**Supplementary material**

***PPCPs analysis***

A 2 μL sample was injected into GC (splitless mode 0.1 min, purge flow 60 mL min-1, at 250 °C). The GC oven was programmed as follows: an initial temperature of 60 °C, held for 3 min, and then was ramped at 5 °C/min to 300 °C, held for 10 min. Samples were analyzed using an GC/MS (Agilent Technologies 7890B, with 5977A MSD). Separation was achieved using a HP-5 MS 30 m×0.25 mm×0.25 μm (Agilent J&W, CA, USA) capillary column with helium as the carrier gas.

***Toxicity tests***

***Daphnia magna* acute toxicity test**

Less than 24 h-old daphnias (neonates) were used in tests. Neonates were transferred into 50-mL glass vessels containing 25 mL of test solution and controls – standard M4 medium,30 and the filtered river Danube sample, in four replicates (5 animals per test vessel) each. Immobilisation of the neonates was recorded after 24 and 48 h, and the results were compared to the controls. Test acceptability criterion for *D. magna* acute toxicity tests is ≥ 90 % survival rate in the control. Dissolved oxygen concentration (mg L-1) was measured prior to *D. magna* toxicity tests, in control / test solutions. Measured values were in the range 4.45-6.19 mg L-1, and did not change considerably over *D. magna* toxicity test.

***Vibrio fischeri* luminescence inhibition test**

*V. fischeri* culture (strain NRRL B-11177), supplied by Macherey-Nagel GmbH&Co. KG, Duren, Germany as freeze-dried bacteria (BioFix®Lumi), was reconstituted, prior to testing, using the commercial reactivation solution from the same manufacturer. Initial luminescence (*I*0) was measured in reactivated bacterial suspension after 15 min long adjustment period at 15 °C. To achieve a minimum required quantity of bacteria for accurate luminescence measurement,31 0.2 mL of the bacterial test suspension was added into 0.8 mL of test solution / control. The final luminescence (*I*30) was measured after 30 min exposure of the bacterial suspension to the test solutions / controls. Tests were carried out in triplicates. The results were calculated as the percentage of luminescence inhibition (*H*30) in test solutions relative to the corresponding controls (standard 2 % NaCl solution / filtered river Danube sample (FD) with addition of 2 % NaCl for salinity adjustment).

The correction factor (*fk*30) was calculated as follows:

**  (1)

where:

*Ik3*0 stands for the bioluminescence in control after 30 min;

*I*0 stands for the initial bioluminescence.

The corrected values of *I*0were calculated as follows:

*Ic*t = *I*0 *fk*30 (2)

The inhibition in each test solution was calculated as follows:

 (3)

where:

*H*30 stands for the inhibition of bioluminescence after 30 min, %;

*I*3stands for the bioluminescence after 30 min.

**Table SI. List of PPCPs studied and their properties**

|  |  |  |  |
| --- | --- | --- | --- |
| Name | Abbreviation | Molecular weight (g mol-1) | Octanol / water partition coefficient |
| Benzophenone1 | BP | 182.222 | 3.18 |
| Benzophenone-32 | BP-3 | 228.247 | 3.79 |
| Carbamazepine3 | CBZ | 236.274 | 2.45 |
| Caffeine4 | CF | 194.194 | -0.07 |

1;25

2;26

3;27

4;28

**Table SII. List of target ions (m / z) of compounds used for analysis**

|  |  |
| --- | --- |
| Analyte | Target  Ion, m / z |
| BP | 105 |
| BP-3 | 151 |
| CBZ | 193 |
| CF | 194 |

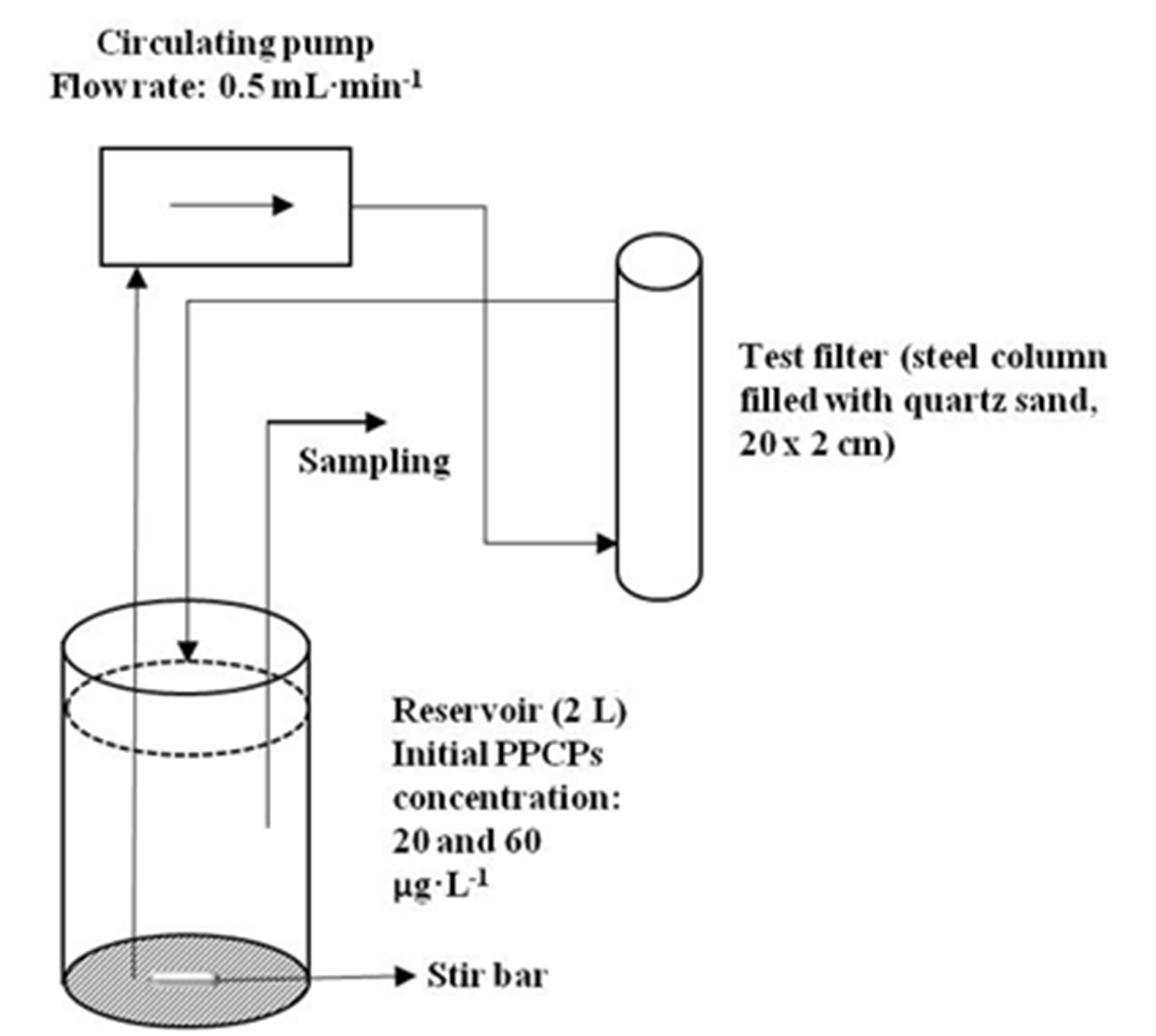
**Table SIII. Parameters for river water during phase of biofilm development**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Parameters | I cycle | | II cycle | | III cycle | | IV cycle | |
| I day | VII day | I day | VII day | I day | VII day | I day | VII day |
| pH | 8.1 | 8.2 | 8.3 | 7.5 | 8.02 | 8.1 | 8.1 | 8.02 |
| Conductivity, µS cm-1 | 390 | 396 | 394 | 392 | 377 | 393 | 351 | 342 |
| Temperature, °C | 17 | 23 | 26.3 | 24.2 | 23.5 | 26 | 19 | 26 |
| KMnO4 consumption , mg L-1 | 5.8 | 2.5 | 9 | 6.3 | 14 | 12.6 | 11.4 | 10 |

**Table SIV. Results of weathering tests in glass beakers**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Phase of experiment | BP | | BP-3 | | CF | | CBZ | |
| Removal, % | Night-day circle | Dark | Night-day circle | Dark | Night-day circle | Dark | Night-day circle | Dark |
| B1 | 83 | 25 | 15 | 54 | 54 | 47 | Not calculated | Not calculated |
| B2 | 90 | 29 | 7 | 53 | 54 | 51 | 0 | 0 |

**Figure S1. Biologically test filter set-up**



**Figure S2. Images of stained samples showing bacteria (coccal and rod forms) and diatoms**

Figure 2.tif

**Figure S3. Images of native samples showing diatoms present in the biofilm**

Figure 3a.tif

*a) Synedra sp.*

Figure 3b.tif

*b) Cyclotella sp.*

Figure 3c.tif

*c) Melosira sp.*

Figure 3d.tif

*d)* *Asterionella sp.*

Figure 3e.tif

*e) Nitzschia sp.*