



# Modelling micropollutant cycle in Lake Como in a winter scenario: Implications for water use and reuse, ecosystem services, and the EU zero pollution action plan

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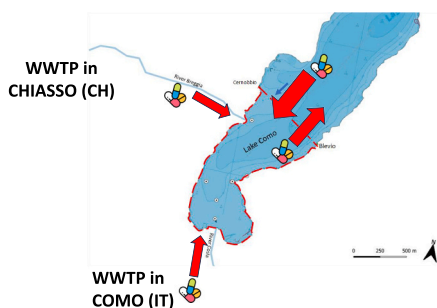
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## HIGHLIGHTS

- Fate and effects of 44 pharmaceuticals were studied in Lake Como (Italy) in winter.
- Diclofenac was the most abundant pharmaceutical in WWTP discharge, rivers and lake.
- Concentrations decreased by two orders of magnitude from WWTP outlet to lake water.
- Little risk was shown for ecosystem and human health for single chemicals, more for mixtures.
- Model simulations revealed water residence time in the bay and most important emission source.

## GRAPHICAL ABSTRACT

### MAIN PHARMACEUTICAL EMISSION FLUXES IN COMO BAY



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## ABSTRACT

The fate and effects of 42 pharmaceuticals was studied in Lake Como (Italy), in wastewater treatment plants delivering water to the lake, in two rivers and in potable water obtained from lake water. Lake Como is one of the deepest and largest lakes in Northern Italy, serving important ecosystem services (i.e., drinking water, recreational, industrial, irrigation uses), some of which are currently at risk giving the current water scarcity and climate change scenarios. The highest concentrations measured in lake water were those of diclofenac, followed by carbamazepine, its metabolite, and clarithromycin. The data measured allowed to calibrate and run a fugacity-based lake model, which showed that the most important chemical load generally comes from the advective water from the north of the lake, rather than from the direct wastewater treatment plant (WWTP) discharges. This indicates that only an important reduction of chemical discharge (reduced use or extensive treatment) at a drainage basin level could significantly reduce concentrations in water. This has strong implications on how to implement the EU zero pollution action plan to significantly improve water ecosystem and human health protection.

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## 1. Introduction

According to the U.S. Environmental Protection Agency (EPA), micropollutants (i.e., pharmaceuticals) are identified as contaminants of emerging concerns (CECs) (US EPA, 2023a, 2023b) and represent a class of organic compounds widespread in ecosystems (Klatte et al., 2017). Pharmaceuticals are typically found in the aquatic compartment at different concentration from ng/L up to few  $\mu\text{g/L}$  (Gunnarsson et al., 2019; Kümmerer, 2010). Their discharge into the environment is of great concern due to the possibility of threat ecosystem services (i.e., drinking water, recreational, irrigation uses) (Kümmerer, 2010).

Municipal and industrial wastewater represent one of the main sources of microcontaminants in aquatic ecosystems since most of the current wastewater treatment plants (WWTPs) (lacking specific removal stages for micropollutants) are incapable to efficiently remove most of them (Elskens et al., 2023; K'oreje et al., 2016; Lonappan et al., 2016; Musolff, 2009; Shraim et al., 2017). Furthermore, increased droughts and water scarcity events, worsened by the global change scenario, increase the ecosystem vulnerability since they directly affect the capacity of freshwater ecosystems to dilute pollutants (Arenas-Sánchez et al., 2016; Azevedo et al., 2018; European Environment Agency, 2021; Kaltenborn et al., 2012).

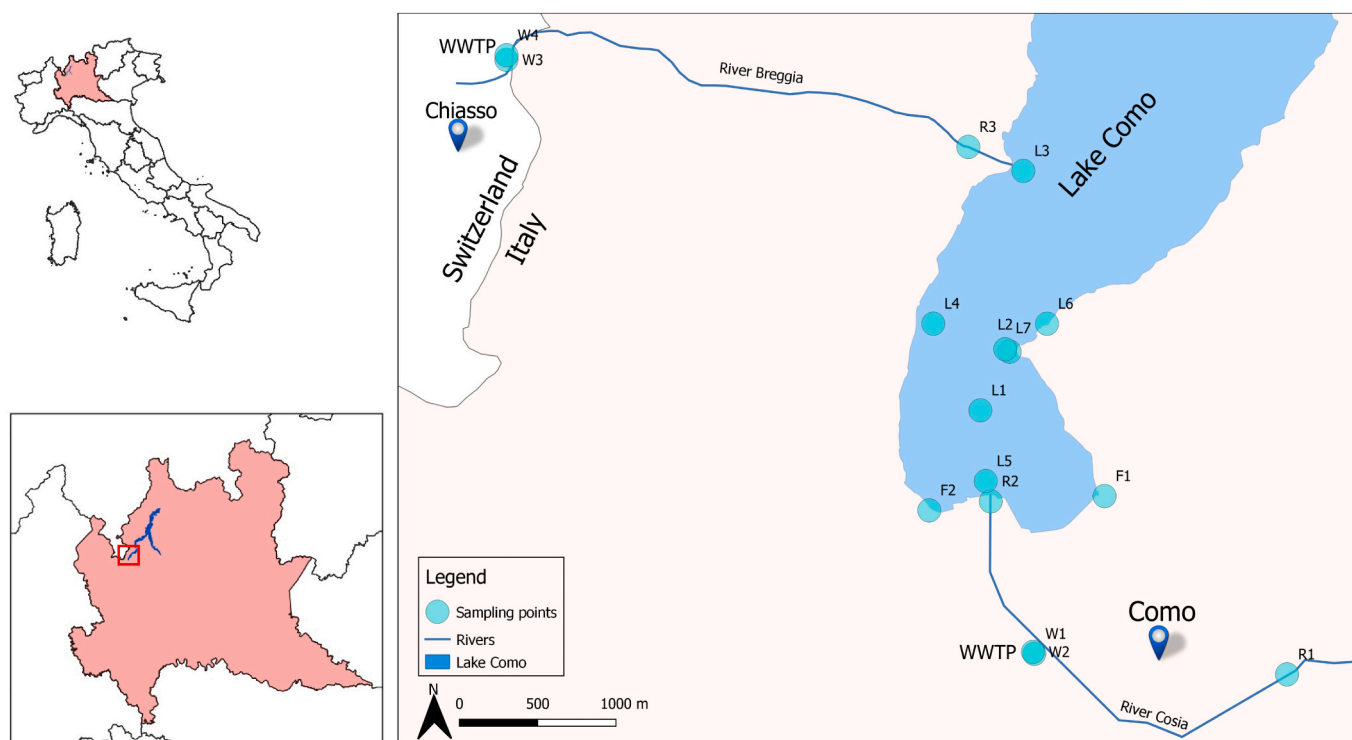
From a European Union (EU) regulatory perspective, the Water Framework Directive (WFD, Directive 2000/60/EC) aims at monitoring of surface-water status considering the ecological and chemical status (WFD art.8). More recently, the European Commission “zero pollution action plan” (EC, 2021) aims at reducing pollution of air, soil and water in European Union at levels “no longer considered harmful to health and natural ecosystems, that respect the boundaries with which our planet can cope, thereby creating a toxic-free environment”, the so called “zero pollution vision for 2050”. This includes a number of objectives to be reached generally within 2030 (European Commission, 2021a, 2021b). Additionally, the ongoing revision of the Urban Wastewater Treatment Directive (European Commission, 2022) calls for new limit values for

micropollutants that require additional treatment, including pharmaceutical and personal care products.

The occurrence of pharmaceuticals has been investigated worldwide in several rivers, lakes and reservoirs (Kleywegt et al., 2011; Wilkinson et al., 2022; Zhou et al., 2019). This group, in a previous paper (Castiglioni et al., 2020), measured 38 pharmaceuticals in Lake Como (or Lario) area. This was done, during the summer period, starting from two WWTPs (Como and Chiasso) and following the water path: rivers (Cosia and Breggia), Como Bay in Lake Como (hypolimnion and epilimnion), and to two drinking water fountains. Lake water is captured within the bay, treated, and distributed as drinking water in the town of Como. Also, another study (Tröger et al., 2021) recently reported the presence of contaminants in Como Bay, including pharmaceuticals, in raw water taken at hypolimnion level (−40 m depth) as well as in treated drinking water.

The Como Bay of Lake Como fits within a specific geopolitical context, since it receives treated water from Switzerland and Italy through the load of two rivers (Breggia and Cosia) (Fig. 1). In addition, this Bay has no outflowing streams, and this implies a relatively long water residence time and, possibly, chemical persistence. For these reasons, this Bay could be considered a good example of a large water reservoir use and reuse, in a context deeply influenced by the political and technical decisions of Switzerland (a non-EU state) and European Union (being Italy subject to EU legislation) on the measures to reduce micropollutant discharge by WWTPs. Therefore, this scenario is crucial both for managing and ensuring the good water quality status, as well as to support decision makers (i.e., potable use of water, irrigation uses, etc.) in national and more international paradigms. In this latter case, it is important to determine the extent (local, regional, international) of reduction measures needed to achieve desired ecotoxicological and toxicological goals, such as those of the “zero pollution action plan” (European Commission, 2021a).

Therefore, the aim of the research is to evaluate the mass balance of pharmaceuticals in a large, deep lake in a winter scenario (using Lake



**Fig. 1.** Study area. Symbols represent sampling points while letters the different types of water sample: L = lake; F = fountain; R = river; W = wastewater treatment plant. (W1 and W2 are at Como WWTP, W3 and W4 at Chiasso WWTP). The sample from point L7 is obtained from a pipe located at −40 m depth which collects lake water for drinking use.

Como as a case study), and their ecotoxicological and toxicological relevance. While other studies mostly focused on river water contamination, the novelty of this study lies in evaluating the risk for ecosystems and human health in a large water reservoir, whose water is used and reused, in full mixed water conditions, and with contaminants reaching the bay from two different countries. The final objective is to calculate the most important source for pharmaceutical pollution in the bay and therefore indicate the level of intensity of measures to reduce such contamination. The results of this exercise have relevant implications on how to implement the EU zero pollution action plan (EC, 2021) to significantly improve water ecosystem and human health protection.

## 2. Materials and methods

### 2.1. Study site

This study was conducted in Lake Como (Fig. 1) one of the deepest Italian lake (mean depth: 161 m; maximum depth, near Argegno: 425 m) and the third in terms of surface area (145 km<sup>2</sup>) and volume (23,372 km<sup>3</sup>) (Salmaso and Mosello, 2010).

Its drainage basin occupies an area of 4552 km<sup>2</sup>, of which 487 km<sup>2</sup> is in Swiss territory. Lake Como has a distinguishing y-like reversed shape, which can be divided into 3 different sub-basins: northern, south-western (referred as western) and south-eastern (referred as eastern). The lake has various tributaries, but the main ones are the Adda and Mera rivers in the northern basin, the Cosia and Breggia rivers in the western basin and Rio Torto and Caldene in the eastern basin (Copetti et al., 2020). River Adda, in this latter arm, is the only outflowing river of the lake. Lake Como is considered as a warm monomict lake, which implies that the lake has a thermal stratification much of the year and may mix thoroughly (full mix) during cold winters. For Lake Como such mixing generally affects the first 150–200 m of depth (Copetti et al., 2020). As regards the hydrodynamics of the lake, the presence of a fresher water plume coming from the north of the lake was recorded, corresponding to Adda and Mera rivers inflows, which tends to move towards the western basin. Moreover, a counterclockwise movement of surficial water currents was noticed, carrying Breggia and Cosia streams waters in the northeastern part of Como Bay, where a pipe collecting water for drinking use is located, at 40 m depth (point L7 in Fig. 1) (Copetti et al., 2020; Guyennon et al., 2014; Laborde et al., 2012, 2010; Morillo et al., 2009).

### 2.2. Sampling

This study was carried out in the winter of year 2022 (8th of February 2022), by means of two parallel (one lake- and one land-based) sampling campaigns. During the first sampling campaign, 5 different points (L1-L5) in Lake Como were first sampled, collecting lake water at three different depths (Fig. 1 and Table SI-1). Sampling was performed with a motorboat, with the help of Guardia Costiera Ausiliaria del Lario, (Dongo, CO), in the southernmost part of the western basin of the lake (the so-called Como Bay). Lake water samples were collected using a 1.7 L Niskin bottle (General Oceanics Inc.) dropped from the boat, at 3 different depths (Table SI-1), hypothetically corresponding to hypo-, meta- and epilimnion, based on the maximum depth of each point. Moreover, lake water temperature and conductivity were measured with a Levelogger 5 Model 3001 probe (Solinst Canada Ltd.). The conductivity sensor was calibrated in the laboratory, using a 1413 µS/cm certified calibration solution (Hanna Instruments, Inc.) prior to sampling in the lake. The probe was submersed from the boat at the 5 sampling points and depth, temperature, and conductivity were measured every 2 s when the probe was pulled back to the boat.

The second campaign was carried out by collecting water from the WWTPs of Como (Como Acqua, points W1 (influent) and W2 (effluent) of Table SI-2 and Fig. 1) and of Chiasso (CDA, CD, points W3 (influent) and W4 (effluent) of Table SI-2 and Fig. 1), rivers Cosia (upstream of the

WWTP of Como and near the mouth, points R1 and R2 of Table SI-2 and Fig. 1) and Breggia (near the mouth, point R3 of Table SI-2 and Fig. 1), public drinking fountains (F1, west and F2 east, Table SI-2 and Fig. 1). In addition, two points of lake water collected from the shore were also sampled. Surface water near the bank of Villa Geno (points L6 Table SI-2 and Fig. 1), and water deriving from hypolimnion (L7) collected at a Lerei Company pumping station before being sent to the depuration plant to obtain drinking water. The day before sampling was characterized by cold temperatures and high wind speed, reaching up to 103 km/h in the nearby meteorological station of Como Villa Gallia (ARPA Lombardia). Water samples were collected in 1 L polyethylene bottles and were stored at –20 °C for a maximum of 30 days, in the dark, in order to avoid any stability issue. Typical protocols are used to assess the stability of analytes before analyses (especially if samples are stored for >30 days) (Riva et al., 2020). This information is also merged with the available data on stability during treatment processes that may reflect the general stability of a substance at environmental conditions (Castiglioni et al., 2018).

For the two WWTPs, composite 24-h samples of influent and effluent wastewater were collected for 1 day (February 7–8, 2022): influent water was collected February 7 while effluent waters were collected the following day (February 8) after the hydraulic residence time (14 h for Como WWTP and 12 h for Chiasso WWTP).

### 2.3. Chemicals

Forty-two pharmaceuticals of different classes were investigated. Reference standard of ranitidine was purchased from Glaxo SmithKline (Philadelphia, USA); ramiprilat from Spectra 2000 s.r.l. (Rome, Italy); 10,11-dihydro-10,11-dihydroxycarbamazepine and rosuvastatine from Santa Cruz Biotechnology (Dallas, TX, USA). All the other standards from Sigma-Aldrich (St. Louis, MO, USA). The labeled compounds salbutamol-D3 (99.1 % D), ibuprofen- D3 (99.7 % D), sulfamethoxazole-D4 (98% D), and bezafibrate-D6 (99% D) were purchased from CDN Isotopes (Quebec, Canada); atenolol-D7 (>97 % D), carbamazepine-D10 (99 % D), ketoprofen D3 (98% D) and 17-β-estradiol- D3 (98% D) were purchased from Sigma-Aldrich (St. Louis, MO, USA); Valsartan-D3 and ramipril-D5 from Spectra 2000s.r.l (Rome, Italy). Only four chemicals were measured in common with the Swiss indicator compounds: carbamazepine, clarithromycin, diclofenac, irbesartan. Stock solutions were prepared at 1 mg/L with methanol and stored at –20 °C in the dark up to a maximum of three months. Working solutions (10, 1, 0.1 and 0.01 ng/µL) were prepared before each analysis by successive dilution with methanol and stored at –20 °C in the dark. Methanol, acetone, hydrochloric acid (37 %), formic acid (98 %) and acetonitrile for LC-MS were acquired from Carlo Erba (Italy); ammonium hydroxide solution (25 %) was purchased from Merck (Darmstadt, Germany), and triethylamine (>99.5 %) was acquired from Fluka (Buchs, Switzerland). HPLC grade (ultrapure) Milli-Q water was obtained with a MILLI-RO PLUS 90 apparatus (Millipore, Molsheim, France).

### 2.4. Analytical methods

Water samples were analyzed by solid phase extraction (SPE) and liquid chromatography tandem mass spectrometry analysis (LC-MS/MS) adapting methods previously developed and validated in our laboratory (Castiglioni et al., 2005). Samples were treated as in (Castiglioni et al., 2020). Briefly, water samples were double filtered on glass microfiber filters GF/A 1.6 µm and on a mixed cellulose membrane filter 0.45 µm (Whatman, Kent, U.K.). According to the type of matrix, different volumes were prepared: 25 mL for influent wastewater, 50 mL for effluent wastewater, 200 mL for river water and 500 mL for lake and drinking water and were acidified to pH 2–2.5 with HCl 37 %.

SPE was performed through an automatic device GX-274 ASPEC (Gilson, Middleton, WI, USA) using 3-mL disposable mixed reversed-phase/cation-exchange cartridge (Oasis MCX – 60 mg, Waters Corp.,

Milford, MA, USA) (Castiglioni et al., 2020).

Mass spectrometric analyses were done in both positive and negative ionization mode using the Selected Reaction Monitoring (SRM) mode and setting a time window of 180 s. Analyses were performed choosing the two most abundant fragmentation products of the protonated pseudo-molecular ions for each analyte and one product for each deuterated analog used as internal standard (IS). Pharmaceuticals were quantified using the isotope dilution method; the most abundant precursor/product ion transition was the quantifier ion, and the other the qualifier. Selected transitions, together with the corresponding optimized instrumental parameters, retention times, and IS used for quantification are reported in (Castiglioni et al., 2020). Calibration curves were prepared freshly before each analytical run. The first calibration point, containing only the labeled compounds, was used as instrumental blank. The limits of quantification (LOQs) were estimated as the concentration giving peaks for which the signal-to-noise ratio (S/N) was 10. LOQs were directly calculated from chromatograms obtained in wastewater, lake and drinking water at the lowest concentration measured, and when necessary (i.e., the substance was not found in none of the samples), a spike of the analyte was done. Generally, LOQs were below 5 ng/L in wastewater and 1 ng/L in lake and drinking water (Tables SI-3 and SI-4). LODs are 1/3 of LOQs. Method repeatability was calculated as the relative standard deviation (RSD) from the analyses of three replicates and was generally below 10 %, as reported in Castiglioni et al. (2020).

## 2.5. Model scenario settings

Dyna model (Di Guardo et al., 2006), a fugacity based water sediment surface water model, was used to evaluate the environmental fate of chemicals in Como basin. The steady state version of this model is based on the original Don Mackay's QWASI model (Mackay et al., 1983) and was first used in a reverse mode to back calculate the advective water fluxes (G) (m<sup>3</sup>/h) in the Como Bay of Lake Como. Atenolol, a hydrophilic chemical (Log K<sub>ow</sub> = 0.16, Table SI-9) given its affinity for water compartment was used as a conservative chemical to estimate the advective fluxes running the model using the annual discharges from the two WWTPs and considering as advective concentrations those measured in point L3. This is because the water currents flow in a counterclockwise manner and therefore the L3 point can be assumed as representing the water inflow concentrations (see also Fig. 3). The model was then run calculating the advective water fluxes in (and out) to match the predicted concentration in water for Atenolol in point L1 (center of the Bay). The model was parametrized using a typical perialpine lake scenario (Table SI-8), developed for Lake Maggiore (a deep water lake located west of lake Como and characterized by similar features) (Di Guardo et al., 2006). Water surface, depth, and volume were updated according to the studied area: surface area of Como Bay in Lake Como was calculated using QGIS (QGIS, 2023), using a nautical chart detailed with bathymetry boundaries provided by the authorities of Lario basin (Autorità di Bacino, 2023). The average water depth was obtained by calculating the weighted average of the depths of the study area (Fig. SI-1). For each depth class (0–50; 50–100; 100–150; 150–200), the corresponding areas and depths were calculated. The average depth was then obtained as the sum of the individually weighted average depths.

Once the advective water fluxes were calculated, the model was applied on selected chemicals to illustrate their environmental fate within Como Bay.

Model performance was evaluated comparing measured and predicted results calculating the efficiency factor (EF) (Infantino et al., 2008; Mayer and Butler, 1993):

$$EF = \frac{\sum (O - \bar{O})^2 - \sum (P - O)^2}{\sum (O - \bar{O})^2} \quad (1)$$

where  $O$  are the observed (measured) concentrations,  $\bar{O}$  the average of the observed concentrations,  $P$  are the predicted (modelled) concentrations. Model performance is best when EF is close to 1.

## 2.6. Risk assessment

An environmental risk assessment (ERA) was performed for river and lake waters considering the risk of single contaminants and the risk derived from the mixtures for freshwater species: cyanobacteria, algae, *Daphnia* sp., and fish. To calculate the Risk Quotients (RQs) the highest measured concentrations (MEC) of pharmaceuticals were divided by the Predicted No Effect Concentration (PNEC). PNEC was estimated from ecotoxicological data considering acute EC<sub>50</sub> data (Table SI-14), given the lack of chronic data, following the guidelines of the Technical Guidance Document for Risk Assessment (TGD Part II) (European Commission, 2003). RQs were finally compared with the threshold value of 1 (European Medicines Agency, 2018). EC<sub>50</sub> values were obtained from laboratory studies collected from the available literature and ECOTOX database (US EPA, 2023b). In absence of experimental data for pharmaceuticals, acute EC<sub>50</sub> were calculated using QSARs (Quantitative Structure-Activity Relationships) equations provided by the TGD. Measured environmental concentrations were estimated as LOQ/2 when the concentration was below LOQ.

In addition, the risk derived from mixtures was estimated using the available experimental or calculated acute toxicity data for the same species. The ERA for mixtures was performed following the Toxic Unit (TUs) approach for each taxonomic group, calculated as the ratio between the measured environmental concentration and the acute median concentration (EC<sub>50</sub> or LC<sub>50</sub>) values. TUs for chemical mixtures were calculated according to the concentration addition approach (CA) as the sum of TUs for individual pharmaceuticals mathematically expressed as in Eq. (2) (Backhaus et al., 2000):

$$TU_m = \sum_{i=1}^n TU_i = \sum_{i=1}^n \frac{C_i}{EC_{x,i}} = 1 \quad (2)$$

where TU<sub>m</sub> is the toxic unit value for the mixture,  $n$  is the number of chemicals in the mixture,  $C_i$  is the measured concentration of each chemical, EC<sub>x</sub> is the Effective Concentration (acute) at the desired percentile ( $x$ ).

Human Risk Assessment (HRA) was evaluated for drinking water samples obtained from lake water, since the water obtained at Point L7 is purified and distributed as drinking water. HRA was evaluated for both individual compounds and mixtures following the available guidelines and procedures as in (Riva et al., 2018). To assess the cumulative risk arising from the co-exposure to all chemicals measured in drinking water samples, we followed the Hazard Index (HI) approach, a method derived from the concept of Concentration Addition, reported by U.S. EPA (USEPA/NCEA, 2000) and by U.S Department of Health and Human Services –Agency for Toxic Substances and Disease Registry (Wilbur et al., 2004). This was used as a worst case scenario considering negligible potential synergistic responses.

Hazard Quotients (HQs) were calculated for each pharmaceutical dividing the MECs by the Drinking Water Guidelines Levels (DWGLs). When the HQ is below 1, the risk for human health can be excluded; otherwise, further investigation is needed. DWGLs is a theoretical concentration below which the risk for humans can be considered negligible, even considering a long-term exposure. DWGLs were calculated considering the acceptable daily intake of a substance and safety factors as reported by the available guidelines (World Health Organization, 2011). The DWGL (ng/L) for a compound was calculated as follows (Eq. (3) (Snyder et al., 2008)):

$$DWGLs = \frac{ADI \times BW}{V} \quad (3)$$



where ADI is the Acceptable Daily Intake calculated as reported in the Supporting information. BW is the body weight (70 kg) and V is the volume of water intake (2 L).

In addition, the cumulative risk of mixtures of pharmaceuticals was assessed calculating the Hazard Index (HI), as the sums of the HQs, by applying, also in this case, the Concentration Addition approach. Acceptable values were those below 1, for which no risk is foreseen. HQ and HI were calculated only for pharmaceuticals which were present in drinking water samples.

Among the 42 chemicals investigated, nine are included in a list of chemicals identified as candidate Priority Substances for the Water Framework Directive (WFD) for which Environmental Quality Standards (EQSs) are proposed (European Commission, 2022). These nine chemicals are: azithromycin, clarithromycin, erythromycin, diclofenac, ibuprofen, carbamazepine, estradiol, estrone, ethinylestradiol. The proposed EQSs are reported in Table SI-19.

### 3. Results and discussion

#### 3.1. Water layer conditions

The measured lake parameters (temperature and conductivity with depth) revealed the absence of stratification and a completely fully mixed layer. The maximum depths measured with the depth probe in the 5 sampling points were, respectively, 48 m, 40 m, 22 m, 50 m, and 10 m. As regards water temperature (Fig. SI-2), the average ranged from 7.6 °C to 8.2 °C in all the sampling points, suggesting well-mixed conditions of the water column in the southern part of the western basin of Lake Como. Also, conductivity (Fig. SI-3) was constant with depth and similar in all points, close to 140  $\mu\text{S}/\text{cm}$ , confirming the presence of a well-mixed water column. Previous study reported a conductivity of 193  $\mu\text{S}/\text{cm}$  during the circulation in Cernobbio, (in the North-west part of the Como Bay), although in 1992. The observed situation of full mixing,

typical of late winter conditions, was confirmed by other studies (Guyennon et al., 2014).

#### 3.2. Overview of pharmaceutical concentrations in samples

Fig. 2 reports the total pharmaceutical concentrations among therapeutic classes in the different group of samples: Como WWTP (IN and OUT), Chiasso WWTP (IN and OUT), five points in Lake Como at three different depths (L1, L2, L3, L4 and L5), shore sampled water lake (L6 and L7), drinking water (F1 and F2), river Cosia (R1 and R2), river Breggia (R3) (Tables SI-3 to -6 and Figs. SI-3 to -19).

Total pharmaceutical concentrations in rivers were 228 ng/L (R1-Before Como WWTP), 7706 ng/L (R2-After Como WWTP) and 2560 ng/L (R3-Breggia), highlighting the large WWTP contribution to river load (Fig. 2b and Table SI-4). This trend was less evident in river Breggia (R3), with a decrease in concentration between Chiasso WWTP effluent and the river (R2) of a factor of 2–4 depending on drugs. This difference could be ascribed to dilution, due to the higher flow rates of river Breggia compared to river Cosia, as already mentioned by (Castiglioni et al., 2020). Anti-inflammatory chemicals were the most abundant class of drugs found in river with the highest concentration found in Cosia river (R2) (4797 ng/L) (mainly diclofenac) as also shown by other studies (Loos et al., 2009). Cardiovascular (i.e., atenolol), antidepressants (i.e., carbamazepine and its metabolite dihydro-carbamazepine), diuretics (i.e., furosemide), antibiotics (azithromycin and clarithromycin) and antihypertensives (irbesartan) were the other largest class of drugs found in rivers (mainly at Cosia-R2 and Breggia-R3). Amoxicillin instead, whose concentration was < LOQ in both WWTPs, was found, at different concentrations, in rivers (from 19 to 44 ng/L), and in River Cosia at R1, before the contribution of Como WWTP effluent (Table SI-4). This presence could be related to non-regulated direct discharge into rivers and not mediated by Como WWTP.

These results are consistent with those measured in summer and

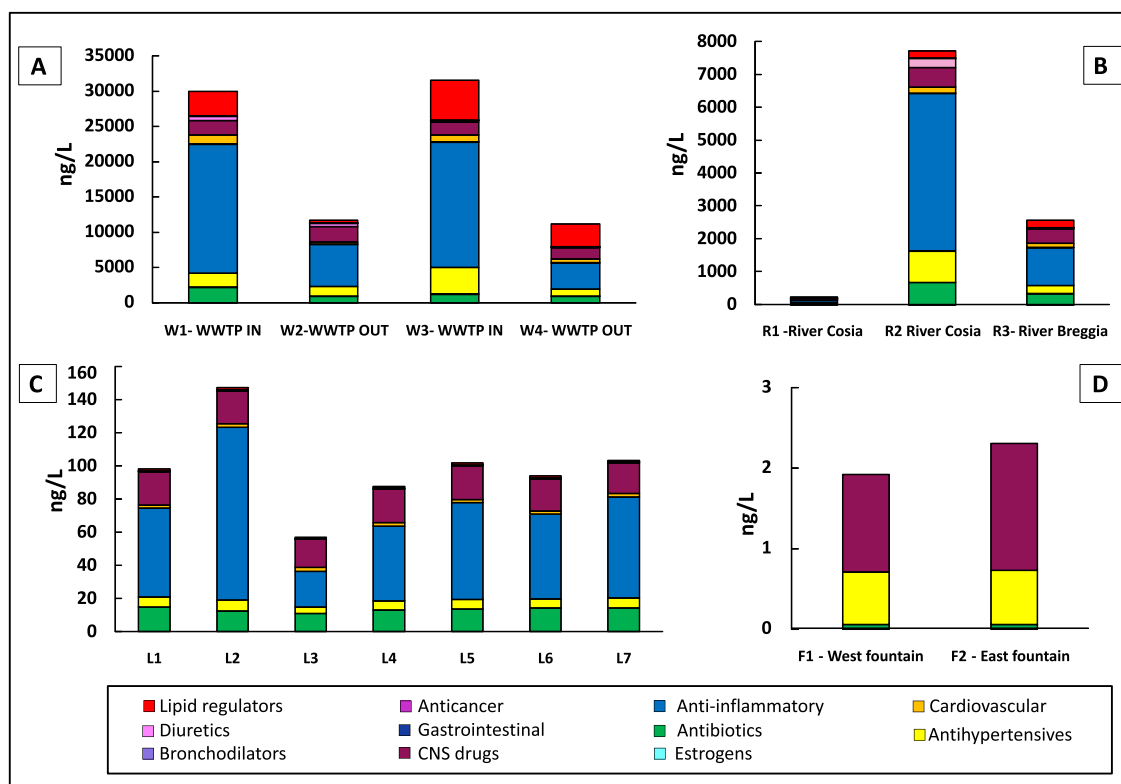


Fig. 2. Total pharmaceutical concentrations in water samples (ng/L): (a) WWTPs, (b) rivers, (c) lake and (d) drinking water. In chart 2c, the bar graphs have been sorted according to the order of the sampling points in the lake. For WWTPs and surface water the single most concentrated chemical was diclofenac, except for paracetamol in WWTP IN for both plants. For fountains the most concentrated chemical was dihydro-carbamazepine.

those reported in other studies for the same winter period in other European countries, also affected by high tourism loads (Mandarić et al., 2017). Similarly, the chemicals found at the highest concentration were diclofenac, carbamazepine and its metabolites, due to their recalcitrance to wastewater treatment (Leverett et al., 2021; Petrie et al., 2015).

Pharmaceutical concentrations in WWTPs (Como and Chiasso) are reported in Fig. 2a and Table SI-3. Both WWTPs registered a similar concentration, with sum of concentrations up to 29,978 ng/L at the influent wastewater and 11,712 ng/L at the effluents. Anti-inflammatories were the most abundant group of drugs (mainly paracetamol, ibuprofen, and diclofenac), followed by lipid regulators (mainly rosuvastatin), cardiovascular and antidepressants (mainly dihydro-carbamazepine), as also shown in other studies (Castiglioni et al., 2020; Loos et al., 2013).

As regards drinking water, antiepileptics-antidepressants were the most abundant class of pharmaceuticals in which dihydro carbamazepine was the only metabolites traced (Table SI-6 and Figs. SI-17 and -18), followed by antihypertensives and antibiotics. About the latter, only clarithromycin was found at F2. This chemical showed two orders of magnitude decrease in concentrations from summer (Castiglioni et al., 2020) to winter (0.06 ng/L); while in F1 it was found only during winter (0.06 ng/L) (Table SI-6 and Fig. SI-17). Lower results (<0.02 ng/L) were measured previously in Como potable water (Tröger et al., 2021), while higher values were measured in China (Ben et al., 2022). This situation clearly demonstrates how this chemical can resist to its elimination during the potabilization treatment: other studies show how clarithromycin can survive to the pre-ozonation step while it is completely removed with the ozonation (Borrull et al., 2021).

Concentrations in Lake Como water were studied taking samples at five points at three different depths together with two more samples: L6 and L7 were collected from land corresponding to epilimnion (surficial water) and hypolimnion, through a collection pipe, respectively.

Pharmaceutical concentrations in lake water (Figs. 2c and 3) decreased, on average, by 2 orders of magnitude in respect to rivers.

Overall, water samples collected at each point showed generally the same concentration values along the water column (Figs. SI-10 to -16), confirming the absence of stratification and a full mixed layer (Fig. 2c). However, L3 showed the lowest concentration in respect to the other lake sampling points. Anti-inflammatories drugs were the most abundant class of pharmaceuticals with the highest value in L2 (209 ng/L) and the lowest in L3 (14 ng/L). Within this category diclofenac was by far the prevalent drug with highest values in L2 (204 ng/L) and the lowest values in L3 (13 ng/L) (Table SI-5 and Fig. 3). Such abundance was often very important, since diclofenac concentrations were larger than the sum of all other chemicals in many lake water samples (Fig. 3), being the most representative chemical. However, when removing diclofenac from the overall average, lipids regulators became the most abundant group of pharmaceuticals followed by antibiotics (Fig. SI-18), situation also shown at European wide level for rivers (Loos et al., 2009). Anti-depressives were the second most representative class of drugs, with dihydro carbamazepine being the most abundant drugs. These results are in line with those reported by summer data by (Castiglioni et al., 2020) (for L6-L7) with slight differences for diclofenac which was 30 times higher in L7 (59 ng/L) and 6 times in L6 (49 ng/L) comparing to the concentration of summer 2018 (about 4 and 8 ng/L respectively) (Table SI-5 and Figs. SI-15 and -16). Overall, the pharmaceutical fingerprint found in Lake Como samples is in line with the contamination reported for surface water by several studies of the European area (Zhou et al., 2019). Indeed, anti-inflammatories drugs (i.e., ibuprofen and diclofenac), lipid regulators, (i.e., gemfibrozil and bezafibrate), CNS drugs (i.e., carbamazepine) and antibiotics (sulfamethoxazole, clarithromycin) were frequently reported in most of the European countries (Zhou et al., 2019).

Water lake circulation was investigated to understand the fate of pharmaceuticals once in the environment. Drug concentration decreased by 3 orders of magnitude from the WWTPs to the lake. The counterclockwise current (Fig. 3) moves first southward along the western shore of Como Bay taking the water coming from the northern

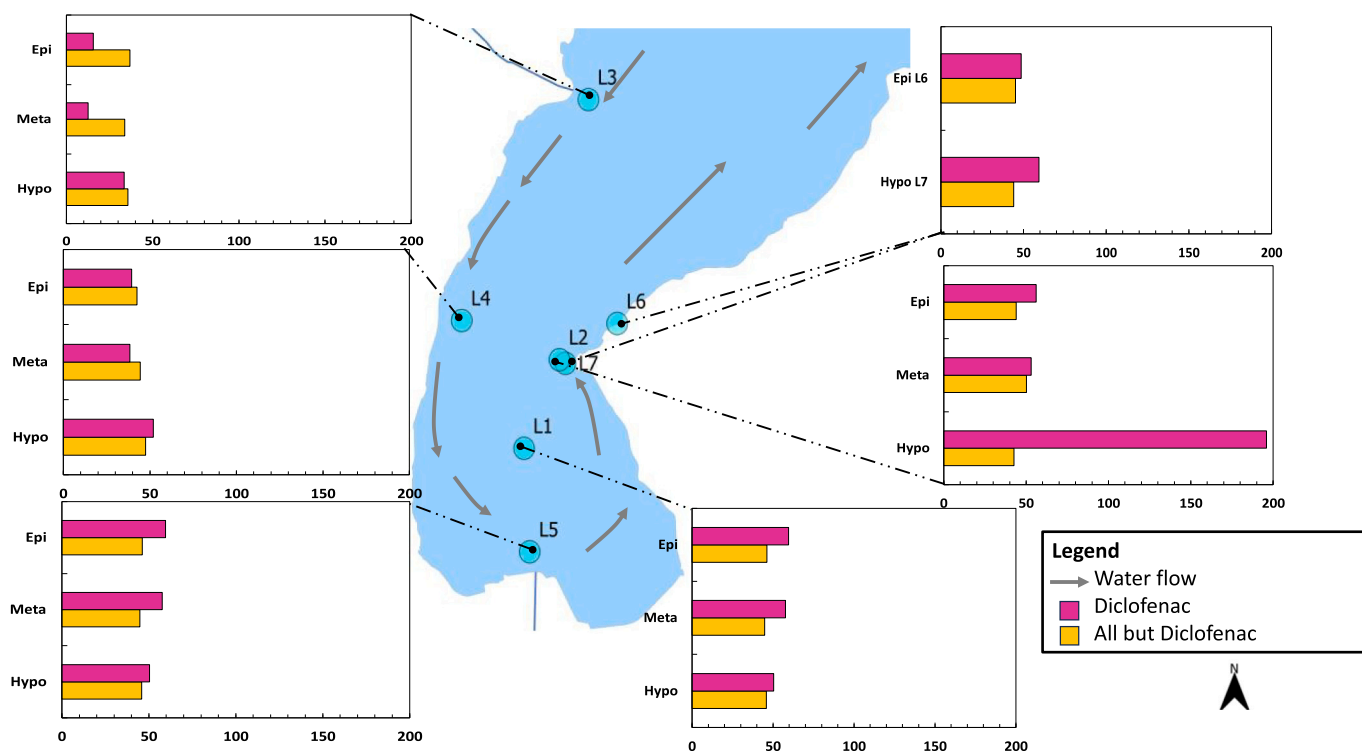


Fig. 3. Concentrations (ng/L) of diclofenac alone and of the sum of all the other pharmaceuticals (excluding diclofenac) in the lake (average of the three sampled depths for each point). Please note that points L6 and L7 are referred to land-based sampled epilimnion and hypolimnion (obtained by the pumping station) samples. Grey arrows indicate the water current direction.

part to enrich in chemical contamination from L3 to L4 (possibly receiving the contribution from River Breggia) and then to L5 (receiving the contribution of River Cosia) and then to L2, where some drugs reach a peak of concentration (i.e., diclofenac, hydrochlorothiazide). The lower concentrations measured in L3, seem to show instead that these waters can be considered as the incoming water fluxes from the north part of the lake.

### 3.3. Seasonal variation: Comparison of winter vs. summer concentrations

The values for winter 2022 are generally comparable to those reported in the same sampling area by (Castiglioni et al., 2020) during the summer period. However, when comparing the ratios of concentrations, it can be shown that they were generally higher in winter than in summer (by a factor of 2 to 3) for example in WWTPs (Fig. 4) and rivers (Fig. 5). Paracetamol was lower in winter by a factor of 3, similarly to ranitidine, probably due to a decrease in their use (not for Chiasso WWTP and River Cosia before the WWTP, point R1) or increased deconjugation in summer (Azuma et al., 2017) (Figs. 4 and 5). By contrast dihydro-carbamazepine, atenolol and rosuvastatin, were 2 up to 40 (for rosuvastatin) times higher respect to those found by Castiglioni et al. (2020). Antibiotics vary in input and output generally by a factor of 2–3, with tendentially higher values for the winter campaign, probably due to their wide use during this period of the year, the COVID 19 pandemic, and/or a reduced degradation (Bijlsma et al., 2021; O'Flynn et al., 2021). Clarithromycin, azithromycin (Como WWTP) and

sulfamethoxazole (Chiasso WWTP) were the most abundant antibiotics found in WWTP. These results are comparable to those of other countries (Petrie et al., 2015; Ramirez-Morales et al., 2020) nonetheless, variations of the compounds mostly present may depend on the origin of the incoming water, e.g., urban, veterinary, hospital wastewater, among others (Lucas et al., 2016). As expected, in the case of WWTPs (Fig. 4), the winter ratios are generally higher for the effluent compared to the influent, probably due to the reduced degradation during the winter period within the WWTP.

When looking at lake water samples, it is apparent that the summer samples present higher ratios, especially in the epilimnion: this can be explained by the summer stratification, which conveys most of the discharges by the rivers in the epilimnion, considerably thinner than the hypolimnion. However, more studies are required in summer stratification conditions to precisely account for this situation.

Regarding rivers, concentration ratios between winter and summer (Castiglioni et al., 2020) also varied up to two orders of magnitude (Fig. 5). This means that rivers typically receive higher concentrations in winter for most of the investigated chemicals. Being pharmaceutical tracers for other micropollutants poorly degraded or retained in WWTPs, it is expected that this behaviour could be generalized for many other contaminants discharged in WWTPs.

### 3.4. WWTP removal efficiency

Removal efficiencies are largely variable among studies due to many

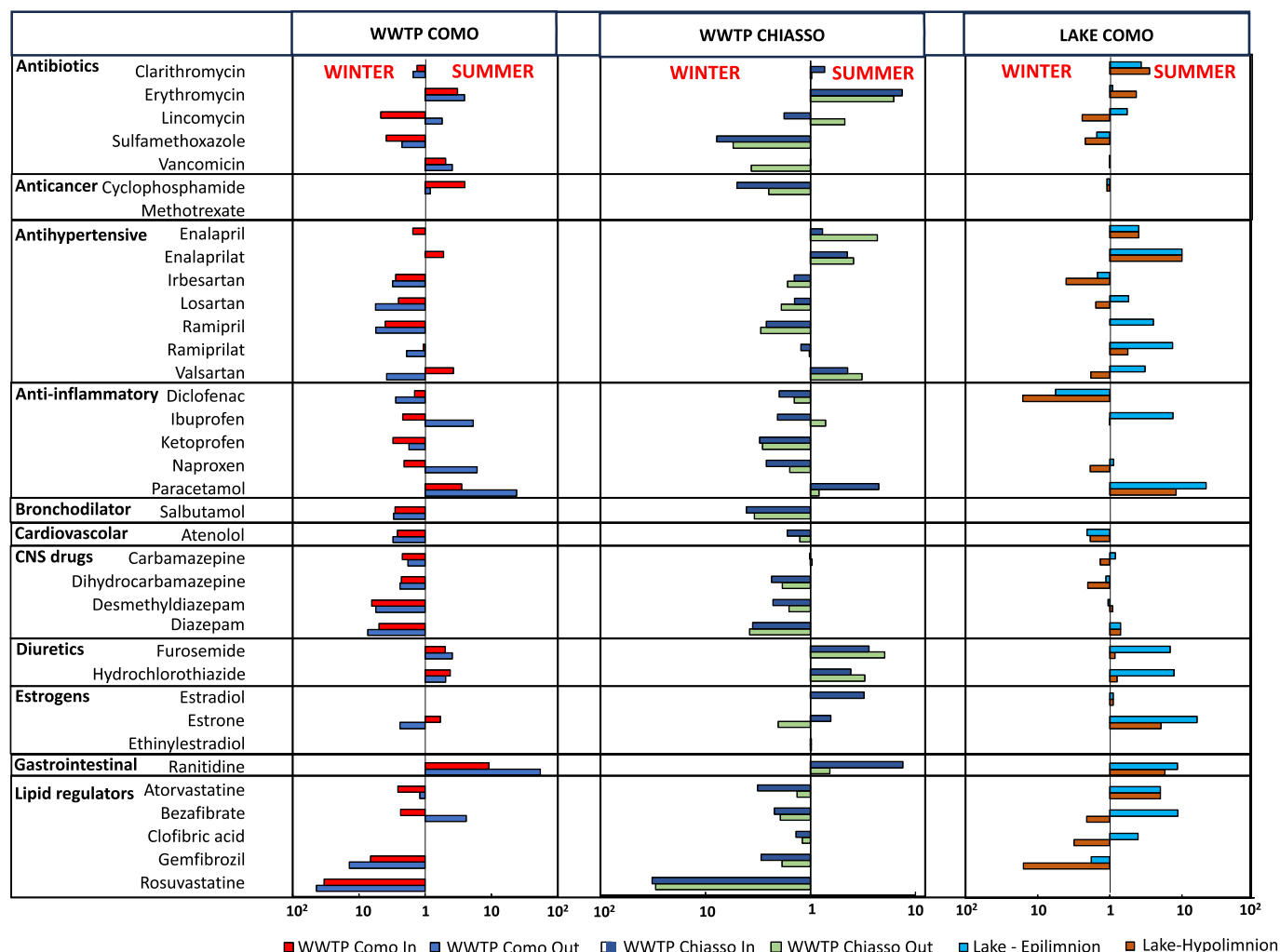


Fig. 4. Ratios of winter vs. summer pharmaceutical concentrations in WWTPs (in and out), Lake epilimnion and hypolimnion.

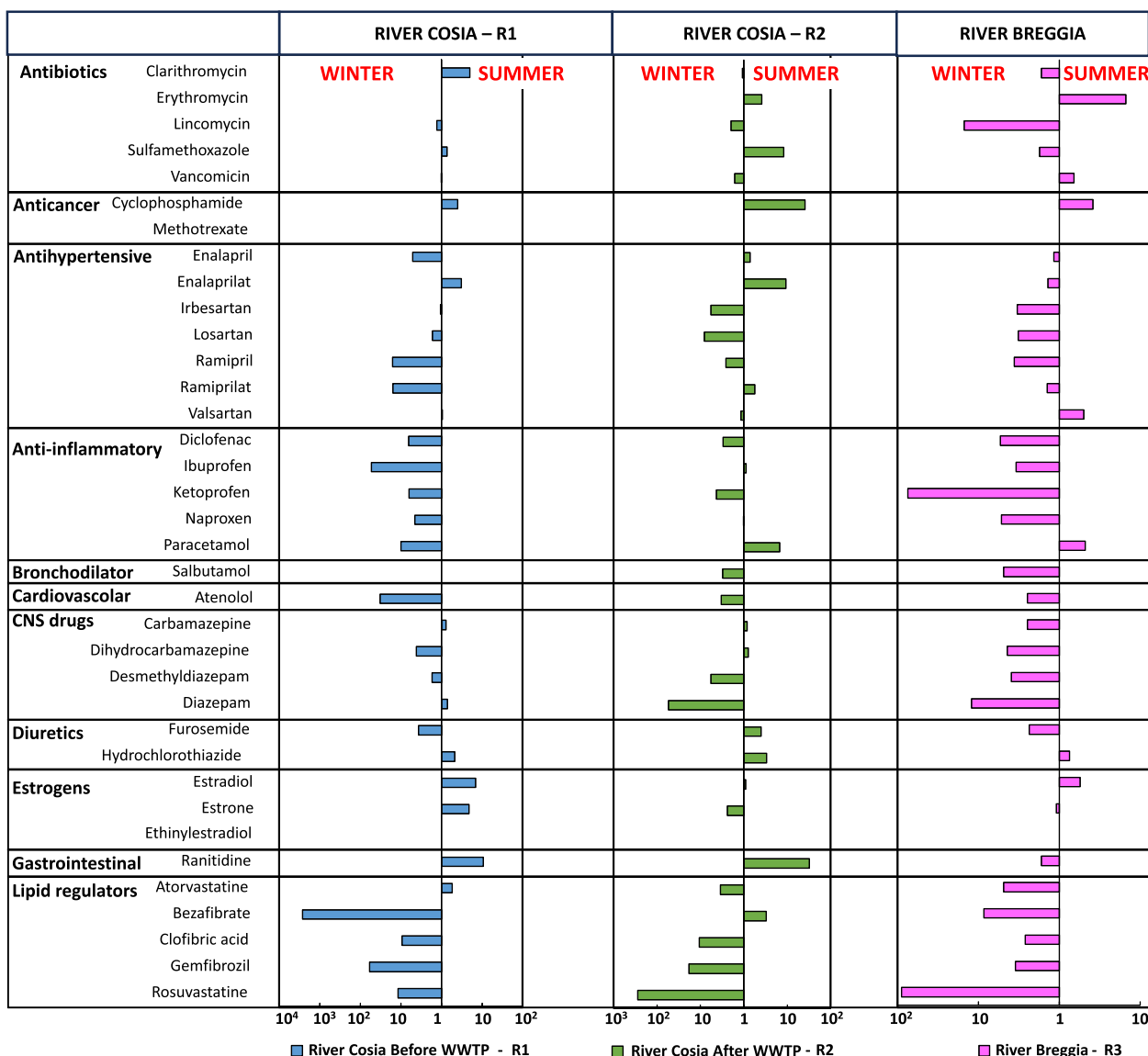


Fig. 5. Ratio of winter vs. summer pharmaceutical concentrations in River Cosia and Breggia.

factors (concentrations, characteristics of the plant, treatments involved etc.) (Besha et al., 2017; Luo et al., 2014; Radovic et al., 2023). However, evaluating the performance of WWTPs is extremely important to decrease the load of micropollutants in water bodies and pushing for implement strategies to reuse water (ie., agriculture reuse of effluents). The total loads of pharmaceuticals in and out of Como and Chiasso WWTPs were obtained sampling input water and output water, and the mean daily average flow rates in the sampling period. At the inlet it varies from tens of grams up to kilograms for some pharmaceuticals, such as diclofenac (54 kg at Como WWTP) and (10 kg at Chiasso WWTP), dihydro-carbamazepine at WWTP of Como (16 kg) and rosuvastatine at Chiasso WWTP (12 kg). The total load for Como Bay accounts for about 150 kg of pharmaceutical discharge considering both WWTPs, being two third of the total amount derived from Como WWTP (Table 1).

WWTPs removal efficiencies (Table SI-7) were obtained calculating the pharmaceuticals mass loads (kg/y) going in and out the WWTPs, multiplying the measured concentration (kg/L) by the influent and effluent wastewater flow rates (L/d) measured the day of the sampling and then multiplying by 365 to have the annual value. The mass loads led also to evaluate the possible different use of pharmaceuticals in

Table 1

Annual fluxes at WWTP inlet and outlet.

Como WWTP In (W1) (kg/y)	Como WWTP Out (W2) (kg/y)	Chiasso WWTP In (W3) (kg/y)	Chiasso WWTP Out (W4) (kg/y)
270.16	100.05	141.59	54.49

Como and Chiasso, with the knowledge of the population served by each WWTP (about 105,000 inhabitants for Como and 29,000 inhabitants for Chiasso (CDA, CD, 2022)). Most chemicals had the same order of magnitude load for both WWTPs. The remaining part had higher loads in Como WWTP (1 order of magnitude, not considering the chemicals which were <LOD or <LOQ in Chiasso) compared to Chiasso. The total removal efficiency for the chemicals measured in both WWTPs was slightly higher for Chiasso WWTP (62 %) compared to Como WWTP (53 %). Diclofenac showed increased concentrations at the outlet (2127 ng/L inlet and 5938 ng/L outlet) followed by carbamazepine, a notoriously poorly degradable molecule (Luo et al., 2014), and its metabolite dihydro-carbamazepine, which maintained the same concentrations at the output. The increase of concentration in effluent could reflect the seasonal changes in WWTPs removal efficiency due to temperature. The



winter total removal efficiency was 20 % lower for Chiasso WWTP and 30 % lower for Como WWTP if compared to the summer one (Castiglioni et al., 2020), although a clear evidence cannot be established due to the limited dataset. However, other authors (Castiglioni et al., 2006; Kosma

et al., 2014; Ma et al., 2013; Ulvi et al., 2022; Vieno et al., 2005) also found a decrease in removal efficiency during cold seasons, as a results of lower biodegradation kinetics during the winter period in respect to summer.

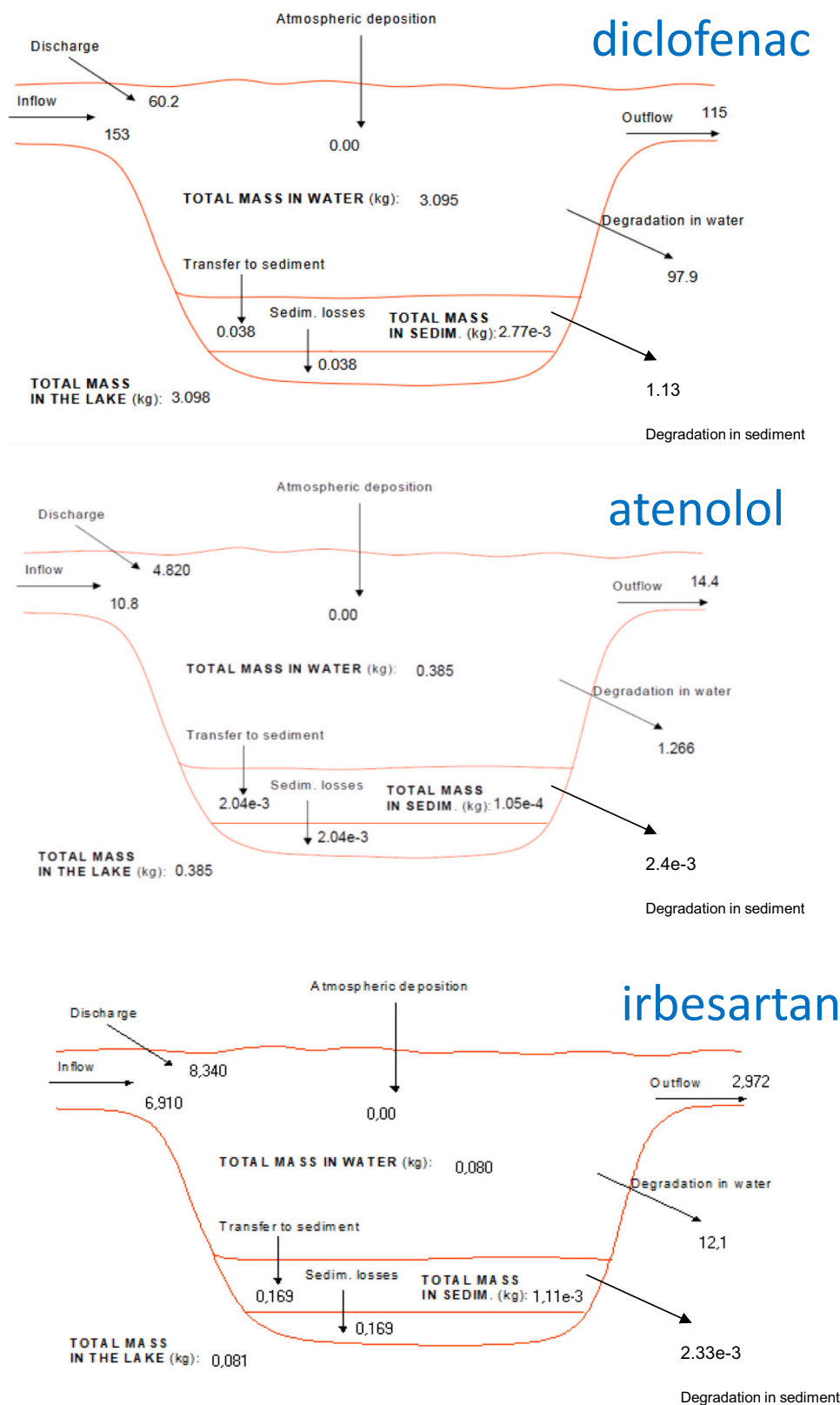


Fig. 6. Model mass balance for three selected chemicals (diclofenac, atenolol, and irbesartan) of variable Log Kow and half-life. All numbers indicate chemical fluxes (kg/y), except for total mass in the lake, water, and sediment (kg).

### 3.5. Modelling the fate of pharmaceuticals in Lake Como Bay

In a perspective of circular economy and water scarcity scenario, water reuse is of fundamental importance. The Como Bay of Lake Como is a particular case of water cycle contamination and reuse since lake water is contaminated by two main WWTPs, receives advective contaminated waters from the northern part of the lake, and it is withdrawn for drinking water purposes in correspondence of the point L2 (Castiglioni et al., 2020). One of the tools that can be employed to evaluate the environmental fate of micropollutants in the basin and estimate the importance of direct (WWTPs) discharges versus the advective flows from the lake, is a mass balance model. The Dyna model was therefore used in steady state mode, with the scenario depicted before, first to estimate the incoming (and outgoing) advective water flows, and later to evaluate the fate of selected, illustrative chemicals. Using atenolol as a chemical water flow marker resulted in water fluxes of  $7.45 \times 10^9 \text{ m}^3/\text{y}$  (considering the well mixed conditions of the lake). These fluxes are in the same order of magnitude as the flows resulting considering the average speeds reported by Laborde et al. (2010), although in stratified conditions. With the calculated advective water fluxes, the hydraulic residence time in Como Bay can be obtained being of about 10 days. Once were the advective water fluxes achieved, the model was also applied to other chemicals for which reliable physical chemical properties could be obtained (Table SI-9 in the Supporting Information). The mass balances (kg/y) of diclofenac, atenolol and irbesartan are illustrated in Fig. 6 and show that advective fluxes of chemicals (coming from northern part of the lake) are generally more than double of the direct discharges (for diclofenac and atenolol), while direct discharge from WWTPs prevail for irbesartan. For diclofenac and for irbesartan, the chemical mass balance is characterized by a reduced net flow out of the system: these chemicals are degraded in water to a large extent (46 % and 79 % respectively) due to their relatively short water half-lives, while atenolol, as expected from its physical chemical properties and half-lives, enters and leaves the water system with about the same chemical fluxes, with little contribution of degradation in water (about 10 %). Fig. SI-20 reports the mass balance graphs for other simulated chemicals, while Table SI-10 reports the calculated chemical residence times, generally comprised between <1 and 10 days. Also, for these chemicals the contribution of the direct discharges by Como and Chiasso WWTPs is generally small compared to the advective input from the entering waters (comprised between <1 and 39 % of the total input), with the exception of hydrochlorothiazide and bezafibrate, for which the direct discharge is 75 % and 57 % respectively. This means that concentrations of pharmaceuticals in Como Bay are mostly driven by the incoming lake water instead of the direct discharges. In other terms, a reduction of discharge concentrations in Como and Chiasso WWTPs would only produce minor reductions of concentrations (about 20–25 % on the average, even in zero discharge conditions). This confirms that the only way to significantly reduce concentrations in water and potential effects is to either reduce emissions (using less pharmaceuticals or implementing additional concentration reduction stages in existing WWTPs) at a watershed level or use chemicals with significantly shorter half-life, feature which is not always feasible since pharmaceuticals require a certain degree of stability to provide proper biological half-lives to ensure a pharmacological effect.

To verify the correctness of the steady state assumptions, in other terms confirming that the prediction of concentrations in water really depended on constant emission, calculated concentrations were plotted against the measured ones (Fig. 7). As shown in Fig. 7, the model was able to predict the concentrations within the Como basin with good accuracy, with values that mostly vary from the measured concentrations by a factor of 2.

Three pharmaceuticals showed larger discrepancies around the 1:1 line (indicating perfect fit between predicted and measured concentrations) (Fig. 7): irbesartan (a factor of 5), valsartan (a factor of 17), losartan (a factor of 4). This variability can be probably caused by

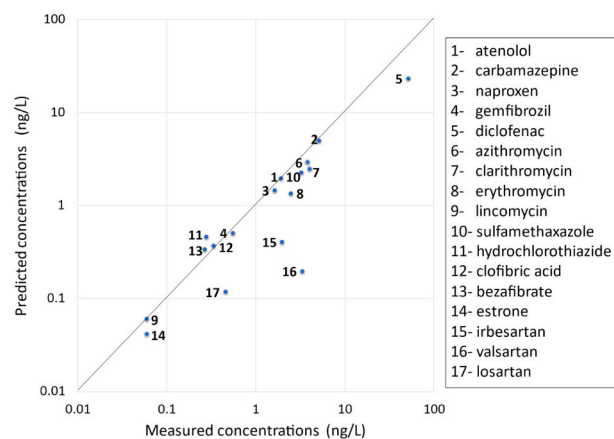


Fig. 7. Comparison of measured vs. Dyna model predicted concentrations of pharmaceuticals in Como Bay. The line represents the situation when measured concentrations are equal to predicted concentrations, indicating perfect fit (1:1). All data are reported on a Log-Log scale.

uncertainty in the physical chemical properties (Table SI-9 in the Supporting Information), such as  $K_{ow}$  (PubChem, 2023a, 2023b; Siddiqui et al., 2011) and model calculated organic carbon/water ( $K_{oc}$ ) and sediment/water ( $K_d$ ) partition coefficients. Since irbesartan, valsartan, and losartan have pKa between 4 and 5 (PubChem, 2023a, 2023b, 2023c), they will be dissociated at environmental pH and therefore the available (nominal)  $Kow$  (of the undissociated form) might not represent the partitioning properties of the dissociated form at environmental pHs. Diclofenac also showed a certain distance from the 1:1 line, although much smaller than the previous three chemicals, and this might be related to potential intermittent use in the past weeks or days (emissions predicted from measured discharge concentrations might be underestimated), circumstance also shown by the large variability of concentrations in the different points and depth (Fig. 3). In a modelling exercise, although of a river system in UK (Johnson et al., 2007), a relatively large variability in diclofenac concentrations was attributed on drug consumption variability in time. Similarly, (Nurmi et al., 2019) simulating diclofenac behaviour in a Finnish lake with another fugacity based model showed that several parameter could alter diclofenac concentrations in water, such as local water flow velocity, which may also depend, for Lake Como bay, from variation with space of lake bottom depth (Fig. SI-1 and Table SI-1 in Supporting Information). However, more studies are needed to better identify the source of such variability.

Model performance was calculated on the non-calibrated model, showing  $EF = 0.64$  when all the data were included. When removing the data of the four chemicals described above (diclofenac, irbesartan, valsartan, and losartan) from model efficiency calculation,  $EF$  increases to 0.84, a value which indicates a reasonably good agreement, as previously obtained in a calibration and validation exercise of the same model (Dyna Model) (Infantino et al., 2008). These results confirm the correctness of the simulation predictions for most of the chemicals while underlying those for which more studies are necessary to understand their behaviour.

### 3.6. Ecotoxicological evaluation of single chemicals and mixtures

The calculated RQs were <1 for most of the measured pharmaceuticals. However,  $RQ > 1$  was obtained for atorvastatin, ketoprofen, irbesartan and spiramycin in river Cosia (R2) while RQ for clarithromycin exceeded in both rivers Cosia and Breggia (R3) (Table SI-12).

Acute TU values were calculated for the total mixture of pharmaceuticals grouped by therapeutic class (Tables SI-15 to -18 and Figs. SI-21 and 22) for cyanobacteria, algae, daphnia, and fish. A threshold of

0.01 and 0.001 (Arenas-Sánchez et al., 2016) was set to identify the potential acute and chronic risk respectively. The results indicated that most of TU values were below these thresholds indicating toxicity is unlikely. However, in site R2 (Cosia river) and R3 (Breggia river), potential chronic risk are reported for daphnia and fish, due to the presence of anti-hypertensive drugs and lipid regulators. On the other hand, TU values for algae exceeded the acute and chronic thresholds, in the same sites. Also looking at lake samples (L1-L7), TUs for Algae were not too far from chronic toxicity levels, indicating potential chronic toxicity. Toxicity to algae was dominated by antibiotics followed by antihypertensive, lipids regulators and anti-inflammatories.

Regarding cyanobacteria all the three river samples exceed chronic thresholds while only R2 and R3 exceed acute threshold, being antibiotics the major contributors to cyanobacteria toxicity. By contrast toxicity was unlikely for Lipid regulators, anticancer and anti-inflammatories.

Pharmaceuticals are biologically active compounds by definition and, except for antibiotics, they are designed to have effects on mammals. Therefore, in general, they are not expected to have strong harmful effects on aquatic organisms. However, the long-term effects, as well as chronic sublethal effects, should not be neglected. A particular case is those of antibiotics that, besides a possible toxic effect to environmental bacteria, are recognized as chemicals of concern due to the development of antibiotic resistance (Brooks and Brooks, 2014). Results for the HRA are reported in Table SI-13, The HQs for individual chemicals, as well as the index for cumulative risk (HIs), were always far below 1. Therefore, no significant risks for consumers seem to be present, either for single pharmaceuticals or mixtures.

An additional possible assessment is the comparison of the measured concentrations of the nine Priority Chemical candidates with the proposed environmental quality standards (EQSs) (Table SI-19). In river samples, the MAC-EQS is exceeded by azithromycin and clarithromycin in R2, while the AA-EQS is exceeded by azithromycin, diclofenac and estradiol in R1, by diclofenac in R2. In lake samples, the MAC-EQS is never exceeded, while the AA-EQS is exceeded only by diclofenac in L1 (at depth of -25 and -48), L2 (at all sampled depth), L4 (at depth of -50), L5 (at all sampled depth), and L7.

#### 4. Conclusions

The presence of contaminants in the water cycle, including water use and reuse in Como Bay of Lake Como was additionally studied, after an initial research in 2020 (Castiglioni et al., 2020) which underlined the specific peculiar situation of the area: different source of pollution (domestic, industrial) managed by WWTPs located in EU (Italy) and in a non-EU country (Switzerland), lack of an effluent in this branch of the lake, lake water collected for potable use. The relevance is high since Lake Como, being a deep lake and one of the largest lakes in Northern Italy, is an important water reservoir, serving important ecosystem services (i.e., drinking water, recreational, industrial, irrigation uses), some of which are currently at risk giving the water scarcity and climate change scenarios. This situation is a good example for evaluating the needs and tools required to reduce water contamination within the future “zero pollution action plan”, in a relatively large and complex water reservoir. To reach this goal, many options are possible. Some are technological, such as the implementation of better removal techniques in WWTPs (such as additional stages), some are political and involve choices on chemical use.

The result of the present study allows to obtain information on seasonal variation of pharmaceutical concentrations, which includes different use of chemicals (some prevailing in winter, some in summer), the additional load of individuals (e.g., in summer) since the town of Como is increasingly visited by tourist, the decreasing chemical removal efficiency of WWTPs in winter. Although the environmental risk assessment at a single chemical level did not raise significant issues, when evaluating mixture toxicity some concerns were raised for river

water but also for lake water, especially for primary producers (algae). Moreover, some exceedances of the EQS have been observed for some of the proposed WFD Priority Chemicals, particularly for diclofenac. So, diclofenac, that shows the highest concentrations in almost all river and lake samples, also appears as the most concerning for regulatory purposes. The application of a fate model allowed to calculate the average water residence time in Como Bay in full mixed conditions, a very important parameter which could regulate the presence of chemicals in the bay. Additionally, the model showed that generally, the most influent chemical loading comes from the advective water from the north of the lake rather than from the direct WWTP discharges. The important point of this finding is that only an action at a drainage basin level could significantly reduce concentrations in water and therefore only a massive implementation of the EU zero pollution action plan could provide significant results in improving water ecosystem and human health protection.

More research is needed to better evaluate and confirm the role of changing scenario conditions: e.g., the influence of seasonal factors, both related to the physical scenario such as temperature, rainfall peaks, etc. as well as the social and political factors (presence of tourism, use of chemicals, etc.). This could be done by collecting more information and measuring concentration to build an unsteady state fate model which could relate many of these time changing parameters.

Also, it will be relevant to reconstruct the role of other sources (e.g., WWTPs) within the lake drainage basin and more importantly, the presence of additional chemicals (e.g., PFAS) and metabolites (also potentially deriving from WWTP upgrades) which should be considered in evaluating mixture toxicity to ecosystems.

#### CRediT authorship contribution statement

**Antonio Di Guardo:** Funding, Resources, Data curation, Writing - Reviewing and Editing, supervision.

**Sara Castiglioni:** Investigation, Data curation, Writing, Reviewing and Editing.

**Isabella Gambino:** Investigation, Writing - Original draft preparation, Writing- Reviewing and Editing.

**Alessia Sailis:** Investigation, Data curation.

**Giulia Salmoiraghi:** Investigation, Data curation.

**Silvia Schiarea:** Investigation, Data curation.

**Marco Vighi:** Investigation, Writing - Reviewing and Editing.

**Elisa Terzaghi:** Investigation, Writing - Reviewing and 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.

#### Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

A file with tables and figures reporting additional detail on sampling and concentrations and other data can be found at <https://doi.org/10.1016/j.scitotenv.2023.167594>.

## References

- Arenas-Sánchez, A., Rico, A., Vighi, M., 2016. Effects of water scarcity and chemical pollution in aquatic ecosystems: state of the art. *Sci. Total Environ.* 572, 390–403. <https://doi.org/10.1016/j.scitotenv.2016.07.211>.
- Autorità di Bacino, 2023. Autorità di Bacino del Lario e dei Laghi Minori [WWW document] (URL). <http://www.autoritabacino.lario.it/it> (accessed 8.2.23).
- Azevedo, L.S., Pestana, I.A., Rocha, A.R.M., Meneguelli-Souza, A.C., Lima, C.A.I., Almeida, M.G., Bastos, W.R., Souza, C.M.M., 2018. Drought promotes increases in total mercury and methylmercury concentrations in fish from the lower Paraíba do Sul river, southeastern Brazil. *Chemosphere* 202, 483–490. <https://doi.org/10.1016/j.chemosphere.2018.03.059>.
- Azuma, T., Ishida, M., Hisamatsu, K., Yunoki, A., Otomo, K., Kunitou, M., Shimizu, M., Hosomaru, K., Mikata, S., Mino, Y., 2017. A method for evaluating the pharmaceutical decontamination potential in river water environments. *Chemosphere* 180, 476–482. <https://doi.org/10.1016/j.chemosphere.2017.04.040>.
- Backhaus, T., Altenburger, R., Boedeker, W., Faust, M., Scholze, M., Grimme, L.H., 2000. Predictability of the toxicity of a multiple mixture of dissimilarly acting chemicals to *Vibrio fischeri*. *Environ. Toxicol. Chem.* 19, 2348–2356. <https://doi.org/10.1002/etc.5620190927>.
- Ben, Y., Hu, M., Zhong, F., Du, E., Li, Y., Zhang, H., Andrews, C.B., Zheng, C., 2022. Human daily dietary intakes of antibiotic residues: dominant sources and health risks. *Environ. Res.* 212, 113387. <https://doi.org/10.1016/j.envres.2022.113387>.
- Besha, A.T., Gebreyohannes, A.Y., Tufa, R.A., Bekele, D.N., Curcio, E., Giorno, L., 2017. Removal of emerging micropollutants by activated sludge process and membrane bioreactors and the effects of micropollutants on membrane fouling: a review. *J. Environ. Chem. Eng.* 5, 2395–2414. <https://doi.org/10.1016/j.jece.2017.04.027>.
- Bijlsma, L., Pitarich, E., Fonseca, E., Ibáñez, M., Botero, A.M., Claros, J., Pastor, L., Hernández, F., 2021. Investigation of pharmaceuticals in a conventional wastewater treatment plant: removal efficiency, seasonal variation and impact of a nearby hospital. *J. Environ. Chem. Eng.* 9, 105548. <https://doi.org/10.1016/j.jece.2021.105548>.
- Borrull, J., Colom, A., Fabregas, J., Borrull, F., Pocurrull, E., 2021. Presence, behaviour and removal of selected organic micropollutants through drinking water treatment. *Chemosphere* 276, 130023. <https://doi.org/10.1016/j.chemosphere.2021.130023>.
- Brooks, B.D., Brooks, A.E., 2014. Therapeutic strategies to combat antibiotic resistance. *Adv. Drug Deliv. Rev.* 78, 14–27. <https://doi.org/10.1016/j.addr.2014.10.027>.
- Castiglioni, S., Bagnati, R., Calamari, D., Fanelli, R., Zuccato, E., 2005. A multiresidue analytical method using solid-phase extraction and high-pressure liquid chromatography tandem mass spectrometry to measure pharmaceuticals of different therapeutic classes in urban wastewaters. *J. Chromatogr. A* 1092, 206–215. <https://doi.org/10.1016/j.chroma.2005.07.012>.
- Castiglioni, S., Bagnati, R., Fanelli, R., Pomati, F., Calamari, D., Zuccato, E., 2006. Removal of pharmaceuticals in sewage treatment plants in Italy. *Environ. Sci. Technol.* 40, 357–363. <https://doi.org/10.1021/es050991m>.
- Castiglioni, S., Davoli, E., Riva, F., Palmiotto, M., Camporini, P., Manenti, A., Zuccato, E., 2018. Mass balance of emerging contaminants in the water cycle of a highly urbanized and industrialized area of Italy. *Water Res.* 131, 287–298. <https://doi.org/10.1016/j.watres.2017.12.047>.
- Castiglioni, S., Zuccato, E., Fattore, E., Riva, F., Terzaghi, E., Koenig, R., Principi, P., Di Guardo, A., 2020. Micropollutants in Lake Como water in the context of circular economy: a snapshot of water cycle contamination in a changing pollution scenario. *J. Hazard. Mater.* 384, 121441. <https://doi.org/10.1016/j.jhazmat.2019.121441>.
- CDA, CD - Consorzio Depurazione Acque [WWW Document], 2022. URL <https://www.cdacd.ch/> (accessed 10.7.22).
- Copetti, D., Guyennon, N., Buzzi, F., 2020. Generation and dispersion of chemical and biological gradients in a large-deep multi-basin lake (Lake Como, north Italy): the joint effect of external drivers and internal wave motions. *Sci. Total Environ.* 749, 141587. <https://doi.org/10.1016/j.scitotenv.2020.141587>.
- Di Guardo, A., Ferrari, C., Infanton, A., 2006. Development of a dynamic aquatic model (DynA model): estimating temporal emissions of DDT to Lake Maggiore (N. Italy). *Environ. Sci. Pollut. Res. Int.* 13, 50–58. <https://doi.org/10.1065/espr2006.01.009>.
- EC, 2021. Zero pollution action plan [WWW document] (URL). [https://environment.ec.europa.eu/strategy/zero-pollution-action-plan\\_en](https://environment.ec.europa.eu/strategy/zero-pollution-action-plan_en) (accessed 8.3.23).
- Elskens, M., Van Langenhove, K., Carboneel, V., Brion, N., Eisenreich, S.J., 2023. Dynamics of estrogenic activity in an urban river receiving wastewater effluents: effect-based measurements with CALUX. *Water Emerg. Contam. Nanoplast.* 2, 9. <https://doi.org/10.20517/weecn.2023.15>.
- European Commission, 2003. Technical guidance document on risk assessment - environmental risk assessment (EUR 20418 EN No. PART II-Chapter 3). <https://publications.jrc.ec.europa.eu/repository/handle/JRC23785.JRC-EU>.
- European Commission, 2021a. Communication From the Commission To The European Parliament, The Council, The European Economic And Social Committee And The Committee Of The Regions Pathway to a Healthy Planet for All EU Action Plan: “Towards Zero Pollution for Air, Water and Soil.”.
- European Commission, 2021b. Annexes to the Communication From the Commission to the European Parliament, The Council, The European Economic And Social Committee And The Committee Of The Regions Pathway to a Healthy Planet for All EU Action Plan: “Towards Zero Pollution for Air, Water and Soil.”.
- European Commission, 2022. Proposal for a Directive Concerning Urban Wastewater Treatment (Recast). pdf. [https://environment.ec.europa.eu/publications/proposal-amending-water-directives\\_en](https://environment.ec.europa.eu/publications/proposal-amending-water-directives_en) (accessed October 4, 2023).
- European Environment Agency, 2021. Water Resources across Europe: Confronting Water Stress: An Updated Assessment. Publications Office, LU.
- European Medicines Agency, 2018. Draft Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use- Revision 1 (accessed July 31, 2019). <https://www.ema.europa.eu/en/environmental-risk-assessment-medicina/products-human-use>.
- Gunnarsson, L., Snape, J.R., Verbruggen, B., Owen, S.F., Kristiansson, E., Margiotta-Casaluci, L., Österlund, T., Hutchinson, K., Leverett, D., Marks, B., Tyler, C.R., 2019. Pharmacology beyond the patient – the environmental risks of human drugs. *Environ. Int.* 129, 320–332. <https://doi.org/10.1016/j.envint.2019.04.075>.
- Guyennon, N., Valerio, G., Salerno, F., Pilotti, M., Tartari, G., Copetti, D., 2014. Internal wave weather heterogeneity in a deep multi-basin subalpine lake resulting from wavelet transform and numerical analysis. *Adv. Water Resour.* 71, 149–161. <https://doi.org/10.1016/j.advwatres.2014.06.013>.
- Infanton, A., Pereira, T., Ferrari, C., Cerejeira, M.J., Di Guardo, A., 2008. Calibration and validation of a dynamic water model in agricultural scenarios. *Chemosphere* 70, 1298–1308. <https://doi.org/10.1016/j.chemosphere.2007.07.047>.
- Johnson, A.C., Keller, V., Williams, R.J., Young, A., 2007. A practical demonstration in modelling diclofenac and propranolol river water concentrations using a GIS hydrology model in a rural UK catchment. *Environ. Pollut.* 146, 155–165. <https://doi.org/10.1016/j.envpol.2006.05.037>.
- Kallenborn, R., Halsall, C., Dellong, M., Carlsson, P., 2012. The influence of climate change on the global distribution and fate processes of anthropogenic persistent organic pollutants. *J. Environ. Monit.* 14, 2854. <https://doi.org/10.1039/c2em30519d>.
- Klatte, S., Schaefer, H.-C., Hempel, M., 2017. Pharmaceuticals in the environment – a short review on options to minimize the exposure of humans, animals and ecosystems. *Sustain. Chem. Pharm.* 5, 61–66. <https://doi.org/10.1016/j.scp.2016.07.001>.
- Keywegt, S., Pileggi, V., Yang, P., Hao, C., Zhao, X., Rocks, C., Thach, S., Cheung, P., Whitehead, B., 2011. Pharmaceuticals, hormones and bisphenol A in untreated source and finished drinking water in Ontario, Canada — occurrence and treatment efficiency. *Sci. Total Environ.* 409, 1481–1488. <https://doi.org/10.1016/j.scitotenv.2011.01.010>.
- K'oreje, K.O., Vergeynst, L., Ombaka, D., De Wispelaere, P., Okoth, M., Van Langenhove, H., Demeestere, K., 2016. Occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater of Nairobi and Kisumu city, Kenya. *Chemosphere* 149, 238–244. <https://doi.org/10.1016/j.chemosphere.2016.01.095>.
- Kosma, C.I., Lambropoulou, D.A., Albanis, T.A., 2014. Investigation of PPCPs in wastewater treatment plants in Greece: occurrence, removal and environmental risk assessment. *Sci. Total Environ.* 466–467, 421–438. <https://doi.org/10.1016/j.scitotenv.2013.07.044>.
- Kümmerer, K., 2010. Pharmaceuticals in the Environment. *Annu. Rev. Env. Resour.* 35, 57–75. <https://doi.org/10.1146/annurev-environ-052809-161223>.
- Laborde, S., Antenucci, J.P., Copetti, D., Imberger, J., 2010. Inflow intrusions at multiple scales in a large temperate lake. *Limnol. Oceanogr.* 55, 1301–1312. <https://doi.org/10.4319/lo.2010.55.3.1301>.
- Laborde, S., Imberger, J., Toussaint, S., 2012. Contributions of local knowledge to the physical limnology of Lake Como, Italy. *Proc. Natl. Acad. Sci. U. S. A.* 109, 6441–6445. <https://doi.org/10.1073/pnas.1113740109>.
- Leverett, D., Merrington, G., Crane, M., Ryan, J., Wilson, I., 2021. Environmental quality standards for diclofenac derived under the European water framework directive: 1. Aquatic organisms. *Environ. Sci. Eur.* 33, 133. <https://doi.org/10.1186/s12302-021-00574-z>.
- Lonappan, L., Brar, S.K., Das, R.K., Verma, M., Surampalli, R.Y., 2016. Diclofenac and its transformation products: environmental occurrence and toxicity - a review. *Environ. Int.* 96, 127–138. <https://doi.org/10.1016/j.envint.2016.09.014>.
- Loos, R., Gawlik, B.M., Locoro, G., Rimaviciute, E., Contini, S., Bidoglio, G., 2009. EU-wide survey of polar organic persistent pollutants in European river waters. *Environ. Pollut.* 157, 561–568. <https://doi.org/10.1016/j.envpol.2008.09.020>.
- Loos, R., Carvalho, R., António, D.C., Comero, S., Locoro, G., Tavazzi, S., Paracchini, B., Ghiani, M., Lettieri, T., Blaha, L., Jarosova, B., Voorspoels, S., Servaes, K., Haglund, P., Fick, J., Lindberg, R.H., Schwesig, D., Gawlik, B.M., 2013. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. *Water Res.* 47, 6475–6487. <https://doi.org/10.1016/j.watres.2013.08.024>.
- Lucas, D., Barceló, D., Rodríguez-Mozaz, S., 2016. Removal of pharmaceuticals from wastewater by fungal treatment and reduction of hazard quotients. *Sci. Total Environ.* 571, 909–915. <https://doi.org/10.1016/j.scitotenv.2016.07.074>.
- Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S., Wang, X.C., 2014. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Sci. Total Environ.* 473–474, 619–641. <https://doi.org/10.1016/j.scitotenv.2013.12.065>.



- Ma, C., Yu, S., Shi, W., Heijman, S.G.J., Rietveld, L.C., 2013. Effect of different temperatures on performance and membrane fouling in high concentration PAC-MBR system treating micro-polluted surface water. *Bioresour. Technol.* 141, 19–24. <https://doi.org/10.1016/j.biortech.2013.02.025>.
- Mackay, D., Joy, M., Paterson, S., 1983. A quantitative water, air, sediment interaction (QWASI) fugacity model for describing the fate of chemicals in lakes. *Chemosphere* 12, 981–997. [https://doi.org/10.1016/0045-6535\(83\)90251-5](https://doi.org/10.1016/0045-6535(83)90251-5).
- Mandarić, L., Diamantini, E., Stella, E., Cano-Paoli, K., Valle-Sistac, J., Molins-Delgado, D., Bellin, A., Chiogna, G., Majone, B., Diaz-Cruz, M.S., Sabater, S., Barcelo, D., Petrovic, M., 2017. Contamination sources and distribution patterns of pharmaceuticals and personal care products in Alpine rivers strongly affected by tourism. *Sci. Total Environ.* 590–591, 484–494. <https://doi.org/10.1016/j.scitotenv.2017.02.185>.
- Mayer, D.G., Butler, D.G., 1993. Statistical validation. *Ecol. Model.* 68, 21–32. [https://doi.org/10.1016/0304-3800\(93\)90105-2](https://doi.org/10.1016/0304-3800(93)90105-2).
- Morillo, S., Imberger, J., Antenucci, J.P., Copetti, D., 2009. Using impellers to distribute local nutrient loadings in a stratified Lake: Lake Como, Italy. *J. Hydraul. Eng.* 135, 564–574. [https://doi.org/10.1061/\(ASCE\)HY.1943-7900.0000048](https://doi.org/10.1061/(ASCE)HY.1943-7900.0000048).
- Musolf, A., 2009. Micropollutants: challenges in hydrogeology. *Hydrogeol. J.* 17, 763–766. <https://doi.org/10.1007/s10040-009-0438-y>.
- Nurmi, T.M.A., Kiljunen, T.K., Knuutinen, J.S., 2019. A fugacity model assessment of ibuprofen, diclofenac, carbamazepine, and their transformation product concentrations in an aquatic environment. *Environ. Sci. Pollut. Res.* 26, 328–341. <https://doi.org/10.1007/s11356-018-3485-x>.
- O'Flynn, D., Lawler, J., Yusuf, A., Parle-McDermott, A., Harold, D., Mc Cloughlin, T., Holland, L., Regan, F., White, B., 2021. A review of pharmaceutical occurrence and pathways in the aquatic environment in the context of a changing climate and the COVID-19 pandemic. *Anal. Methods* 13, 575–594. <https://doi.org/10.1039/D0AY02098B>.
- Petrie, B., Barden, R., Kasprzyk-Hordern, B., 2015. A review on emerging contaminants in wastewaters and the environment: current knowledge, understudied areas and recommendations for future monitoring. *Water Res.* 72, 3–27. <https://doi.org/10.1016/j.watres.2014.08.053>.
- PubChem, 2023a. Irbesartan [WWW document]. URL: <https://pubchem.ncbi.nlm.nih.gov/compound/3749> (accessed 9.24.23).
- PubChem, 2023b. Losartan [WWW document]. URL: <https://pubchem.ncbi.nlm.nih.gov/compound/3961> (accessed 9.24.23).
- PubChem, 2023c. Valsartan [WWW document]. URL: <https://pubchem.ncbi.nlm.nih.gov/compound/60846> (accessed 9.24.23).
- QGIS, 2023. Welcome to the QGIS project! [WWW document]. QGIS a free and open source geographic information system. URL: <https://qgis.org/en/site/> (accessed 8.2.23).
- Radović, S., Pap, S., Niemi, L., Prodanović, J., Turk Sekulic, M., 2023. A review on sustainable technologies for pharmaceutical elimination in wastewaters — a ubiquitous problem of modern society. *J. Mol. Liq.* 383, 122121 <https://doi.org/10.1016/j.molliq.2023.122121>.
- Ramírez-Morales, D., Masis-Mora, M., Montiel-Mora, J.R., Cambronero-Heinrichs, J.C., Briceño-Guevara, S., Rojas-Sánchez, C.E., Méndez-Rivera, M., Arias-Mora, V., Tormo-Budowski, R., Brenes-Alfaro, L., Rodríguez-Rodríguez, C.E., 2020. Occurrence of pharmaceuticals, hazard assessment and ecotoxicological evaluation of wastewater treatment plants in Costa Rica. *Sci. Total Environ.* 746, 141200 <https://doi.org/10.1016/j.scitotenv.2020.141200>.
- Riva, F., Castiglioni, S., Fattore, E., Manenti, A., Davoli, E., Zuccato, E., 2018. Monitoring emerging contaminants in the drinking water of Milan and assessment of the human risk. *Int. J. Hyg. Environ. Health* 221, 451–457. <https://doi.org/10.1016/j.ijheh.2018.01.008>.
- Riva, F., Castiglioni, S., Pacciani, C., Zuccato, E., 2020. Testing urban wastewater to assess compliance with prescription data through wastewater-based epidemiology: first case study in Italy. *Sci. Total Environ.* 739, 139741 <https://doi.org/10.1016/j.scitotenv.2020.139741>.
- Salmaso, N., Mosello, R., 2010. Limnological research in the deep southern subalpine lakes: synthesis, directions and perspectives. *Adv. Oceanogr. Limnol.* 1, 29–66. <https://doi.org/10.1080/19475721003735773>.
- Shraim, A., Diab, A., Alsuhaibi, A., Niaz, E., Metwally, M., Amad, M., Sioud, S., Dawoud, A., 2017. Analysis of some pharmaceuticals in municipal wastewater of Almadinah Almunawarah. *Arab. J. Chem.* 10, S719–S729. <https://doi.org/10.1016/j.arabj.2012.11.014>.
- Siddiqui, N., Husain, A., Chaudhry, L., Alam, M.S., Mitra, M., Bhasin, P.S., 2011. Pharmacological and pharmaceutical profile of valsartan: a review. *J. Appl. Pharm. Sci.* 12–19.
- Snyder, E.M., Bruce, G.M., Pleus, R.C., Hemming, J.D.C., 2008. Toxicological Relevance of EDCs and Pharmaceuticals in Drinking Water. AWWA Research Foundation, Denver, CO (accessed October 4, 2023). <https://www.waterrf.org/research/projects/toxicological-relevance-edcs-and-pharmaceuticals-drinking-water>.
- Tröger, R., Ren, H., Yin, D., Postigo, C., Nguyen, P.D., Baduel, C., Golovko, O., Been, F., Joers, H., Boleda, M.R., Polesello, S., Roncoroni, M., Taniyasu, S., Menger, F., Ahrens, L., Yin Lai, F., Wiberg, K., 2021. What's in the water? – Target and suspect screening of contaminants of emerging concern in raw water and drinking water from Europe and Asia. *Water Res.* 198, 117099 <https://doi.org/10.1016/j.watres.2021.117099>.
- Ulvi, A., Aydin, S., Aydin, M.E., 2022. Fate of selected pharmaceuticals in hospital and municipal wastewater effluent: occurrence, removal, and environmental risk assessment. *Environ. Sci. Pollut. Res.* 29, 75609–75625. <https://doi.org/10.1007/s11356-022-21131-y>.
- US EPA, 2023a. Contaminants of emerging concern including pharmaceuticals and personal care products [WWW document]. URL: <https://www.epa.gov/wqc/contaminants-emerging-concern-including-pharmaceuticals-and-personal-care-products>.
- US EPA, 2023b. ECOTOX Knowledgebase [WWW Document]. US EPA United States Environmental Protection Agency (URL). <https://cfpub.epa.gov/ecotox/>.
- USEPA/NCEA, 2000. Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures (No. EPA/630/R-00/002). U.S. Environmental Protection Agency Washington.
- Vieno, N.M., Tuhkanen, T., Kronberg, L., 2005. Seasonal variation in the occurrence of pharmaceuticals in effluents from a sewage treatment plant and in the recipient water. *Environ. Sci. Technol.* 39, 8220–8226. <https://doi.org/10.1021/es051124k>.
- Wilbur, S.B., Hansen, H., Pohl, H., Colman, J., McClure, P., 2004. Using the ATSDR Guidance Manual for the Assessment of Joint Toxic Action of Chemical Mixtures. Environmental Toxicology and Pharmacology, Risk Assessment of Chemical Mixtures, ICCM-2002 Symposium, 18, pp. 223–230. <https://doi.org/10.1016/j.etap.2003.03.001>.
- Wilkinson, J.L., Boxall, A.B.A., Kolpin, D.W., Leung, K.M.Y., Lai, R.W.S., Galbán-Malagón, C., Adell, A.D., Mondon, J., Metian, M., Marchant, R.A., Bouzas-Monroy, A., Cuni-Sanchez, A., Coors, A., Carriquiriborde, P., Rojo, M., Gordon, C., Cara, M., Moermond, M., Luarte, T., Petrosyan, V., Perikhanian, Y., Mahon, C.S., McGurk, C.J., Hofmann, T., Kormoker, T., Iniguez, V., Guzman-Otazo, J., Tavares, J. L., Gildasio De Figueiredo, F., Razzolini, M.T.P., Dougnon, V., Gbaguidi, G., Traoré, O., Blais, J.M., Kimpe, L.E., Wong, M., Wong, D., Ntchantcho, R., Pizarro, J., Ying, G.-G., Chen, C.-E., Páez, M., Martínez-Lara, J., Otamonga, J.-P., Poté, J., Ifo, S. A., Wilson, P., Echeverría-Sáenz, S., Udikovic-Kolic, N., Milakovic, M., Fatta-Kassinos, D., Ioannou-Tfofa, L., Belušová, V., Vymazal, J., Cárdenas-Bustamante, M., Kassa, B.A., Garric, J., Chaumot, A., Gibbs, P., Kunchulia, I., Seidensticker, S., Lyberatos, G., Halldórsson, H.P., Melling, M., Shashidhar, T., Lamba, M., Nastiti, A., Supriatin, A., Pourang, N., Abedini, A., Abdullah, O., Gharbia, S.S., Pilla, F., Chefetz, B., Topaz, T., Yao, K.M., Aubakirova, B., Beisenova, R., Olaka, L., Mulu, J. K., Chatanga, P., Ntuli, V., Blama, N.T., Sherif, S., Aris, A.Z., Looi, L.J., Niang, M., Traore, S.T., Oldenkamp, R., Ogunbanwo, O., Ashfaq, M., Iqbal, M., Abdeen, Z., O'Dea, A., Morales-Saldaña, J.M., Custodio, M., de la Cruz, H., Navarrete, I., Carvalho, F., Gogra, A.B., Koroma, B.M., Cerkvenik-Flajs, V., Gombač, M., Thwala, M., Choi, K., Kang, H., Ladu, J.L.C., Rico, A., Amerasinghe, P., Sobek, A., Horlitz, G., Zenker, A.K., King, A.C., Jiang, J.-J., Kariuki, R., Tumbo, M., Tezel, U., Onay, T.T., Lejju, J.B., Vystavna, Y., Vergeles, Y., Heinzen, H., Pérez-Parada, A., Sims, D.B., Figy, M., Good, D., Teta, C., 2022. Pharmaceutical pollution of the world's rivers. *Proc. Natl. Acad. Sci. U. S. A.* 119, e2113947119 <https://doi.org/10.1073/pnas.2113947119>.
- World Health Organization, 2011. Guidelines for Drinking-Water Quality, 4th ed. World Health Organization, Geneva.
- Zhou, S., Di Paolo, C., Wu, X., Shao, Y., Seiler, T.-B., Hollert, H., 2019. Optimization of screening-level risk assessment and priority selection of emerging pollutants – the case of pharmaceuticals in European surface waters. *Environ. Int.* 128, 1–10. <https://doi.org/10.1016/j.envint.2019.04.034>.