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Quantitative target and systematic non-target analysis of polar organic micro-pollutants along the river Rhine using high-resolution mass-spectrometry – Identification of unknown sources and compounds

Matthias Ruff, Miriam S. Mueller [1], Martin Loos, Heinz P. Singer*

Eawag, Swiss Federal Institute of Aquatic Science and Technology, 8600 Dubendorf, Switzerland

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ABSTRACT

In this study, the contamination by polar organic pollutants was investigated along the Rhine River, an important source of drinking water for 22 million people in central Europe. Following the flow of the river, a traveling water mass was sampled using weekly flow-proportional composite samples at ten different downstream sites, including main tributaries. Using a broad analytical method based on solid phase extraction and high-resolution mass spectrometry, the water was analyzed for more than 300 target substances. While the water in Lake Constance contained only 83 substances in often low concentrations, the number of detects found in the water phase increased to 143 substances and a weekly load of more than 7 tons at the last sampling site, the Dutch-German border. Mostly present were chemicals originating from wastewater treatment plants, especially the artificial sweetener Acesulfam and two pharmaceuticals, Metformin and Gabapentin, which dominate the weekly load up to 58%. Although the sample campaign was performed in a dry period in early spring, a large variety of pesticides and biocides were detected. Several industrial point sources were identified along the waterway's 900 km journey, resulting in high concentrations in the tributaries and loads of up to 160 kg. Additionally, an unbiased non-target analysis was performed following two different strategies for the prioritization of hundreds of potentially relevant unknown masses. While for the first prioritization strategy, only chlorinated compounds were extracted from the mass spectrometer datasets, the second prioritization strategy was performed using a systematic reduction approach between the different sampling sites. Among others, two substances that never had been detected before in this river, namely, the muscle relaxant Tizanidine and the solvent 1,3-Dimethyl-2-imidazolidinone (DMI), were identified and confirmed, and their loads were roughly estimated along the river.

1. Introduction

The Rhine River is one of the most important rivers in central Europe. Its water is abstracted in nine countries, and the six direct neighboring countries (Switzerland, Austria, Germany, France, Belgium and the Netherlands) produce drinking water for approximately 22 million people by river-bank filtration. In contrast, the Rhine's water is intensively used by different groups of interest: more than 2800 municipal and industrial wastewater treatment plants (WWTPs) (European Environment Agency and Federal Office for the Environment, #1) release the wastewater of 58 million people living in the catchment area (ICPR, 2012); numerous industrial plants and power plants use water for cooling circuits; the water body is an important international waterway for million tons of trading goods; finally, 53% of the catchment area is used by agriculture (ICPR). With all those utilizations a high variety of chemicals can directly or indirectly reach the aquatic environment. The emissions can be continuous and discontinuous caused by constant discharges and accidental spills, respectively. For some of these chemicals, their sources, structures and effects on aquatic ecosystems are well known and described (Kidd et al., 2007), but the identity and effect of most of the compounds are still unknown. Hence, the ecotoxicological effects, especially of mixtures of this vast array of chemicals, cannot be assessed (Chevre et al., 2008). To guarantee a high quality of drinking water, a network of monitoring

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* Corresponding author.

E-mail address: Heinz.Singer@eawag.ch (H.P. Singer).

[1] Actual address: CONACYT, Consejo Nacional de Ciencia y Tecnología, Del. Benito Juárez, C.P. 03940 D.F Mexico.

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stations was established along the river, which is organized by the International Commission for the Protection of the Rhine (ICPR).

Until the 1990s inorganic pollutants, such as heavy metals, salts and nutrients, were the focus of the monitoring programs; today, however, organic micro-pollutants have become a focus of interest because of their potential risk to aquatic ecosystems. To detect organic micro-pollutants, methods that use gas chromatography (GC) coupled to mass spectrometry (MS) were widely established for the screening of targeted (Schwarzbauer and Heim, 2005) and non-targeted (Schwarzbauer and Ricking, 2010) organic compounds. However, this technique only detected volatile, thermostable and rather non-polar substances. Approximately 10 years ago liquid chromatographic (LC) analytic methods coupled to mass spectrometers complemented the GC–MS technique and increased the analytical spectrum towards semi-polar and polar substances, which are more relevant for the dissolved water phase due to their high solubility, persistence and mobility. A broad variety of environmentally relevant substances and their transformation products (TPs) have been investigated in recent years and were detected along the Rhine. In these studies, single substances (Buerge et al., 2009; Funke et al., 2015) or substance groups such as flame retardants (Reemtsma et al., 2008), artificial sweeteners (Scheurer et al., 2009) or antiviral pharmaceuticals (Prasse et al., 2010) have been analyzed. In the work of Scheurer et al. (2011), six anthropogenic wastewater tracers were studied along the Rhine River. Most of these studies were performed using grab samples, which allowed first load estimations, but due to the high dynamic behavior of large river systems (in-situ degradation along the river, local emission, weak horizontal and vertical mixing) grab samples are prone to over-interpretation. LC coupled to high-resolution mass spectrometers (HRMS) enables the simultaneous screening of a large number of substances (several hundreds). Such multi-component screenings have become increasingly more established in recent years (de Jongh et al., 2012; Heeb et al., 2012; ter Laak et al., 2012), but they rarely find their way into routine analysis or have been used to systematically evaluate the contamination situation of the Rhine. However, even multi-component methods include the detection of only known substances (targets), those compounds that are part of national or institutional screening lists. Using the LC-HRMS technique, unknown substances (non-targets) can also be covered and systematically evaluated (Hug et al., 2014; Schymanski et al., 2014b). The unknown analysis ‘without any a priori information’ (Krauss et al., 2010) can be used to identify new emerging organic substances in the water phase of the Rhine River. Given the fact that completely unbiased non-target screenings (Ibáñez et al., 2014) lead to huge datasets, dedicated approaches for the reduction and prioritization of data have to be applied.

In this work a comprehensive target analysis was combined with a systematic non-target analysis by using the analytical possibilities provided by the LC-HRMS technology. Therefore, full scan measurements detected in high-resolution mode are used to collect the information of all ionizable compounds while following data-dependent fragmentation spectra-supplemented structural information. Because the water of the Rhine River originates from different countries and regions with different customs, the contamination pattern might change along the river. River monitoring stations along the Rhine generate data about how the water quality changes over time at a certain point. In contrast to that temporal variation of the contamination, the goal of this work was a detailed investigation of the spatial variation. Therefore, we followed a water-wave downstream according to its travel time and sampled the same water at different locations. To keep the study representative the water was collected at each site as a seven-day composite sample. This allowed for a reliable calculation of the loads going downstream. For the non-target screening, a new prioritization approach was applied for the numerous unknown components observed in the Rhine samples.

2. Methods and materials

2.1. Sampling and sites

The Rhine River covers on a length of 1233 km and a catchment area of approximately 200,000 km^2. After a distance of 160 km the Alpine Rhine reaches Lake Constance, with a total area of 536 km^2 and a volume of 48 km^3 (Moschet et al., 2013). Because of a time delay of several years, the outflow of Lake Constance is officially defined as km 0 (ICPR). The sample campaign was performed along the river including its main tributaries (Fig. 1). The samples consisted of 7-day composite samples at 10 destinations along the river, whereas the sampling start was shifted in accordance with the travel time. The significance is that the same water mass was always sampled, but at different locations. The travel time of the river was calculated using a model provided by the ICPR (Rhine-alarmmodel – Version 3.08.01; Netter-Delft Hydraulics, Int. Comm. for the hydrology of the Rhine basin). The water was taken from sampling stations along the riverbank or national/international monitoring stations located on the route from Lake Constance to the Rhine delta at the border of Germany and the Netherlands (Table SI1). The 7-days-water-mass was sampled at the following sampling stations all equipped with cooled (4 °C) automated samplers: Diepoldsau (before Lake Constance; outflow of the lake is defined as km 0), Oehningen (km 25; March 6–12), Reckingen (km 90; March 7–13), Albruck (km 110; March 7–13), Weil (km 171; March 9–15), Karlsruhe (km 360; March 14–20), Mannheim (km 425; March 15–21), Mainz (km 495; March 16–22), Koblenz (km 590; March 17–23) and Bimmen-Lobith (km 865; March 20–26). Additionally, the main tributaries were sampled: river Thur (km 65; CH; February 28–March 14), river Glatt (km 80; CH; February 28–March 14), Aare (km 100; main tributary in CH, 1:1 water-ratio Aare and Rhine; March 16), Neckar (km 425; GER; March 15–21), Main (km 495; GER; March 16–22) and Mosel (km 590; F/GER; March 17–23). Except for the samples from Oehningen, Glatt and Thur (premixed flow-proportional 14-day composite samples) and Aare (grab sample), all 7 days composite samples (1 L) were prepared by flow-proportional mixing of 24 h composite samples (i.e., the maximum resolution of a sample was ±1 day). All samples were stored in glass bottles in the dark at 4 °C until analysis.

The sampling period in spring 2011 (exact sampling dates given in Table SI1) was a remarkably dry period with constant low flow conditions along the entire catchment area. For example, the mean flow for Basel in March 2011 was 580 m^3/sec (min 514 m^3/sec; max 742 m^3/sec). Between the first station we sampled, Diepoldsau (before Lake Constance) and Bimmen-Lobith, the last station of the sampling campaign, the flow increased by a factor of 13, due to the tributaries, from 110 m^3/sec to 1450 m^3/sec, respectively.

2.2. Chemicals

All organic solvents were of HPLC grade purity and were supplied by Acros Organics (Geel, Belgium) and Sigma Aldrich (Buchs, Switzerland). Ammonia 25% and formic acid 98–100% were purchased from Merck (Darmstadt, Germany). Nanopure water was generated by a lab water purification system (Millipore, Bedford, USA).

For the target analysis of 302 compounds and the confirmation of 7 unknown compounds, reference standards were purchased as pure substances or concentrated solutions from Sigma–Aldrich (Buchs, Switzerland), Dr. Ehrenstorfer (Augsburg, Germany), Toronto Research Chemicals (North York, Canada), ReseaChem