the concentration that causes a 50% reduction in AM fungal germination percentage. A total of nineteen a.i. (6 fungicides, 7 herbicides, 6 insecticides) with different mode of action were tested.

Among insecticides, only 3,5,6-Trichloro-2-pyridinol exhibited an EC50 lower than 20 times the FC. Similarly, among herbicides, only glyphosate presented an EC50 below 20 times the FC. In contrast, for fungicides, flutolanil and hymexazol were the only compounds with an EC50 exceeding 20 times the FC, whereas fludioxonil completely suppressed the germination percentage (< 0.01x the FC) of *R. intraradices*. Overall, insecticides and herbicides demonstrated relatively minor effects, whereas fungicides showed the most significant impact on the germination of *R. intraradices* among the three families of pesticides evaluated.

Our results also highlight the efficient and fast approach of the in vitro assay for investigating the toxicity of pesticides on these important soil microorganisms.

Keywords: arbuscular mycorrhizal *fungi*, fast-track *in vitro* system, pesticides, endpoint values (EC50), spore germination percentage.

The MicrobMonitor holobiont project: aquaculture ecosystem microbiome status as a link between the environment and fish health

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In nature, there is a link between the health status of all organisms in ecosystems through shared microbes, known as the concept One Health. Microbial diversity is a key factor regulating the health status of the host: eubiosis/dysbiosis condition of the associated microbiota strongly influences health and disease status of the host in a specific ecosystem. Given that in mind we have started a multidisciplinary One Health research holobiont project MicrobMonitor with a primary mission to tackle the subject of a “healthy” microbiome and to give answers to human, animal and environmental health problems. The project includes sampling in a closed-loop system freshwater aquaculture basin Ribnjak 1961 (Sišćani, Croatia) rearing the common carp (Cyprinus carpio). The project will integrate microbiome research, in which changes in bacterial assemblages in one aquaculture pond (poultry manure, water, sediment and fish tissue) in the hatchery/nursery system during the first 8 months of carp breeding year. Additionally, the climate change heatwave impact on the aquaculture ecosystem dynamic in regarding to microbiota-host-environment interactions will be investigated during summer period. Methodologically the MicrobMonitor holobiont project will include metagenomic NGS and network analysis to analyze alternations in microbiome community structure and function. The MicrobMonitor holobiont project intends to provide us with the answers to an important scientific question: What is a connection force between the health status of a host and the environment in a closed-loop aquaculture ecosystem through shared microbiomes, known as the concept of One Health?

Keywords: holobiont project MicrobMonitor; One Health; aquaculture ecosystem; microbiome; common carp

MICROSENSE : Development of a novel microbial bioindicator/biomarker to evaluate biodegradation potential of micro- and macro pollutants in environmental matrices (soil and water)

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Numerous harmful chemical compounds from urban, agricultural and industrial activities are continuously released into the terrestrial environment. The scientific community is increasingly focused on detecting, understanding, and controlling these agents that harm human health and ecosystem sustainability. Monitoring these toxic substances is challenging and costly, mainly due to the complexity and expense of identifying the chemicals involved. Despite many analytical methods, collecting sufficient samples promptly remains a significant obstacle in assessing environmental damage. Moreover, traditional chemical analyses have limited environmental applicability because they do not account for the effects on organisms or the interactions between substances (additive, antagonistic, or synergistic), nor their bioavailability. Researchers, therefore, emphasize the need for biological methodologies to achieve a comprehensive ecosystem approach.

The MICROSENSE project aims to investigate interactions and links between pollutants, nutrients, and the intrinsic capacity of microbiomes to metabolize these substances. To achieve this goal, we combined analytical, enzymatic, and metagenomic methods to identify microorganisms, genes, and enzymes involved in the metabolization processes of these compounds. The complementarity of these technologies enables the detection of the dynamics and functioning of microbial communities in the different studied matrices (soil and water/wastewater) and the identification of relevant bioindicators/biomarkers. The first step of the project was to conduct a state-of-the-art review and select several micro and macro pollutants of interest in Wallonia (Belgium). A pilot test with several bioreactors, in which activated sludge was treated with a dose of previously selected pollutants, was developed. Several monitoring campaigns were carried out. Various physico-chemical, enzymatic, and microbiological parameters are analyzed to determine the presence of a unique pollution indicator (gene, enzyme, indicator species, etc.). The final objective of the project is to develop a field kit to evaluate the potential contamination of the environmental matrix of interest.

Keywords: Micropollutants ; phytopharmaceuticals ; bacterial bioindicator ; bacterial biomarker

Algal-bacterial interactions increase toxicant sensitivity in algae

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Biological complexity and species interactions play a pivotal role when assessing toxicity, however, it is commonly overlooked in bioassays. We investigated if species interactions (*i.e.* algal-bacterial interactions) alter toxicity in algae. The green microalgae *Chlorella vulgaris* was exposed for 72h to the herbicide terbutryn and to the antibiotic ciprofloxacin in single populations and in algal-bacterial consortia with the bacteria *Pseudomonas putida*. Effect data on algal growth inhibition concentration-responses tests were obtained using by chlorophyll fluorescence measurements as proxy of algal biomass. For terbutryn, algae in single-species tests showed an EC50 of 11.6 [10.9, 12.2] nM and in algal-bacteria consortia tests an EC50 of 8.4 [8.01, 8.4] nM. These results indicates that algae are more sensitive to terbutryn when exposed in algal-bacteria consortia, which is explained by interspecific competition. Algae and bacteria compete for the nutrient resources, when algae present the receptor of the target of the toxicant (*i.e.* photosystem II) its growth is inhibited and, in turn, its capacity to uptake nutrients. In these unfavorable conditions algae are less competitive to uptake nutrients, and the growth of the surviving cells might be limited by the lack of nutrients that the major competitor (*i.e.* bacteria) is able to uptake. For ciprofloxacin, algae showed the same sensitive when exposed in single populations (i.e. EC50=19.1 [18.8, 19.3] µM) and in consortia (i.e. EC50=19.9 [19.6, 20.2] µM), which is explained for the lack of interspecific competition at the tested concentrations since ciprofloxacin killed all bacteria at the tested concentrations. In aquatic ecosystems where algae live in consortia with other bacteria and other microorganisms forming ecological communities, such competitive processes might increase the sensitive of algae to toxicants. Hence, natural phytoplanktonic communities might be more impacted by toxic exposure than predicted from single-species tests alone.

Keywords: competition, phytoplankton, terbutryn, ciprofloxacin, EC50 values

Evaluating the potential impact of pesticides on soil microbial communities: Integrating *in vitro* single species tests of soil nitrifiers with in soil tests

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Soil microbial communities are crucial for maintaining ecosystem functions and services, yet their sensitivity to pesticide toxicity remains underexplored. Outdated protocols hinder accurate assessment, necessitating a shift towards modern methodologies. Recent benchmarking research highlights ammonia-oxidizing microbes (AOM) as valuable indicators due to their functional significance and sensitivity to stressors. Building upon prior *in vitro* investigations by our group on liquid cultures of soil representative nitrifiers, our study extended this research to examine pesticide toxicity on AOM (in parallel with effects on the diversity of broad phylogenetically distinct microbial domains) in three soils with varying physicochemical properties. Our research aimed to provide a more ecologically relevant assessment of the toxicity of pesticides on natural soil microbial communities using classic and advanced omic tools in an ecotoxicological context. The soils were exposed to the recommended (1x) and 5x and 10x the recommended dose of a fungicide (pyraclostrobin) and two herbicides (glyphosate, metsulfuron-methyl), which exhibited notable toxicity in prior in vitro single-species tests with a range of nitrifier strains. Key toxicity endpoints, such as NO3- levels, were monitored, and the abundances of the amoA, nxrB, 16S rRNA, and ITS genes for AOM, nitrite-oxidizing bacteria (NOB), total prokaryotes, and fungi, respectively, were quantified using q-PCR. Amplicon sequencing analysis monitored the effects of pesticides on the diversity of ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), total bacteria and fungi. These data are pending and will be used for the construction of Species Sensitivity Distributions (SSDs) that will allow us to obtain group-specific Hazard Concentration values 5% (HC5) for AOA, AOB, bacteria and fungi but also to compare and contrast with SSDs constructed for AOM using single-species derived EC50 values. By intergrating single-species tests and amplicon sequencing data, we aim to implement new approaches and tools for more ecologically relevant pesticide toxicity assessments on soil microorganisms.

Keywords: ammonia-oxidizing microorganisms, microbial indicators, pesticides toxicity, risk assessment scheme

Pesticide mixture effects on bioaccumulation and toxicity in biofilms

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Pesticides exert a significant risk to aquatic ecosystems, including biofilm communities composed of microorganisms (microalgae, bacteria and fungi) embedded in an extracellular matrix. Biofilms are at the basis of food webs. They are good indicators for water quality assessment as they respond quickly to contamination, and pesticides are toxic to biofilms. Pesticide properties (logKOW and mode of action), influence their biological responses and bioaccumulation. However, the toxicity was mainly shown for single compound exposures, whereas pesticides always occur in mixtures. Moreover, bioaccumulation is not always a linear function of exposure concentration, implying that concentration measurements do not provide an accurate risk indicator. There is a need to understand the mixture effects (competitive, additive or synergistic) of pesticides in biofilms, because the pollution linked to pesticides is considerable and continues to increase. In order for current regulations to be as close as possible to the environmental reality and to consider the real toxic effect of pesticides on biofilms, it is essential to understand the inherent mechanisms.

We, therefore, propose establishing a predictive model to determine pesticide mixture impact on bioaccumulation and toxicity in biofilms according to pesticide properties and biofilm composition. Given the high number of pesticides on the market, it is impossible to study all possible combinations of mixtures in the environment. This is why the main mechanisms that govern bioaccumulation and toxicity caused by pesticides need to be parameterised and simplified in a predictive model. The mixture effect will be assessed and added as a factor in the predictive model. Ultimately, the predictive model will be adjusted with the biochemical composition of the biofilm. The fact that bioaccumulation and toxicity are linked to measurable parameters makes the use of this model universal.

Keywords: Pesticide mixtures, Biofilms, Predictive model

Effect of herbicide exposure on ammonia and nitrite oxidizing microorganisms and their subsequent resistance to drought

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Soil microorganisms are important for ecosystem functioning, where their involvement in nutrient cycling makes them an essential part in agricultural systems. However, agrochemicals like pesticides can affect these non-target organisms and we currently lack standardized test methods targeting soil microbial function and diversity in environmental risk assessments of pesticides. Bacteria and archaea involved in nitrification have been previously identified as relevant indicator organisms for the effects of anthropogenic disturbances. Nitrification is the two-step process, where ammonia is first oxidised to nitrite and then further to nitrate by different microbial groups. The aim of this study is to assess the effect of herbicides on the abundance and activity of ammonia and nitrite oxidising microorganisms and if pesticide exposure affected their resistance and resilience when subject to an additional stressor. For this purpose, we set up a microcosm experiment in a climate chamber under controlled conditions where an arable soil was first exposed to the herbicides clopyralid, an auxin mimicking substance, metribuzin, a photosystem II inhibitor, and tembotrione, a 4-Hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor and monitored during ten weeks and then to osmotic stress caused by drying and rewetting followed by a recovery phase. The active ingredients were applied at 1 ▯ and 10 ▯ of the recommended dose according to EFSA guidelines. Microbial abundances of the functional guilds as well as the soil nitrate pools indicating nitrification activity was not affected by pesticide exposure regardless of dose three and ten weeks after application. By contrast, the following exposure to drying-rewetting stress had a comparably much stronger effect, but the effects of the osmotic stress were similar in the contaminated and uncontaminated soil. This study highlights the use of combining end-point measurements on indicator organisms with effects on ecological processes (e.g. resistance and resilience) in environmental risk assessment of pesticides on soil microorganisms.

Keywords: soil microbial community, pesticides, nitrification, drought

Developing the first fungal toxicity test for Antarctica using an endemic fungal species

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Antarctica, once an untouched wilderness, now faces escalating anthropogenic pressures, particularly in its limited ice-free regions. Station operations, scientific research and tourism have led to increased pollution in Antarctic soil, particularly from hydrocarbons and metals. There are no Antarctic specific environmental guidelines for contaminants available. This means there are currently no standards to determine risk to environments, or in contaminated sites, if remediation has been sufficient for the soil to be returned to the environment. We are helping to address this gap through the development of the first native Antarctic fungal toxicity test.

The use of traditional single-species toxicity assays in Antarctica are challenging due to the lack of standard test taxa, the extreme conditions, and strict biosecurity regulations, necessitating in-house solutions for ecological risks assessments. While toxicity tests have been performed using several Antarctic species, including plants, invertebrates, and algae, a major gap exists in understanding fungal sensitivities. Fungal communities are critical to a healthy ecosystem, even in Antarctica. Thus, it is vital that fungal decomposers and nutrient cyclers are included in regulatory frameworks, enhancing the ecological relevance of future risk assessments in Antarctica.

We screened 110 Antarctic fungi isolated from the soils of Robinson Ridge, in eastern Antarctica, for their sensitivity to hydrocarbons. Of these, 62 isolates maintained stable growth, 16 were stimulated by hydrocarbons, and 25 showed no clear response. A concentration-response relationship was shown in seven fungal isolates, which are now the focus for single-species toxicity test development. In addition, we are using amplicon sequencing to understand how fungal communities respond to active bioremediation at Australia’s Casey station. This research will contribute significantly to understanding Antarctic ecology, enhancing risk assessment methods, and understanding the impacts of contaminants and human activities in the Antarctic environment.

Keywords: Bioremediation, Hydrocarbons, Metals, Soil, Risk Assessment

Assessing the health of marine ecosystems through microbial communities: Evidence from sediments of the eastern Adriatic coast

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Despite the importance of microorganisms in functioning of the marine ecosystems, as well as their known responsiveness to environmental change, they have been overlooked in the Marine Strategy Framework Directive (MSFD). As an instrument for protection of the marine ecosystems MSFD aims to achieve good environmental status through 11 descriptors, but biologically focuses on macroorganisms for monitoring. The MicroLink project, funded by the Croatian Science Foundation, investigates the potential of microbial communities residing within polluted coastal sediments, as indicators of marine health, a concept that has not yet been considered in EU directives. Our study involved collection of 67 sediment samples from 7 bays and ports within the eastern Adriatic coast classified as “ecosystems at high risk“ with bad or poor ecological status but also from respective areas which are considered as reference sites based on the limited anthropogenic interventions they are exposed to. As sediments act as sinks for pollutants they provide a stable image of the effects of contamination on the whole ecosystem. The sediments in certain areas showed high levels of contamination, including metals and tributyltin, which led to a 5- level classification according to “anthropogenic disturbance”. To establish a link between benthic environmental conditions and microbial community structure and diversity, we analyzed both the chemical and microbiological components of sediment samples. The use of DESeq2 and classification and regression tree methods provided a better understanding of microbial responses to the anthropogenic disturbance. Prokaryotes were identified as the most promising group of microbes for further research, with specific bacterial families (*Rhodobacteraceae*, *Ectothiorhodospiraceae*, *Cyclobacteriaceae*) and genera (*Boseongicola*, B2M28, Subgroup 23, Sva0485, *Thiogranum*) proposed as potential indicators of environmental status. Finally, predictive models were developed to identify key indicator variables for assessing anthropogenic impact in sediments. This study advances the discussion on the integration of microbial indicators in the assessment of marine ecosystem status.

Keywords: microbial community, marine sediment, anthropogenic disturbance, biomonitoring, Adriatic Sea

Understanding Variability in Arbuscular Mycorrhizal Fungi Responses to Pesticides: Impact of Fungicide Application Methods and Dosages on AMF sensitivity

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Research on arbuscular mycorrhizal fungi (AMF) responses to pesticides reveals significant variability due to factors like type of chemical, dosage, soil conditions, timing, and species involved. Therefore, standardized tests enabling a risk assessment are needed. Such tests must evaluate both, direct and indirect effects of AMF response to pesticides within regulatory frameworks.

In a controlled greenhouse study, we examined how fungicide application affected AMF by analyzing its impact on colonization rate and plant growth using corn as a host plant (*Zea mais*). Pelletized AMF inoculum (*Rhizophagus irregularis*) was used to deliver precise doses, ensuring consistency across treatments. Our aim was to assess AMF sensitivity to Azoxystrobin at three application rates (1x, 2x, and 20x recommended dose), using three application types (mixing into substrate, foliar application, drenching) combined with varying AMF introduction timing.

Plant growth (shoot and root biomass) remained unaffected by introduction of inoculum or fungicide across all treatments in foliar and drench applications, including controls. However, plants exhibited delayed initial growth with soil-mixed fungicide. Foliar application with initial AMF introduction resulted in no significant deviation in mycorrhizal colonization compared to controls, suggesting plant-induced physiological impacts rather than substrate effects. Simultaneous application of fungicide and AMF at 1x dose showed no difference, but higher doses increased colonization rates. Drench application with initial AMF introduction showed no notable effects at the lowest dosage but reduced colonization at 2x, and 20x doses. Simultaneous application of pesticide and AMF did not change the colonization rate. Mixing of Azoxystrobin into the substrate from the start suppressed colonization even at the lowest dose, with no colonization observed at higher doses.

The results highlight the need to consider application methods and dosages in pesticide risk assessments for AMF, as the sensitivity depends on how and when the mycorrhiza interacts with the pesticide and the plant.

Acknowledgment: This work has been performed in the frame of the MSCA-ITN-EID-Horizon2020 project ARISTO Grant No. 956496, funded by the European Commission.

Keywords: Arbuscular mycorrhizal fungi, pesticide, risk assessment

Evaluating PFAS Impacts on Mytilus edulis via Spiked Microalgae: Integrating qPCR, Biochemical and Lipidomic Approaches for Coastal Monitoring and Risk Assessment

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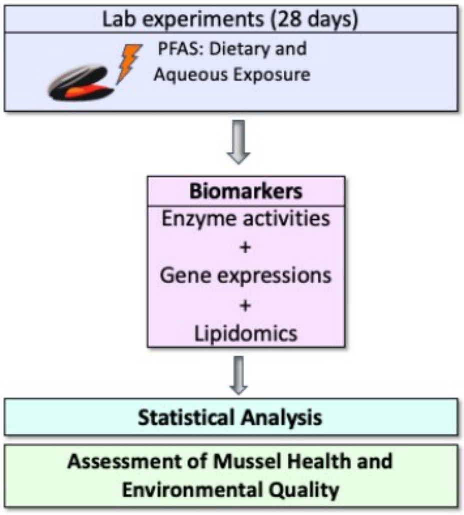
Per- and polyfluoroalkyl substances (PFAS) are highly persistent and harmful chemicals with complex environmental transport behaviour. These substances, used in various industrial and commercial applications, are eventually released into the environment. With over 4000 non-naturally occurring chemicals, PFAS have emerged as a concerning class of widespread environmental contaminants. Despite growing concerns due to their bio accumulative and toxic properties, there remains a significant knowledge gap regarding biota exposure to PFASs.

In this research, the impact of PFAS is assessed by exposing the blue mussels, Mytilus edulis, to PFAS mixtures, mimicking environmental contamination through water and microalgae- *Tisochrysis lutea*, in controlled tanks. The analysis compared control mussels with PFAS-exposed ones, evaluating health effects using various biomarkers for oxidative stress, detoxification and neurotoxicity. Early molecular responses were investigated by measuring the relative expression of genes, associated with cell proliferation, apoptosis, cellular stress, and energy metabolism through RT-qPCR analysis. Furthermore, a lipidomic analysis was performed to investigate alterations in lipid profiles in response to varying exposure conditions.



This research sheds light on the biochemical and molecular responses of blue mussels to simultaneous exposure to PFAS through waterborne and foodborne routes. By enhancing our understanding of the risks associated with these contaminants, the findings contribute significantly to the field of ecotoxicology and aid in the development of more effective management strategies.

Keywords: PFAS, micro algae, multi -biomarker approach

Benthic diatoms as indicators of pesticide pollution in Swiss watercourses.

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Protecting aquatic ecosystems from pesticide pollution and other micropollutants is a critical environmental priority. Benthic diatoms, commonly used to indicate nutrient pollution, exhibit variable species sensitivities to pesticides. Therefore, we ask if they could also serve as effective bioindicators for pesticide pollution.

To identify potential benthic diatom taxa that are specifically sensitive or tolerant to pesticides, we conducted a laboratory study using flow chambers as microcosms for periphyton colonisation in a closed and strictly controlled system. We continuously exposed the periphyton to 44 treatments, consisting of combinations of pesticide stress levels (36 levels of Toxic Unit mix ranging from 0 to 6.02) and three environmental factors (i.e., light intensity, temperature, and nutrient levels) over a 23-day colonisation period.

Following colonisation and exposure, we analysed the diatom community composition and structure using both morphological and molecular identification techniques. The morphological assessment of the diatom community composition, based on Principal Component Analysis, revealed no significant correlation with pesticide stress, while light intensity had a significant impact. At the species level, preliminary results based on a beta regression analysis indicate the potential to identify indicator species for pesticide stress. Community level periphyton functions and structures (i.e. effective photosynthetic activity, chlorophyll-a content, ash-free dry weight and bacterial abundance) showed no significant differences among pesticide treatments. We hypothesise that the lack of effects on these periphyton descriptors this is due to functional redundancy happening through compensatory dynamics and species complementarity.

These findings suggest that certain diatom species could be promising bioindicators for pesticide pollution. Further experiments at the community level and on single species are required to validate this hypothesis. Ultimately, this research could serve as a step towards developing a diatom based- indicator for pesticide pollution, which might improve the assessment and management of pesticide impacts on aquatic ecosystems.

Keywords: Benthic diatoms, Bioindicators, Pesticides

Bacterial biodiversity distribution in the Beauce aquifer: towards defining new indicators of water quality

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Groundwater accounts for nearly 99% of the liquid fresh water circulating on our planet. In the Water Framework Directive, groundwater quality assessment is carried out through targeted chemical analysis but does not consider its ecological state. Microorganisms are present up to great depths and contribute to the geochemical state of water and to pollution mitigation as well as harboring biodiversity, thus contributing to many above ground ecosystem services. However, we lack extensive knowledge of microbial diversity and activities in these environments and few significant studies are available although knowledge of groundwater microbial biodiversity and its drivers could lead to a better understanding of the biogeochemistry of aquifers, the impact of pollution and the identification of new indicators, thus assisting groundwater management.

The work presented here aimed to analyze groundwater microbial diversity on a large scale to establish the existence of a biological signature linked to the hydrogeochemistry with the hypothesis that differences could indicate disturbances. Sixty samples were collected from the Beauce aquifer (France). This aquifer, located between approximately 50 and 70 m depth, includes areas connected to the surface and confined zones and various anthropic and geogenic pressures (pesticides, nitrates, arsenic, etc.). Samples were collected in spring 2021, DNA was extracted from the filtered biomass and microbial diversity assessed through 16S rRNA gene metabarcoding (Illumina). Physico-chemical data highlighted three hydrogeochemical signatures, one with oxygenated conditions and high nitrate contents, one in the confined zone with anoxic conditions and high dissolved iron and manganese contents and one at the interface. The analysis of sequencing data alone and then in conjunction with hydrogeochemical data makes it possible to accentuate the differences between the three zones and highlight points whose signature does not correspond to that expected, highlighting possible dysfunctions linked to anthropogenic pressures and opening new possibilities for water quality assessment.

Keywords: groundwater, biodiversity, indicators, anthropogenic pressures

Characterisation of Cu Bioreporter and Optimisation of Bioassay

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Heavy metals, such as copper (Cu), are essential for living organisms, their normal growth and metabolism. However, at elevated concentrations they can be harmful to biota and the environment. In recent years, bioluminescent whole-cell microbial bioreporters have been successfully used as biological tools for the assessment of heavy metal availability and toxicity. However, the sensitivity of bioassays can be affected by the physiological status of microbial cells and the bioassay conditions. These influences are rarely examined. This study investigated the influence of growth stage, cell density, washing media and addition of glucose on Cu bioreporter sensitivity. The effects were explored through two endpoints, microbial respiration and bioluminescence.

The growth phase and cell density of bioreporter had a significant impact on the bioluminescence response and the bioreporter's sensitivity to Cu. Washing the bioreporter cells with Modified Minimal Medium (MMM) and Davis Minimal Medium (DMM) increased the sensitivity of the bioassay. The Lowest Observed Effective Concentration (LOEC) for the bioreporter cells washed by MMM medium was 0.005 mg l -1 Cu, indicating the highest sensitivity of washed bioreporter to Cu solution. The addition of glucose to washing media caused a noticeable improvement in the sensitivity of the Cu bioreporter to Cu2+. To better understand the effects of different glucose concentrations on the bioassay performance, respiration was measured and compared with the luminescence response. The statistical analysis indicated a positive linear relationship between the bioluminescence and respiration. This study demonstrated that the sensitivity of the bioassay to Cu could be improved by washing media and addition of glucose. Further studies are needed to test the effects of bioassay conditions on its performance and sensitivity.

Keywords: Bioreporter , bioassays, bioluminescence

Assessing the efficiency and the side effects of atrazine-degrading biocomposites amended to contaminated soil

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Even decades after being banned in Europe, atrazine can still be found in soils. While bioaugmentation using pesticide-degrading bacteria is already employed as a strategy for remediating polluted soils, there is still a need to improve its efficiency. Therefore, investigating the use of carrier materials to deliver and stabilize pesticide-degrading microorganisms *in situ* emerges as a promising approach for further exploration. Here, we generated atrazine-degrading biocomposites by cultivating either a single strain or a 4- species bacterial consortium as biofilms on zeolite, which serves as the carrier material. Using a microcosm experiment, we then evaluated their efficiency to mineralize 14C-atrazine in an agricultural soil comparing to free-living cells, and assessed the side effects of the two inoculation methods on the native soil bacterial community using 16S rDNA amplicon sequencing. We showed that, right after inoculation, the atrazine mineralization potential of the free-living cells was higher than that of the biocomposites. However, microcosms inoculated with the biocomposites exhibited significantly higher atrazine mineralization potential than those inoculated with free-living cells after 15 and 35 days of incubation, indicating a greater stability in the soil environment. This finding was further supported by an increase in the abundances of atrazine-degrading genes in soils treated with biocomposites after 35 days of incubation. We also found that the inoculation of free-living cells and biocomposites influences the diversity and composition of the native microbial community differently, and that these effects are modulated by the scenario of atrazine contamination during soil inoculation. Altogether, our results provide a thorough evaluation of the efficiency and the ecotoxicological impact of atrazine-degrading biocomposites in soil.

Keywords: Bioremediation, Atrazine, Microbial Consortia, Zeolite, Ecotoxicological Impact

Efficiency, stability and side-effects of a 2,4-D degrading strain grown on zeolite as a carrier material in three agricultural soils

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While bioaugmentation using pesticide-degrading bacteria is a possible strategy for remediating polluted soils, there is still a need to improve its efficiency to make it fully operative. Therefore, investigating the application of carrier materials to deliver and stabilize pesticide-degrading microorganisms *in situ* emerges as an interesting approach for further exploration. The application of pesticide-degrading biocomposites might support long-term microbial activity and limit unintended impacts on native soil microbial communities. Herein, we generated two 2,4-D degrading biocomposites using *Cupriavidus necator* JMP134 grown as biofilms on two carrier materials, raw zeolite and modified (surface covering with Ca/Fe- Layered Double Hydroxides (LDH)) zeolite. We hypothesized that the LDH-modified zeolite would increase the efficiency and the stability of the 2,4-D degrading strain in soils. The performances of these biocomposites were assessed in three different agricultural soils and compared to inoculations with free-living cells at three sampling times: right after inoculation,14 days, and 55 days post-inoculation. Right after inoculation, the mineralization rate of 2,4-D measured by radiorespirometry was significantly increased in all tested soils when inoculated with either free-living cells or biocomposites. However, the extent of this increase was found to be soil-dependent. After 14 days of incubation, no clear difference was detected between biocomposites or free-living cell inoculations in 2,4-D exposed soil microcosms. The experiment is still ongoing and the long-term efficiency of the different inoculation types will be assessed. Further molecular analyses based on direct soil DNA extraction and 16S rDNA amplicon sequencing will be conducted to investigate the unintended effects of the two biocomposites on the structure and diversity of the soil native bacterial communities. Altogether, our results will provide a thorough evaluation of the 2,4-D mineralizing efficiency and the unintended effects of 2,4-D-degrading biocomposites on soil bacterial communities.

Keywords: pesticide, biodegradation, ecotoxicological effects, zeolite, soil microbiome

Degradation of chloromethane by microorganisms under anaerobic conditions

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Chloromethanes are short chlorinated aliphatic hydrocarbons, widely used as solvents or as intermediates in chemical syntheses (e.g. production of silicones) and constitute, after hydrocarbons, the most common pollutants of water and soil. Most chloromethanes are naturally produced by biotic and abiotic processes, but the increase in their concentration is linked to human activity.

Little is known about the microbiological degradation of chloromethane CH 3Cl in the environment, with only a few bacterial strains identified, capable of degrading chloromethane under aerobic conditions, via the *cmu* degradation pathway. Only two additional strains have been found able to degrade CH 3Cl without O2, *Acetobacterium dehalogenans* strain MC, under acetogenesis conditions, and *Pseudomonas aeruginosa* strain NB1, under nitrate-reducing conditions.

Our project focuses on the study of new microorganisms able to degrade chloromethane under anaerobic conditions, through bioprospecting of sediments from a brackish pond on the French Mediterranean coast. Enrichment cultures were established from the sediments, to target anaerobic microorganisms, in particular sulfate-reducers. These cultures exhibit degrading chloromethane activity. The microbial diversity of these cultures was analysed. This will involve isolating strains of interest from these cultures, which could be used in biotechnological processes. The longer-term aim of this research is to highlight new pathways for the degradation of chlorinated hydrocarbons.

Keywords: chloromethane degradation, marine environment, bacterial diversity, anaerobiosis

Bacterial diversity : a new tool to better trace the origin of arsenic transported in in mine-impacted river during floods?

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Flood is the main vector for particulate metal and metalloid pollution of hydrosystems downstream of mining sites. In Salsigne (Orbiel catchment, France), a century of gold mining has generated multiple tailings piles, galleries and an open-cast quarry, which are potential sources of arsenic to the hydrosystem. Identifying the origin of the contaminated particles transported in the Orbiel during floods and assessing the relative contribution of the different sources of contamination is a major challenge for the management of this site and the protection of water resources. Due to the limitations of the geochemical approaches conventionally used, the identification of the sources of contaminated particles transported during flood events requires the implementation of new methodologies. Given the selection pressure arsenic exerts on bacterial communities, we hypothesised that the study of bacterial diversity associated with particles transported in the Orbiel river during flood could contribute to better discriminate between sources of contamination. The aim is to seek a proof of concept on the potential of the proposed approach for tracing these sources of contamination. Waters, sediments and soils were sampled during low flow conditions at various stations located directly downstream of potential arsenic sources. Water was also sampled during two flood events at the outlet of the catchment. Bacterial diversity was determined by metabarcoding. A quantification of genes involved in arsenic resistance and biotransformation was performed. This study provides new information on (i) spatial and temporal dynamics of bacterial diversity at the scale of a mining district and (ii) the relevance of using bacterial diversity associated with the particles transported during flood as innovative tracers to identify the sources of mobilised contamination. In this respect, our results suggest that a highly contaminated tributary draining one of the tailings piles significantly contribute to the particles transported dowstream during the flood events studied.

Keywords: bacterial diversity, arsenic pollution, tracing, mining district, flood

Mechanisms of tolerance and bioremediation potentials of bacteria in contaminated marine sediments

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Historically, the port of Šibenik was one of the most powerful maritime ports, specialized in transshipment of phosphates and artificial fertilizers, but also the general cargo such as lead, aluminum, and wood. The bioavailable fraction of metals was determined, and compared to control (clean sites), the concentrations of Li, Mn, Co, Cu, Zn, As, Cd, Sb and Pb were increased. Due to long-term and constant exposure to heavy metals and other types of pollutants (e.g. TBT), this site was expected to harbor communities resistant to heavy metal pollution. Microorganisms adapted to survive in such environments present a potential base for novel bioremediation strategies. Here, we present the results of the

- Isolation of bacteria from the sediment of polluted harbor
- Identification of the isolates using 16S rDNA
- Measurement of the total and bioavailable fraction of heavy metals the bacteria are exposed to
- Determination of the tolerance of the isolates towards a range of concentrations (10-5000 ug/L?) of following metals; Ni, Cu, Cd, Cr, Sn, Pb, Co, Zn, Hg, and TBT, compared to control (E. coli K12 strain)
- Testing of the biosorption abilities of isolates by determining the residual metals in the media after incubation, and removal of bacteria
- Determination of the genetic basis of metal tolerance in the most prominent strains.

As the culmination of this work, we will propose a consortium of microorganisms (combinations of several different bacteria) capable of bioremediation of polluted sediments, depending on the types of pollution present. Engineering microbial communities to perform a specific function is currently a major challenge in modern biology. For this purpose, we will use a newly developed predictive framework, „ecological structure-function landscape modeling”. for predicting which combination among a given set of microbes will optimize a given function as a community.

Keywords: Bioremediation, sediment, metal resistance

Screening for PFAS co-metabolizing bacteria

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Per- and polyfluoroalkyl substances (PFAS) constitute a group of anthropogenic chemicals that are manufactured due to their distinctive characteristics. Recent studies have shown that PFAS ingestion is linked to several health issues. Firefighting foam from exercise facilities is a major source of environmental PFAS pollution in extreme concentrations. Microbial bioremediation for PFAS treatment has been challenging due to their persistence and the absence of fully capable degrading organisms. However, this study, rather than isolating new degraders that rely on PFAS for carbon and energy, screened for bacteria that co-metabolize PFAS. Twenty-two soil and water samples were collected from PFAS contaminated firefighter training areas in Denmark (Korsør and Værløse). Dilutions of bacterial communities from the environmental samples were screened for indications of perfluorooctanoic acid (PFOA) degradation and subsequently the detection of fluoride using a colorimetric high-throughput defluorination assay (F- release). Samples showing defluorination were subsequently selected for further investigation through genome sequencing of microbial communities. Several notable bacterial genera, previously associated with degradation of recalcitrant pollutants, were identified in the defluorinating samples, including Sphingomonas, Pseudomonas, and Stenotrophomonas. Preliminary metabolomic analyses of samples indicate that shorter chain PFAS accumulate in defluorinating samples, supporting microbial defluorination of PFAS. It was not yet possible to cultivate defluorinating strains in the presence of PFOA, likely due to the cellular stress imposed by released fluoride. Future work within the project will work towards isolation of pure defluorinating bacterial isolates.

Keywords: PFAS, bioremediation, genomics, metabolomics

Endogenous methylmercury production: implications for biomagnification in pelagic food webs

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Mercury (Hg) is a contaminant of global concern, which poses risks to human and ecosystem health due to its toxicity and bioaccumulation capacity. The biotransformation of Hg in the environment is mainly controlled by microorganisms carrying hgcAB genes and/or merAB genes, which have primarily been linked to anoxic sediment or settling detritus. However, endogenous methylation/demethylation by the gut microbiome of animals is another possibly important – yet largely unexplored – source.

This project aims to evaluate the endogenous MeHg production capacity of sediment-living invertebrates using benthic amphipods as model organism, and its contribution to the MeHg load in predators higher in the food web. Using a combination of molecular biology techniques such as digital PCR and shotgun metagenomics, experiments with isotopic tracers and statistical modelling, we will quantify the occurrence of Hg-cycling genes in amphipod guts, identify their bacterial carriers, assess the functionality of these genes, and investigate a potential correlation with MeHg levels in animals higher in the food web, using the Baltic Herring as key species.

Preliminary results show that the hgcA gene is present in the gut microbiome of amphipods in the Baltic Sea, with significant variability in the gene abundance across the species. Further results will allow us to comprehensively investigate primary consumers' endogenous Hg methylation potential (sum of methylation and demethylation capacity) and provide new knowledge on host-microbiome interactions in the ecological context of mercury bioaccumulation and biomagnification.

Keywords: Endogenous methylmercury production, Gut microbiome, Invertebrates, Bioaccumulation, Baltic Sea

Bioremediation of isoproturon and sulfamethazine : linking microbial community composition and structure to degradation capacity

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The large use of xenobiotics in current agricultural practices calls for methodological developments aiming at lowering their persistence in soil and aquatic environments. Bioaugmentation with complex microbial communities has been proposed as a more efficient alternative to single strain inoculation, however this method still needs further developments. Specifically, a better understanding of how degrading microorganisms must overcome environmental filtering when introduced into a new environment, and how biotic interactions with the autochthonous microbial communities might shape their colonization trajectories is needed in order to design efficient bioremediation strategies.

Here we focused our research on two xenobiotics used in agricultural practices : a recently banned herbicide still frequently retrieved in soil, Isoproturon (IPU), and a veterinary antiobiotics detectable in rivers and sediments, Sulfamethazine (SMZ). To evaluate the importance of environmental filtering, IPU and SMZ degrading microbial communities were extracted from their initial matrix, respectively soil and sediment samples, using a suspension-dilution approach, and reciprocally inoculated into the other sterilized environmental matrix. Promisingly, the extracted degrading microbial communities successfully colonized the environments in which they were introduced and their degrading potential was maintained.

We then coupled biocidal treatments with serial dilutions to construct microbial community compositional variants in order to infer associations between specific bacterial OTUs and the two xenobiotic degrading capacities. Cluster-based statistical methods were used to group community variants based on their mineralization dynamics. Different mathematical models were then trained to predict group membership based solely on community composition. Predictive power of the models was then assessed and significant associations between OTU abundances and mineralisation potentials were detected.

Next we looked at community structure and co-occurrence networks, to identify microbial groups and potential interactions - antagonist and cooperative - that play a role in the xenobiotics biodegradation. The structure of cooccurrence networks differ significantly between IPU and SMZ degrading communities, underlying compound-specific interaction patterns potentially shaped by degradation pathways. Upcoming work will evaluate the possibility of mixing degrading communities based on microbial community properties to obtain multi-degrading communities for bioremediation perspective.

Keywords : bioremediation, microbial communities, herbicide, antibiotic

Breaking news: Carbon-fluoride bond cleavage by gut microbes

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Fluorinated compounds including per- and polyfluoroalkyl substances (PFAS), are used in numerous applications, resulting in global contamination. To date, few enzymes are reported to be capable of carbon-fluorine bond cleavage. This resilience stems not only from the high strength of the carbon- fluorine bond, but also to the scarcity of fluorinated natural products and the toxic effects of fluoride on microorganisms.

Our study investigates the previously unknown enzymatic capabilities of human gut bacteria in carbon-fluorine bond cleavage in fluorinated amino acids and organic acids. Through metagenome mining and in vitro experimentation, we identified that certain gut commensals, including Clostridia, Bacilli (Firmicutes), and Coriobacteriia (Actinobacteria), encode enzymes capable of cleaving multiple carbon-fluorine bonds including polyfluorinated (-CF 2) moieties. Our findings show that these gut microbial enzymes, particularly those in the L-2-haloacid dehalogenase family, can perform both dechlorination and defluorination. To distinguish these activities, we used alanine scanning and developed a rapid 96-well fluoride detection assay to screen almost 500 single protein variants for defluorination activity. Molecular dynamics simulations, protein engineering, and machine learning revealed the crucial role of the protein carboxyl-terminal region in defluorination. Notably, we converted an inactive enzyme into a defluorinating enzyme by swapping the terminal 41 amino acids from an active counterpart.

Understanding the critical differences in enzymatic carbon-fluorine bond cleavage lays the groundwork for engineering proteins aimed at environmental remediation, particularly in addressing the persistence and toxicity of PFAS. Our discovery that gut microbial enzymes can cleave carbon- fluorine bonds provides new insights into the metabolism of (poly)fluorochemicals, with significant implications for the health of the host, microbial communities, and the environment.

Keywords: PFAS, Human gut microbes, Defluorination, Enzyme engineering, Fluorochemical toxicity

Microorganisms play an important role in the development of new nanotechnologies for water remediation

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Clean water and life below water are two of the Sustainable Development Goals established by all United Nations member states in 2015. These goals aim to ensure the availability and sustainable management of water and sanitation for all, as well as to conserve and sustainably use the oceans, seas, and marine resources for sustainable development. They play a central role in policymaking and form the basis for the European Green Deal, which includes the Chemical Strategy for Sustainability towards a toxic-free environment. These goals are strategically important but can also be seen as utopian, as achieving a completely toxic-free environment may be unrealistic. One central approach to addressing the issue of unwanted toxicants in the environment is to clean contaminated water using well-proven water remediation techniques. Here, nanotechnology can play a crucial role, offering a wide variety of new techniques and materials that can be both efficient and selective, targeting a much broader range of contaminants than traditional methods. However, a major drawback of many experimental studies is that they are performed in controlled laboratory settings without the presence of aquatic organisms. Our study indicates that the adsorption capacity of nanomaterials can be substantially lowered when algae are present due to a shift in the equilibrium state. Additionally, the toxicity could be increased due to "Trojan horse" effects. Therefore, we suggest that microorganisms should be included as a standard component in these initial tests, as they will inevitably be present in real aquatic systems where these technologies will be applied.

Keywords: Nanotechnology, water remediation, algae, microorganisms

Specialized bacterial populations and mechanisms in the mitigation of risk of polar transformation products in PAH-contaminated soils

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Genotoxicity of PAH-contaminated soils can increase after bioremediation despite effective PAH removal, which has been attributed to the formation and accumulation of polar transformation products, such as oxygenated PAHs (oxy-PAHs). These compounds are generally regarded as more toxic and leachable than parent PAHs, easily migrating to groundwaters. Biodegradation of oxy-PAHs has been reported under biostimulation conditions; however, the underlying microorganisms and mechanisms determining their fate in the environment remain to be elucidated. To understand the mechanisms involved in the biodegradation of oxy-PAHs, we stablished microcosms systems with either 9,10-anthraquinone (ANTQ) or 7,12-benz(a)anthraquinone (BaAQ) as carbon sources and creosote-contaminated soil as inoculum. Sphingobium sp. AntQ-1 was isolated from these microcosms for its ability to grow on ANTQ. The combined metabolomic, genomic and transcriptomic analysis of strain AntQ-1 allowed a comprehensive reconstruction of the ANTQ biodegradation pathway. Specific mechanisms for oxy-PAH degradation were revealed, involving the cleavage of the central ring catalyzed by Baeyer-Villiger monooxygenases (BVMO). Essential genes for the ANTQ biodegradation pathway were encoded in megaplasmid pANTQ-1. Abundance of strain AntQ-1 16S rRNA and its BVMO genes in the microcosms correlated with maximum ANTQ biodegradation rates, supporting the environmental relevance of this mechanism. A specialized BaAQ-degrading bacterial consortium was obtained by enrichment from the microcosms with BaAQ as sole carbon source. The integration of data from metabolomic and metagenomic functional gene analyses revealed that the BaAQ metabolic pathway was initiated by BVMOs with identical genetic sequences to those encoded in pANTQ-1, indicating horizontal gene transfer phenomena between members of the soil. Our results demonstrate the existence of highly specialized microbial communities in contaminated soils responsible for processing oxy-PAHs. Also, they suggest that Baeyer-Villiger oxidations, infrequent in PAH-biodegradation routes, could play a key role as a detoxification mechanism to mitigate the risk posed by the presence of oxy-PAHs in contaminated sites.

Keywords: oxy-PAHs, bacterial degradation, Baeyer–Villiger, horizontal gene transfer

In vitro and *in situ* bacterial and fungal PCB transformation

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Polychlorinated biphenyls (PCBs) are synthetic organochlorine compounds recognized as persistent organic pollutants. Present in terrestrial and aquatic environments and biomagnified throughout the food chain, they are recognized as endocrine disruptors, carcinogens, embryotoxic, immunotoxic and neurotoxic. Therefore, PCB dissemination in environments poses both an ecological and health problem. Studies have shown that PCBs can be transformed by some soil microorganisms, suggesting that bioremediation of polluted sites is potentially possible, offering a more efficient, less expensive and more eco-responsible alternative to already existing physical and chemical decontamination techniques. However, if certain microbial strains have the ability to transform these pollutants in vitro, their effectiveness for in situ biotransformation remains to be demonstrated. Furthermore, PCB transformation products are rarely analyzed and could potentially be ecotoxic.

This study aims to test the hypothesis that bioremediation of PCB polluted sites would be possible via the application of one or several microorganisms possessing enzymatic biotransformation activities. For this, we isolated a collection of 29 fungi and 17 bacteria from a polluted site and tested them for their capacities to transform Aroclor (i.e. a mixture of PCBs) and to secrete ligninolytic activities in vitro. Genomic characterization of some of these isolates is currently underway. Preliminary studies of microbial interactions between taxa and the development of molecular tools making it possible to explore, in contaminated soil, their capacities to transform PCBs in situ by bio-augmentation, applying them: alone, with bio-surfactants or in consortia. The ecotoxicity (phyto- and zoo-toxicity) of the transformation products of PCBs by these microorganisms alone is as well currently in progress.

Keywords: Polychlorinated biphenyls (PCBs), Soil microorganisms, Microbial bioaugmentation, Ecotoxicity

Bioplastics: trick-or-treat for freshwater ecosystems in the context of global change

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Plastics are present in our daily life, but their high persistence and degradation into microplastics make them a great environmental threat. Alternatively, bioplastics (BioP) have been developed. BioP are biodegradable at the end of their life and/or produced from renewable sources. They have become an alternative since they can degrade into water and carbon dioxide. In fluvial ecosystems, the inherent microbial communities could potentially decompose BioP due to their great capacity for recycling and decomposition of nutrients such as carbon. However, it is still unknown how the degradation of BioP will affect the carbon and nutrient cycles, and consequently, the effects it will have on essential ecological services such as water purification. In addition, global change, especially rising temperatures, may further accelerate their degradation. Thus, this study aims to explore and provide new knowledge on the decomposition and effects of BioP on freshwater ecosystems under different freshwater temperatures using microbial communities as a bioindicators.

Results from a microcosm study measurements of different materials decomposition and effects on functional and structural biofilm performance under different temperatures will be presented. To achieve this aim, a microcosm study is being performed at 20oC and 30oC using different materials: conventional fossil plastic, different BioPs, and leaves. After biofilm colonisation, samples were taken over a week to analyse water physicochemical parameters, nutrients, DOC, CDO, as well as functional and structural biofilm changes using several endpoints - total biomass, algal and bacterial biomass, extracellular polymeric substances (EPS), enzyme activities, photosynthetic activity. SEM observations of materials and biofilm community were also evaluated.

The results will serve to evaluate the effects of BioP on rive ecosystems and on water quality. Moreover, results will provide evidence of the effects of BioP as a potential source of anthropogenic organic matter, especially nutrients and their potential effects on carbon cycle.

Keywords: Bioplastics, biofilm, global change, freshwater ecosystems

Bacterial degradation of the toxic halogenated solvent dichloromethane in aquifer microcosms: impact of disturbances in physico-chemical parameters

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The ability of a microbial population to degrade a pollutant in situ also depends on the physico- chemical parameters it is exposed to. Dichloromethane (DCM, CH2Cl2) is a toxic industrial solvent, frequently detected in groundwater and biodegradable under both oxic and anoxic conditions. Previous work with laboratory-scale aquifers demonstrated that DCM biodegradation was more pronounced under fluctuating water level conditions. We hypothesized that matrix porosity, which strongly impacts water content and redox conditions in aquifers, affects DCM bioremediation in natural environments. To test this, we studied the effect of matrix granulometry, water content and oxygen concentration, and the response of a drastic change in oxygen concentration on DCM biodegradation kinetics and bacterial community composition.

Laboratory microcosms with beads of 2, 0.5 or 0.1 mm of diameter were set up with contaminated industrial groundwater showing DCM biodegradation. Experiments involved addition of 7 successive pulses of 1 mM DCM at different water content, i.e. water saturation, water holding capacity (WHC) or ½ WHC, and under different oxygen conditions, i.e. stable oxic or anoxic conditions, or disturbed with a sudden switch in oxygen content. The obtained results showed that oxygen concentration was the main parameter shaping both the function and the composition of the bacterial community, with anoxic conditions being the main limiting factor for DCM biodegradation and taxonomic richness. The impact of low water content was more severe at higher matrix grain size. Finally, the effect of a disturbance in oxygen content differed depending on the initial conditions, and ranged from susceptibility to disturbance to the emergence of the DCM degradation function.

Taken together, our results suggest that granulometry informs the effect of water content on DCM biodegradation and bacterial community composition. In contrast, the effect of oxygen content on DCM biodegradation was independent of grain size.

Keywords: Disturbance, laboratory microcosms, matrix grain size, oxygen content

Carbon-source dependent gene expression dictates glyphosate degradation in *Ochrobactrum sp.* strain GPr1 13

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Glyphosate-based herbicides are used extensively and globally due to their ability to target weeds in a non-specific manner. Glyphosate inhibits the production of aromatic amino acids, which not only restricts plant growth but can also prevent microbial growth. Key microorganisms, who are resistant to the herbicide, are capable of glyphosate biodegradation and, ultimately, reduce glyphosate impact on the soil microbiome. However, the specific conditions required for glyphosate biodegradation remain unconstrained. Here we show, through the RNA-sequencing of a glyphosate biodegrader *Ochrobactrum sp*. strain GPr1 13 grown under varying conditions, that glutamate is required for glyphosate biodegradation. Glyphosate was biodegraded by strain GPr1 13 as a source of phosphate and this finding was corroborated by the overexpression of the *phn* operon in the presence of glyphosate compared to the phosphate only condition. Prior work demonstrated that strain GPr1 13 was unable to use glyphosate as its sole source of carbon and the little to no expression of the sarcosine degradation genes, *soxABD*, highlights that the glyphosate degradation pathway likely ends with an accumulation of sarcosine. Notably, the ABC permease genes (*aapJQMP*) – known to facilitate the transport of glutamate into the cell – were highly and differentially expressed in the presence of glyphosate compared to the phosphate only condition, regardless of the presence or absence of glutamate. This suggests that glyphosate is transported into the cell through the amino acid permease transporter. However, despite the overexpression of these transport genes, strain GPr1 13 could only perform complete glyphosate biodegradation when glutamate was provided as a carbon source. Collectively, these data indicate that multiple mechanisms (*e.g*., transport, carbon substrate availability) directly impact glyphosate biodegradation and highlight the knowledge gaps that still exist regarding glyphosate biodegradation in a single microorganism, let alone in a complex soil microbial community.

Keywords: Glyphosate biodegradation, glutamate, RNA-sequencing

Inferring Pollutant Biotransformation Potential of Microbial Environmental Communities by Genome-resolved Metagenomics Analysis

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Our understanding of microbial pollutant biotransformation remains incomplete and is often based on studies using cultivated bacterial strains. Many environmental microorganisms cannot be cultured in laboratory conditions, and in some cases, pollutant biotransformation results from the collective actions of a microbial community rather than a single strain.

In this study, we investigate environmental microbial communities holistically by integrating metagenomic data from environmental samples with pollutant biotransformation evidence of 169 common pollutants, including pharmaceuticals, food additives, and pesticides. Our unique dataset, collected during a field campaign in Switzerland, encompasses seven wastewater treatment plants. Samples were collected from activated sludge, influent, and effluent wastewater, and riverine biofilms upstream and downstream of the treatment plant.

State-of-the-art metagenomic analysis revealed 47 million genes and approximately 15,780 medium- and high-quality metagenome-assembled genomes (MAGs), with only 20% of the genes confidently annotated through database matching. One of our key objectives is to increase the number of annotated genes to enable a comparative analysis of genotypes associated with biotransformation "phenotype" across samples. To this end, we have curated a collection of biotransformation-related genes from various sources, to construct Hidden Markov Models (HMMs) to identify distant homologs. For the remaining unannotated genes, we will apply AI-based models (e.g., CLEAN, DeepFRI) to perform functional annotation using sequence-based and structure-based predictions.

Through genome-resolved analysis, we aim to identify candidate enzymes responsible for the biotransformation of pollutants with no previously known associated genes. We anticipate that this project will significantly advance our understanding of microbial biotransformation potential and pave the way for methodologies that can harness this potential from (meta)genomic data.

Keywords: Pollutants, Metagenomics, Biotransformation

Biodegradation of PCE and 1,2-DCP by a natural groundwater bacterial community: kinetics, isotopic fractionation and evolution of the microbial community

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This study aims to characterize the biodegradation of tetrachloroethylene (PCE) and 1,2- dichloropropane (1,2-DCP) in a contaminated aquifer beneath an industrial site in the Jura region in France. These compounds are notable for their persistence and widespread presence at contaminated sites [1,2]. While PCE and 1,2-DCP degradation pathways are known [3], their degradation mechanisms specifically under anaerobic conditions and in aquifer systems have been less studied [4]. The study utilizes a multidisciplinary approach, combining molecular biology and isotope geochemistry.

The biodegradation kinetics of PCE, and 1,2-DCP were studied under anaerobic conditions using microbial communities from two on-site piezometers. Contaminant concentrations and their degradation metabolites were monitored. The microbiological analysis consisted of qPCR, amplicon sequencing of the 16s rRNA gene and metagenomics sequencing to track the diversity, abundance and activity of microbial communities capable of reductive dechlorination. Compound-specific isotope analysis (CSIA) is used to establish the initial isotopic ratios of pollutants and monitor their evolution, thereby quantifying the extent of biodegradation. A combined, carbon and chlorine CSIA approach is applied to evaluate reaction pathways involved. The changes in major ions concentrations (e.g., nitrate, nitrite, ammonium, sulfate, etc.) were measured using ion chromatography. The physicochemical conditions such as pH, conductivity redox potential and oxygen concentration were also monitored.

PCE and 1,2-DCP concentrations decreased over time, in conjunction with isotopic enrichment. The pollutant reduction rate (%) for PCE and 1,2-DCP was significant. Their degradation products were also monitored, as they were further biodegraded.

The objective was to characterize the microbial communities indigenous to the site's groundwater that are involved in PCE and 1,2-DCP degradation under aquifer-like conditions. Specifically, a focus is set on the degradation efficiency, the microorganisms involved and the genes linked to degradation. In addition, the study establishes the link between isotopic fractionation and the microbial activity responsible for pollutant degradation on this site.

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Keywords: biodegradation, isotopic fractionation, aquifer, chlorinated solvents, reductive dehalogenase

Arsenic-rich mining residues phytostabilisation: impact of microbial activities on arsenic and metals mobility and bioavailability for plants

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Securing mining residues represents a major environmental challenge, and phytostabilisation is a moderate cost option for mitigating erosion and contaminated-particles dispersion. Within the interdisciplinary oMIMo project (ANR-22-CE04-0012-0), a controlled scenario of assisted phytostabilisation was set-up in 20-L pots on two As-rich residues from former mines of Ag-Pb (Pontgibaud) and Sn (Abbaretz). Residues were amended with compost and limestone to provide organic nutrients to plants and buffer pH, planted or not with a herbaceous plant Festuca rubra found on both sites, and watered regularly with spring water mimicking natural rain. Experiment will last for two years in a greenhouse, and experimental data, acquired monthly then every six months, combined the evolution of geochemistry (pore water pH, Eh, conductivity, and chemistry, including speciation (AsIII and AsV) of As), microbial processes (most probable number of soil prokaryotes oxidising or reducing As, Fe and S, active community diversity), and bioavailability and toxicity of As and metals for plants (As and metal content in leaf, Omega-3 index). After seven months of monitoring, results showed the development of Festuca rubra on both amended residues and the establishment of an active prokaryote community in the pots. Increased numbers (as MPN) of heterotrophs, FeII oxidisers, and AsIII oxidisers were found. The later especially increased in Abbaretz pots in which Fe and As, mostly as AsV, were leached from the residue, while they both remained trapped in the Pontgibaud solid compartment.

One year monitoring of geochemical and biological (microbiology and plants) parameters, including taxonomic diversity of active prokaryotes, will be discussed according to their evolution. A deeper understanding of mechanisms (notably microbial) influencing the fate of As and metals in the residues is expected, with the final aim to develop a reactive transport model to predict As and metals mobility and availability to plants in phytostabilised As-rich mining wastes.

Keywords: microbial processes, arsenic and metals fate, mining residues, phytostabilisation

Bacteria versus PFAS: let the defluorination begin!

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Per- and poly-fluoroalkyl substances (PFAS) are a large class of often toxic xenobiotic synthetic molecules widely used in industry for a wide variety of applications. Their chemical stability leads to their ubiquitous accumulation in the environment. Microbial degradation of PFAS represents an attractive possibility for treatment of PFAS contamination, in complement to physical and chemical approaches currently being developed.

We aim to identify PFAS-degrading microorganisms and corresponding defluorination enzymes by specifically detecting the fluoride ion released by cleavage of the carbon-fluorine bond using a fluoride- specific biosensor and high-throughput microfluidics.

Ongoing work involves previously described defluorinating strains, environmental samples contaminated with PFAS from soil, rivers and groundwater, and laboratory cultures derived from them. Analyses of microbial diversity also explore the potential of microbial composition dynamics upon PFAS exposure for bioindication of the toxic effects of this class of chemicals.

Keywords: : PFAS, defluorination, toxicity assessment, bioindication

Evidence for the advantage of sequential anaerobic-aerobic transformation of toxaphene

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The insecticide toxaphene is a complex mixture of approximately 1000 highly chlorinated bicyclic terpenes. It was banned by the Stockholm Convention on Persistent Organic Pollutants. Toxaphene is more susceptible to anaerobic transformation via reductive dehalogenation and dehydrohalogenation, resulting in the accumulation of congeners with lower numbers of chlorine. The environmental fate of these anaerobic transformation products is still unknown. Previously, we demonstrated that in the presence of camphor, toxaphene may also undergo aerobic transformation, in which camphor probably served as a co-substrate in a cometabolic process. Therefore, in the present study we questioned if the products of anaerobic bioremediation pre- treatment of toxaphene (denominated PAT-toxaphene) may be further transformed under aerobic conditions. To answer this question, we used biosolids to establish an enrichment culture with camphor as the sole carbon source. Next, we evaluate the transformation of PAT-toxaphene by this culture with and without camphor. Significant transformation of PAT-toxaphene was observed both in the presence and the absence of camphor. Since cytochrome P450cam catalyses the first step of camphor degradation, we tested if metyrapone, a specific inhibitor of P450 hinders the transformation of PAT-toxaphene. Interestingly, transformation was inhibited only in the presence of camphor suggesting that transformation of PAT-toxaphene is carried by at least two different enzymes. Metagenomic analysis of these cultures reaffirmed the establishment of distinct communities in these two cultures. Vineibacter terrae and Afipia felis were dominated in the cultures that received only PAT-toxaphene (above 65% of the population) while Alicycliphilus denitrificans and Sphingopyxis soli dominated the cultures that also received camphor (above 60% of the population). Currently, we are trying to identify which enzymes may participate in the process by metagenomic analysis as well as to characterize the transformation products using gas chromatography with electron capture negative ion mass spectrometry.

Keywords: biotransformation of Toxaphene

Microbial transformation of pesticides in agricultural soils and drinking water catchments

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Intensive agricultural practices lead to high inputs of pesticides in soils, which can leach along degradation products to the aquifers, potentially contaminating drinking water sources. Microbial communities in soil and water play a crucial role in the fate and transformation of these pesticides, with an impact on water quality. A site in southwestern France (Saint-Gein, Landes) was selected as reference due to its drinking water catchment’s past and present high concentrations in nitrogen- based pesticides. In situ concentrations found in late 2023 go up to 3.4 µg/L of metolachlor ESA and 0.04 µg/L of atrazine-desethyl. Soil and underground water samples taken from this site have served as inocula for enrichments of communities, isolations of strains and in situ diversity assessment. Targeted molecules are S-metolachlor, atrazine, chlorothalonil and dimethenamid-P.

Bacterial strains from soil and water have been obtained after an enrichment process in vials with a minimum medium and pesticides as the only carbon source (400 mg.L-1 and 800 mg.L-1), followed by Sanger sequencing. Multiple strains belonging to several genera have been retrieved, such as Pseudomonas, Sphingomonas, Variovorax, Sphingopyxis, Rhodanobacter and Burkholderia. Information on communities’ diversity derived from the same enrichment process was acquired through MiSeq sequencing. Fungal strains were also obtained from an enrichment process in microplates, in a carbon enriched medium with the addition of pesticides (500 mg.L-1 and 1 g.L-1) and antibiotics.

Representatives of isolated strains were evaluated for their degradation potential. The most promising strains were then combined to create consortia, which were further tested for enhanced pesticide degradation. The most effective consortium has then been used in microcosms experiments to compare degradation rates and pathways with and without the addition of the consortium. This approach allowed us to investigate the overall fate of pesticides in soil and underground water.

Keywords: pesticides, microbial degradation, strains, consortium reconstitution

Improving monitoring and environmental risk assessment of pharmaceuticals, antimicrobial resistance and pathogens from terrestrial to aquatic environments

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Global contamination of soil and aquatic ecosystems by pharmaceutical and microbiological pollutants (such as antimicrobial-resistant microorganisms and/or pathogens) raises severe concerns about impacts on ecosystem health and repercussions on humans and animals. Preserving ecosystems from adverse ecotoxicological effects of pharmaceuticals and their transformation products and limiting the environmental spread of antimicrobial resistance and pathogens is imperative to reach several UN Sustainable Development Goals as well as the European Green Deal, Water Framework Directive and Biodiversity Strategy for 2030. To meet these huge environmental and societal challenges, public and private stakeholders and EU policy makers require improved monitoring and Environmental Risk Assessment to protect microbial diversity and functions in contaminated ecosystems.

In this context, the MSCA Doctoral Network Pharm-ERA funded by the European Union (Grant Agreement 101119261) proposes a high-level interdisciplinary and intersectoral research and training network based on 10 doctoral projects covering scientific disciplines including environmental and analytical chemistry, microbial ecology, ecotoxicology, molecular biology (incl. multi-omics approaches) and chemical fate/effect modelling. Pharm-ERA involves 9 Beneficiaries (including 2 non-academics) and 6 Associated Partners (including 5 non-academics), committed to contribute to research, training, dissemination, communication and exploitation of results targeting end-users such as Environmental consultancies and agencies. Pharm-ERA will provide EU with high-level scientific experts who will further shape and implement the next generation of environmental management strategies, EU guidelines and regulations to reduce the adverse environmental effects and risks of pharmaceuticals and the spread of antimicrobial resistance and pathogens in terrestrial and aquatic environments. This will ensure sustainability of our ecosystems, fostering positive impacts on human and animal health and well-being far beyond the Pharm-ERA project.

Keywords: Microbial ecotoxicology, Soil-water-sediment continuum, Doctoral network, Interdisciplinary

ecotoxicomic

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