Science of the Total Environment 576 (2017) 785–795

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Integrating chemical analysis and bioanalysis to evaluate the contribution of wastewater effluent on the micropollutant burden in small streams

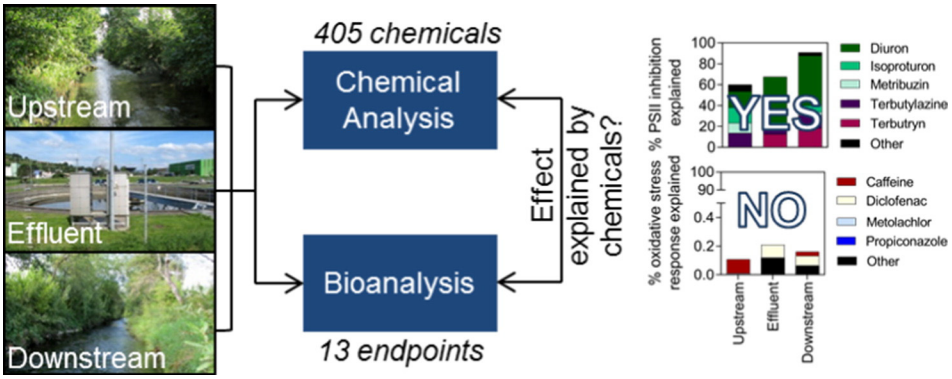
Peta A. Neale [a,b,1], Nicole A. Munz [c,d,1], Selim Aït-Aïssa [e], Rolf Altenburger [f], François Brion [e], Wibke Busch [f], Beate I. Escher [a,b,f,g,*], Klára Hilscherová [h], Cornelia Kienle [i], Jiří Novák [h], Thomas-Benjamin Seiler [j], Ying Shao [j], Christian Stamm [c], Juliane Hollender [c,d]

[a] Australian Rivers Institute, Griffith School of Environment, Griffith University, Southport, QLD 4222, Australia
[b] The University of Queensland, National Research Centre for Environmental Toxicology (Entox), Brisbane, QLD 4108, Australia
[c] Eawag, Swiss Federal Institute of Aquatic Science and Technology, CH-8600 Dübendorf, Switzerland
[d] Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich, 8092 Zürich, Switzerland
[e] Institut National de l'Environnement Industriel et des Risques INERIS, Unité d'Ecotoxicologie, 60550 Verneuil-en-Halatte, France
[f] UFZ – Helmholtz Centre for Environmental Research, 04318 Leipzig, Germany
[g] Eberhard Karls University Tübingen, Environmental Toxicology, Center for Applied Geosciences, 72074 Tübingen, Germany
[h] Masaryk University, Research Centre for Toxic Compounds in the Environment (RECETOX), Kamenice 753/5, 62500 Brno, Czech Republic
[i] Swiss Centre for Applied Ecotoxicology Eawag-EPFL, Überlandstrasse 133, CH-8600 Dübendorf, Switzerland
[j] Department of Ecosystem Analysis, Institute for Environmental Research, RWTH Aachen University, 52074 Aachen, Germany

HIGHLIGHTS

- Chemical analysis and bioassays showed WWTP effluent as major micropollutant source.
- Pesticides mainly upstream, with pharmaceuticals and consumer chemicals in effluent.
- Chemical composition and effects downstream explained by mixing of upstream sources.
- Only small fraction of effect in most bioassays was explained by detected chemicals.
- Chemical and bio-analysis gave complementary information for pollutant assessment.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 August 2016
Received in revised form 3 October 2016
Accepted 19 October 2016
Available online xxxx

Editor: D. Barcelo

ABSTRACT

Surface waters can contain a range of micropollutants from point sources, such as wastewater effluent, and diffuse sources, such as agriculture. Characterizing the source of micropollutants is important for reducing their burden and thus mitigating adverse effects on aquatic ecosystems. In this study, chemical analysis and bioanalysis were applied to assess the micropollutant burden during low flow conditions upstream and downstream of three wastewater treatment plants (WWTPs) discharging into small streams in the Swiss Plateau. The upstream sites had no input of wastewater effluent, allowing a direct comparison of the observed effects with and without

* Corresponding author at: UFZ – Helmholtz Centre for Environmental Research, 04318 Leipzig, Germany.
E-mail address: beate.escher@ufz.de (B.I. Escher).
[1] Both Neale and Munz contributed equally to the manuscript and are co-first authors.

http://dx.doi.org/10.1016/j.scitotenv.2016.10.141

Keywords:
Wastewater
Micropollutant
Chemical analysis
Bioassays
Surface water
Mixture modeling

the contribution of wastewater. Four hundred and five chemicals were analyzed, while the applied bioassays included activation of the aryl hydrocarbon receptor, activation of the androgen receptor, activation of the estrogen receptor, photosystem II inhibition, acetylcholinesterase inhibition and adaptive stress responses for oxidative stress, genotoxicity and inflammation, as well as assays indicative of estrogenic activity and developmental toxicity in zebrafish embryos. Chemical analysis and bioanalysis showed higher chemical concentrations and effects for the effluent samples, with the lowest chemical concentrations and effects in most assays for the upstream sites. Mixture toxicity modeling was applied to assess the contribution of detected chemicals to the observed effect. For most bioassays, very little of the observed effects could be explained by the detected chemicals, with the exception of photosystem II inhibition, where herbicides explained the majority of the effect. This emphasizes the importance of combining bioanalysis with chemical analysis to provide a more complete picture of the micropollutant burden. While the wastewater effluents had a significant contribution to micropollutant burden downstream, both chemical analysis and bioanalysis showed a relevant contribution of diffuse sources from upstream during low flow conditions, suggesting that upgrading WWTPs will not completely reduce the micropollutant burden, but further source control measures will be required.

1. Introduction

Surface waters can contain a wide range of micropollutants, including pesticides, pharmaceuticals, personal care products and industrial compounds (Loos et al., 2009; Moschet et al., 2015), which have the potential to adversely impact exposed ecosystems (Malaj et al., 2014; Stalter et al., 2013). To mitigate the effect of micropollutants on the aquatic environment, it is important to identify their sources, which can be either from point sources, such as wastewater effluent discharges, or diffuse sources, such as agriculture (Eggen et al., 2014; Maletz et al., 2013). This can help to inform solutions to reduce the concentration and bioactive fraction of micropollutants, *i.e.*, the micropollutant burden, in surface waters, which may include upgrading wastewater treatment plants (WWTPs) or regulatory changes, such as banning certain chemicals (Schwarzenbach et al., 2006).

Water quality monitoring programs, such as requested under the European Union Water Framework Directive (European Commission, 2011; European Commission, 2012), typically focus on chemical analysis, which can provide useful information about the concentration and type of chemicals present in a sample. However, targeted chemical analysis alone has some limitations. It is unable to detect unidentified chemicals and transformation products or account for the mixture effects that can occur between the many compounds present in water. For a comprehensive view of the micropollutant burden, chemical analysis should be combined with bioanalysis. While bioassays cannot identify individual chemicals, they can provide information about the joint effect of all bioavailable active chemicals present in a sample, with more potent chemicals showing a greater effect in the assay (Escher and Leusch, 2012; Prasse et al., 2015; Wernersson et al., 2015). The development of bioanalytical tools for water monitoring requires adequate choice of biological endpoints and quality measures (Altenburger et al., 2015; Busch et al., 2016), with applied bioanalytical test batteries ideally including assays indicative of induction of xenobiotic metabolism, endocrine disruption, reactive modes of action, adaptive stress responses and cytotoxicity (Escher et al., 2014).

The complementary approach of chemical analysis and bioanalysis has been applied to monitor water quality and to evaluate WWTP and advanced water treatment plant efficiency (Creusot et al., 2014; Jálová et al., 2013; Margot et al., 2013; Tang et al., 2014). Applying bioassays and chemical analysis in parallel overcomes the limitations associated with the individual approaches and can reveal the presence of potent undetected chemicals and identify chemicals that contribute to the observed effect (Escher and Leusch, 2012). Mixture toxicity modeling can be used to determine the fraction of the observed effect that can be explained by detected chemicals using the bioanalytical equivalent concentration (BEQ) approach (Neale et al., 2015a). Bioanalytical equivalent concentrations from chemical analysis (BEQ_{chem}) are calculated using the detected chemical concentration and their relative potency, and can be compared to bioanalytical equivalent concentrations from bioassays (BEQ_{bio}). For example, detected chemicals can often explain a high percentage of estrogenic activity (Leusch et al., 2014; Murk et al., 2002), while only a small fraction of non-specific effects or adaptive stress responses can typically be explained (Tang et al., 2013; Yeh et al., 2014).

In this study, both chemical analysis and bioanalysis were applied to assess the micropollutant burden in small streams upstream and downstream of three WWTPs, with the upstream sites not being affected by inputs of treated wastewater. The water samples were collected under low flow conditions to minimize the impact of diffuse sources. The analyzed chemicals were primarily pharmaceuticals and pesticides, with the other analyzed chemicals including biocides, food additives, illicit drugs, industrial chemicals and estrogens. The biological effects were evaluated using a suite of *in vitro* assays, which represent different cellular toxicity pathways, including xenobiotic metabolism, receptor-mediated effects, adaptive stress responses and cytotoxicity, as well as whole organism assays with algae and zebrafish embryos. Assays indicative of xenobiotic metabolism, such as activation of the aryl hydrocarbon receptor (AhR), and adaptive stress responses, such as the oxidative stress response, can respond to a range of compounds with different modes of action (Martin et al., 2010; US EPA, 2015). In contrast, assays indicative of receptor-mediated effects can provide information about the presence of chemicals with a common specific mode of action. For example, hormone-mediated effects including activation of the estrogen receptor (ER) and activation of the androgen receptor (AR) can detect natural and synthetic hormones, as well as other environmental endocrine disrupting compounds, which are often associated with wastewater (Vethaak et al., 2005). Further, assays indicative of inhibition of photosystem II (PS*II*) and of acetylcholinesterase (AChE) are more suitable to detect chemicals of an agricultural origin as they can detect PS*II* inhibiting herbicides (Escher et al., 2008a) and organophosphate and carbamate insecticides (Hamers et al., 2000), respectively.

The current study aimed to assess the impact of wastewater effluent on the micropollutant burden in small streams using a complementary chemical analysis and bioanalysis approach. Four hundred and five chemicals were analyzed and the applied test battery included assays indicative of activation of AhR, activation of AR, activation of ER, PS*II* and algal growth inhibition, AChE inhibition, mutagenicity and adaptive stress responses for oxidative stress, genotoxicity and inflammation, as well as assays indicative of estrogenic activity and developmental toxicity in zebrafish embryos. A mass balance approach was used to calculate the fraction of effluent downstream based on both chemical analysis and bioanalysis, while mixture toxicity modeling was applied to assess whether the detected chemicals were contributing to the biological effect.