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Sorption of selected pharmaceuticals on river benthic biofilms formed on artificial substrata

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ABSTRACT

The dissolved organic and inorganic contaminants in rivers, lakes and seas are distributed among the aquatic phase, biota, sediments and biofilms formed on different artificial and natural substrata. Since the biofilms play an important role in the food web of aquatic ecosystems, it is necessary to clarify what kind of contaminants are bounded to these biological surfaces. In this study the concentration of eight pharmaceuticals (carbamazepine, ciprofloxacin, clarithromycin, diclofenac, metoprolol, sitagliptin, sulfamethoxazole and tetracycline) was determined in the Danube water at Budapest (Hungary) and in the biofilms formed on glass and polycarbonate substrata during a six weeks long growing period at the same sampling site. The target compounds were extracted from the dried biofilms by microwave (MW) assisted hot water treatment, however, the recovery of tetracycline was extremely low, indicating damage and loss of this constituent caused by MW treatment. The concentrations of the other seven pharmaceuticals were determined by LC-MS following the solid phase extraction of analytes. Clarithromycin, ciprofloxacin, diclofenac, metoprolol and sitagliptin were detectable in the biofilms due to biological uptake and electrostatic-mediated adsorption on the negatively charged biofilms, however, carbamazepine and sulfamethoxazole with neutral charge were not detected. The bioaccumulation factors of biofilms grown on glass or polycarbonate substrata changed between 175 and 614 and 148–314 L/kg, respectively, and increased in order of diclofenac < sitagliptin < clarithromycin < ciprofloxacin < metoprolol. These values are about 2–3 orders of magnitude lower than the published data for different metal cations which form chemical complexes or chelates with carboxyl and hydroxyl groups of extracellular polymer matrix. Due to the higher amount of adsorbed pharmaceuticals and the higher biodiversity of diatom species in the biofilms formed on glass substrata, compared to polycarbonate, as artificial substrata the glass carrier plates can be recommended for biofilm studies.

1. Introduction

The release of pharmaceutical residues by conventional wastewater treatment plants (WWTPs) into the rivers became a widely studied environmental issue during the last twenty years (Jelić et al., 2012; Gabet-Giraud et al., 2014; Aubertheau et al., 2017). The concentration of these contaminants in the aqueous phase decreases steadily away from the discharge point of WWTPs, generally from μg/L to ng or pg/L. In spite of their relatively low concentrations, pharmaceuticals and their degradation products have undesirable effects on the freshwater ecosystems, e.g., influencing the fish population (Sanchez et al., 2011) or altering the microbial communities by suppressing algal growth and microbial respiration in biofilms (Ricart et al., 2010; Proia and Osorio, 2013; Vasselon et al., 2017, Chonova et al., 2016; Chonova et al., 2018, Hagberg et al. 2021).

The periphytic, leaf litter or sediment biofilms (Battin et al., 2016) play an important role in the "self-purification" of surface water due to 1) abiotic (passive physical) adsorption processes of dissolved inorganic

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and organic contaminants on their sorption sites e.g., extracellular polymer substances, cell walls, cell membranes and cell cytoplasm (Flemming, 1995; Flemming and Leis, 2003) or 2) biotic processes e.g. active biological uptake by microorganisms or biodegradation by bacteria (Bighiu and Goedkoop, 2021). Interactive sorption is a dominant pathway for adsorption of pharmaceutical compounds because these molecules contain various active sites such as functional groups (–COOH, –OH, –NH_2, –CHO, etc.) and other electrostatic points containing heterogenous atoms e.g., fluor or chlorine (Akhtar et al., 2015). Physical adsorption of a pharmaceuticals may occur via interactive mechanisms such as van der Waals forces, electrostatic interactions, protonation, ion exchange, dipole–dipole interactions, hydrogen bonding and complex formation. Considering the negative charge of biofilms at quasi neutral pH (Bryers, 2000), all pharmaceuticals having positive charge at this pH can be adsorbed on the biofilms due to electrostatic interactions. The ionization state of pharmaceuticals is controlled by the solution pH and their acidic dissociation constants (pKa). Knowing the dissociation constants, the major species of pharmaceuticals in the aquatic phase can be estimated usually in neutral pH range (Qiang and Adams, 2004; Babić et al., 2007). It appears that speciation (i.e., charge) and molecular size of the antibiotics are important in explaining their sorption to typically negatively charged biofilm (Wunder et al., 2011). In addition to sorption of organic pollutants on the surface layer of biofilm matrices, their biological uptake processes by microorganisms embedded in the extracellular polymer matrix play also a potential role in the partitioning of dissolved pharmaceuticals between the aquatic phase and biofilms (Torresi et al., 2017). In their study, a moving bed biofilm reactor was applied to study the sorption of 23 different pharmaceuticals in biofilms. They established that only three macrolides (clarithromycin, erythromycin, roxithromycin), three beta-blockers (atenolol, propanolol, metoprolol) and two psycho-active (citalopram, venlafaxine) pharmaceuticals showed higher sorption potential due to their positive charge at pH of 7.5. To extend the laboratory investigations to field experiments, Tien et al. (2009) suggested to apply artificial substrata. They found that the freshly formed colonization biofilms are more suitable for biomonitoring the water quality than the relatively old, so called accumulation biofilms. In spite of their suggestion the researchers preferred the natural old biofilms collected from stones with unknown surface properties. Huerta et al. (2016) determined the concentration of 44 pharmaceuticals in the water phase of Segre River, however, only seven compounds (diclofenac, diltiazem, norverapamil, verapamil, gemfibrozil, venlafaxine and hydroxycarbamazepine) were detected in biofilms formed on rocks in the riverbed. Aubertheau et al. (2017) collected biofilms also from the rocks located near the riverbank at depth of 50–100 cm in the Vienne River. In these biofilms several negatively charged (diclofenac, sulfamethoxazole, levofloxacin, ofloxacin), uncharged (carbamazepine) and positively charged (propranolol) pharmaceuticals were detected. It means that not only the ionization of dissolved pharmaceutical molecules and the physical adsorption were dominant for the bioaccumulation but also the active biological uptake of pharmaceuticals by microorganisms living in the biofilms. The distribution of antibiotics in water, sediments and biofilm was studied in an urban river (Córdoba, Argentina) by Valdés et al. (2021). The biofilms were grown on etched glass substrata. Depending on the sampling sites and the distance from the WWTP the bioaccumulation factor of biofilms for ciprofloxacin and clarithromycin changed in the range of 5389–12258 and 729–2584 L/kg, respectively. The bioaccumulation of these antibiotics was also investigated in urban lowland rivers by Mastrángelo et al. (2021). The biofilms were collected from stones nearby the stream banks. They found that bioaccumulation factors for ciprofloxacin and clarithromycin varied in the range of 481–725 and 83–178 L/kg depending on the different aquatic environment. In case of carbamazepine these values changed at lower level between 10 and 141.

Since the benthic biofilms represent an important food resource in the aquatic environment, their potential role in the trophic transfer of adsorbed pharmaceuticals or metal contaminants is inevitable (Ruhi et al. 2016, Bonnineau et al. 2021). On basis of literature data discussed above it can be hypothesized, that pharmaceuticals that are detectable around the year in the aquatic phase of the Danube River are also present in the benthic biofilms allowing their trophic transfer to biofilm consumers. In order to investigate the potential bioaccumulation of eight, frequently used pharmaceuticals (carbamazepine, ciprofloxacin, clarithromycin, diclofenac, metoprolol, sitagliptin, sulfamethoxazole and tetracycline) in biofilm matrices, benthic biofilms were grown on two different artificial substrata in a six weeks long field experiment that was carried out in the Danube River at Budapest (Hungary). The concentration of pharmaceuticals was determined weekly in the aquatic phase during the growing period of biofilms, and at the end of the field experiment in the biofilms, applying solid phase extraction and liquid chromatography – mass spectrometry (LC-MS) analytical techniques following the microwave (MW) assisted hot water extraction of biofilms.

2. Materials and methods

2.1. Chemicals and reagents

All analytical standards (95% ≥ purity), namely, carbamazepine (CARB), ciprofloxacin (CIPR), clarithromycin (CLAR), diclofenac (DICL), metoprolol (METO), sitagliptin (SITA), sulfamethoxazole (SULF) and tetracycline (TETR) were purchased from Sigma-Aldrich (Sigma-Aldrich Ltd., Budapest, Hungary). The structure and physico-chemical properties are listed in Supplementary Materials (Table S1). Isotopically labelled internal standards (ILISs, 98% ≥ purity), namely, ciprofloxacin-d8 (CIPR-d8), clarithromycin-N-methyl-d3 (CLAR-d3), diclofenac-d4 (DICL-d4), racemic metoprolol-d7 (METO-d7), sitagliptin-d4 phosphate (SITA-d4) and sulfamethoxazole-d4 (SULF-d4) were purchased from Toronto Research Chemicals (Toronto Research Chemicals Inc., North York, Canada). The methanolic solution (100 mg/L) of carbamazepine-d10 (CARB-d10) was obtained from Sigma-Aldrich.

From the analytical standards, 1 g/L standard stock solutions were prepared on a weight basis in methanol. Multicomponent standard solutions at appropriate concentration levels were prepared daily by dilution of the standard stock solutions with methanol. From the ILISs, individual stock solutions of 1 g/L were prepared in methanol except for CARB-d10 (see above). A multi-component ILIS solution containing 0.5 mg/L of all ILISs was prepared and 250 μL was added to each water sample prior to SPE. All standard stock solutions were stored at − 14 °C for a maximum time period of two months.

An ELGA Purelab Option-R7 unit (ELGA LabWater/VWS Ltd., High Wycombe, UK) was used for production of ultrapure water (UPW) with resistivity of 18.2 MΩ cm. Acetonitrile, methanol and formic acid (99%) of LC-MS grade were purchased together with 2 M hydrochloric acid and ethylenediaminetetraacetic acid disodium salt dihydrate ($Na_2EDTA·2H_2O$) (99.6% purity) from VWR International Ltd. (Debrecen, Hungary). For ion chromatographic measurements, ammonium chloride, calcium chloride, magnesium chloride hexahydrate, potassium chloride, sodium nitrate and sodium sulphate were purchased from Sigma-Aldrich Ltd. (Budapest, Hungary).

2.2. Water sampling and growing of benthic biofilms on artificial substrata

The benthic biofilms were grown in the Danube River at Budapest (Hungary) on 5 glass and 5 polycarbonate plates (size 10x23 cm) fixed in vertical position in plex-glass holders and submerged into the Danube River at a depth of 20–30 cm using a plastic unit which was connected to the "Green Island" boat dock (GPS coordinates: 47°28′50.2″N, 19°03′32.1″E) at 15 m distance from the riverside in time period of August 18 – September 29, 2020. The position of the plex-glass holders was parallel to the water stream. The experimental setup, its fixation and position during the growing period in the river and the glass substrata

covered with biofilm are demonstrated in Supplementary Material (Figs. S1–S3). This sampling site is located about 10 km away downstream from the WWTP. After six weeks, the substrata were removed from the plex-glass holders and the biofilm was scraped into sterile plastic vessels using plastic knife. The samples were transported to the laboratory in a cold box. The biofilms removed from one glass and one polycarbonate carrier plate were freshly prepared for biological investigations. The biofilms formed the other four glass and four polycarbonate plates were separately dried at 60 °C and their dry mass was measured to assess the substrate effect on the biofilm production. During the growing period of biofilms, 5 L water samples were collected weekly from the 50 cm surface layer of Danube River and transported in amber glass bottles to the laboratory located only 300 m distance from the sampling site.

2.3. Characterization of water matrix

The pH, conductivity and turbidity were measured on site applying portable Hanna Multi Meter (HI 98129, Hanna Instrument, USA), and turbidimeter (Lovibond 210 IR, VWR International, USA), and the total organic carbon and total nitrogen content were determined in the laboratory by using a Multi N/C 3100 analyser (Analytik Jena, Jena Germany). The concentration of several inorganic cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, NH_4^{+}) and anions (Cl^{-}, SO_4^{2-}, NO_3^{-} were determined by using a dual channel ion chromatograph (Dionex ICS 5000+, Thermo Fisher Scientific, USA). The concentration range of measured chemical parameters can be found in Table S2.

For LC-MS analysis, the water samples were filtered using Whatman nylon membrane filters with pore size of 0.45 μm and 0.20 μm (GE Healthcare, Little Chalfont, UK). Then, $Na_2EDTA*2\ H_2O$ was added in concentration of 1 g/L and sample pH was set to 4 with formic acid. For preconcentration of the analytes solid phase extraction method (SPE) was applied using 500 mg Oasis HLB 6 cc cartridges (Waters Corporation, Milford, Massachusetts, USA). The SPE cartridges were conditioned with 3 mL of methanol and 3 mL of UPW. Samples of 500 mL were loaded onto the cartridges with an approximate flow rate of 3 mL/min. After loading the sample, cartridges were washed with 3 mL of UPW and dried under vacuum for 5 min. Elution and pooling of the retained analytes in amber-colored glass vials were achieved with 2 × 2 mL of methanol. Eluates were evaporated to dryness under a gentle stream of nitrogen, then reconstituted in 0.4 mL of methanol (Krakkó et al., 2019).

Determination of target compounds was carried out on an Agilent 1260 high performance liquid chromatograph coupled to an Agilent 6460 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, USA). A Zorbax Eclipse XDB-C18 (50 mm × 4.6 mm, 1.8 μm particle size) analytical column equipped with a 5 mm long guard column of the same type was purchased from Agilent Technologies Inc. The mobile phase consisted of 0.1% (v/v) formic acid in ultrapure water (eluent A) and acetonitrile (eluent B). The following gradient elution program was used: 0 min, 10% B; 0–1 min, 10% B; 1–4 min, 50% B; 4–10 min, 99% B, 10–14 min, 99% B with 4 min post-run equilibration (Krakkó et al., 2019). Injection volume was 2 μL. Positive ESI was applied for measurement of the target molecules with exception of diclofenac, and quantification was carried out using internal standard calibration with the respective ILISs listed in Section 2.1. The experimental conditions of the mass spectrometer are given in Table S3.

2.4. Characterization of benthic biofilms

For biological characterization of biofilms grown on glass and polycarbonate substrata, diatom counting was conducted. The frustules were cleaned with hydrochloric acid and hydrogen peroxide, subsequently washed in distilled water and mounted on microscope glass slides in Naphrax (CEN, 2014). At least 400 frustules were counted by replicate. The investigation of diatom species was carried out by a Zeiss Z2 Axio Imager microscope equipped with differential interference contrast (DIC) optics. For identification of diatom species Diatom Guide Books (Lange-Bertalot, 2001; Lange-Bertalot et al., 2017; Bey and Ector, 2013) were used and their relative abundances were determined according to the EN 14,407 standard.

Before the chemical investigation, the four dried biofilm samples/substrate were homogenized in a mortar. To extract the pharmaceuticals from the dried biofilms, a MW-assisted hot water extraction procedure was applied using a Milestone Start MW equipment (Bergamo, Italy). Considering our former experiences (Tatár et al., 1994) related to the potential degradation of organic target molecules during MW-heating, at first a model solution of the 8 selected target compounds with concentration of $2x10^{-6}$ M were heated in MW field at temperature of 100 °C for 5, 10 and 20 min. After cooling and applying the SPE method, the recovery values were determined by LC-MS technique discussed in subchapter 2.3. In the next step 0.5 g dried biofilm in 50 mL ultrapure water was extracted applying the workflow of analytical procedure (Fig. 1.). After extraction the samples were filtrated at 70 °C to minimize the readsorption of target molecules on the surface of "biofilm sludge". The biofilm residues were washed with 2 × 10 mL UPW then $Na_2EDTA*2\ H_2O$ and formic acid for pH adjustment were added to the solutions. The solid phase extraction of the target compounds from nearly 75 mL solutions and analysis by LC-MS technique were carried out similarly to water samples (see Section 2.3). The validation method parameters are listed in Table S4.

2.5. Calculation of bioaccumulation factor of benthic biofilms for pharmaceuticals

The bioaccumulation factors were calculated as ratios of concentrations determined in dried biofilms (ng/kg) and Danube River (ng/L) water. This factor determined for various contaminants in a well-defined aquatic environment help us to estimate the rate of a possible trophic transfer for biofilm consumers.

3. Results and discussion

3.1. Biomass production on artificial substrata and algological characterization of biofilms based on diatom counting and identification

The dry mass of biofilms grown on glass and polycarbonate substrata amounted to 9.4 ± 0.4 and 10.1 ± 0.5 mg/cm^2, respectively. It should be mentioned, that the polycarbonate surface seems to be slightly more favourable for biomass production than the glass, as it was also observed previously in the Tisza River (Kröpfl et al., 2006). On basis of diatom counting in the biofilms formed on polycarbonate and glass substrata, 37 and 48 taxa were identified, respectively (Table S5). It means the biodiversity of biofilms formed on glass substrate is considerably higher compared to biofilms grown on polycarbonate. In both cases, the *Navicula recens* (Lange-Bert.) Lange-Bert., *N. antonii* Lange-Bert. and *Nitzschia dissipata* (Kütz.) Rabenhorst were the dominant species (Fig. 2). All of them are able to move on the polysaccharide matrix excreted and to find nutrient rich habitats. *Nitzschia dissipata* is one of the most frequently detected species in flowing and standing freshwater habitats with medium to high trophic levels. Massive growths occasionally develop, particularly in larger running waters. Length of the valve: 12.5–85 μm, width of the valve: 3.5–5 μm. *Navicula antonii* and *N. recens* also prefer large rivers, with more or less increased electrolyte contents and organic material. They are eutrophic indicator species (van Dam et al., 1994). Length of *N. recens*: 16–51 μm, width of the valve: 5.5–9 μm. Length of *N. antonii*: 11–30 μm, width of the valve: 6–8 μm (Lange-Bertalot et al., 2017).

Summarizing it can be stated that the biofilm matrices formed on different substrata contain various diatom species having different biological uptake and emitting different metabolites, and thus influencing the surface properties of biofilms and the bioaccumulation processes. The analytical results confirm this assumption that the amount of

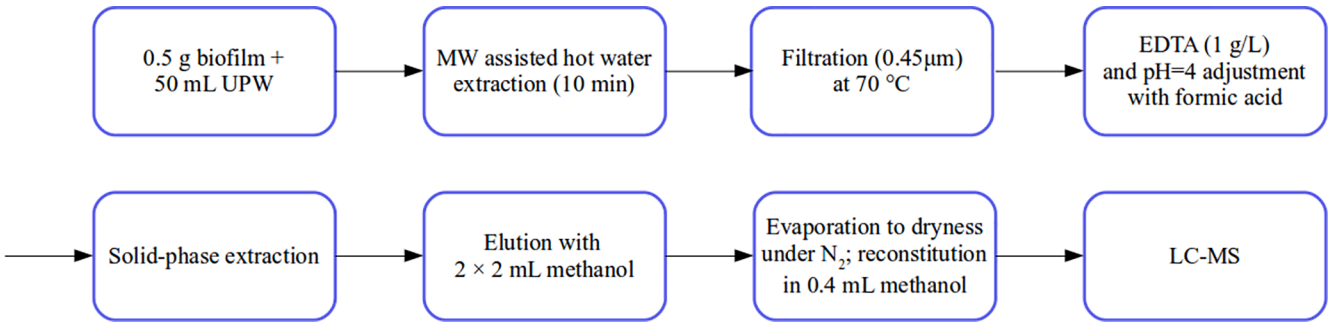

Fig. 1. Workflow of analytical procedure developed for determination of pharmaceuticals in benthic biofilms.

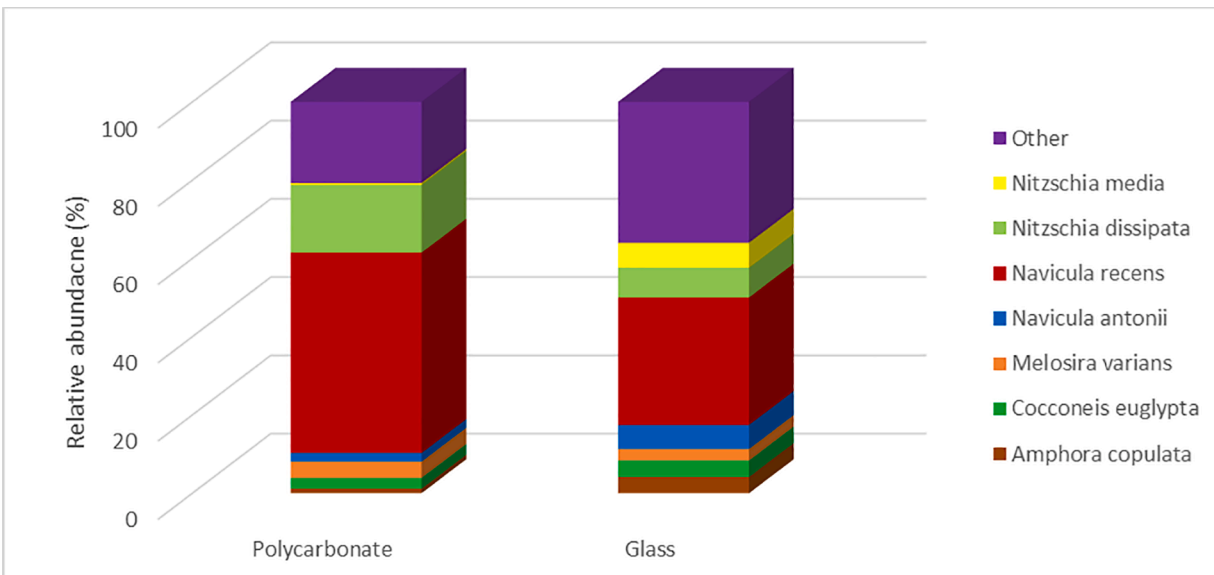

Fig. 2. Distribution of diatom species in biofilm grown on polycarbonate and glass substrata.

adsorbed pharmaceuticals is by factor 1.1–2.0 higher for biofilms with lower biomass but higher biodiversity (Table 1.).

3.2. Effect of MW treatment on recovery of target compounds from their aquatic solution

As demonstrated in Fig. 3, the recovery values of seven target compounds after 5, 10 and 20 min MW-treatment of their aquatic solutions varied between 97 and 109%, 98–106% and 80–108%, respectively. However, the concentration of tetracycline considerably decreased during the MW treatment of its model solution. This phenomenon can be explain by epimerization of this compound at elevated temperature (Pena et al., 1998), because we observed that the peak area of tetracycline decreased and a second peak with the same exact mass appeared in the chromatogram with higher retention time. Considering this fact, we later omitted to examine tetracycline. It should be noted that recovery of ciprofloxacin showed also a decreasing tendency with increasing MW-treatment time, however, degradation products were not detectable in the mass spectra. Therefore we included a shorter, only 5 min MW treatment time at 100 °C.

3.3. Effect of biofilm matrices on the recovery of target pharmaceuticals

Dried biofilm samples were spiked with multi-component standard solution containing the target molecules in concentration of 2×10^{-6} M. As the Fig. 4 demonstrates there is a considerable matrix effect and the recoveries changed in the range of 53 and 95% increasing in the following order: clarithromycin, sitagliptin, diclofenac, ciprofloxacin, sulfamethoxazole, metoprolol, carbamazepine. Huerta et al. (2016) observed also the lowest matrix effect in case of carbamazepine and metoprolol.

Table 1
Mean concentrations of target pharmaceuticals determined in Danube River water during the growing period of biofilms and in the dried biofilms, as well as the calculated bioaccumulation factors from dried biofilms.

	CARB	CIPR	CLAR	DICL	METO	SITA	SULF
Danube water ng/L	23 ± 5	8 ± 2	2 ± 1	21 ± 4	7 ± 1	16 ± 4	12 ± 2
Biofilms on glass ng/g	n.d.	3.3 ± 2.7	0.8 ± 0.2	4.2 ± 1.1	4.3 ± 1.5	3.2 ± 1.3	n.d.
Bioaccumulation factor (glass) L/kg	–	413	400	175	614	200	–
Biofilms on polycarbonate ng/g	n.d.	2.3 ± 1.8	0.5 ± 0.1	3.1 ± 0.9	2.2 ± 0.8	3.0 ± 1.8	n.d
Bioaccumulation factor (polycarbonate) L/kg	–	288	250	148	314	187	–

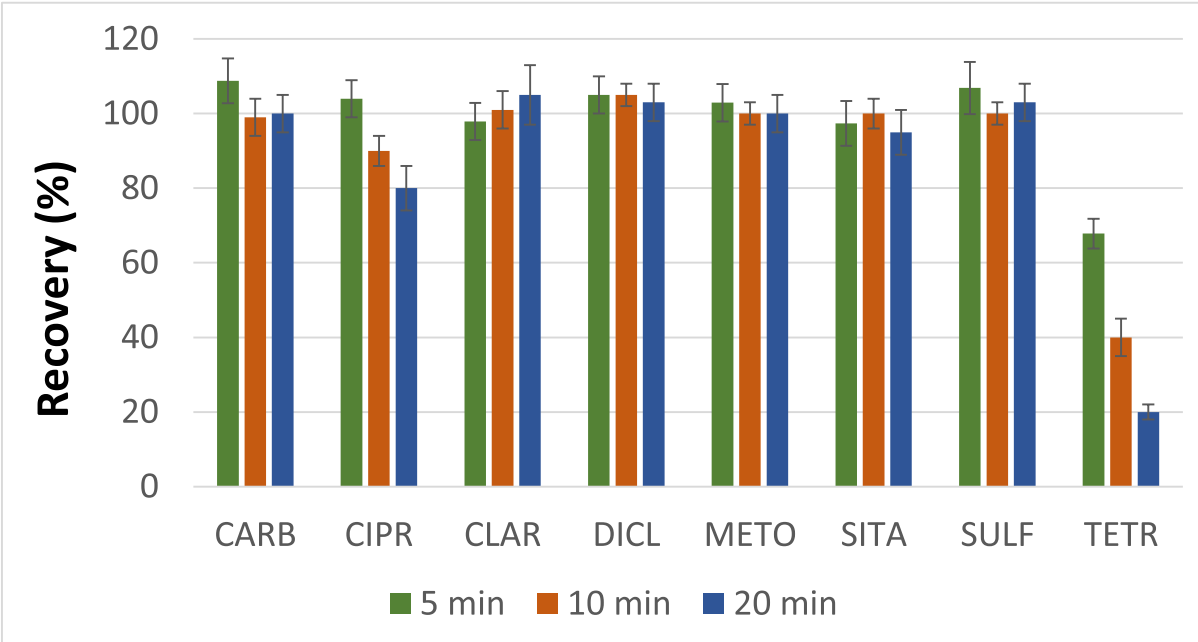

Fig. 3. Recovery values of pharmaceuticals after MW-treatment of their aquatic solution with concentration of $2x10^{-6}$ M for 5, 10 and 20 min.

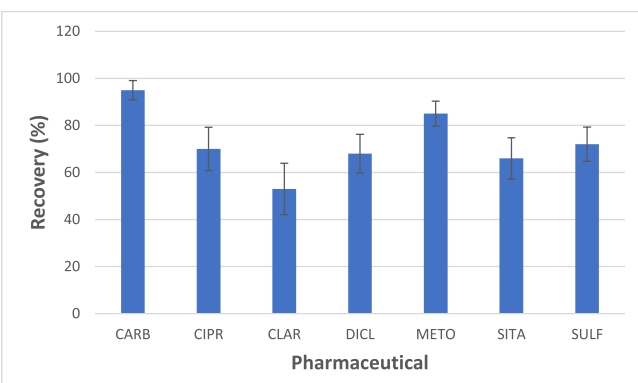

Fig. 4. Recovery values for the target molecules in presence of biofilm matrix determined after MW-assisted hot water extraction at 100 °C, for 5 min.

3.4. Determination of bioaccumulation factors of biofilms for target pharmaceuticals

The concentration of seven target pharmaceuticals in the Danube water changed in the range of 2–23 ng/L. Clarithromycin had the lowest and carbamazepine the highest concentration in the water phase (Table 1). From the seven analytes, carbamazepine and sulfamethoxazole were not detectable in the river biofilms, in spite of their relatively high concentration in the Danube water (23 ± 5 ng/L and 12 ± 2 ng/L, respectively). Huerta et al. (2016) had similar observation during their field experiment conducted in the River Segre. These results could be explained by the neutral properties of carbamazepine and the negatively charged sulfamethoxazole molecules at a quasi-neutral pH range since these properties are not favourable for electrostatic-mediated physical adsorption. However, Aubertheau et al. (2017) detected both of these pharmaceuticals in biofilms in the River Vienne. In case of diclofenac our observations harmonize with the results of Huerta et al. (2016) and Aubertheau et al. (2017). It means this molecule was detectable in all biofilms originated from three different rivers in spite of its negative charge at the neutral pH inhibiting the electrostatic- mediated adsorption. Antibiotics (ciprofloxacin and clarithromycin) were detected in both the Danube water and the biofilms, however, our concentration values are considerably lower than the measured data of Valdés et al. (2021). At this point is necessary to emphasize that these pharmaceuticals are extremely important to follow their fate and effect on the development of bacterium resistance in the aquatic environment. On the measured concentration of metoprolol and sitagliptin in river biofilms we did not find experimental data in the literature. It should be emphasized that while the pharmaceutical molecules have well-defined chemical structure and physiochemical properties, our knowledge on the biofilm properties (age, thickness, composition of algal and bacterial community, chemical composition of extracellular matrix etc.) is extremely deficient to clarify these inhomogeneous observations. In addition as mentioned above we have not any information on the active biological uptake by different microorganisms existing in the biofilms.

The bioaccumulation factors of biofilms calculated for the investigated pharmaceuticals are listed in Table 1 and increased in the following order diclofenac < sitagliptin < clarithromycin < ciprofloxacin < metoprolol. However, this order is characteristic only for the freshly formed "Danube biofilms" developed on the selected artificial substrata.

Although the diatom composition of biofilms grown on polycarbonate and glass substrata are different (Fig. 2 and Table S5), the bioaccumulation factors of these biofilms for the detected target compounds are similar and the differences can be characterized only by factor 1.07–2.02. The bioaccumulation factors of both biofilms are increasing in the same order as listed above. It means that the surface properties of biofilms and the biological uptake of the target molecules by microorganisms are partly similar in both cases, i.e. the substrata have only a moderate effect on the accumulation of the investigated pharmaceuticals in benthic biofilms. To clarify the observed phenomena it would be necessary to collect more information on the structure, the physicochemical characteristics and the adsorption capacities of benthic biofilms having well- defined microbiological composition.

4. Conclusion

On bases of our experimental results, it can be established that there is a moderate substrate effect influencing the biomass production and the biodiversity of diatom species in the biofilms grown at the same

environmental conditions, and thus these biofilms have different physicochemical properties. Since the bioaccumulation factors of biofilms determined for different pharmaceuticals strongly depend on their surface properties and their biological composition, the comparison of literature data representing various aquatic environments is extremely difficult. It means among the actors in the interaction between a pharmaceutical and biofilm only the pharmaceutical have well-defined chemical structure and physicochemical properties, the biofilms have only partly known and seasonally changing biological and physicochemical properties. Despite our incomplete knowledge, the regular monitoring of pharmaceuticals in biofilms help us to receive information on the potential trophic transfer of pharmaceuticals endangering the biofilm consumers.

CRediT authorship contribution statement

Borbála Dömölki: Writing – original draft, Formal analysis, Investigation. **Dániel Krakkó:** Methodology, Investigation. **Péter Dobosy:** Methodology, Investigation. **Zsuzsa Trabert:** Methodology. **Ádám Illés:** Investigation. **Dávid Stefán:** Investigation. **András Székács:** Methodology. **Éva Ács:** Methodology. **Gyula Záray:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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