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Deliverable D3.3

Micropollutant loads at focus sites and link to abundance of key microorganisms and ecological & biogeochemical processes

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PU	Public	**X**
CO	Confidential, only for the members of the consortium (including the Commission Services)	

Content

Micropollutant loads at focus sites and link to abundance of key microorganisms and ecological & biogeochemical processes

1. Analysis of micropollutants in water from the River Erpe

Samples of surface water (SW) and hyporheic pore water (HPW) were obtained from the River Erpe, a medium sized, eutrophic lowland river (Lewandowskei et al. 2011) at the eastern edge of Berlin, Germany. The Erpe receives effluent water (EW) from several smaller waste water treatment plants (WWTPs) and one large WWTP (Muenchehofe) that has a dry weather capacity of 42,500 m^3 d^{-1} treating approximately 15% of Berlin's wastewater. The EW content of the stream underlies strong diurnal fluctuations and can reach more than 80% of the total discharge. Water samples were collected during field campaigns in April (HPW 24 h time series + SW) and June (SW 48 h time series), 2016. Surface water samples were collected upstream (USW) and downstream (SW) of the EW inlet of the WWTP Muenchehofe. SW time series samples were taken hourly for 48 h using an autosampler (ISCO 3700 portable sampler, Teledyne Isco, Lincoln NE) that was installed 700 m downstream of the inlet. Grab samples of USW were collected to test for background micropollutant concentrations. For the collection of HPW, modified minipoint samplers (Duff et al. 1998) connected to syringe pumps were employed 800 m downstream of the EW inlet and water was extracted in depths of 10, 20 and 30 cm. O_2, conductivity and pH were monitored to control for surface water infiltration. Additional SW grab samples were taken at the same location every 4 h for comparison. All samples collected in the field were immediately frozen on dry ice and stored at -20°C until further processing.

Prior to micropollutant analysis, samples were defrosted in a 25°C water bath and then analyzed using a recently developed direct injection-ultra performance liquid chromatography tandem mass spectrometry method (Posselt et al. *In preparation*). The method is capable of analyzing a total of 44 targets which were selected based on literature review and included 6 analgesics (4 non-steroidal anti-inflammatory drugs (NSAIDs), acetaminophen, and the opioid analgesic tramadol), 3 β-blockers, 3 lipid regulators, 2 Angiotensin II receptor antagonists, 2 antihyperglycemics, 2 diuretics, 2 corrosion inhibitors, 1 antianxiety agent, 1 antibiotic, 1 anticonvulsant, 1 stimulant, 1 antidepressant, 1 artificial sweetener and 18 transformation products. Details of the method can be found elsewhere (Posselt et al. *In preparation*).

A total of 35 out of 44 targets including 14 transformation products (TPs) were detectable in at least two matrices (mostly USW and HPW; Figure 1). Only 9 substances were above the limit of quantification (LOQ) in USW, possibly due to inputs from smaller WWTPs or diffuse discharges further upstream. Hence, the largest proportion of micropollutants in HPW and SW originates from the WWTP Muenchehofe. Micropollutant concentrations in SW and HPW were similar, with the exception of guanylurea, valsartan, acesulfame, metoprolol, alpha-hydroxy-metoprolol, and 2,4-chlorobenzoic acid, which had significantly higher concentrations in SW relative to HPW ($p<0.05$; ANOVA), possibly due to transformation in the HZ. Valsartan acid was also unusual in that it was the only target to display significantly higher concentrations in HPW relative to SW, suggesting its formation in the HZ.

Concentrations of parent compounds reported here are considerably higher than reported previously in comparable environments (Kasprzyk-Hordern et al. 2009; Bendz et al. 2005), possibly due to the high EW ratio in this river. In fact, levels of diclofenac, bezafibrate, 1H-benzotriazole and ketoprofen reached or exceeded reported maximum concentrations from a comprehensive European survey that included data from 122 sampling stations (Loos et al. 2009). Concentrations of carbamazepine, hydrochlorothiazide, metoprolol and bezafibrate exceeded levels found in comprehensive WWTP effluent studies (Kasprzyk-Hordern et al. 2009; Kostich et al. 2014). Elevated concentrations of the TPs valsartan acid, o-desmethylvenlafaxine and guanylurea were also observed, in some cases exceeding their respective parent component (PC) concentrations by over an order of magnitude. Most notably, concentrations of guanylurea reported here (>200 μg L^{-1}) are among the highest levels reported in the aquatic environment, to our knowledge. In comparison, a literature review from 2014 reported that concentrations of guanylurea in small streams with high effluent load reached up to 30 μg L^{-1}, (Ter Laak et al. 2014) while concentrations in EW were up to 100 μg L^{-1} (Kosma et al. 2015). Notably, 40 μg L^{-1} of metformin was reported to induce significant adverse effects in male fish (Niemuth et al. 2015). To our knowledge there are no data on the ecotoxicity of guanylurea.

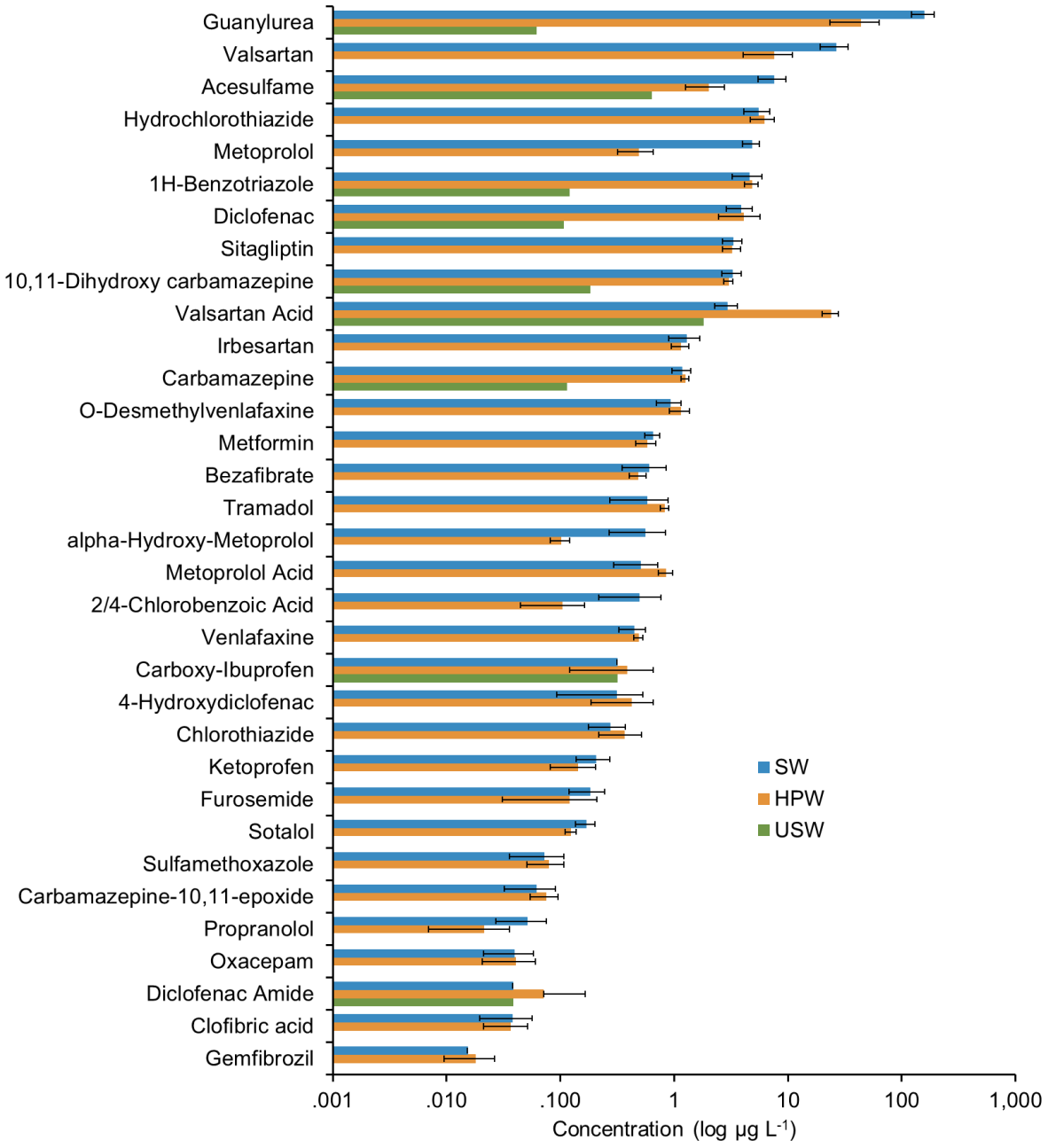

Figure 1. Average Erpe surface water (SW, N=6), hyporheic pore water (HPW, N=72) and upstream surface water (USW, data>LOQ) concentrations measured over 24 h in April 2016. Levels of 1-methyl-1H-benzotriazole, 4-hydroxy-1H-benzotriazole, acetaminophen, acridine, acridone, ibuprofen, naproxen and sulfamethoxazole-β-D-glucuronide were below LOQ in all samples and are not presented. Error bars indicate standard deviations.

2. Flume experiment to assess the biodegradation of micropollutants in River Erpe

The degradation potential of micropollutants previously detected in River Erpe, was investigated in a joint experiment with flume mesocosms based primarily on Li, et.al. (2015). The experiment was designed to elucidate the influence of microbial communities and hyporheic flow on the degradation half-lives of the micropollutants, as described in Deliverable 2.1 (Set-up and validation of flume systems to study the link between ecological and biogeochemical processes in the hyporheic zone).

Using a central composite face design (CCF), 20 flumes were set up with sediment from River Erpe and artificial river water according to the two factor variables:

a) Microbial diversity: in high, medium and low levels (1, 0, –1 respectively) implemented by dilution of River Erpe sediment with industrial sand.
b) Hyporheic flow: in high, medium and low levels (1, 0, – 1 respectively) encouraged by the induction of six, three or zero bedforms.

The flumes were fortified with a mix of the target micropollutants at a concentration comparable to the levels found in River Erpe (Figure1). In addition, two additional flumes were set up as controls for sorption and two flumes were not spiked with micropollutants to monitor the flume dynamics in the absence of xenobiotics.

Samples of SW were collected in 10 different time points along 78 days and analyzed with the method mentioned previously (Posselt et al. *In preparation*). The micropollutant concentrations were fitted to a first-order kinetic function, from which the dissipation half-life (DT50) was calculated as shown in Figure 2A. If more than 7 time point concentrations were below LOQ, the DT50 was not calculated. The DT50s obtained for the 20 flumes set included in the CCF design were fitted to a second-order response surface model (RSM) to search for linear and non-linear effects of our two variables. For quality control, more than 50% of the DT50s had to be available.

The RSM was implemented for the micropollutants: acesulfame, benzotriazole, bezafibrate, gemfibrozil, hydrochlorothiazide, irbesartan, ketoprofen, metformin, metoprolol, propanolol and sotalol. Preliminary results show that microbial diversity levels have a significant quadratic and/or linear effect on the DT50 of all the micropollutants, whereas hyporheic flow (or bedforms) showed only a significant (linear) effect on sotalol ($p < 0.05$), as illustrated in Figure 2B. Microbial diversity and bedforms had a significant interaction term for sotalol ($p < 0.005$), metoprolol ($p < 0.005$) and acesulfame ($p < 0.05$).

Bacterial community composition for each of the flumes are currently analyzed using Illumina MiSeq sequencing of 16SrRNA genes and will aid in the interpretation of the different DT50 obtained. Metagenomes will be predicted based on 16S rRNA gene data and putative drivers of micropollutant biodegradation will be delineated by an in silico pathway analysis. The results will contribute to clarify the effect of microbial biodiversity on the biodegradation in flume beds and evaluate flow conditions as a potential driver of microbial biodiversity.

Further, the association of micropollutant loads at focus sites and link to abundance of key microorganisms has been studied by artificially manipulating micropollutant concentrations in lab-based microcosm setups. In one such study, biodegradation potential of a model compound, ibuprofen (non-steroidal anti-inflammatory drug) and associated key degraders were determined using HPLC-MS and amplicon Illumina MiSeq sequencing targeting the 16S rRNA genes and transcripts respectively. Concentration-dependent increase in activity of specific taxa was observed indicating utilization of the micropollutant by some indigenous microbial communities as an energy source. Moreover, to understand the influence of biogeochemical factors such as redox gradients on the biodegradation potential of micropollutants in specific zones of the hyporheic zone, metoprolol (beta-blocker) removal was investigated in both oxic and anoxic conditions and analysis of the associated microbial community dynamics is ongoing.

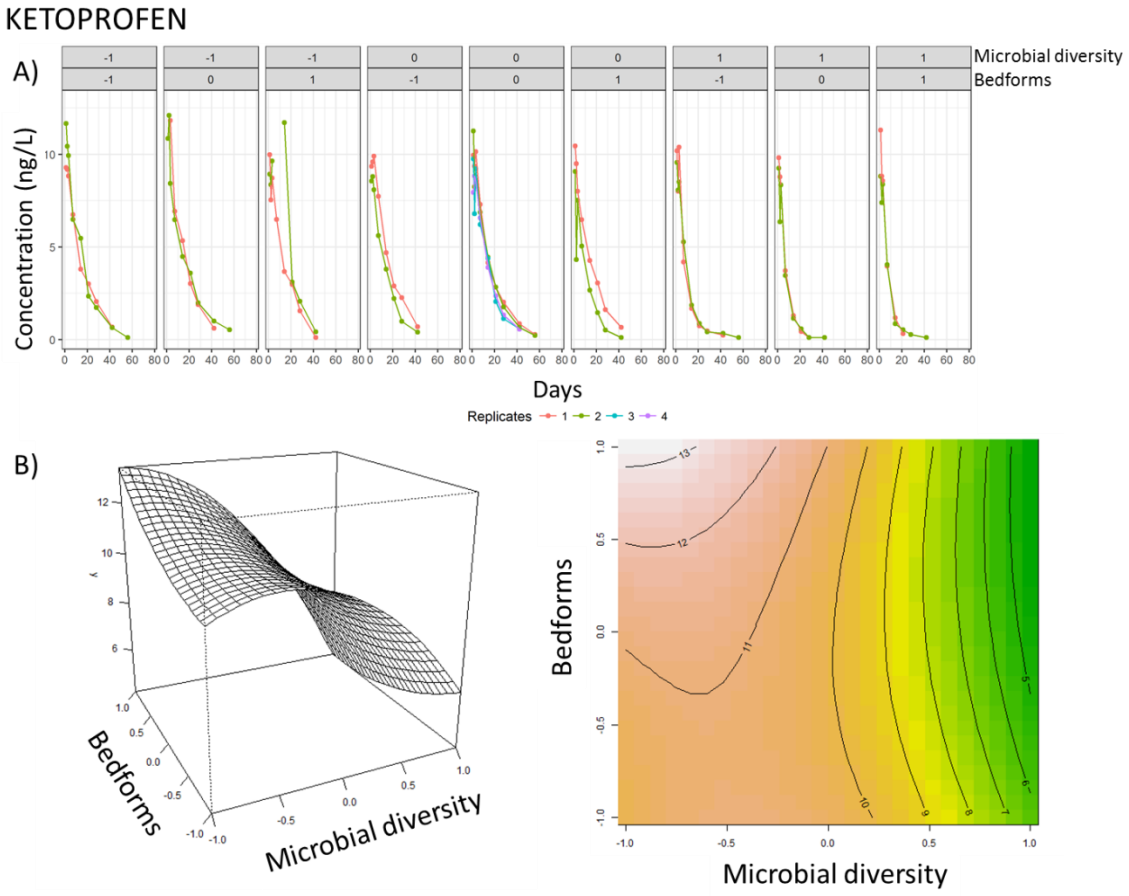

Figure 2. A) Concentration measurements in ng/L of the pharmaceutical ketoprofen for each of the 20 flumes in the CCF design. B) 3D and heat map graph of the response surface model fitted to the DT50s obtained for ketoprofen, showing a significant quadratic ($p < 0.05$) and linear ($p < 0.001$) response to the variable: microbial diversity, a non-significant effect of the variable: bedforms and a non-significant interaction term between the two variables. Level -1, 0 and 1 indicate the low, medium and high levels of each of the variables.

3. References

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