Environmental Specimen Bank. © Fraunhofer IME | Kappest

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Welcome

This time we focus on molecular analytical methods. The *TrendDNA* project examines the development of the biodiversity using samples from the Environmental Specimen Bank (ESB). Further topics are the newly developed toxicogenomic methods for monitoring sewage treatment plants and test systems for understanding the soil microbiome. The portrait introduces Dr. Bernd Göckener, the new project manager of the ESB.

Yours sincerely

Prof. Dr. Christoph Schäfers

TrendDNA: a time travel into Biodiversity

More than 400,000 environmental snapshots are stored in the German Environmental Specimen Bank (ESB) archives. The samples come from various ecosystems and sampling locations throughout Germany. Since they are stored in tanks cooled by liquid nitrogen at ultra-low temperatures, all chemical and biological information is preserved, including the genetic material. Therefore, the samples can still be analyzed even after decades.

Up to now, all investigations have been focused on identifying and monitoring chemical substances as consequences of pollutant emissions. However, the presence of pollutants may have an impact on the biodiversity in an ecosystem, which has not yet been investigated in the samples.
Led by the University of Duisburg-Essen (UDE), researchers from the UDE, the University of Trier, the Senckenberg Nature Research Society and the Fraunhofer Institute for Molecular Biology and Applied Ecology IME are working together on the 1.2 million euro project *TrendDNA* funded by the German Federal Ministry for the Environment, Nature Conservation, Nuclear Safety and Consumer Protection, in developing genetic methods that will gain information on the biodiversity of German ecosystems in the future.
The project aims to use preserved DNA of the ESB samples to have a comprehensive look at temporal biodiversity trends.

In this issue you can read:

Who stays, who goes? Initial studies suggested that there have been significant declines in species numbers in areas with strong human influence in recent decades. Therefore, the experts are for example investigating fish communities from riverine sites in Germany by analyzing DNA extracted from suspended particular matter. By using high-throughput sequencing they aim to reveal the extinction of insect species and the occurrence of newly immigrating species associated with e.g. leaf and needle samples. "The potential of monitoring changes in biodiversity, using PCR-based techniques, in communities and individual populations is enormous", says Dr. Cecilia Díaz leading the project for the Fraunhofer team.
One of the greatest strengths of PCR-based techniques is sensitivity. Starting with a single molecule, millions or billions of copies can be synthesized after 32 cycles of amplification. This sensitivity is a strong tool for monitoring environmental samples containing low and degraded amounts of DNA. However, this extreme sensitivity can lead to amplification of nonconforming DNA, if appropriate quality assurance protocols, to avoid contamination with exogenous DNA, are not implemented. In *TrendDNA* we aim to incorporate DNA analysis as part of the routine monitoring of the environmental specimen bank samples. We need to establish protocols to get quality DNA from all the different specimen types, in order to guarantee the quality of the findings.

Toxicogenomic method to monitor adverse effects in water samples from wastewater treatment plants

Treated and untreated wastewater treatment plant effluent induces differential gene expression after exposure of zebrafish embryos

View of a sewage treatment plant.
Photo: © shutterstock.com 279357398

The availability of quality water in sufficient quantity is a limiting factor for the industrial location Germany as well as worldwide. Integrated and innovative approaches to process water recycling with the recovery of raw material are in demand.
At the Bitterfeld-Wolfen Chemical Park model site, a model project on the use of innovative technologies for the recovery of process chemicals and raw materials as well as water from complex industrial wastewater was carried out and included the participation of several Fraunhofer institutes (Innopush project PROWA).
By analyzing the substances prioritized in the project, it was possible to characterize the samples taken from the wastewater treatment plant process with regard to their chemical contamination. By estimating the site-specific environmental exposure during the reuse of industrial process water, this example demonstrates how the previously measured loads of the treated wastewater can be integrated into a comprehensive water assessment concept.
"However, information on the ecotoxicologically relevant mechanisms of action of the substances remaining in the wastewater treatment plant effluent has been lacking up to now", says Prof. Dr. Christian Schlechtriem, Head of Bioaccumulation and Animal Metabolism.
In this project, a modified fish embryo test was used for the first time to determine such mechanisms in differently treated effluents. The method is able to detect

specific adverse effects, such as endocrine disruption in vertebrates, using toxicogenomic fingerprints, even in environmental samples with complex compositions such as wastewater. The specific signatures can be integrated into screening approaches to monitor the efficiency of novel additional purification process steps in wastewater treatment. The results of the transcriptome studies are very promising and have demonstrated the potential of the test for future use in monitoring programs.
The toxicogenomic method was developed by the Fraunhofer Attract group Eco'n'OMICs led by Dr. Sebastian Eilebrecht.

Understanding soil microbiomes: where we are? Identification of missing test systems and endpoints

Soil microorganisms play a significant role in major processes driving nutrient cycling and bioavailability, therefore are essential for aboveground ecosystems. The tremendous diversity of soil microbiomes represents a great challenge to understand their complex ecology, hence it appears difficult to predict shifts in soil microbiome functions and ecosystem services they provide.

In the risk assessment of active substances used in plant protection products, biocides or pharmaceuticals, effects on the summary processes of nitrogen and carbon transformation (OECD 216, OECD 217) are important. However, substance influences, which remain undetected due to the functional redundancy of the microbial population, are not detected within the framework of these test methods. However, such changes in community structure may represent impairments that can reduce resilience to additional chemical and non-chemical stressors. Since soil microorganisms have a multitude of special functions, it therefore seems insufficient to assess the risks for soil microorganisms only on the basis of the results of the two previously mentioned test systems. This issue is being explored in a UBA project, headed by Dr. Schlich, where alternative systems are under evaluation to be included in the risk assessment.

Soil sample.
Photo: © shutterstock.com 575991601

Additionally, under the leadership of Prof. Dr. Bodo Philipp, Dr. Cecilia Díaz and Dr. Karsten Schlich are seeking to combine information of standard test systems and microbiome amplicon sequencing, using rRNA transcripts. "Our idea is to develop mathematical models that integrate existing genomic, physiological, and physicochemical information with metagenomic data so as to maximize information content and predictive power, in the direction of environmental protection goals", says Dr. Díaz.
For this, the use of model compounds which are readily metabolized by known specialized bacteria in soil could be used to determine the soil fitness.

If the degradation of this model compound is reduced in the presence of certain stressors (e.g. herbicides) this could be indicative of the inhibition of the bacteria involved. As a first approach, it is planned to test bile acids, which are steroid molecules that enter the soil via excretion from vertebrates. Interestingly, most of the bacterial genera that are able to degrade bile acids are also known to be potent degraders of pollutants. "In the best case, we could use a simple bile-acid degradation assay to generate genera-specific information about the resilience of a microbial population in a stressed soil", says Prof. Philipp.

»With the Environmental Specimen Bank and new, more sensitive detection methods, we can describe the environmental burden from the past and presence more and more comprehensively.«

Dr. Bernd Göckener...

...took over responsibilities as the project manager of the Environmental Specimen Bank (ESB) this year. He succeeded Heinz Rüdel, who started his retirement. Since June 2022, Bernd Göckener and his colleague Mark Bücking now share the lead of the department for Trace Analysis and Environmental Monitoring at Fraunhofer IME.

Bernd Göckener studied Food Chemistry at the University of Wuppertal und finished his Master thesis at Fraunhofer IME. In this context he worked in the field of per- and polyfluoroalkyl substances (PFAS) and optimized an analytical method which enables the PFAS burden in complex samples to be more comprehensively determined. After this, he started his PhD thesis at Fraunhofer IME, where he investigated the effects of different food processing steps on residue levels and the degradation of different plant protection products. From 2018 onwards, Bernd Göckener headed the laboratory for Environmental and Food Analysis at Fraunhofer IME. In this role, he supervised various environmental monitoring studies, for example for the German Federal Environmental Specimen Bank or different regional authorities. Additionally, he supervised accompanying chemical analyses in ecotoxicological studies under GLP.

Without the use of chemicals, our modern life would be impossible. Nevertheless, many chemicals may be of oncern if they are released into the environment. Therefore, the release of chemicals into the environment should be minimized as much as possible.
The German ESB was initiated in the 1980s to describe the environmental burden of chemicals and to store samples for future analyses. The samples are stored in a unique archive that is operated at Fraunhofer IME on behalf of the German Environment Agency. For this purpose, experts are taking samples from different ecosystems such as rivers, oceans and forests on a regular basis all over Germany and store them at cryo-temperatures. These temperatures exclude any chemical/biological alteration of the samples during decades of storage. With the help of the ESB and recent suitable analytical detection methods, trends in the environmental burden of specific chemicals can be described over several decades. Due to the cryogenic storage, this can also be done retrospectively. If the environmental burden of a chemical falls below a threshold value in the long term, regulatory restrictions by authorities can be considered as being successful.
An increasing burden, for example of newly introduced replacements of banned chemicals, indicates that regulatory measures have to be implemented or adapted.
Due to an increasing number of chemicals coming to the market the assessment of environmental quality is becoming more and more complex. Developing new and more sensitive detection methods helps to better describe the joint environmental burden of legacy and new chemicals. Thereby, we at Fraunhofer IME and our cooperation partners are actively working on environmental protection.

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