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Legacy and Emerging Pollutants in an Urban River Stretch and Effects on the Bacterioplankton Community

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Abstract: River contamination is due to a chemical mixture of point and diffuse pollution, which can compromise water quality. Polycyclic Aromatic Hydrocarbons (PAHs) and emerging compounds such as pharmaceuticals and antibiotics are frequently found in rivers flowing through big cities. This work evaluated the presence of fifteen priority PAHs, eight pharmaceuticals including the antibiotics ciprofloxacin (CIP) and sulfamethoxazole (SMX), together with their main antibiotic resistant genes (ARGs) and the structure of the natural bacterioplankton community, in an urbanized stretch of the river Danube. SMX and diclofenac were the most abundant chemicals found (up to 20 ng/L). ARGs were also found to be detected as ubiquitous contaminants. A principal component analysis of the overall microbiological and chemical data revealed which contaminants were correlated with the presence of certain bacterial groups. The highest concentrations of naphthalene were associated with *Deltaproteobacteria* and *intI1* gene. Overall, the most contaminated site was inside the city and located immediately downstream of a wastewater treatment plant. However, both the sampling points before the river reached the city and in its southern suburban area were still affected by emerging and legacy contamination. The diffuse presence of antibiotics and ARGs causes particular concern because the river water is used for drinking purposes.

Keywords: PAHs; sulfamethoxazole; ciprofloxacin; antibiotic resistance genes; bacterioplankton community; surface water quality

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1. Introduction

Freshwater is a precious and limited resource for humans and ecosystems. Most of the world's major cities were built on or around areas of freshwater, especially rivers [1]. For this reason, most lotic ecosystems have been suffering various anthropogenic impacts for a long time (e.g., organic load, fertilizers, organic and inorganic contaminants) and their water quality has been harmed [2]. Rivers are open and hydrodynamic systems and are strongly influenced by variable biotic and abiotic factors and by surrounding compartments (air, soil). Several pollutants of different chemical classes are transported from point (e.g., municipal and industrial wastewater treatment plants), and diffuse sources (e.g., agricultural areas) to surface water [3]. A mixture of legacy and emerging pollutants has been found in rivers, causing particular concern for their possible effects on the ecosystem and human health. For example, Polycyclic Aromatic Hydrocarbons (PAHs) and some pharmaceuticals have been identified in several works as common contaminants in lotic waters [4,5]. The WFD (Water Framework Directive) commits European Union

member states to achieving a good qualitative and quantitative status for all water bodies [6]. However, several water bodies have still not achieved this goal, and emerging contaminants (e.g., antibiotics) are not yet regulated [7]. Due to their diffuse occurrence and intra-species and inter-species mobilization, ARGs can be also considered emerging contaminants [8–11]. Particular concern relies on the spread of ARGs from pathogens and non-pathogens bacteria, through horizontal gene transfer occurring in wastewater [12–14].

PAHs are among the most widespread legacy pollutants and are commonly found in water and sediment as well as in soil and air. PAHs are toxic for both humans and biota [15,16]. Depending on their concentration and organisms' exposure, they can cause acute or chronic, such as carcinogenic, mutagenic and teratogenic, effects [17]. For example, PAHs have toxic effects on fish, including their early development and bone and liver metabolism; moreover, they can have an endocrine-disruptive action [18]. PAHs can derive from natural processes or can be formed as products of incomplete combustion from either natural (forest and brushfires) or anthropogenic sources (vehicle emissions, domestic heating and cigarette smoke) [19]. PAHs can reach surface waters through atmospheric deposition, urban run-off, municipal and industrial effluents, and oil spillage or leakage [20]. Depending on their number of aromatic rings, they are divided into low molecular weight (LMW) PAHs, with two or three benzene rings, and high molecular weight (HMW) PAHs, with four or more benzene rings [21]. Due to their low aqueous solubility and strong hydrophobic nature, some PAHs (e.g., pyrene) tend to combine with particulate material in the aquatic environment, which causes a limited bioavailability, and consequently makes them recalcitrant to biodegradation [22]. Organisms living in PAH-contaminated environments can absorb these compounds through their tissues or by ingestion of contaminated sediment or particles and then transfer contamination through the aquatic food web [20]. Among PAHs, 16 are included in the Environmental Protection Agency (EPA) priority list (USEPA, 2008) and seven of these are regulated by Directive 2013/39/EU, which establishes environmental quality standards for surface water. The maximum allowable concentrations of these PAHs are: 100,120, 130,000, 27, 17, 17 and 0.82 ng/L for anthracene, fluoranthene, naphthalene, benzo(*a*)pyrene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene and benzo(*ghi*)perylene, respectively.

Antibiotics are emerging contaminants commonly found as river micro-pollutants [23,24] downstream from wastewater treatment plant outlets. Owing to their antimicrobial activity, they can kill or inhibit natural microbial populations involved in specific ecosystem functions (e.g., denitrification) [25] and, at the same time, they can select antibiotic resistant bacteria (ARB) [26]. Antibiotics, ARBs and ARGs can be found in wastewater treatment plant (WWTP) effluents [27], and once discharged to surface water, these contaminants reach the environment. Consequently, ARBs can spread in surface water and transfer their ARGs to natural microbial populations [28]. There is particular concern about ARGs being acquired in the human population through drinking water originating from rivers [29]. Although in clinical settings bacterial pathogens causing infections difficult to treat in humans (e.g., *Enterococci* and coliforms) have been identified, common environmental bacteria, which contribute significantly to the antibiotic resistance spread in freshwater, have been poorly studied so far [30–32]. In fact, the selection of resistant bacteria can occur [33] and the presence of other contaminants (e.g., metals, biocides, pharmaceuticals, etc.) can also increase ARG mechanisms, which act as homeostatic responses of microbial populations to toxic substances [34]. A watch list has been proposed at the European level for defining new priority substances to be included in the Water Framework Directive. Currently, the latest version of this watch list (2020) [35] contains three antibiotics, such as ciprofloxacin (CIP), amoxicillin (AMX) and sulfamethoxazole (SMX). The latter has been found in most worldwide surface water investigated with quite variable concentrations, from a few ng/L to µg/L [36–40].

In a four year study of the river Tiber from its source to its mouth, Saccà et al., (2019) [4] found SMX concentrations varying depending on the season and anthropogenic disturbance at each sampling site (e.g., pristine water: lower than the detection limit of 0.1 ng/L and at the most urbanized point: between 29.3 and 79 ng/L). In other works,

much higher amounts were found, e.g., 1920 ng/L in the river Llobregat (Spain) [39] and 1483.9 ng/L in the river Liao (China) [40].

Similarly, ciprofloxacin has also been commonly found in lentic and lotic waters. For example, a concentration of 191 ng/L in the river Ter [37] in Spain, 2745 ng/L in the river Reda in Poland[41] and concentrations ranging from 70 to 125 ng/L in the river Tiber in Italy [38,42] were found. In Chinese surface waters, 106.2 ng/L in Lake Honghu [43] and 185.14 ng/L in the Liaoning area of the river Liao [40] have been found.

It is generally recognized that antibiotic contamination in surface water is linked to a traditional WWTP incapability to remove them [44,45]. Antibiotic load is generally higher immediately downstream WWTPs and depending on human consumption. In this context, sulfamethoxazole and ciprofloxacin have been selected in this work, because they are commonly prescribed worldwide and representative of the entire class of antibiotics in terms of behavior in the environment. SMX has a polar nature and is significantly more degradable than CIP; the latter has low solubility and it is an intrinsically persistent compound [38,42].

The antibiotics SMX and CIP are intrinsically toxic for microbial communities [25], and damages to natural aquatic biofilms has been demonstrated [46]. An antibiotic selection pressure on environmental microbial communities can lead to reservoirs of antibiotic resistance bacteria and genes in the environment [38,47]. Moreover, if the water is used for drinking and bathing purposes, the presence of a mixture of these chemicals can also be hazardous for human health. The World Health Organization (WHO) highlighted the potential implications from involuntary drug intake via drinking water [48]. The concerns about human health concern ingestion not only of antibiotic residues but also non-pathogenic bacteria carrying antibiotic resistance genes. Once ingested, antibiotic residues and ARBs can alter the human microbiome and select for resistant bacteria [47]. Antibiotic resistance can lead to treatment failure and fatality by rendering antibiotic therapy ineffective [49].

Moreover, emerging contaminants include other pharmaceuticals, which are commonly used in human medicine, such as non-steroidal anti-inflammatory agents, hormones, lipid regulators, etc. Among these, diclofenac, a nonsteroidal anti-inflammatory drug with pain-relieving properties, has been utilized for both humans and domestic animals since 1970 [50]. Pharmaceutical industries, hospitals, and household drainage have been continuously introducing diclofenac or its metabolites into surface water [51]. Based on in vitro/in vivo studies, diclofenac toxicity in birds, mammals, aquatic species and plants has been found. Diclofenac has been reported to be hazardous to aquatic organisms even at low environmental concentrations (ng/L) [52,53]. Moreover, biomagnification in the food chain may constitute an ecological risk to non-targeted organisms [54]. Its intrinsic hydrophilicity and stability make it quite persistent in the aquatic environment [55]. In European surface waters, variable concentrations are reported: e.g., 445.2 ng/L in the river Llobregat in Spain [56], 53 ng/L in the river Danube in Serbia [57] and concentrations ranging from 0.9 to 849 ng/L in the river Tiber in Italy [4]. In China surface waters, 717 ng/L in the river Liao [58] and 121.6 ng/L in the river Beiyun Basin [59] have been found.

The current work aimed to evaluate the contamination of a stretch of the river Danube passing through Budapest, with particular emphasis on emerging contaminants, such as antibiotics and ARGs. Three monitoring campaigns were performed at three different points, i.e., one sampling site inside and two outside the city. Three hormones (17β-estradiol, 17α-ethinylestradiol, estrone), four nonsteroidal anti-inflammatory drugs (fenoprofen, naproxen, ibuprofen, diclofenac) one lipidic regulator (gemfibrozil), two antibiotics (sulfamethoxazole and ciprofloxacin), fifteen ubiquitous PAHs, four ARGs (*sul1*, *sul2*, *qnrS* and *qepA*) and one mobile genetic element (MGE) *intI1* were analyzed. The effects of the chemical mixture on the natural river water microbial community were also evaluated in terms of alteration of its structure.

2. Materials and Methods

2.1. Sample Collection and Processing

River water was collected along the river Danube from 3 points affected by anthropogenic activities nearby (1 and 3) and inside Budapest (2) (Figure 1). Point 1 was located north from the city and Point 3 in the south. The sampling point 2 was immediately downstream from a Sewage Treatment Plant (processing 180,000–200,000 m^3 sewage per day). The sampling procedures adopted for this study were those applied in several works on European rivers [60,61]. The samplings were performed in April 2017, November 2017 and October 2018. For organic pollutant analysis (PAHs and pharmaceuticals) 2.5 L surface water samples were collected in triplicate (0–20 cm depth) and stored in previously cleaned (HNO_3, pH < 2 and washed in ultrapure water to neutralize the pH) glass bottles. Water samples for the bacterioplankton characterization (Fluorescence In Situ Hybridization: FISH Analysis) were collected in sterile bottles (3 bottles, 1 L each). They were immediately fixed with formaldehyde (2% final concentration) and volumes of 4 mL for each sample were filtered through a 25 mm white polycarbonate membrane with a porosity of 0.2 µm (Merck Millipore) using a gentle vacuum (<0.2 bar). Other samples were immediately filtered for DNA extraction (see Section 2.4). All samples were transported to the lab using a freezer bag with an ice pack.

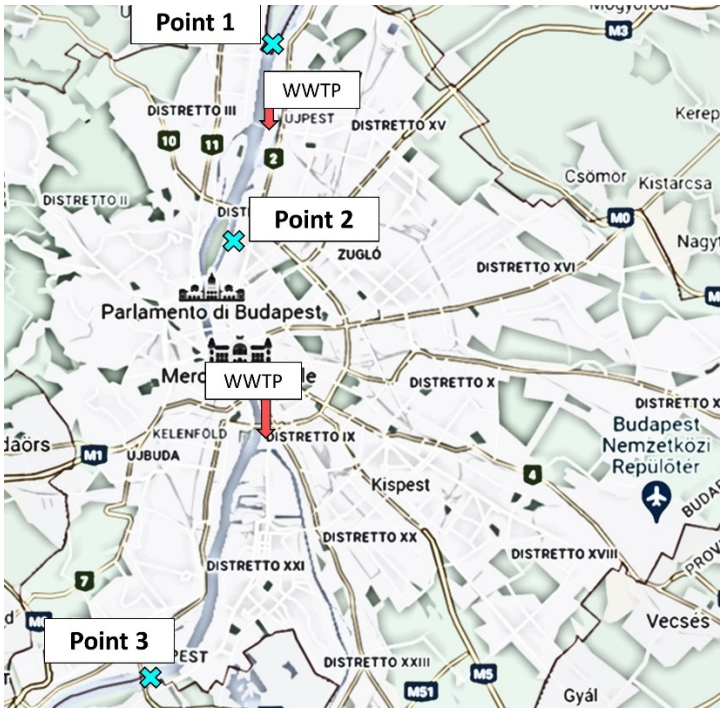

Figure 1. Map showing the location of the sampling points (P1, P2 and P3) and WWTPs.

2.2. Analysis of PAHs and Pharmaceuticals from Water

Fifteen PAHs (listed in Table S1), eight pharmaceuticals (17β-estradiol, 17α-ethinylestradiol, estrone, fenoprofen, naproxen, ibuprofen, diclofenac, gemfibrozil) and two antibiotics (SMX and CIP) were searched for in river water samples, by combining solid phase extraction (SPE) and instrumental analysis using high-performance liquid chromatography (HPLC, Perkin Elmer, Milan, Italy LC 100 Colum Oven connected to Perkin Elmer Serie 200 micropump) coupled with a fluorescence (FLD) or mass spectrometer detector.

Specifically, PAHs analytical determinations were obtained by coupling LC with programmable FLD (Perkin Elmer 200 a), and a triple quadrupole mass spectrometer detector (mod. API 3000, AB Sciex, Darmstadt, Germany) was used for the pharmaceuticals and antibiotics determinations, as described in detail in Barra Caracciolo et al., (2019) [5].

All target compound analytical standards (98% purity) and solvents (HPLC-grade purity) used for the chemical determinations were purchased by Sigma Aldrich, Steinheim, Germany.

2.3. Microbiological Analysis

Water samples for the bacterioplankton characterization were analyzed with the Fluorescence In Situ Hybridization method (FISH), using Cy3-labelled oligonucleotide probes (Biomers.net, Ulm, Germany) targeting the dominant bacterial taxa found in freshwater ecosystems [62], as described in detail in Saccà et al., (2019) [4].

2.4. DNA Extraction

Freshwater samples (100 mL for each sampling point) were filtered on polycarbonate filters (Osmonic INC, porosity 0.22 µm, diameter 47 mm) and frozen at −20 °C until DNA extraction. The latter was performed using the DNeasy PowerSoil kit (QIAGEN Venlo, The Netherlands, Cat No./ID: 12888-100) in line with the manufacturer's protocol and in accordance with how reported in Garner et al., (2016) [63]. The evaluation of the extracted DNA quantity and quality was performed using a Multiskan Sky Microplate Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

2.5. Quantification of ARGs and intI1 Sequences by qPCR

The qPCR was performed with a CFX96 real-time PCR detection system (Bio-Rad, Hercules, CA, USA), as reported in another work [64], to target the *sul1*, *sul2*, *qnrS*, *qepA* and *intI1* genes. The primer list used is reported in detail in Table S1. All ARGs and *intI1* qPCR results were normalized per mL of river water filtered.

2.6. Statistical Analysis

The microbiological and chemical results are reported as the average of a triplicate analysis of triplicate samples. Pairwise comparison of mean values (following unpaired *t*-test) was performed with R software. The Principal Component Analysis (PCA) was run to graphically synthesize the bacterioplankton community structure at each sampling point by considering the relative abundance of the main bacterial groups and the concentrations of legacy and emerging contaminants. PCA was performed with R software, using the packages "*FactoMineR*" and "*factoextra*".

3. Results

Figure 2 shows the average pharmaceutical concentrations at Point 1, Point 2 and Point 3 over April 2017, November 2017 and October 2018 samplings. Pharmaceuticals were found in higher amounts ($p < 0.05$) in April than November and October samplings. Antibiotics were among the most abundant pharmaceuticals and SMX was in higher concentrations (5–35 ng/L) than CIP (3–16 ng/L). The highest SMX amounts were found at Point 2 (inside Budapest), (20.9 ± 4.9 ng/L).

Figure 3 shows the average PAH concentrations at Point 1, Point 2 and Point 3 detected during the April 2017, November 2017 and October 2018 samplings. PAHs were in significantly higher amounts in the April sampling than the other ones (November and October). A peak (12.2 ± 2.3 ng/L) in naphthalene concentration was found in the October sampling.

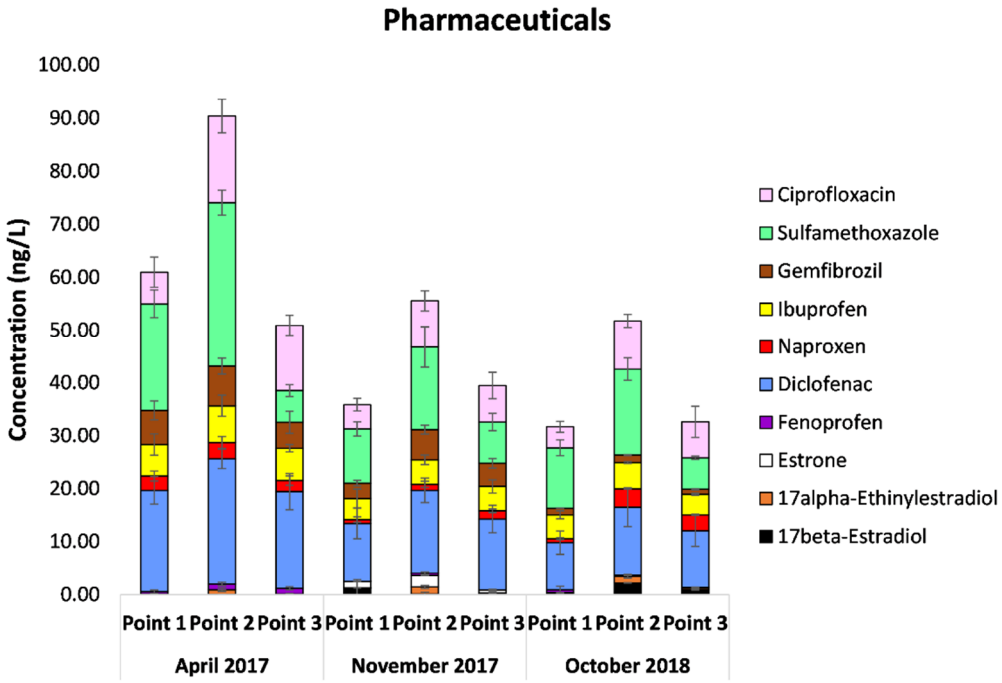

Figure 2. Concentration values (ng/L) for the eight pharmaceuticals and two antibiotics measured at the three sampling points (1, 2 and 3) during the three sampling times.

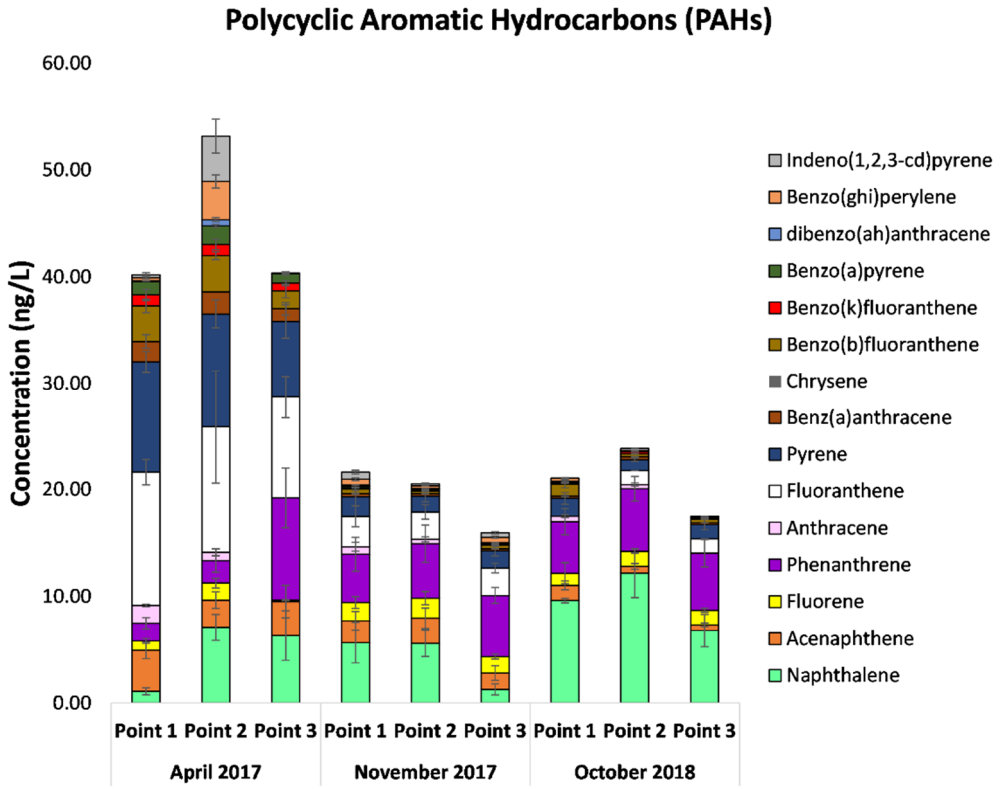

Figure 3. Concentration values (ng/L) for the fifteen PAHs measured at the three sampling points (1, 2 and 3) during the three sampling times.

Figure 4 shows the average ARG concentrations at Point 1, Point 2 and Point 3 in the April 2017, November 2017 and October 2018 samplings. The most abundant ARGs were detected in April 2017 and the highest values were found at Point 2 (Figure 4). Interestingly,

in all samplings SMX-genes (*sul1*, *sul2*) were more abundant than CIP-genes (*qnrS*, *qepA*), in line with the higher SMX concentrations.

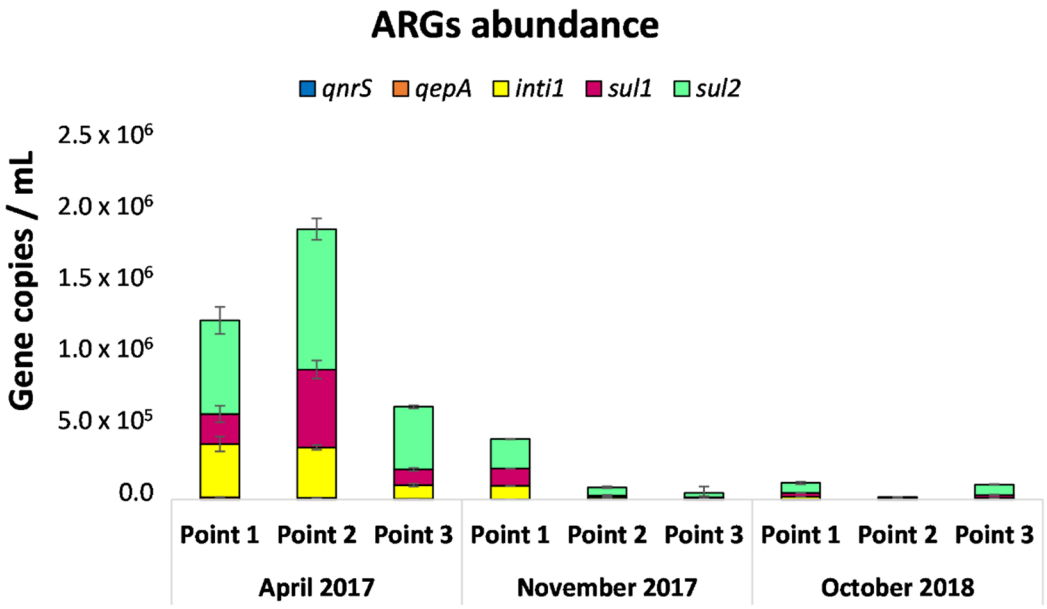

Figure 4. Gene abundance (gene copies/mL) of the five ARGs measured at the three sampling points (1, 2 and 3) during the three sampling times.

The structure of the bacterioplankton community at Point 1, Point 2 and Point 3 in April 2017, November 2017 and October 2018 samplings are reported in Figure 5. The *Bacteroidetes* and *Alphaproteobacteria* groups were dominant in April 2017; *Gammaproteobacteria* and *Actinobacteria* increased significantly in November 2017. Finally, a further shift in the bacterial community was observed in October 2018, with an increase in *Betaproteobacteria* and *Deltaproteobacteria*.

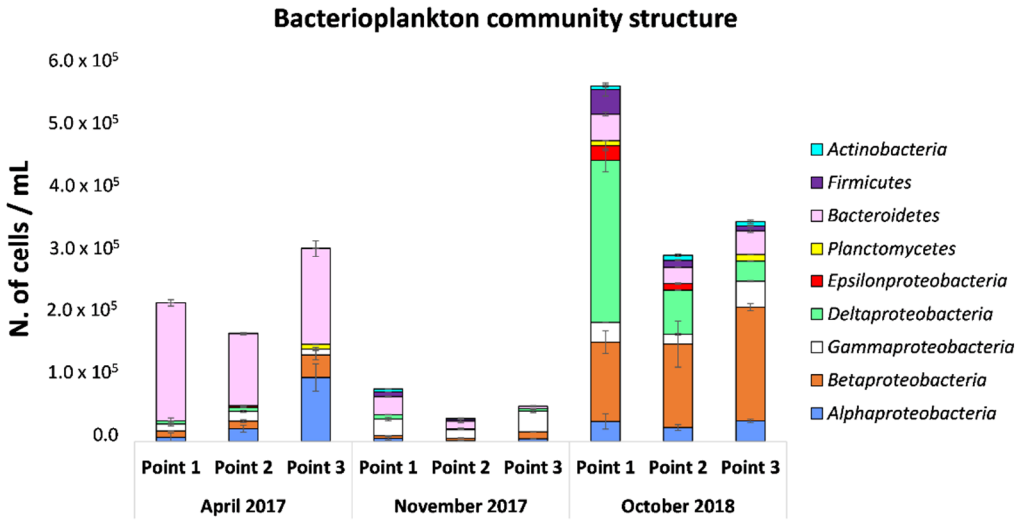

Figure 5. Bacterioplankton characterization (N. of cells/mL) evaluated using Fluorescence In Situ Hybridization at the three sampling points (1, 2 and 3) over the three sampling times (April 2017, November 2017 and October 2018).

A PCA analysis was performed using pharmaceutical, PAH and ARG data and the relative abundances of the bacterial groups (*Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Epsilonproteobacteria*, *Planctomycetes*, *Bacteroidetes*, *Firmicutes* and *Actinobacteria*) as variables, to reduce the dimensionality of the dataset and pinpoint the

most important factors causing the overall variability. The PCA results (Figure 6) explained 57.3% of the total variance. Dimension 1 accounts for 42% of the variance; the positive segment of the plot for this dimension is closely related to the levels of many contaminants (e.g., diclofenac r = 0.92; sulfamethoxazole r = 0.71; pyrene r = 0.98, $p < 0.05$). Dimension 2 explained 15.3% of the variance; this dimension is significantly related to *Alphaproteobacteria* (r = −0.66, $p < 0.05$), fluorene (r = 0.69, $p < 0.05$) and the MGEintI1 (r = 0.62, $p < 0.05$).

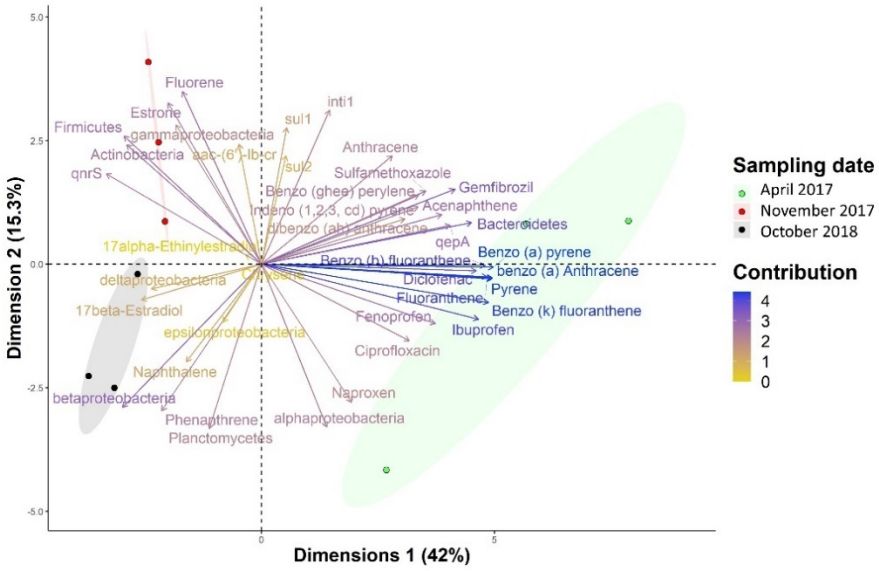

Figure 6. Principal component analysis (PCA) performed on overall data regarding pharmaceuticals, PAHs, ARGs and the main bacterioplankton groups obtained from the different sampling times and points. Green dots represent Point 1, Point 2 and Point 3 in the April 2017 sampling; red dots represent Point 1, Point 2 and Point 3 in the November 2017 sampling; and black dots represent Point 1, Point 2 and Point 3 in the October 2018 sampling. The gradient contribution represents the contribution of the variables to each dimension (from yellow: weak to blue: strong).

4. Discussion

In the present work, pharmaceuticals, PAHs, ARGs and bacterioplankton community structure were analyzed in a stretch of the river Danube, at three different sampling sites and times. The overall concentration of contaminants (legacy and emerging ones) found in this stretch of the river was quite low in comparison with other European lotic systems [4,36,38,39,56,57]. River contamination is the result of the contaminant load from various anthropogenic sources (WWTP effluents, vehicles, house heating, industrial activities, power plants, etc.), which are directly connected to human population density, and of a river's capacity to attenuate overall contamination. However, a key factor is the "dilution effect", which in turn depends on river discharge and seasonal rainfall [65] and is particularly significant when WWTP effluents enter natural river water. Moreover, abiotic (e.g., photodegradation) and biotic degradation times and sorption onto particles can also determine contaminant concentrations [5,24]. In this study, emerging contaminants, and in particular pharmaceuticals, were found in higher amounts than PAHs, and this can be ascribed to the different chemical-physical properties of these molecules and different emission sources. Indeed, pharmaceutical input into freshwater is mainly related to continuous WWTP effluent discharge, while PAH diffusion in the environment depends on variable anthropogenic sources (e.g., home heating, vehicle emissions) and atmospheric transport dynamics. Some authors report that PAH contamination can involve WWTPs where they are not removed and consequently can also enter in river water from this source [66–68].

In this work, SMX and CIP were found as the most abundant contaminants at all the points investigated. The amounts of these antibiotics were lower (SMX up to 30.93 ng/L, CIP up to 16.31 ng/L), than those found in a smaller river, such as the Tiber [4,60], presum-

ably because of the "dilution effect". The river Danube is in fact longer and wider than the Tiber. Interestingly, SMX concentrations were always higher than CIP. Although CIP is more persistent than SMX [38,42], the latter is being continuously introduced into the aquatic ecosystem (owing to its widespread use among the human population) and was detected in higher amounts, suggesting its pseudo-persistence [69].

SMX and CIP resistance genes were detected at all sampling points, confirming they are widespread emerging contaminants. SMX-resistance genes (*sul1*, *sul2*) were significant ($p < 0.05$) and more abundant than CIP ones (*qnrS*, *qepA*) in line with the antibiotic concentrations. Overall the ARGs and MGE found in this study (*sul1* 10^5, *sul2* 10^5, *intI1* 10^5 copies/mL) were comparable to those reported in other works (from *sul1* 10^4, *sul2* 10^3, *intI1* 10^3 to *sul1* 10^6, *sul2* 10^6, *intI1* 10^6 copies/mL) [70,71].

The different inputs (e.g., the use among the human population) of the chemicals and the distance from their source determined the different concentrations found in the Danube at each sampling point. Although they are not prescribed in Hungary, fenoprofen, estrone and gemfibrozil were found (at quite low concentrations) in this stretch of the river. This means that the Danube in Budapest is also contaminated by pharmaceuticals used in southern Germany and northeast Austria. In any case, at Point 2 inside the city and immediately downstream from a WWTP, the concentrations of antibiotics and diclofenac were the highest. Interestingly, even though the WWTP located immediately before Point 2 (processing 180,000–200,000 m^3 of sewage per day) is smaller than that before Point 3 (processing 350,000–500,000 m^3 of sewage per day), the location of Point 2 made it the highest impacted by the WWTP contamination, (Figure 1). Moreover, because the river water before reaching Point 3 shapes two branches, only one will have a contaminant load.

Finally, the highest concentrations of legacy and emerging pollutants were detected in April sampling. These results were presumably due to the highest consumption of these chemicals in this season, as found in other studies [64,72]. In line with the highest river chemical load, the highest ARG abundance was also found, suggesting also a possible influence of PAHs in ARG selection. Although how environmental antibiotic resistance can be selected, maintained or decreased in natural environments is a complex issue and far from being completely understood, it has been recognized that a variety of mechanisms (e.g., horizontal gene transfer, genetic mutation and recombination) and selective pressures, including the co-presence of other contaminants, can be involved. [73]. For example, Rodgers et al., (2019) [74] demonstrated the role of PAHs in the propagation of ARGs by bacteria conjugation and inhibiting bacteria transformation, which is the uptake and stabilization of extracellular DNA by a competent cell [75]. Chen et al., (2017) [76] also found that PAHs can operate as a selective stress for ARGs, and some PAH-selected *Proteobacteria* could be involved in ARG enrichment.

The present work also analyzed the overall structure of the bacterioplankton, showing how it responded differently to the various chemical concentrations. Different bacterial groups dominated depending on the overall contaminant pressure, which presumably induced "resistance mechanisms" or degradation capabilities versus the various chemicals. For example, in the October sampling, *Betaproteobacteria* and *Deltaproteobacteria* dominated and were associated with naphthalene (PCA results). In fact, some sulfate-reducing bacteria, belonging to *Deltaproteobacteria*, are reported to metabolize naphthalene [77,78]. Similarly, *Bacteroidetes*, including several genera able to resist antibiotics [79,80], were the most abundant group in April when a higher antibiotic load was also detected.

The overall results showed that the river Danube represents an example of legacy and emerging contaminated ecosystem, where the contaminant mixtures affect the microbial community structure and ARG abundance in different ways. Because Danube river water is also used for drinking and irrigation purposes, the presence of emerging contaminants such as antibiotics and ARGs does not exclude their unwanted ingestion by humans, pets and livestock animals. These findings support the One Health approach, which suggests considering ecosystem and human health at the same time.

5. Conclusions

This work showed widespread and variable pollution by legacy and emerging contaminants in the urbanized stretch of the river investigated. SMX, CIP and diclofenac were the most abundant pharmaceuticals found. The Bacterioplankton community showed a structural plasticity (e.g., shifts in the dominant groups) and different functional response (e.g., ARG abundances) to the different pressures from the chemical mixture. The widespread presence of PAHs, pharmaceuticals, antibiotics and ARGs causes particular concern because the river water is used for drinking purposes. The diffuse detection of ARGs in water samples highlights the need of urgent strategies for facing this issue. In Europe, the EU4Health program, initially planned for preventing COVID-19 pandemic diffusion, have been funding health organizations and NGOs also for proposing prevention measurements and solutions for reducing the number of antimicrobial-resistant infections. At the same time, additional scientific studies and monitoring data are necessary for supporting a possible legislation for defining water AB concentration limits.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/w13233402/s1, Table S1: List of PAHs analyzed and primers used for ARGs quantification.

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Conflicts of Interest: The authors declare no conflict of interest.

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