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# Contaminants of Emerging Concern in the Seine River Basin: Overview of Recent Research



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**Abstract** For over 30 years, the sources and the transfer dynamics of micropollutants have been investigated in the PIREN-Seine programme. Recent works included a wide range of chemicals and biological contaminants of emerging concern (i.e. contaminants whose occurrence, fate and impact are scarcely documented). This chapter presents a brief overview of research recently conducted on contaminants as diverse as macro- and microplastics, poly- and perfluoroalkyl substances (PFASs), pathogenic protozoa, antibiotics and the associated antibiotic resistance. The multiscalar study of plastics and PFASs at a large spatial scale is rare; the results produced in recent years on the Seine River catchment have provided an original contribution to the investigation of the dynamics of these contaminants in urban environments. The results also highlighted that pathogenic protozoa are ubiquitous in the Seine River basin and that the contamination of bivalves such as *Dreissena polymorpha* could reflect the ambient biological contamination of watercourses. The widespread occurrence of antibiotics in the Seine River was demonstrated, and it was shown that the resistome of biofilms in highly urbanised rivers constitutes a microenvironment where genetic support for antibiotic resistance (clinical integrons) and resistance genes for trace metals are concentrated.

**Keywords** Antibiotic resistance, Antibiotics, Bioaccumulation, Contaminants of emerging concern, Macro- and microplastics, Pathogenic protozoa, PIREN-Seine, Poly- and perfluoroalkyl substances, Sediment, Water, Zone Atelier Seine

## 1 Introduction

The Seine River basin is under severe anthropogenic pressure for a number of reasons, including the emission of micropollutants by industrial, agricultural and urban activities in combination with relatively low river water flow per capita (cf. [1]). Thus, the sources and the transfer dynamics of both organic micropollutants and trace metals have been investigated over several decades within the PIREN-Seine programme. As regards organics, previous studies addressed this issue at different temporal and spatial scales, focusing, for instance, on polycyclic aromatic hydrocarbons (PAHs) (see [2]) or legacy organohalogenes [3, 4]. However, due to evolving chemical regulations, improved analytical capabilities and progress in

ecotoxicological assessment, recent studies have included a wide range of chemicals and biological contaminants of emerging concern. A small selection of studies conducted since the 2010s is presented hereafter to illustrate this work, with a brief overview of recent studies on macro- and microplastics, fluoroalkyl substances, pathogenic protozoa as well as antibiotics and antibiotic resistance.

## 2 Macro- and Microplastics

### 2.1 Context

Water pollution by plastics and microplastics is often described as an emerging concern. However, in the Seine River basin, boatmen have been complaining since the 1990s about the presence of plastic bags blocking the cooling circuits of barges. Prior to 1994, SIAAP (Greater Paris Sanitation Authority) had installed floating booms to trap macro-waste, including plastics floating on the water surface. However, this highly visible pollution did not attract the attention of public authorities or aquatic environment managers (e.g. it was not mentioned in the 2000 European Water Framework Directive) or even environmental protection organisations. However, within the EU Marine Strategy Framework Directive released in 2008, litter was listed as one of the descriptors of good ecological status. Following observations in the ocean environment (e.g. “great garbage patch” in 1997), this issue, both abroad and in France, has become a social issue and a research subject for scientists.

Plastics observed in freshwater reach several orders of magnitude in size and a wide spectrum of shapes. Microplastics were brought to light in 2004 [5] and were defined as plastic particles whose longest dimension is less than 5 mm [6]. As a consequence, macroplastics have their longest dimension greater than 5 mm. Macroplastics are either manufactured plastic items (primary plastics) or fragments (secondary plastics), and microplastics are mostly secondary plastics categorised depending on their dimensions: fibres (length  $\gg$  diameter) and fragments (characteristic length  $\gg$  thickness) composed of different irregular shapes and spheres [6]. More generally, anthropogenic particles (APs) cover a very broad category of particles produced directly or indirectly by human activities. In the case of the Seine River, APs are mostly small pieces, fragments or fibres and, regardless of their size, originating from plastics, dyed particles or textile fibres. Special attention is being paid to plastic fibres whose production increases by approximately 4% per year (60,000 tons in 2016, 20% of the world plastic production [7]). Although fibres are often not included in the key figures concerning plastic materials, they are used in several industrial sectors including the textile industry. Plastic synthetic fibres represent the main fraction of the world fibre production, which also includes other artificial fibres made from natural raw material (e.g. rayon made from cellulose) and natural fibres (e.g. cotton and wool). Fibres are present in the environment and are produced by the wear and tear of larger items.

## 2.2 Objectives

Since the beginning of the 2010s, research on plastic litter in continental environments has developed, even if it remains limited compared to work on the marine environment. This research has revealed the widespread occurrence of plastic debris in environmental compartments including the atmosphere [8] and food: salt [9], possibly honey [10], mussels [11, 12] and also bottled water [13, 14]. These findings have raised concerns about the human health effects of plastics [15] but also receiving systems [16] and aquatic ecosystems [17, 18].

In 2013, the Leesou research unit launched a research project on microplastics in urban hydrosystems, in partnership with the PIREN-Seine programme and the Observatoire des Polluants Urbains en Ile-de-France (OPUR), which has expanded to include the issue of macro-pollution by plastics. This project had several objectives, including (1) the estimation of macroplastic mass fluxes from Paris to the mouth of the Seine and their dynamics and (2) the identification of the sources and fluxes of microplastics at the scale of the Parisian Metropolis, from the atmosphere to the Seine River and the assessment of its plastic contamination from the Paris conurbation to its mouth.

## 2.3 Macroplastics in the Seine River

In contrast to microplastics, macroplastic pollution is highly visible. There is a need to investigate the occurrence and dynamics of macroplastics, in order to implement efficient multiscale reduction policies in the Marine Strategy Framework Directive [19]. Various methods have been proposed recently [20, 21], based on waste production and different leakage rates into the environment; these approaches have the major interest to allow, relatively easily, for the estimation of estimating macroplastic flux to the ocean at the global scale.

The evaluation of these methods on data collected in watersheds is obviously the next step and is currently underway in the Seine River basin. A first rough attempt estimated the yearly macroplastic mass fluxes between 1,000 and 10,000 metric tons [22]. More recently, two conceptual modelling approaches, based on the data available in the Seine catchment, have been implemented [23]. The first one (CM1) was based on the extrapolation of the retention efficiency of a network of floating booms installed by the Greater Paris Sanitation Authority since 1994, which removes 27 tons of plastics yearly [24], accounting for 0.9–6.3% of the total mass of debris. A significant proportion of these macroplastics consists of food wrappers and containers and plastic cutlery, most likely associated with recreational activities. The second approach (CM2), based on the Jambeck and Lebreton methodologies [20, 21], has calculated the amount of mismanaged plastic waste (MMPW) at the basin scale, of which 15% and 40% are assumed to be transferred to the English Channel. MMPW is based on (1) the population (GIS data), (2) the economic level of

**Table 1** Annual plastic mass flux to the English Channel from the Seine catchment areas

	Dris [22]	CM1	CM2	Lebreton et al. [21]
Annual flux estimate (metric ton year <sup>-1</sup> )	1,000–10,000	1,800–5,400	1,800–5,900	~20

the territory considered (World Bank data), (3) per capita plastic waste production (ADEME and ORDIF reports) and (4) an estimated 2% of littering [20].

The corresponding estimates (Table 1) were compared to the Lebreton theoretical estimate [21]. Despite their simplicity, these methods yielded similar results, especially the two conceptual models, based on completely different conceptual representations, i.e. between 1,800 and 5,900 metric tons a year.

At the same time, non-governmental organisations that harvest plastic litter on river banks in the estuary only collect up to 88–128 metric tons per year [23]. The discrepancy between these values raises new questions: does the fraction stranded on river banks really account for such a small fraction of the total flux transported by the river? Is the harvested fraction only a very small fraction of the stranded fraction? Are the two conceptual models and the Lebreton approach totally erroneous?

To provide preliminary answers to these fundamental questions, these annual fluxes were first converted to per capita fluxes. Over 1 year, plastic leakage into the Seine River reached 0.01–0.4 kg of plastic per capita, which is far less than estimations for the Nhieu Loc–Thi Nghe River, a tributary of the Saigon River in Vietnam, with a median load equal to 1.6 kg per capita. It is also lower than the average annual input of plastic for the coastal population worldwide, which reaches 0.7–2 kg per capita, with the highest values observed in South-East Asia. These values only confirm the fact that plastic leakage in Western countries is small compared to that reported for other parts of the world, Asia especially [20]; they do not, however, provide any relevant information to solve the questions mentioned above.

Other approaches are presently under investigation, which aim to understand more precisely the dynamics and the trajectories of macroplastics in the Seine River and especially in the Seine estuary. Such approaches might help in the implementation of new plastic harvesting strategies as well as new stringent regulations regarding plastic litter to drastically reduce ocean plastic pollution. Pathways and routes relevant to plastic debris remain partially unknown; in particular, the role of floods, runoff, combined sewer overflows (CSO) and bypass of wastewater treatment plants (WWTP) in plastic leakage must be investigated.

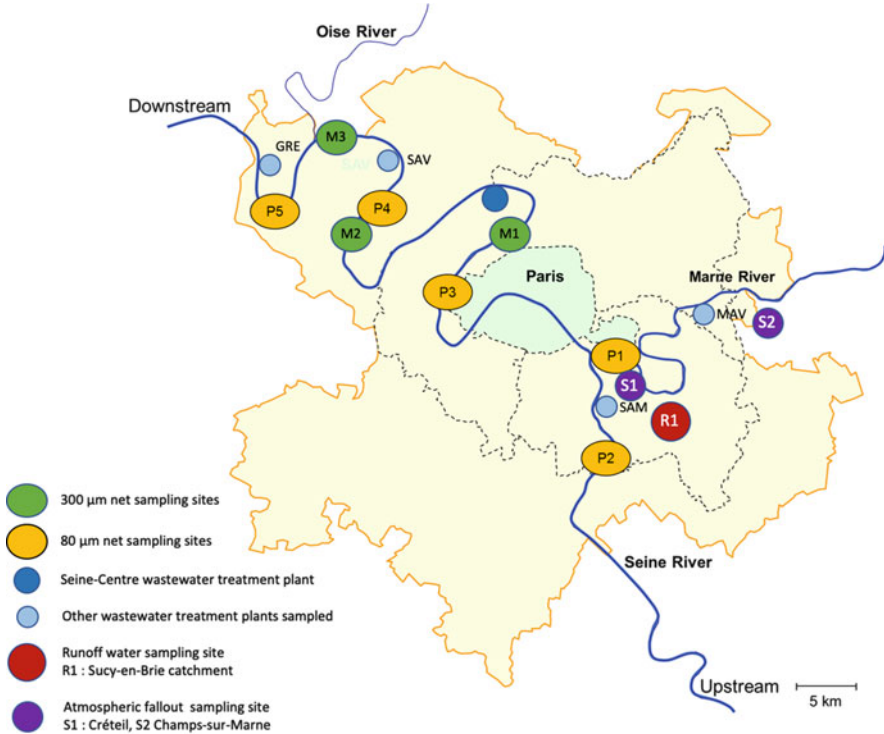


Fig. 1 Map of Paris megacity and location of the various sampling sites

## 2.4 Microplastic Sources and Fluxes in Greater Paris and the Seine River

Between 2014 and 2016, an investigation of sources and fluxes of microplastics was carried out on the Paris megacity. The following sources were investigated: atmospheric fallout, runoff water, grey water, wastewater outfall and CSO. Moreover, from April 2014 to December 2015, monthly monitoring was carried out at four sampling stations (P2–P5) on the Seine River from upstream to downstream of Paris plus one station on the Marne River (P1) [11] (Fig. 1).

This survey aimed at (1) estimating the various annual fluxes of microplastics in a large urban area and (2) linking the urban fluxes to the microplastic concentration in the Seine River and estimating the annual flux transported by this river.

Various sampling techniques (using nets or bulk water samples) were used. All analytical details are provided elsewhere [22]. The results are summarised in Fig. 2, which presents a first attempt at a mass balance of microplastics at the Greater Paris scale. Concentrations of fibres and fragments (in items  $L^{-1}$ ) are provided, as well as plastic fluxes (metric tons  $year^{-1}$ ).

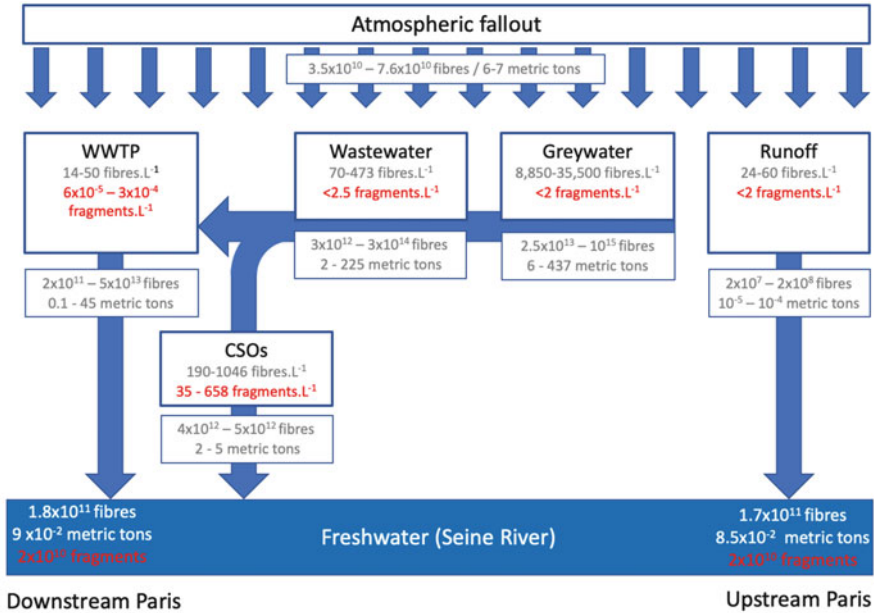


Fig. 2 Mass balance of microplastics at the Greater Paris scale (fibres and fragments)

Unexpected results were obtained. First of all, the number of plastic fibres (30% of the total number of fibres) exceeded the number of fragments by several orders of magnitude. The atmospheric fibre fallout represents a significant flux of plastic, around 10 metric tons per year at the Greater Paris scale. Fibres are also mainly present in grey and wastewater, and the input to WWTPs is estimated at several hundred tons per year. WWTPs contribute significantly to the reduction of plastic fluxes from the urban hydrosystem, and only 10% of the incoming flux is released into the receiving system (i.e. the Seine River). During wet weather periods, CSOs discharge huge fluxes of plastic fibre, which, based on a yearly average, are greater than the fluxes associated with treated wastewater discharge. In separate sewer system sectors, runoff exhibits concentration similar to those observed in WWTP effluents, but the corresponding flux is smaller by several orders of magnitude.

Microplastic fragment concentrations are small compared to fibre concentrations in the various compartments sampled, except in CSOs where the highest fragment concentration is observed: their concentration reaches 50% of the plastic fibre concentration. Thus, concerning the microplastic concentration and flux in the Seine River, two main conclusions can be drawn:

- There is no significative difference between the upstream and downstream concentration or flux.
- The flux observed in the Seine River is much smaller than the sum of urban incoming fluxes for fibres and much larger for fragments.



Additional surveys are necessary to decrease the uncertainties in the concentration and flux estimates. However, these results clearly show that additional significant sources of plastic fragments occur within the urban area, such as the inputs linked to storm water or the fragmentation of plastic litter on the river banks. Their relative contributions are undetermined so far.

This type of multiscale study of plastics at the catchment scale is rare, and the results produced in recent years in the Seine catchment are therefore original. However, additional studies are clearly required to achieve a more comprehensive overview of the dynamics of both micro- and macroplastics in the environment and further insight into the ecotoxicological consequences of their presence in freshwater ecosystems. From a water quality management point of view, the relevant figures and numbers necessary to engage efficient actions are still missing.

### **3 Poly- and Perfluoroalkyl Substances (PFASs)**

#### ***3.1 Context***

Poly- and Perfluoroalkyl Substances (PFASs) constitute a vast family of molecules bearing a fluorinated aliphatic chain ( $C_nF_{2n+1}$ ) [25]. The industrial synthesis of these compounds began around 1950, and world production exceeded three million tons in 2000. The numerous applications of PFASs include additives in the synthesis of fluoropolymers, water and oil repellents for textiles, firefighting foams, lubricants, coatings and food packaging [25]. Less than 20 years ago, perfluorooctane sulfonate (PFOS) was found to be globally distributed in wildlife [26] and humans [27], while concerns were raised about its adverse effects [28] before it was officially classified as a Persistent Organic Pollutant in 2009 [29]. Since then, a large number of studies have addressed the issues of PFAS sources and environmental fate [30]. Besides airports and military bases, industrial sites such as fluorochemical facilities, metal plating industries, textile mills and power plants, urban areas are also considered as key sources of PFASs to hydrosystems [31, 32], due to either point source contamination (e.g. wastewater discharge) or diffuse contamination (e.g. urban runoff). However, the dynamics of these chemicals in urban rivers still remain poorly understood. In this context, the aims of the studies conducted within the PIREN-Seine programme since 2010 were twofold: (1) investigate the occurrence and the spatio-temporal dynamics of PFASs in the Seine River under contrasted hydrological conditions and (2) investigate the transfer of these chemicals to biota in urban rivers.

### 3.2 Dynamics of PFASs in the Seine River

A longitudinal upstream–downstream concentration gradient was previously observed for various contaminants (e.g. PAHs, organohalogens, phthalates) in relation to the impact of the Greater Paris conurbation [33]. The dynamics of selected PFASs in the Seine River were investigated using a dual strategy: (1) the time trends and mass flows were determined for a single study site during a flood cycle, and (2) both the seasonal and spatial fluctuations at the water year scale were studied at the regional scale.

Changes in river flow may have a large impact on the concentration of chemical point sources, and coordinating water quality monitoring with the analysis of hydrological conditions is essential to understand the fate and transport of trace organics in surface waters [34]. Thus, water samples were first collected weekly over a 4-month period in 2011 (January–May), in the centre of Paris (Quai d’Austerlitz), right at the heart of the conurbation. This sampling site was deemed representative of the impact on water bodies of urban inputs. Selected PFASs, including C<sub>4</sub>–C<sub>14</sub> carboxylates (PFCAs) and C<sub>4</sub>–C<sub>10</sub> sulfonates (PFSAs), were analysed in both the particulate and dissolved phases of water samples using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) [32].

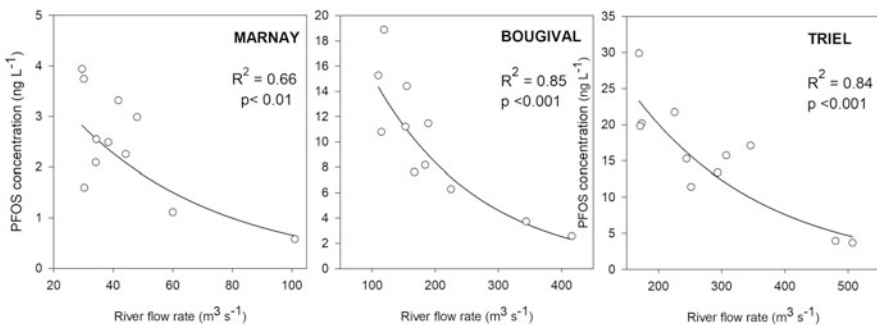
Over the period considered, the river flow rate ranged between 150 and 640 m<sup>3</sup> s<sup>-1</sup>, and several spate events were recorded. Suspended solid (SS) levels were generally low, in good agreement with previous reports [4]; these levels exhibited large variations (3–60 mg L<sup>-1</sup>) and were strongly correlated with the river flow rate [32]. Among the targeted PFASs, perfluorooctane sulfonate (PFOS) and perfluorohexane sulfonate (PFHxS) were the dominant compounds.  $\Sigma$ PFASs varied between 30 and 90 ng L<sup>-1</sup> (average, 55 ng L<sup>-1</sup>), and PFASs were mainly found in the dissolved phase due to the relatively low SS levels. These PFAS concentrations were in good agreement with those determined in the Seine downstream of Paris during low-flow periods [35] and were close to the average value determined for 122 watercourses at the European level (59 ng L<sup>-1</sup>) [36], which suggests that the Seine River in Paris is not a hotspot of contamination of PFASs by European standards. However, the median  $\Sigma$ PFASs for this site was approximately three times higher than that determined at a nationwide level in France (i.e. 7.8 ng L<sup>-1</sup>) [31]. In addition, PFOS levels (10–40 ng L<sup>-1</sup>) were lower than the maximum allowable concentration (EQS-MAC, 35  $\mu$ g L<sup>-1</sup>) but consistently above the environmental quality standard expressed as an annual average value (EQS-MA) of 0.65 ng L<sup>-1</sup> in Directive 2013/39/EU.

$\Sigma$ PFASs and the river flow rate were negatively correlated, which suggests a dilution of the contributions from point sources when flow increases. However, the selected linear model explained only 25% of the observed variation of concentration. Other contributions via the sewerage network or urban runoff are therefore likely to influence the levels observed in the Seine River. In addition, the PFHpa to PFOA concentration ratio, indicative of the contribution of direct (i.e. non-atmospheric) inputs [37], was also positively correlated with flow rate. This suggests an increase

of indirect inputs such as atmospheric deposition and subsequent urban runoff during high-flow events, which also correspond to periods of heavier rainfall. The cumulative PFAS mass flow was estimated based on the relationship between PFAS concentrations and the river flow rate. The weekly PFAS mass flow ranged between 4 and 15 kg, and the total flow over the 15-week study period was estimated at about 140 kg (i.e. approximately  $500 \text{ kg year}^{-1}$ ). Such figures are in reasonable agreement with previous estimates for the Seine River, but nearly 200 times lower than for the Po River (Italy) [35].

To achieve further insight into the dynamics of PFASs in this system, PFAS concentrations were monitored at the regional scale over a longer time period [38]. For sample collection, three sites previously used as pilot sites for nearly two decades were selected (Marnay, Bougival and Triel, from upstream to downstream; see [1] for site location). A wider range of chemicals was analysed: 11 PFCAs, 5 PFASs, FOSA and its *N*-alkylated derivatives (MeFOSA, EtFOSA) and 1 fluorotelomer sulfonate (6:2 FTSA). Four 1-month campaigns were undertaken over a 1-year period (September 2011 to December 2012), and the results based on grab samples (2–4 per campaign) confirmed the ubiquitous character of PFASs, since they were detected in all samples.

In the water column, total PFAS levels ranged from 2 to  $90 \text{ ng L}^{-1}$ . A significant upstream–downstream gradient was observed, associated with the increase in anthropogenic pressure on the fluvial ecosystem (Fig. 3). The levels observed at Triel were on average ten times higher than those observed at Marnay, with intermediate levels being observed at Bougival. In accordance with previous observations, PFOS, PFHxS and PFOA were the dominant compounds at Marnay and Bougival, but a non-negligible contribution of the shorter-chain ( $C_5$ – $C_7$ ) carboxylic acids was also reported. A notable feature was the sharp increase in the relative abundance of 6:2 FTSA at Triel. This compound has been used as an alternative to PFOS (metal plating), but it may also result from the degradation of more complex fluorotelomer-based compounds used in firefighting foams or food packaging [25]. The high levels of 6:2-FTSA observed at Triel, on average nine times higher than those observed at Bougival, could also result from the influence of an industrial source located in the nearby Oise basin. The hypothesis of the existence of distinct



**Fig. 3** Correlation between PFOS levels and river flow rate in the Seine River

sources for this compound downstream of the basin is reinforced by the lack of correlation between its levels and those of major PFASs (e.g. PFOS).

During this monitoring study, the river flow rate was in the range 30–100 m<sup>3</sup> s<sup>-1</sup> at Marnay and 170–500 m<sup>3</sup> s<sup>-1</sup> at Triel. At each site, PFAS levels appeared constant under stable hydrological conditions over short monitoring periods (e.g. 4 weeks), while the molecular pattern remained unchanged for a given site. Seasonal variations were examined at the water year scale, and the highest PFAS concentrations appeared to be associated with low-flow conditions. At the downstream sites (e.g. under the influence of the Paris conurbation), all major PFASs displayed negative correlations with flow rate, strongly suggesting that point sources were predominant. However, at the farthest upstream site, a few compounds only were negatively correlated with flow rate (e.g. PFOS; see Fig. 3), and no significant trend was found between  $\Sigma$ PFASs and flow rate. Thus, the upstream site (Marnay), located in a rural area between the urban centres of Troyes and Paris, appeared to be rather under the combined influence of point and diffuse PFAS sources.

The average PFAS daily flow in the Seine River increased by a factor of about 80 between Marnay (upstream) and Triel (downstream), thereby providing evidence of the major contribution of the Paris conurbation. The order of magnitude of the annual total PFAS mass flow estimate was 10 and 800 kg year<sup>-1</sup> at Marnay and Triel, respectively. These figures are in good agreement with those previously determined in the centre of Paris (around 500 kg year<sup>-1</sup>), especially considering the methodological differences between the two studies (e.g. sampling frequency).

### 3.3 *Transfer of PFASs to Biota in the Seine River Basin*

Considering the current mechanistic understanding of PFAS bioaccumulation, experimental studies are still needed to characterise the transfer of these chemicals within food webs. In the PIREN-Seine programme, the bioaccumulation of PFASs was investigated through several field studies that addressed this issue at different levels of biological organisation. In particular, two model organisms were selected, namely, (1) common chub, *Leuciscus cephalus*, a fish species widely used for water quality monitoring, and (2) periphytic biofilm. Note that only the results obtained with the latter are shown below.

Periphytic biofilms are mainly composed of both heterotrophic and autotrophic microbial cells embedded in an exopolymer matrix comprising polysaccharides and proteins [39]. At the interface of the water column and solid substrates such as bed sediment, they may play a central role in controlling contaminant bioavailability and transfer to consumers such as invertebrates or fish that graze on it.

Biofilm samples (3–4 per site,  $n = 11$ ) were collected on artificial support (low-density polyethylene) colonised in situ. High detection frequencies (80–100%) were observed for C<sub>8</sub>–C<sub>12</sub> PFCAs, PFHxS and PFOS.  $\Sigma$ PFASs were in the range 4.3–33 ng g<sup>-1</sup> dw, and concentrations in biofilm samples largely exceeded those of sediment samples [38]. The linear isomer of PFOS dominated

**Table 2** Log BCF (mean  $\pm$  SD) calculated for compounds detected in both water (dissolved phase) and biofilm [38]

Log BCF	PFOA	PFNA	PFDA	PFHxS	PFUnDA	PFOS	6:2 FTSA
Marnay	2.9 $\pm$ 0.1	4.0	3.6 $\pm$ 0.6	2.0 $\pm$ 0.2	–	3.8 $\pm$ 0.1	3.6
Bougival	2.3 $\pm$ 0.1	3.5 $\pm$ 0.4	3.0 $\pm$ 0.6	1.4 $\pm$ 0.2	–	3.1 $\pm$ 0.2	1.5
Triel	2.3 $\pm$ 0.1	3.6 $\pm$ 0.6	3.2 $\pm$ 0.1	1.6 $\pm$ 0.1	3.9	3.2 $\pm$ 0.1	1.4 $\pm$ 0.2

the molecular pattern (>50% of  $\Sigma$ PFASs), in agreement with profiles commonly reported for aquatic wildlife [40]. It can be speculated that PFASs were not only absorbed at the biofilm cell surface, but that they may also have been incorporated within the extracellular polymeric matrix or undergo intracellular accumulation [41]. These results imply that periphyton may constitute a key entry point for PFASs at the base of riverine food webs and a major source of PFASs for grazers.

The bioconcentration factor (BCF) is a useful metric to assess the bioaccumulation potential of a chemical. BCFs were calculated for PFASs detected in both dissolved phase and biofilm samples. The log BCF values ranged between 1.0 and 4.1 (Table 2), which is consistent with observations for other trace organics, e.g. pesticides [42].

Moderate  $BCF_{\text{biofilm}}$  was reported for PFNA, PFDA and linear PFOS, larger than those of shorter-chain compounds such as PFHxS (Table 2). This highlights the importance of perfluoroalkyl chain length and functional group on PFAS bioaccumulation potential; the influence of such structural features on PFAS bioaccumulation in fish, as well as on the sediment–water partitioning, was extensively investigated in a tributary of the Seine River and is discussed in detail elsewhere [43]. The upstream–downstream gradient of PFAS levels in biofilm was comparable to the contamination gradient observed for water samples, i.e. displaying maximum values downstream of Paris. However, BCFs were significantly higher at the upstream sites than at the downstream sites (Table 2). Principal component analysis revealed the dependence of BCF on the dissolved phase concentration, which would be consistent with the conceptual model developed by Liu et al. [44] (i.e. adsorption-like process or PFAS–protein interaction). In addition, negative correlations were also observed between BCF and major cations (except for  $Ca^{2+}$ , unaffected by the longitudinal gradient), as observed for another model organism, the planktonic crustacean *Daphnia magna* [45]. However, this does not provide evidence of a causal relationship, and this result may be coincidental due to the collinearity between PFAS levels and major cations. Finally, the organic C/N ratio (i.e. proxy of bacteria/algae relative abundance) [46] was the only descriptor of biofilm characteristics positively correlated with BCF. Altogether, these results suggest that biofilm community characteristics may also be a determinant of PFAS bioaccumulation in periphyton.

## 4 Pathogenic Protozoa

### 4.1 Context

The increase of anthropogenic pressures on ecosystems has led to the increased frequency of pollution episodes by biological agents. Among these pollutions, the faecal contamination of aquatic environments affects numerous regions of the world, with proven risks to human health [47].

Three protozoan parasites are clearly identified as public health priorities: *Cryptosporidium* spp., *Giardia duodenalis* and *Toxoplasma gondii*. *Cryptosporidium* and *Giardia* are responsible for cryptosporidiosis and giardiasis, respectively. They can cause significant morbidity in immunocompetent patients, and *Cryptosporidium* can lead to death in immunocompromised patients [48]. *T. gondii* is responsible for toxoplasmosis, and 30% of the entire human population is chronically infected. An infection during pregnancy may lead to serious malformations of the foetus. In humans, the main vector of these biological agents is water contaminated by human or animal faeces, subsequently used for drinking or irrigating crops [49]. Their parasitic stages of transmission, i.e. oocysts and cysts, are very robust under environmental conditions, and they are ubiquitous in aquatic habitats. *Cryptosporidium* spp. and *G. duodenalis* are the protozoan parasites most often involved in water-related epidemics (i.e. due to the ingestion of drinking water or the accidental ingestion of contaminated water during recreational activities).

The assessment of the microbiological water quality is based on the monitoring of the occurrence of two bacterial indicators of faecal contamination – *Escherichia coli* and *Enterococcus* – according to the World Health Organization and European regulations (2006/7/EC) [50]. However, they can be quickly removed from the environment and are more sensitive than protozoa to environmental stresses (e.g. temperature variations, pollutants) and disinfection treatments [51]. Consequently, the abundance of these bacterial indicators does not reflect, or very little, the overall sanitary quality [52, 53]. Indeed, previous studies conducted on the Seine River demonstrated the lack of correlation between *Cryptosporidium* and *Giardia* concentrations and bacterial indicators in wastewater and river water [54, 55]. The authors suggest that viral and bacterial indicators are not appropriate to predict parasite loads in surface waters.

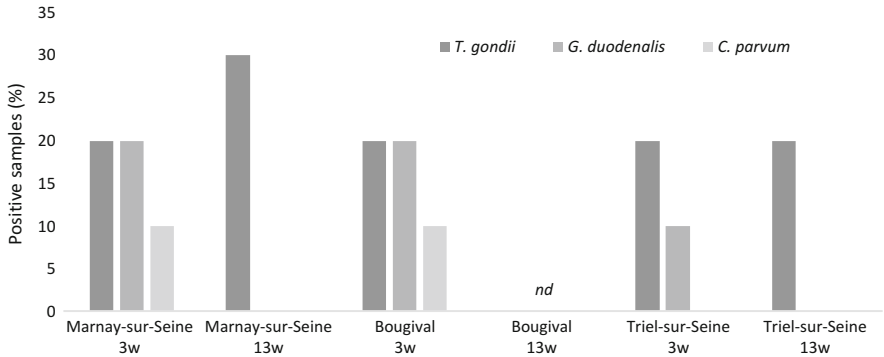
To investigate the occurrence of *Cryptosporidium* and *Giardia* in filtered water samples, the AFNOR NF T 90-455 standard (July 2001) proposes a detection technique based on both immunocapture on beads and immunofluorescence revelation. This detection is therefore highly specific, but it has not been applied yet to the detection of *T. gondii*. Other limitations have been identified: this method requires large volumes of water and high concentrations of parasites; it is expensive, and it does not allow for the rapid routine detection of parasites. It is therefore urgent to improve analytical tools for the detection of these biological contaminants for the purpose of monitoring water masses, thereby improving the assessment and management of health risks.

In this context, numerous studies have highlighted the value of bivalves for aquatic environment monitoring. These organisms are sedentary, have a high filtration rate and are characterised by their ability to accumulate environmental contaminants. For example, the use of bivalves revealed the contamination with pathogens, while direct measurements on water samples were negative [56]. In addition, oocysts of *Cryptosporidium parvum* have been detected in mussels (*Mytilus galloprovincialis*) and cockles (*Cerastoderma edule*) from a shellfish-producing region in Spain. The authors counted up to  $5 \times 10^3$  oocysts in the tissues of bivalves [52]. The bioaccumulation of protozoa in bivalves is fairly well documented in marine environments; in contrast, few studies have been conducted on continental aquatic environments, despite their direct connections to pollutant sources (e.g. discharge of effluents from water treatment plants, direct discharge of livestock effluents, runoff or leaching from contaminated soil). The need for a better understanding of the protozoa ecology in freshwater ecosystems is increasingly spotlighted. Therefore, particular interest was focused on the freshwater bivalve *Dreissena polymorpha* (zebra mussel). Laboratory exposures have shown that *D. polymorpha* was capable of (1) bioaccumulating cysts of *G. duodenalis* and oocysts of *C. parvum* and *T. gondii* and (2) retaining *T. gondii* oocysts in its tissues in amounts close to those found in tanks after 14 days of exposure [57, 58].

#### 4.2 Occurrence of Pathogenic Parasites in the Seine River

In a previous study, Mons et al. [54] assessed the protozoan contamination in the Seine River at sampling points located near the entry of drinking water plants (Ivry and Orly) or farther downstream in Paris (Tolbiac, Alma, Garigliano) and its periphery (Suresnes and Clichy). *Cryptosporidium* and *Giardia* were detected in filtered water in 45% (67/149 samples) and 94% (140/149 samples) of samples, respectively. *Giardia* was found more frequently and in larger quantities than *Cryptosporidium*. Thus, downstream of Paris, maximum concentrations reached 245 *Cryptosporidium* oocysts  $10 \text{ L}^{-1}$  and 512 *Giardia* cysts  $10 \text{ L}^{-1}$ . These authors suggested that protozoan contamination in the Seine River was not linked to urban runoff but to land application of cattle manure and heavy rainfalls, which contribute to protozoan runoff from contaminated soils. Thus, *Cryptosporidium* and *Giardia* probably originate from rural areas, not from the Paris conurbation itself.

In this context, a field study was conducted within the PIREN-Seine programme, to further investigate the occurrence and sources of pathogenic protozoa in the Seine River using active biomonitoring with *D. polymorpha*. This field survey was carried out on three sites along the Seine River, following an upstream–downstream gradient: Marnay-sur-Seine (rural site), Bougival and Triel-sur-Seine (urban sites) (see [1]). Zebra mussels were collected in April 2016 at the Lac du Der-Chantecoq (N 48°36'10.0728" E 4°44'57.408"). Mussels measuring  $2 \pm 0.2 \text{ cm}$  were acclimated in the dark in mineral water at 12°C for 2 weeks with two water changes a week to ensure that they were protozoan-free. Bivalves were caged in May 2016 for



**Fig. 4** Percentage of zebra mussels positive for *T. gondii*, *G. duodenalis* or *C. parvum* after 3 and 13 weeks of caging at Marnay, Bougival and Triel ( $n = 10$ ). *nd* not detected

3 and 13 weeks, and three protozoa were quantified in the tissues of bivalves using molecular biology techniques. After 3 weeks of exposure, biological contamination by the three protozoa was observed at Marnay-sur-Seine and Bougival; *T. gondii* and *G. duodenalis* were detected in bivalves caged in Triel (Fig. 4). Thus, these results suggested that a 3-week caging period was sufficient to demonstrate the water contamination by protozoa. After 13 weeks, no protozoa were detected in zebra mussels caged at Bougival, and only *T. gondii* was detected in tissues of mussels caged at Marnay and Triel.

Bougival and Triel-sur-Seine are urban sites, and the biological contamination could be related to the high population density in this part of the Seine River basin. Prevalence rates of cryptosporidiosis in humans range from 1% to 20%; giardiasis is endemic in humans, and the prevalence of *Giardia* ranged from 1 to 5% [59]. Concerning *T. gondii*, felids are the definitive hosts, and toxoplasmosis is present in every country, with human seropositivity rates ranging from less than 10% to over 90% [48].

The biological contamination in Marnay-sur-Seine can be related to substantial agricultural and farming activities in this area. Livestock, particularly cattle, are an important source of *C. parvum*. In a Canadian farm animal analysis, the presence of *Cryptosporidium* was detected in faeces samples of cattle (20%), sheep (24%), pigs (11%) and horses (17%) [60]. Infected calves can excrete up to  $10^7$  oocysts per gramme of faeces [61].

These different studies highlighted the fact that protozoan parasites are ubiquitous in the Seine River and that bivalves, as sedentary organisms, could reflect ambient biological contaminations of watercourses. More specifically, *D. polymorpha* could be used as a new bioindicator in sanitary biomonitoring of freshwater bodies.



## 5 Antibiotics and Bacterial Antibiotic Resistance

### 5.1 Context

Since the first synthesis of antibiotics in 1940s, numerous molecules have been discovered, and nowadays there are about 10,000 antibiotics on the market [62]. Although these molecules have reduced mortality from infectious diseases and thus increased life expectancy, the use of these molecules has also induced environmental contamination. The first detection of pharmaceuticals in surface waters occurred in 1976 with the detection of clofibric acid and salicylic acid in a lake in Nevada. Since then, almost all categories of pharmaceutical substances have been found in surface waters [63].

The actual impact of antibiotic discharge on ecosystem functioning is still unknown. For instance, the concentrations of antibiotics observed in water (on the order of  $\text{ng L}^{-1}$ ) are too low to affect the growth of fish such as Japanese medaka ( $\text{EC}_{50}$   $100 \text{ mg L}^{-1}$  for sulfonamides) or algae (in the range of  $0.1\text{--}1 \text{ mg L}^{-1}$ ). At the microbial community level, concentrations measured in situ are below the minimum inhibitory concentration required to exert selection pressure on environmental microorganisms (on the order of  $\text{mg L}^{-1}$ ); however, subinhibitory concentrations may promote mutagenesis or modify gene expression and may significantly influence bacterial physiology [64–67].

Since 2003, several studies of the PIREN-Seine programme have targeted pharmaceutical residues, particularly antibiotics. The main objectives of these studies are to determine the pathways of contamination in the natural environment, the environmental behaviour of these substances and the potential risk to ecosystems.

### 5.2 Sources of Antibiotics in the Seine Watershed

In rural areas, no contamination by antibiotics is observed in forest streams, but the contamination appears when streams flow through agricultural or breeding areas. Tamtam et al. [68] measured a concentration of  $20 \text{ ng L}^{-1}$  of enrofloxacin in a small river (the Blaise). This compound is exclusively used in veterinary medicine, and therefore the finding shows the contribution of this use to river contamination.

Antibiotic inputs to rivers in agricultural/rural areas may also come from their use in fish farming [69]: in such farms, antibiotics may be mixed with fish food and dispersed directly into the breeding ponds. Thus, the presence of fish farms may generate the discharge of antibiotics into rivers. During antibiotic treatment in fish farms, flumequine was quantified as high as  $7 \mu\text{g L}^{-1}$  in the effluent of the treatment pond, and 2 days after the end of the treatment, flumequine concentrations were below the limits of quantification. However, 20 days later, this molecule was still measurable in the sediments of the river downstream of the fish farm discharge [68].

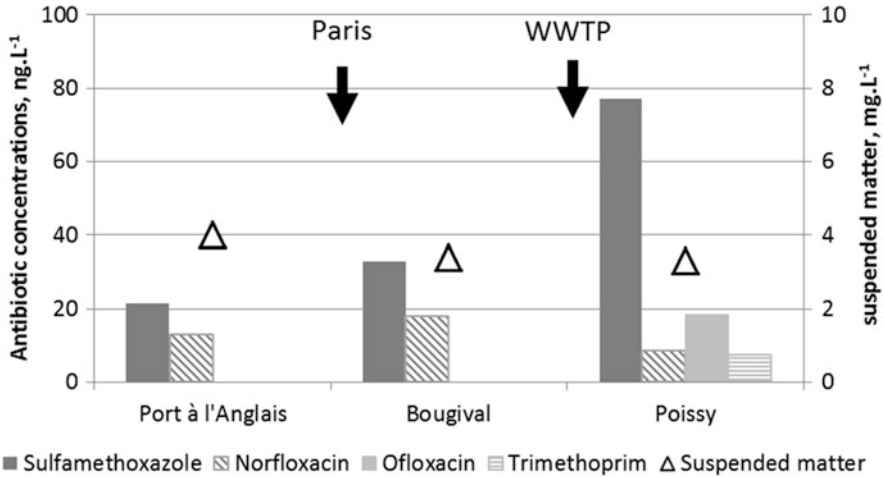
Antibiotics in rivers also have an urban origin, associated with urban or hospital wastewater treatment plants (WWTPs). Hospital effluents may contain numerous compounds, with individual concentrations ranging from a few  $100 \text{ ng L}^{-1}$  up to  $47 \mu\text{g L}^{-1}$  (norfloxacin), and specific antibiotics such as vancomycin that are used exclusively in hospital facilities [70]. These concentrations are very high compared to those observed in the domestic effluents of residential areas. The mean hospital effluent concentration was 90-fold higher than that of the domestic effluent. However, since the volumes of hospital effluents are about six times lower than those of domestic effluents, the mass flow of antibiotics from domestic wastewater was approximately 1.5 times higher at the inlets of WWTPs [71].

WWTPs therefore play an important role in the life cycle of pharmaceutical products. Since these molecules are not completely eliminated by WWTPs, urban effluent outfalls are considered as point sources of antibiotics into the environment [63]. In WWTPs located in a small catchment in the Seine River basin, the antibiotics more frequently detected in influents were sulfamethoxazole, norfloxacin, ofloxacin and trimethoprim [71]. These compounds have different behaviours in WWTPs: fluoroquinolones (norfloxacin and ofloxacin) are mainly eliminated by adsorption onto sludge. Sulfamethoxazole and trimethoprim are, respectively, poorly or not adsorbed on particles, and their elimination through sorption is less efficient [72]. Thus, WWTPs only partly remove antibiotics, and the discharge of treated water into rivers can lead to increased concentrations downstream of the discharge, depending on river flow. Dinh et al. [70] observed the occurrence of fluoroquinolones and sulfonamides (sulfamethoxazole) in water downstream of WWTP discharge outlet. Fluoroquinolones are gradually adsorbed into the sediments, and only sulfonamides are detected far away from the discharge point. Therefore, fluoroquinolones accounted for up to 90% of antibiotics in the sediment [71].

### 5.3 *Antibiotic Contamination in the Seine River*

The main antibiotics quantified in the Seine River are sulfonamides, fluoroquinolones, macrolides and diaminopyrimidines [68]. These compounds are used in human as well as veterinary medicine. For example, sulfamethoxazole is the main sulfonamide, with concentrations ranging from 6 to  $544 \text{ ng L}^{-1}$  throughout the year. Their concentrations of sulfonamide increase from upstream to downstream with a maximum observed downstream of the main WWTP discharge outfalls of the Paris conurbation (Poissy) (Fig. 5). Besides sulfamethoxazole, fluoroquinolones are the main family of antibiotics measured in the Seine. Norfloxacin shows the same pattern as sulfamethoxazole, and ofloxacin is only detected at the farthest downstream site.

Trimethoprim (diaminopyrimidines) is often quantified in the Seine with a background level around  $10 \text{ ng L}^{-1}$  and is mainly present downstream of WWTP discharges because of its low elimination by WWTPs [72]. The decrease of these



**Fig. 5** Antibiotic concentrations ( $\text{ng L}^{-1}$ ) in September 2009 in the Seine River: upstream of Paris (Port à l'anglais), downstream of Paris (Bougival) and downstream of the main WWTP discharge outfalls of the Paris conurbation (Poissy)

antibiotic concentrations during high-flow events seems to confirm that the intake is mainly related to medical uses and the origin of contamination is point sources. Overall, antibiotic concentrations measured in the Seine River are similar to those measured in Europe [73].

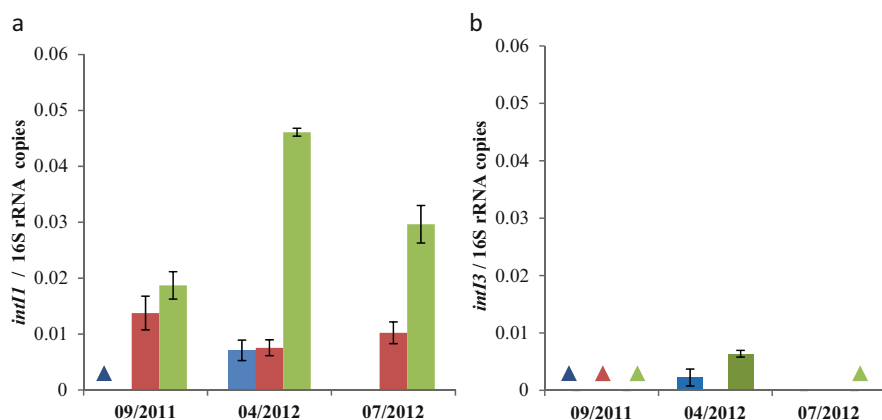
#### 5.4 The Resistome and Antimicrobial Resistance

The contamination of water with antibiotics, which results from their prescription in human or veterinary medicine, is accompanied by a contamination of waterbodies by antibiotic-resistant bacteria. The occurrence of such resistant strains may be explained by the selection pressure exerted on the gut microbiota of humans and animals receiving antibiotic therapy. In the Seine River, Servais and Passerat demonstrated the presence of antibiotic-resistant faecal bacteria, the abundance of which reflects the level of anthropisation of the watershed [74].

In this context, particular attention is focused on biofilms or periphyton, which are microenvironments likely to concentrate chemical and microbiological contaminants [75–77]. In the aquatic environment, biofilms are ecological niches, where microbial communities experience chronic multiexposure to chemical contaminants (organic or metallic) including antibiotics. To this exposure is added a continuous supply of antibiotic-resistant bacteria of human or animal origin and therefore of genetic support involved in the dissemination of antibiotic resistance, such as clinical integrons [78]. Today, clinical integrons, considered as xenogeneic contaminants, are believed to be bioindicators of the risk of dissemination of antimicrobial

resistance in the environment [79, 80]. Moreover, within these biofilms, the presence of metallic contaminants has been shown to be favourable for the spread of antimicrobial resistance [81, 82].

Within the PIREN-Seine programme, the resistome (genes conferring antibiotic and/or trace metal resistance) of microbial communities in biofilms has been studied as an indicator of vulnerability or environmental resilience to chemical or microbiological contaminants. In the Seine River, observations in situ and in the laboratory have shown that the acquisition of a trace metal tolerance of microbial biofilm communities depends on several factors. At the cellular level, the increase of genes encoding resistance to heavy metals, such as silver (*silA* gene) or cadmium/zinc/cobalt (*czcA* gene), suggests a selection of resistant bacteria in response to chronic exposure to toxic thresholds in  $\text{Ag}^+$ ,  $\text{Zn}^{2+}$ ,  $\text{Co}^{2+}$  or  $\text{Cd}^{2+}$ . At the microbial community level, the resistance to antibiotics may be related to a change in microbial diversity, with an increase in the abundance of bacterial genera able to grow in contaminated environments, such as *Burkholderiales*, *Cytophagales* and *Sphingobacteriales* [83]. Within these phyla, there are autochthonous bacterial genera but also bacteria that could be opportunistic pathogens such *Burkholderia*. Moreover, there is a permanent presence of antibiotic resistance genetic supports (class 1 clinical integron), whose abundance increases with the degree of anthropisation of the watershed, the maximum values being observed downstream from Paris and the discharge of the main treatment plant in the Paris region (Triel), regardless of the season (Fig. 6).



**Fig. 6** Seasonal variation of relative abundance of clinical integrons in total DNA extracted from periphyton (artificial disposals) along the upstream–downstream transect, (■) upstream of Paris (Marnay), (■) downstream of Paris (Bougival), (■) downstream of Paris and impacted by the treated effluent of the Seine Aval WWTP (Triel). (a) Class 1 integron (intI1/16S rRNA copy number) and (b) class 3 integron (intI3/16S rRNA copy number). Filled triangle: Detectable but not quantifiable. INSERM UMR 102 Limoges

These results show that the resistome of biofilms in highly urbanised rivers, such as the Seine River, constitutes a microenvironment where genetic support for antibiotic resistance (i.e. clinical integrons) is concentrated. It would be advantageous to determine whether antibiotic concentrations within these biofilms are consistent with increased mutagenesis and genetic transposition events. Biofilms would then constitute micro-niches or “hotspots” that are favourable for the transfer of genes and thus for the dissemination of the genes involved in antimicrobial resistance, within indigenous communities.

## 6 Conclusions and Perspectives

Research undertaken within the PIREN-Seine programme over 30 years has considerably improved the understanding of the sources and dynamics of a wide range of chemical and biological contaminants in the Seine River basin. A few examples of recent research were briefly exposed in this chapter.

In the near future, research prospects should include the investigation of additional contaminants of emerging concern or recently regulated chemicals; upon prioritisation, the list of newly targeted chemicals could encompass, for instance, chlorinated paraffins, novel flame retardants, biocides, antimony, manufactured nanoparticles and nanoplastics, etc. Further studies should better investigate the fate (i.e. transfer processes and fluxes) of micropollutants in relation to the hydrodynamic conditions, especially during extreme events related to climate change, such as floods or low flow/drought. To this end, it is anticipated that modelling approaches (1) would greatly improve the quantitation of gross fluxes transported by the Seine River, (2) could prove useful to better assess future contamination trends based on contaminant emission and hydrological scenarios and (3) would help estimate the exposure of biota and humans while enabling the investigation of key factors controlling this exposure.

Whenever possible, a more systemic approach should be implemented at the river basin scale, including various environmental compartments, to achieve a more holistic view of contaminant fate. In particular, the atmosphere–soil–river–estuarine continuum should be taken into consideration. The global impact of this chronic multi-contamination should be assessed at different levels of biological organisation, and human and social science should also be considered to address such issues in a more holistic way. An original approach, derived from territorial ecology, would consist in interpreting chemical fluxes at the basin scale by considering the connections between material production, trade or consumption of agricultural and household goods, emissions, stocks constituted in environmental compartments and transfers between compartments.

New methodological approaches should also be implemented. Suspect or nontarget screening using high-resolution mass spectrometry would greatly help characterise the chemical fingerprints of diverse environmental compartments and their temporal and spatial variability, thereby contributing to a better understanding of the human and biota

exposome. Using passive sampling, in combination with both target and nontarget methods, would allow for the acquisition of time-averaged, low-frequency data to build up long-term data sets (plurennial or even decadal scale). This approach provides data that are complementary to high-frequency sampling and that are needed to help estimate the efficiency of regulations on the occurrence and dynamics of chemicals of interest. Biota could also be used to this end, provided well-known sentinel organisms are used (e.g. gammarids or freshwater mussels).

Finally, future research should also address emerging issues regarding biological contaminants, especially those that are not or are poorly related to the abundance of faecal bacterial indicator. The study of pathogenic protozoa transfer in freshwater ecosystems, or the emerging pathogens such *Leptospira*, is fundamental to improve the microbial risk assessment of surface waters. In addition, protozoa may lead to the modulation of physiological responses in sentinel organisms (e.g. bivalves), thereby potentially leading to erroneous interpretations in environmental monitoring studies. Thus, the influence of confounding factors such as the infection by protozoa represents a major issue as regards the use of biomarkers for environmental quality assessment (for further details regarding this issue, see [84]). Furthermore, the transfer of genetic element encoding resistance to antibiotics (1) from environmental microbial communities to strains that are pathogenic for humans or (2) from genes of clinical origin to environmental pathogenic bacteria that are opportunistic for humans (e.g. *Pseudomonas*, *Aeromonas* or *Burkholderia*) is identified as a major risk to public health related to the environment. The assessment of such a transfer of environmental resistance to humans (retro-transfer) is crucial to evaluate; it is, however, challenging because it involves rare events occurring on a time scale that remains difficult to determine.

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