

## Proposals for NORMAN Joint Programme of Activities 2025

Title	Joint Danube Survey (JDS)- 5: The WWTP project - An integrated assessment
Type of activity	Pilot study
Leader	UFZ
Topic / activities	<p><b>Background / Justification for the proposed activity:</b></p> <p>Increasing chemical pollution is a threat to sustainable water resources worldwide. Waste water treatment plants (WWTPs) are often suspected to add to this chemical burden. This is because increasing complexity of chemical pollution mixtures entering waste water treatment plants pose challenges to common treatment technologies. As a consequence, effluents containing an unknown number of various contaminants might impact surface water bodies, with unknown effects for aquatic organisms and, ultimately, for humans. Elucidating the chemical complexity of contaminants in waste water (both influents and effluents) is required to understand the contribution of waste water treatment plants to chemical pollution of the aquatic environments and to determine the associated risks.</p> <p>The Danube River is the second largest river in Europe and an important water resource for many European countries. However, the fact that many large cities and waste water treatment plants are located in the (catchment) area of the Danube River, makes it susceptible to the entry of a large number of domestic and industrial pollutants. The Joint Danube Survey (JDS)-3, JDS-4, and many other studies have assessed different types of stressors in waste water individually, but a comprehensive characterization and harmonized evaluation of mixtures of all types of chemicals emitted via WWTP effluents to the Danube catchment are missing. The proposed JPA aims at narrowing this knowledge gap by integrating the analysis of a broad range of organic and inorganic contaminants including metals, rare earth elements, hydrophilic and hydrophobic organic micropollutants, micro- and nanoplastics assessed for example through chemical analysis, bioassays and antibiotic resistance genes.</p> <p>In the framework of the Joint Danube Survey 5, waste water influent, effluent, sludge and combined sewer overflow samples will be taken in ten waste water treatment plants in the Danube catchment. In this JPA, the samples will be analyzed by international experts using complementary tools, combining chemical analysis and bioassays, with the overarching goal being a comprehensive integrated assessment.</p> <p><b>Description of the proposed activity and expected outcomes for 2025:</b></p> <p><b>Activity 1:</b> Scientific coordination of the project An important part of this JPA is to bring together experts from different disciplines, aligning the analyses and ensuring a complementary and holistic assessment of the waste water samples. This includes the compilation of data and evaluation of results from different analyses in a comprehensive and integrated way. Results will be presented to the scientific community and public, and published in internationally recognized journals. Further, this project is committed to giving advice to policy makers. → <b>UFZ</b></p> <p><b>Activity 2:</b> Sample preparation and distribution for chemical analyses and bioassays From the daily taken influent and effluent samples, 7-day composite samples will be prepared. These will be filtered and extracted using solid phase extraction (SPE). Extracts will be concentrated under N<sub>2</sub> steam and reconstituted in methanol. These extracts (enrichment factor 1000) will be the basis for the chemical analyses and bioassays enabling the knowledge transfer between the different analyses. → <b>UFZ</b></p> <p><b>Activity 3:</b> Target and suspect screening of organic substances Analysis of a broad range of target compounds (~580 chemicals) including pesticides, industrial chemicals, polymer additives, surfactants and pharmaceuticals using high-performance liquid chromatography (HPLC) coupled to high-resolution mass spectrometry (HRMS). Results will be supported by findings from suspect screening. → <b>UFZ</b></p> <p><b>Activity 4:</b> Target analysis of the samples with a focus on selected WWTP contaminants of particular concern</p> <ul style="list-style-type: none"> <li>- ~ 150 persistent, mobile (PM) and toxic (Ts) compounds and PFAS (including ultra short chain PFAS) will be analysed using super critical fluid chromatography (SFC)- HRMS and LC-HRMS → <b>UFZ</b></li> <li>- Tire-derived compounds will be analysed using LC-MS/MS → <b>University of Vienna</b></li> <li>- Priority substances (<b>tbc</b>)</li> <li>- Drugs of abuse → <b>IDAEA-CSIC Barcelona</b></li> </ul> <p><b>Activity 5:</b> Analysis of micro-and nanoplastics</p> <ul style="list-style-type: none"> <li>- nanoplastics → <b>UFZ</b></li> <li>- microplastics → <b>UBA</b></li> </ul>

	<p><b>Activity 6:</b> Analysis of inorganic contaminants</p> <ul style="list-style-type: none"> <li>- Rare Earth Elements → <b>University of Lorraine (tbc)</b></li> <li>- Metals → <b>BOKU (tbc)</b></li> </ul> <p><b>Activity 7:</b> Battery of bioassays</p> <p>Besides the chemical analysis of the influent and effluent samples, a batterie of bioassays will be conducted targeting a range of specific endpoints, including endocrine, dioxin-like and neurotoxic effects. Findings from the chemical analysis will complement findings from the bioassays and help to explain mixture effects and identify potential drivers of the effects observed in the bioassays.</p> <ul style="list-style-type: none"> <li>- <i>Neurite Outgrowth inhibition in SH-SY5Y cells</i> → to test developmental neurotoxicity</li> <li>- <i>Multiplex Assay MitoOxTox using AREc32 cells</i> → to quantify cytotoxicity, oxidative stress response and mitochondrial toxicity using the reporter cell line AREc32</li> <li>- <i>CAFLUX AhR</i> using H4G1.1c2 cells → for screening of dioxin-like compounds</li> <li>- <i>Algae test with S. vacuolatus</i> → to assess chemicals affecting photosynthesis activity</li> <li>→ <b>UFZ</b></li> <li>- <i>CALUX bioassay battery</i> using U2OS cells → to assess the activation and inhibition of (anti-)estrogens, (anti-)androgens, progestins and glucocorticoids. The PFAS-Calux assay will be conducted, which is a TRR binding test in combination with the TRβ CALUX assay.</li> <li>→ <b>Goethe-University Frankfurt</b></li> </ul> <p><b>Activity 8:</b> Metaproteomics to explore the microbial community in sewage sludge</p> <p>Proteins extracted from sewage sludge are enzymatically digested into peptides and then analyzed with nano-liquid chromatography coupled to a mass spectrometer (MS). Results will be used to better understand biological processes in waster water treatment plants including the degradation of pollutants.</p> <p>→ <b>UFZ</b></p> <p><b>Added value / Link with other NORMAN activities and / or other projects</b></p> <p>An integrated assessment of waste water influents and effluents is highly needed in order to determine the impact of WWTPs on the pollution of the Danube River with the overarching goal to contribute to maintain the Danube River a safe and clean water resource in the future. The results from this integrative JPA can help to guide the monitoring and prioritization of water pollution, and identify and improve strategies for removal. Thereby this JPA serves as a best practice analysis for the implementation of the EU Urban Wastewater Directive (UWWTD).</p> <p>Regarding other NORMAN activities, results form the chemical analyses and bioassays of the waste water influent and effluent samples conducted within this study can be used to support findings from other JDS-5 activities such as the analysis of the surface water and bank filtrate along the Danube River and provide insights on possible entry pathways of contaminants. In addition, findings from the passive sampler project in JDS-5 conducted near selected waste water treatment plants will provide valuable time-integrated assessments of chemicals which might be below the detection limit and thus overlooked by the analysis conducted in the WWTP study. The here presented JPA is strongly connected to other projects within JDS-5 and contributes to a holistic assessment of the Danube River.</p>
<p><b>Participants</b></p>	<p>Helmholtz Centre for Environmental Research (UFZ), Leipzig (Germany)  Goethe-University Frankfurt, Frankfurt (Germany)  University of Vienna, Vienna (Austria)  BOKU University, Vienna (Austria)  University of Lorraine, Nancy and Metz (France)  IDAEA-CSIC, Barcelona (Spain)  German Federal Environmental Agency (UBA), Dessau (Germany)  Further participants to be confirmed.</p>
<p><b>Proposed in-kind contribution</b></p>	<p>All practical work of on site sampling will be conducted in the framework of JDS-5 organized by the International Commission for the Protection of the Danube River (ICPDR) supported by the NORMAN network. The conduction of the chemical analysis and bioassays itself will be performed by the participating partners free of charge (worth ~150.000 €).  Support for the presented JPA is needed to cover the personnel costs for coordination of the activities, overarching data synthesis, preparation of publications as well as for consumables for chemical analysis and bioassays, and for the shipment of sample extracts from UFZ to the participating laboratories.</p>
<p><b>Contribution needed from NORMAN Association<sup>1</sup></b></p>	<p>Total: 50.000 €</p>

<sup>1</sup> Please, provide here a transparent justification of the requested resources and of the in-kind contribution, thereby distinguishing between the costs associated with “person-months” for the organisation, the “travelling costs” for invited speakers and the costs for the logistics (e.g. meals, room rental etc.)