

DISCUSSION

This project delivers initial data on the sources and fate of microplastics in an urban area and its subsequent impact on surface water. These first investigations confirm the presence of microplastics in sewage, freshwater and total atmospheric fallout and provide knowledge of the type and size distribution of microplastics. Investigating microplastics in urban areas and in surface water needs an updated methodology, since the presence of organic matter and clay matter adversely affect the observation and counting of microplastics. Thus, enzymatic digestion is required. Moreover, from an ecotoxicological point of view, the literature reports that risks of ingestion and ecotoxicological impacts are higher with small-

er microplastics (Wright et al., 2013). In this context, manta trawl sampling campaigns alone cannot characterise this risk but investigations of smaller microplastics are also required. Therefore, both different but complementary sampling approaches developed in this study could be implemented in future studies. While analysing fibres needs the use of the plankton net due to its small mesh size, sampling higher volumes is mandatory to collect other shapes of microplastics.

This work may also contribute to the debate on microplastics sampling and analysis strategies in freshwater. Future research as a part of the LEESU project will also soon be performed on the interaction between microplastics and micropollutants within the receiving water.

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The NORMAN interlaboratory study on biotesting of spiked water extracts

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BACKGROUND

The NORMAN network maintains five Working Groups focusing on specific issues related to emerging substances. The NORMAN working group on Bioassays and Biomarkers (Bio WG) has its focus on the application of biotools for environmental quality monitoring. A main

objective is to provide recommendations for the implementation of effect-based tools in regulatory frameworks.

During the re-launch meeting of the Bio WG in November 2012 (IVM, VU University Amsterdam, the Netherlands), a blind interlaboratory study (ILS) applying biotests to evaluate complex surface water extracts was

proposed as a main activity for 2013–2014. The ILS objective was to verify whether a battery of bioassays conducted in different laboratories following their own methods and protocols would produce comparable results when applied to evaluate spiked water extracts. Another important expected outcome of the ILS was the promotion of the use of biotests for water quality monitoring at the level of European policy-makers.

The lead in planning and organising the ILS was taken by the Department of Ecosystem Analysis (ESA) of the Institute for Environmental Research, RWTH Aachen University, Germany. In parallel, activities towards the validation of a common battery of bioassays were developed within ongoing European monitoring projects, such as the demonstration programme of the Marie Curie ITN EDA-EMERGE (www.eda-emerge.eu), the SOLUTIONS project (www.solutions-project.eu), and the Joint Danube Survey 3 (www.danubesurvey.org). In all these projects, there is close cooperation between the Bio WG and the NORMAN activity on large-volume active sampling for effect-based monitoring, chemical screening and Effect-Directed Analysis (EDA). Such NORMAN activity is organised by the EDA Working Group (EDA WG), led by the Department for Effect-Directed Analysis of the UFZ Helmholtz Centre for Environmental Research, Leipzig, Germany [1].

THE INTERLABORATORY STUDY

During the re-launch meeting of the Bio WG, all participants contributed to the discussion and selection of the biotests that would make up the ILS bioassay battery. The final bioassay selection was done after considering the relevance of different test systems and endpoints, as well as logistic limitations. The selected bioassay battery includes three acute-toxicity assays with organisms representing different trophic levels (Algae assay, Daphnia assay, Fish embryo toxicity test); and mechanism-specific bioassays for estrogenicity (YES assays, ER-Luc cell-based assays) and mutagenicity (Ames fluctuation test) assessment.

To identify which partners could perform which bioassays, a query was sent around to the Bio WG participants. Since a limited volume of the water extract was available for the ILS as described below, a number of institutes were selected to perform each bioassay. In making that selection, an important decision criterion was the inclusion of all interested partners in the ILS. Finally, there was the selection of three to four participants to perform each bioassay (Table 1).

	RWTH (DE)	BfG (DE)	Ifremer (FR)	IVM (NL)	Recetox (CZ)	INERIS (FR)	Entox (AU)	ITM (SE)	Ecotox Centre (CH)	ISSEP (BE)	Water-net (NL)
Algae					X		X		X		X
FET	X		X		X						
Daphnia						X		X		X	X
YES		X			X					X	
ER-Luc	X			X		X	X				
Ames		X		X			X				

Table 1: Bioassays performed by each participant institute

The preparation of the clean water extract was done by the EDA department of UFZ. 180 litres of clean water were collected at a previously studied reference site, followed by concentration using large-volume solid-phase extraction (LVSP) to a final volume of 18 mL, resulting in a 10 000 times concentrated extract.

There was the decision by RWTH and UFZ on four emerging pollutants, i.e. triclosan [2] (CAS 3380-34-5), acridine [3-5] (CAS 260-94-6), 3-nitrobenzanthrone [6] (CAS 17117-34-9) and 17-alpha-ethinylestradiol [7] (CAS 57-63-6), to be used for the spiking of the water extract. The selection of the chemicals considered their relevance as environmental pollutants and their capacity to cause effects on the different bioassays.

Preliminary tests were done by RWTH to evaluate the water extract with the bioassay battery. In addition, the selected chemicals were also tested as single chemical exposure in some of the bioassays whenever previous results were not available.

For the composition of the spiked water extracts, there was decision on spiking with single chemicals and as well with a final chemical mixture

(Table 2). The selected concentrations were aimed at producing full dose-response curves in the bioassays. That was done considering own results and literature data. The composition of chemical spiking of the water extract was designed for each bioassay, resulting in one or two simple mixtures plus a final mixture for each bioassay. The spiked water extracts were prepared, separated in aliquots for the different biotesters, identified with codes, and sent to the biotesting partners. The institutes were therefore not informed of the composition of the spiked extracts throughout the testing procedure.

Bioassay	Code	Chemicals for spiking	Water extract
Algae	A	TCS	10,000 x
	B	ACR	10,000 x
	C	TCS, EE2, ACR	10,000 x
FET	A	TCS	10,000 x
	B	ACR	10,000 x
	C	TCS, EE2, ACR, 3-NBA	10,000 x
Daphnia	A	TCS	10,000 x
	B	ACR	10,000 x
	C	TCS, EE2, ACR, 3-NBA	10,000 x
YES	A	EE2	10,000 x
	B	TCS, EE2, ACR	10,000 x
ER-Luc	A	EE2	10,000 x
	B	TCS, EE2, ACR	10,000 x
Ames	A	3-NBA	10,000 x
	B	TCS, EE2, ACR, 3-NBA	10,000 x

Table 2: Composition of spiked water samples, which consisted of one or two single chemical spiking and a chemical mixture for each bioassay

TCS: triclosan
ACR: acridine
EE2: 17-alpha-ethinylestradiol
3-NBA: 3-nitrobenzanthrone

Regarding biotesting protocols, standardised methods such as OECD or ISO guidelines were recommended, but were not mandatory, so biotesters were free to use their own methods. The only restriction was the limited volume of extract for biotesting. Also, specific dilution series were recommended to the biotesters, but were not mandatory either. During the biotesting period, the RWTH group provided assistance to all participants regarding biotesting.

NORMAN ILS WORKSHOP

The results from the different bioassays were sent to RWTH. When necessary, the participants were requested to provide additional or missing data or information. Finally, the RWTH team grouped the results and prepared a summary of the full ILS, which was provided to the ILS participants. On the 22–23 October 2014, the participants of the ILS and of the Bio WG, as well as external experts, were invited to join a workshop at RWTH Aachen University. The event had participants from the following institutes and countries: BfG (Germany), Waternet (the Netherlands), Waterproof (the Netherlands), INERIS (France), RECETOX (Czech Republic), ISSEP (Belgium), IVM-VU (the Netherlands), Ecotox Centre (Switzerland), LANUV- NRW (Germany), IWW Zentrum Wasser (Germany), and Bio5-RWTH (Germany).

During the workshop, a summary of the ILS and respective results was presented, followed by discussion in small groups of the outcomes from the different bioassays. Additionally, outreach actions and the planning of the 2015 activities of the Bio WG were proposed and discussed.

Bioassays produced mostly highly comparable results, even when protocols differed strongly. For statistical evaluation with respect to a scientific publication of the results, data are currently collected by the RWTH group in a uniform format. This exercise is also the most important next step towards the implementation of bioanalytical monitoring tools, where harmonised methods for data analysis and results evaluation are crucial. Experiences from sampling, bioassay, data analysis and evaluation will then be integrated into a testing strategy outlined by the forthcoming final public report of the ILS, showing the capabilities and advantages – but also the limitations – of bioanalytical water quality monitoring and management.

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NORMAN WG-5: Wastewater reuse and contaminants of emerging concern

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INTRODUCTION – CURRENT STATE OF KNOWLEDGE

In response to the escalating problem of water shortage, treated wastewater is nowadays widely reused and is generally considered as a reliable alternative water source for irrigation and replenishment among other applications. Water demands already exceed supplies in regions with more than 40% of the world's population and it's expected that in the coming years as much as 60% of the world's people may confront water scarcity [1].

Although the reuse practice is accompanied by various benefits relating to the enhancement of water balance and soil nutrition, a number of questions are still unanswered, rising concerns within the scientific community. Besides the lack of knowledge in respect of possible elemental interactions that may influence the accumulation of metals/elements in the soil and the subsequent uptake by plants and crops, organic microcontaminants and antibiotic resistant bacteria and antibiotic resistance genes (ARB&ARG) in treated wastewater require much attention. After conventional treatment, the organic matter remaining in the effluents consists of a number of recalcitrant organic compounds including potential endocrine disrupting compounds and pharmaceutical residues such as antibiotics, since the treatment processes currently applied fail to completely remove such microcontaminants, including ARB&ARG. This leads to their subsequent release in terrestrial and aquatic environments, with major consequences as far as human and environmental health is concerned.

Current open challenges associated with wastewater reuse include (i) the reduction of the emission from urban wastewater treatment plants (UWTPs) of a wide range of microcontaminants including ARB&ARG, as well as their transformation products formed during treatment and while being in the environment after wastewater is discharged or reused, (through biotic/abiotic processes), and (ii) the control of their potential uptake by plants/crops, and the potential transfer of ARG to the indigenous environmental microbiota, which in turn can be transmitted through the food and water network.

It is therefore imperative to assess the impacts that these microcontaminants may have in the environment. In particular, the examination of the evolution of antibiotic resistance after treated wastewater is discharged in the environment or reused is urgently required. To identify the technologies that are most suitable to remove such microcontaminants from wastewater, taking into account their cost-effectiveness is of utmost importance [2-5].

To avoid negative environmental and human impacts, and considering the EU precautionary principle [6], regulatory frameworks are required, based on validated scientific information. The NORMAN Network, through its WG-5, aims to increase the scientific understanding of these crucial issues and to potentially boost technological developments to reduce the emission of microcontaminants from wastewater.

OBJECTIVES

WG-5 addresses critical questions related to the issues discussed above, associated with the release of microcontaminants from wastewater in order to provide deeper insight into the effects of long-term environmental and biota exposure even to sub-lethal levels of microcontaminants, to consolidate data on crop uptake, to propose criteria/specs on technologies/ assessment methods, to suggest advanced effluent quality criteria to mitigate the risks associated with wastewater reuse, and therefore to contribute to and encourage the sustainable reuse of reclaimed wastewater.

More specifically, WG-5 focuses its efforts on: (i) evaluating the risks associated with wastewater reuse in respect of the evolution and spread of antibiotic resistance in the environment and water resources, (ii) evaluating, based on research studies and information available, the risks associated with microcontaminant uptake by crops, (iii) revealing and counteracting weaknesses/knowledge gaps in environmental chemistry and microbiology/toxicology required for the above-mentioned activities,