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1 Ecotoxicological risk assessment of micropollutants from treated urban wastewater effluents for
2 watercourses at a territorial scale: application and comparison of two approaches.

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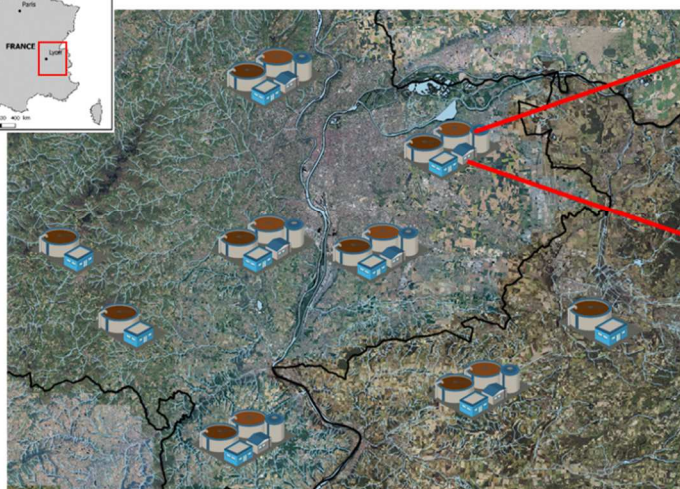
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10 Abstract

11 In most cases, urban Wastewater Treatment Plants (WWTP) only partially abate pollutants occurring
12 in the influent. Treated effluents can thus contain a complex mixture of ecotoxic pollutants, such as
13 heavy metals, detergents, disinfectants, plasticizers, pharmaceuticals residues or pesticides. In this
14 context, Ecotoxicological Risk Assessment (ERA) provide essential decision-making tools to public
15 authorities for establishing environmental policies and conducting territorial planning. The present
16 work aims to develop a territorial-scale ERA methodology using two complementary approaches
17 based on a Risk Quotient (RQ) calculation: (1) the first, based on the risk linked to each individual
18 pollutant (single substances ERA); (2) the second, considering all pollutants present, and the “cocktail
19 effect” (mixture ERA). This research was performed at 33 urban WWTPs of in a highly urbanized part
20 of France (Lyon area). Initial minimum, median and maximum pollutant concentrations in treated
21 effluents were obtained from a literature review of physico-chemical analysis studies, to reconstitute
22 “typical” effluents. The classical approach (single substances ERA) identified the riskiest substances
23 (e.g. endocrine disruptors, as the Estrone with RQ up to 593.75), and showed the risks for each
24 WWTP. The mixture ERA approach revealed new risks, which were not highlighted in the classical
25 ERA approach, thus increasing the number of WWTPs identified as at risk. This study shows the
26 importance of accounting for the cocktail effect, which is not considered in current regulatory
27 decisions. Finally, this methodology allowed us to identify the riskiest situations (often medium sized
28 WWTPs, releasing into small streams), that could worsen in the context of climate change.



Territory-scale approach



Ecotoxicological risk assessment

Substance approach: risks

$$RQ_x = \frac{PEC_x}{PNEC_x} \quad RQ_y = \frac{PEC_y}{PNEC_y} \quad RQ_z = \frac{PEC_z}{PNEC_z}$$

Mixture approach: cumulative risks

$$RQ_{\text{Mix}} = \sum_{x=1}^n \frac{PEC_x}{PNEC_x}$$

Graphical abstract

Keywords

Urban Wastewater
Micropollutants
Pharmaceuticals
Ecotoxicological Risk Assessment
Mixture Risk

Highlights

- Micropollutants are often **not effectively removed and so** released by the urban WWTPs.
- A territorial approach is essential to evaluate the risks linked to WWTP effluents.
- Ecotoxicological Risk Assessment (ERA) for the WWTPs of the Grand Lyon territory was carried out.
- Two different ERA approaches were used for WWTP risk assessment.
- Mixture ERA revealed hidden risks compared to the classic single substance approach.

1. Introduction

In recent decades, many studies have detected and reviewed the presence of (in-)organic (micro-)pollutants in surface water (Luo et al., 2014; Pal et al., 2010). This pollution stems from intense urban and industrial activities leading to the release of liquid effluents. Among the various sources of contamination, the discharge of treated urban wastewater appears to be a major source of potentially ecotoxic pollutants including pharmaceuticals, personal care products, surfactants, plasticizers, heavy metals and pesticides (Deblonde et al., 2011; Loos et al., 2013; Luo et al., 2014; Pal et al., 2010; Teijon et al., 2010). These results can be explained by the frequent low efficiency of urban wastewater treatment plants (WWTP) in micropollutant abatement (Falas et al., 2016; Grand-Clément et al., 2017) and occasionally, the creation of (more) ecotoxic by-products (Bertanza et al., 2013; Rosal et al., 2009; Stalter et al., 2010). The significant toxic potential linked to each of these pollutants has already been evaluated (Orias and Perrodin, 2014; Gosset et al., 2017).

However, the chemical characterization of treated WWTP effluents is not currently sufficient, as these data do not account for the exposure of aquatic organisms. To understand the impact on these organisms, performing Ecotoxicological Risk Assessment (ERA) on WWTP effluents is crucial. These traditionally compare the measured presence of each pollutant within the receiving ecosystems through Predicted Environmental Concentration (PEC) or Measured Environmental Concentration (MEC) values, and their potential effects on the aquatic ecosystems through Predicted No Effect Concentration (PNEC) values (Perrodin et al., 2011). To this end, many investigations have been performed on urban WWTP effluents and receiving watercourses (Kosma et al., 2014; Yan et al., 2014; Perrodin et al., 2013; Pereira et al., 2015; Singh et al., 2014).

But, in many cases, ERA are performed on isolated WWTPs, and ecotoxicological risks at the territorial scale are not taken into account. Loiseau et al. (2012) indicated that finding methods for environmental assessment of a territory are essential, because they provide useful decision support tools and information to help public authorities in designing policies for territorial planning. This is

79 why spatialized environmental assessment methods have been developed for some years (Carafa et al.,
80 2011; Loiseau et al., 2012; [Pereira et al., 2015](#); Stelzenmüller et al., 2015; Vulliet and Cren-Olivé,
81 2011; Yang et al., 2010). One critical development in territorial ERA has been identified: assessing the
82 risk linked to multiple WWTPs for the receiving aquatic watercourses in a territory. An initial study
83 was carried out by Brus and Perrodin (2017). They developed an ERA methodology to evaluate the
84 ecotoxicological risks of 18 WWTPs, scattered over a territory, on receiving watercourses for
85 micropollutants (pharmaceuticals, plasticizers, pesticides, ...) not often examined, at realistic
86 concentrations reviewed from scientific literature. They uncovered a high risk for many of them.

87 One other limitation was highlighted from their methodology: the non-consideration of
88 “cocktail effect” in the regulatory-based risk assessment procedure based on European Technical
89 Guidance Document (TGD, 2003) that they employed. [It has been now widely demonstrated that](#)
90 [aquatic organisms exposed to a mixture of pollutants at low doses and individually not supposed to](#)
91 [induce negative impacts \(concentration under the No Observed Effect Concentration \(NOEC\)\), can](#)
92 [lead significantly intoxicated \(Beyer et al., 2014; Kortenkamp, 2007\). This joint toxic effects have](#)
93 [been for example observed with organism’s exposure to endocrine disruptors, pharmaceuticals,](#)
94 [pesticides, or surfactants \(Beyer et al., 2014; Kortenkamp, 2007; Panouillères et al., 2007; Vasquez et](#)
95 [al., 2014\). Thus,](#) this problematic has emerged for now several years in various worldwide ERA
96 studies (Brus and Perrodin, 2017; Gros et al., 2010; Jean et al., 2012; Kosma et al., 2014; Orias and
97 Perrodin, 2014; Perrodin et al., 2011). Research is currently moving toward the development of new
98 methods to evaluate mixture toxicity (Riva et al., 2019) and risk. Several studies [have recently applied](#)
99 mixture ERA, most of the time through an additive model for risk quotient (RQ) calculation, on
100 different families of wastewater pollutants, and demonstrated its interest (Backhaus and Faust, 2012;
101 Backhaus and Karlsson, 2014; Escher et al., 2011; Kase et al., 2018; Riva et al., 2019; Thomaidi et al.,
102 2015).

103 In summary, the aim of the present study was to evaluate the influence of the “cocktail effect”
104 consideration for the ecotoxicological risk assessment of urban wastewaters for receiving watercourses
105 at a territorial scale. For that, a methodology was developed and applied, employing two compared

approaches based on Risk Quotient (RQ) calculation: (1) the first, studying the risk linked to each individual pollutant (single substance ERA) (2) the second, considering all pollutants present (the “cocktail effect”), into the receiving watercourses (mixture additive ERA). Initial minimum, median and maximum pollutant concentrations in treated effluents used for this study were obtained from a literature review of physico-chemical analyses studies to reconstitute “typical” effluents. This work was then applied to 33 urban WWTPs of the highly urbanized Lyon region in France. Finally, the interest of this territory-scale methodology based on single and mixture ERA was discussed in a context of climatic change that could worsen the identified risk situations.

2. Materials and methods

2.1. Presentation of the territory and WWTPs

This work studies the highly urbanized territory of Grand Lyon (France) and the surrounding suburban municipalities. This area was selected for several reasons: the presence of available data for WWTP and the watercourse flow rates, the diversity (WWTP/watercourse flow rate ratios) of each WWTP and the highly anthropized situation (and so, a priori, high risk).

A total of 85 WWTPs were identified in the territory. Sufficient information (WWTP and watercourse flow rates) to perform an ERA were available for 41 WWTPs. These WWTPs are presented in Table 1. Among them, 32 correspond to WWTPs releasing treated effluents into watercourses, and 9 in the soil. These 32 WWTPs were selected to evaluate the ecotoxicological risk of wastewater discharges for aquatic ecosystems in the Lyon area. The WWTP locations, flow rate and receiving watercourses are presented in Table 1 and Figure 1. These WWTP flows present a large diversity in the territory, from 4 to 215092 m³/day.

2.2 Implementation of the methodologies for ERA evaluation on the selected territory

2.2.1 Pollutant ecotoxicity, and concentration in WWTP effluents and watercourses

Two approaches exist when conducting ERA. The first, theoretical, is based on the compilation of data obtained from peer-reviewed literature, and the calculation of a maximum, median and/or minimal “typical” concentration observed for each researched pollutant in the various studies. The

second, more realistic, is based on the measurement of selected compounds in collected WWTP effluent samples. However, the latter approach is very expensive (Orias and Perrodin, 2014), and particularly when territorial ERA are performed. This is why the first approach was employed to perform the ERA evaluations in the selected territory. It assumes that the pollutant concentration ranges detected in different countries are similar (Thomaidi et al., 2015). Thus, 55 pollutants were examined in the study: 7 analgesic and anti-inflammatory pharmaceuticals (Diclofenac, Ibuprofen, Indomethacine, Ketoprofen, Naproxen, Paracetamol (Acetaminophen), Salicylic acid), 1 antiepileptic (Carbamazepine), 4 lipid regulators (Fenofibrate, Bezafibrate, Gemfibrozil, Clofibrate), 9 antibiotics (Amoxicillin, Ciprofloxacin, Doxycyclin, Erythromycin, Norfloxacin, Ofloxacin, Roxithromycin, Sulfamethoxazole, Trimethoprim), 3 β -blockers (Atenolol, Metoprolol, Propanolol), 4 steroid hormones (Estrone, Estradiol (17β -Estradiol), 17α -Ethinylestradiol, Estriol), 4 antineoplastics and immunomodulant agents (tamoxifène, 5-fluorouracil, Cyclophosphamide, Ifosfamide), 1 disinfectant (Triclosan), 2 detergents (Nonylphenol, Octylphenol), 4 plasticizers (Bisphenol A, DBP (DiButyl Phthalate), DEHP (Di(2-ethylhexyl) phthalate), DMP (Dimethyl phthalate)), 2 fire retardants (TCEP (Tris (2-chlorethyl) phosphate), TCPP (TRIS(2-Chloro-1-methylethyl) Phosphate)), 7 metallic trace elements (Cadmium, Chromium, Copper, Nickel, Mercury, Lead, Zinc), 6 pesticides (Atrazine, Diuron, Diazinon, Cypermethrin, Clotrimazole, Tebuconazole), and **Ammonia**. Their ecotoxicity (through Predicted No Effect Concentration (PNEC) values) and typical concentrations (minimum, median and maximum) in WWTP treated effluents have already been calculated in Brus and Perrodin (2017) and were used in this study to realize the 2 ERA methodologies in the present territory. Briefly, PNEC values were obtained from international database (e.g. INERIS, ECHA or OSPAR Commission), or from scientific literature on pollutant concentrations. These data are supplied in supplementary data (Table S1).

The Predicted Environmental Concentration (PEC) of the selected pollutants in the watercourses of our territory, **which are presented in Figure 2**, were based on WWTP discharge rates, their typical concentration in effluents and watercourse flows during the low-water period (to apply the

precautionary principle in the most critical period of the year) and calculated using the following equation (1):

$$PEC_{x,y} = \frac{WWTP_x \text{ flow rate}}{Watercourse_x \text{ flow rate}} Conc_y \quad (1)$$

With $PEC_{x,y}$: Predicted Environmental Concentration the pollutant y for the WWTP_x in $\mu\text{g/L}$; WWTP_x flow rate: the flow rate of the WWTP_x in m^3/s ; Watercourse_x flow rate: the flow rate of the WWTP_x's receiving watercourse in m^3/s and $Conc_y$: the (minimum, median or maximum) concentration of the pollutant y in the urban WWTP effluent.

2.2.2 Evaluation of the ecotoxicological risk of single micropollutants (classic approach)

The involvement of each pollutant in the ecotoxicological risk of each WWTP discharge was calculated using its ecotoxicity data (PNEC) and its final concentration in the receiving watercourse (PEC), in accordance with the guidelines of the Technical Guidance Document (TGD) of the European Chemical Bureau (ECB, 2003), following the equation (2) below:

$$RQ_{x,y} = \frac{PEC_{x,y}}{PNEC_y} \quad (2)$$

With $RQ_{x,y}$: Risk Quotient of the pollutant y for the WWTP_x; $PEC_{x,y}$: Predicted Environmental Concentration of the pollutant y for the WWTP_x in $\mu\text{g/L}$ and $PNEC_y$: Predicted No Effect Concentration of the pollutant y in $\mu\text{g/L}$. An $RQ_{x,y}$ value below 1 was associated with a nonexistent ecotoxicological risk, and a $RQ_{x,y}$ value above 1 to a potential ecotoxicological risk for watercourses (ECB, 2003 ; Perrodin et al., 2011) following the ranking categories described in Table 2.

2.2.3 Evaluation of the cumulative ecotoxicological risk of the micropollutant mixtures

Several methods have been developed to assess the cumulative ecotoxicological risk of a mixture of micropollutants for watercourses (Backhaus and Faust, 2012; Backhaus and Karlsson, 2014). These have been applied in various water contamination contexts related, for instance, to

pesticide or pharmaceutical **residues** (Palma et al., 2014; Riva et al., 2019). Two concepts of mixture risk assessment have been designed based on the interaction of chemicals in the water samples: The Concentration Addition (CA) and the Independent Action (IA) (Junghans et al., 2006; Backhaus and Faust, 2012). The former (CA) assumes a similar action and an identical site of action for all the chemicals on aquatic organisms. The **latter** (IA) assumes a dissimilarity in the mechanisms of the chemicals' action in the mixture (Junghans et al., 2006; Riva et al., 2019). Consequently, the CA concept can lead to an overestimation of the mixture's risk while the IA concept can lead to an underestimation. Even if the CA concept is considered by some authors to be a conservative method (worst-case scenario), it remains relevant for preliminary mixture risk assessment (Palma et al., 2014) and is usually employed. The CA concept-based risk assessment is based on the summing of all the risk quotients of each pollutant (Riva et al., 2019) detected in a water sample. Finally, mixture risk quotients were calculated in this study following eq. (3):

$$RQ_{Mix,x} = \sum_{y=1}^n \frac{PEC_{x,y}}{PNEC_y} = \sum_{y=1}^n RQ_{x,y} \quad (3)$$

With $RQ_{Mix,x}$: Risk Quotient for the cumulative risk of the pollutant mixture for WWTPx ; $RQ_{x,y}$: Risk Quotient of the pollutant y for the WWTPx; $PEC_{x,y}$: Predicted Environmental Concentration of the pollutant y for the WWTPx in $\mu\text{g/L}$ and $PNEC_y$: Predicted No Effect Concentration of the pollutant y in $\mu\text{g/L}$. Similarly, to the $RQ_{x,y}$ calculated for each pollutant alone, a RQ_{Mix} value below 1 was associated with a nonexistent ecotoxicological risk, and a RQ_{Mix} value above 1 with a potential ecotoxicological risk for watercourses following the ranking categories described in Table 2.

2.3 Data representation and WWTP risk ranking and comparison of the methodologies

For each ERA methodology and each WWTP, Risk quotients (RQ) were determined using minimum, median and maximum concentrations in order to calculate better, median and worst case ecotoxicological risks for receiving watercourses (Palma et al. 2014). For the individual pollutant risk assessment, WWTPs were ranked from the least risky WWTP to riskiest, in order to identify the most significant risk situation in the territory. Cumulative risks were presented cartographically, using a

Geographical Information System (GIS), with the dedicated QGIS free and open-source desktop software (<http://www.qgis.org>). A comparison between the two different approaches was performed, evaluating the number of WWTPs identified as risky for their respective receiving watercourses, and the RQ obtained in each case.

3. Results and discussion

3.1 Pollutant ecotoxicity, and concentration in WWTP effluents and watercourses

3.1.1 Predicted No Effect Concentration (PNEC) values

The ecotoxicity of each of the studied pollutants (PNEC) employed in the present paper is presented in supplementary data Table S1. A wide variability of PNEC values can be noted. The three most toxic compounds recorded were the steroid hormones (Estrone, 17 β -Estradiol and 17 α -Ethinylestradiol), with calculated PNEC values between 0.008 and 0.16 ng/L. Their very high toxicity is directly linked to the significant endocrine disruption properties of these molecules for aquatic organisms, such as fish, with very high reproduction and embryonic development interference at very low concentrations (Lang et al., 2001; Santos et al., 2014). Other very ecotoxic micropollutants families were the pyrethroid pesticides (e.g. Cypermethrin, PNEC = 3 ng/L), the antibiotics (e.g. Amoxicillin, PNEC = 0.78 ng/L), and anticancer drugs (e.g. fluorouracil, PNEC = 1 ng/L). The least ecotoxic pollutants detected were Tris(2-Chloro-1-methylethyl) Phosphate, a fire retardant, and Indomethacine, an analgesic with PNEC values of 260 and 16.14 μ g/L, respectively.

The authors would like to draw attention to the fact that the PNEC (7.4 μ g/L), and RQ values for ammonia nitrogen must be treated with caution in the present work. The toxicity of ammonia is highly dependent on pH and water hardness (Emmanuel et al., 2005; Perrodin et al., 2013), which can influence the equilibrium between ammonia (NH₃, the most toxic form) and ammonium ions (NH₄⁺, the least toxic form) in aqueous samples. In the case in urban effluents, slightly alkaline pH (most of

the time between 7.5 and 8.5 (Teijon et al., 2010; Stalter et al., 2010)) allows the nitrogen to be present in both ammonium and ammonia forms (Clément and Merlin, 1995), and thus to contribute significantly to the ecotoxicological risk linked to WWTP effluents. Following the equation from Clément and Merlin (1995), a 10%/90% $\text{NH}_3/\text{NH}_4^+$ ratio was so considered to calculate the NH_3 PEC into the watercourses and so the RQ values by comparison with its ecotoxicological threshold (PNEC).

3.1.2 Pollutant concentrations in effluents and in watercourses (PEC)

Table S1 presents the minimal, median and maximal pollutant concentrations in the effluents. Ten of the 55 surveyed pollutants were not detected in the best case, with a maximum concentration of 100 ng/L for organic contaminants (Tris(2-Chloro-1-methylethyl) Phosphate). The majority were detected at a concentration below 50 ng/L (e.g. Triclosan, 13 ng/L). A large range has been reviewed for median and maximal concentrations of pollutants. Dimethyl phthalate was detected with the lowest concentrations (resp. 0.19 and 115 ng/L), and conversely, Ammonia was observed with the highest concentrations (resp. 104.44 and 600 $\mu\text{g/L}$). Some heavy metals, such as Nickel and Zinc occurred at high concentrations in treated urban effluents (median concentration of 21 and 105 $\mu\text{g/L}$ respectively). This presence can be explained either by a low removal efficiency in treatment plants, as noticed by scientific community (da Silva Oliveira et al., 2007; Shafer et al., 1998), or by a very important initial concentration into the influent, depending of the WWTP. Pharmaceutical drugs represent the main family of organic micropollutants in the reviewed literature in treated effluents. Incoming raw wastewater concentrations due to their specific consumption by patients and fluctuation of removal efficiency rates lead to a significant variability in the concentration of each of these substances (e.g. Clotrimazole and Salicylic acid have median concentration of 4 ng/L and 1.1 $\mu\text{g/L}$ respectively) (Verlicchi et al., 2014; Yang et al., 2017).

As detailed in a previous section (2.2.1), PEC values were calculated for the lowest flow period of the year. On that basis, dilution factors were calculated comparing WWTP and watercourse

flow rates. The calculation led to a wide range of effluent dilutions (between 0,0042 % of effluent discharged into the freshwater for WWTP N° 30 and 100 % for N°1) depending on the plant. Thus, a wide range of risk situations is to be expected. All the detailed data are presented in Table S2.

Finally, every predicted concentration in each watercourse is provided in Table S2. The comparison between pollutant concentrations remains identical to the one for effluents, as in this study, the “typical” effluent was applied to all WWTPs in the territory. It is worth noting that the final pollutant PEC range calculated for most of the WWTPs is in accordance with the concentrations detected in French and worldwide streams (most of the time between 0 and 500 ng/L depending on the substance and the stream) (Chiffre et al., 2016; Patrolecco et al., 2015; Petrie et al., 2015; Vulliet et al., 2011), showing that the present methodology is environmentally realistic.

3.2 Single substance approach

The ecotoxicological risk quotients for each substance and WWTP are provided in the supplementary data Table S2. The Figures 3a, b and c represent the risk quotients for the 11 main pollutants for the best, median and worst cases (Ammonia, Amoxicillin, Copper, Diazinon Diclofenac, Ethynylestradiol, Estradiol, Estrone, Nickel, Norfloxacin, Roxithromycin, Tamoxifene, Trimethoprim and Zinc) involved in the 15 riskiest WWTPs identified in the Lyon territory for receiving watercourses.

In short, in the most favorable case, 7 of the 33 WWTPs constituted a risk for receiving freshwater, with higher quotients for 17 β -Estradiol (up to an RQ value equal to 37.5 for the riskiest WWTP), 17 α -Ethynylestradiol, Copper, Nickel, Trimethoprim and Zinc. For median concentrations, 16 of the 33 represented a risk with higher values for 17 β -Estradiol (up to an RQ value equal to 187 for the riskiest WWTP), 17 α -Ethynylestradiol, Amoxicillin, Estrone, Nickel, Roxithromycin and Trimethoprim. Finally, in the worst-case scenario, 28 of the 33 WWTPs led to a risk (RQ above 1), with the highest values for 17 β -Estradiol (up to an RQ value equal to 3750 for the riskiest WWTP), 17 α -Ethynylestradiol, Diazinon, Estrone, Nonylphenol and Trimethoprim. On the contrary, the lowest

RQ_{med} and RQ_{max} were computed for Dimethyl phthalate, Clotrimazole, Cyclophosphamide and Ifosfamide.

The present results are in accordance with previous studies. For example, Riva et al. (2019) conducted research on the risks linked to 47 emergent pollutants in 3 rivers in a highly urbanized zone, contaminated by WWTP effluents, runoffs, etc. Among the riskiest pollutants, Amoxicillin, 17 β -Estradiol and Estrone were identified respectively with RQ values up to 11.33, 158 and 903.6 depending of the sampling site. In their case, as in the present study, even if the predicted/measured concentrations of estrogens in the receiving streams were very low, the very high toxicity of these compounds (biocides and endocrine disruptors) resulting in very low PNEC values, led to high RQ values. Our results are also corroborated by several studies that conducted ERA for urban WWTPs, and contaminated rivers by emerging micropollutants (pharmaceuticals, plasticizers, pesticides, ...) in various regions of the world such as Greece, Portugal, Spain and the USA. The authors demonstrated significant risks (RQ above 1) for Diclofenac (Kosma et al., 2014; Pereira et al., 2015), Trimethoprim (Kosma et al., 2014; Valcarcel et al., 2011), 17 α -Ethinylestradiol (Barber et al., 2013) and Amoxicillin (Thomaidi et al. 2015). Zinc, Copper and Nickel risks are linked to their high concentrations in effluents, which can be attributed to the low efficiency of WWTP in removing them. Finally, all of these results show that the current theoretically proposed methodology is in accordance with all the recent research and could be used as an a priori risk tool for WWTP management in a territory.

3.3 Mixture approach

To obtain a more representative view of the risks linked to the pollutants that do not have an independent toxic action, but rather in interaction with one other, a mixture risk assessment was performed. To our knowledge, the present paper is the first to investigate the cumulative risk of a “representative” mixture of micropollutants from urban wastewater treatment plant effluents, based on literature review, for aquatic ecosystems. Results are presented in Figure 4. The smallest circles correspond to the cumulative risk for the minimal concentrations, medium circles for median concentrations and the biggest circles for maximal reviewed pollutant concentrations in effluents.

To identify the number of WWTPs presenting a significant risk (above 1), the supportive situation led to identify 8 WWTPs as compared to 7 for the single substance approach; 19 against 16 for median situation and 32 against 28 for worst-case situation. As expected, in all cases, the number of WWTPs with a significant mixture risk is much greater than for the single substance approach, as well as the RQ values (up to 9270.55 for WWTP N°1 and maximum concentrations). Hence, these results support the hypothesis that classical single substance ERA analyses can mitigate and overshadow some of the risks and thus demonstrate the importance of performing mixture ERA. This observation has already been attested by Riva et al. (2019). A recent study reported the mixture risk of emerging organic micropollutants from several WWTP effluents, for Greek rivers (Thomaidi et al., 2015). Authors evaluated the mixture RQ linked to the maximum concentrations of each pollutant detected (worst-case scenario), and calculated RQ values were up to 1000 for most of the rivers (16/25). These results are precisely in agreement with the present work.

Nevertheless, if the mixture approach has the advantage of taking into account the cumulative risk of many substances, it remains an additive approach that does not consider the synergic and antagonistic ecotoxic effects of pollutants, which have been demonstrated to occur in many surveys, for example, the mixture of antibacterial compounds on the microalgae *P. subcapitata* (Yang et al., 2008), or veterinary intestinal pest control drugs on *D. magna* (Puckowski et al., 2017), and must be refined.

3.4 ERA evaluation at highly urbanized territory scale and further climate change influences

In assessing the territorial risks of WWTP effluents, results highlighted very diverse risks depending on exposure factors. The mixture approach has pointed out that eight (N° 1, 2, 4, 9, 10, 12, 13 and 18) of the thirty-three WWTPs led to a significant risk regardless of the pollutant concentration considered (minimal, median or maximal). Figure 5 represents the RQmix for median pollutant concentrations in relation to each WWTP and watercourse flow rates. It demonstrates that most of the time, the RQmix is higher for small and median WWTPs rejecting effluents into small/very small watercourses (e.g. N° 1, 2, 4, 9, 10, 12, 13 and 18), and at the opposite end, WWTP dumping into the high flow rates lead to limited/very weak risks (e.g. N° 23, 24 and 30). It is important to note that the

riskiest situation corresponds to a WWTP (N°1) discharging into a small dried-up (intermittent) watercourse during the driest period of the year, leading to a flow composed of 100% wastewater, as pointed out in a previous study (Brus and Perrodin, 2017).

The present methodology may be useful for further WWTP placement in a selected territory, and to identify the already installed plants that need to be adjusted, for example strengthening the treatments or reducing the inbound/outbound loads. Nevertheless, one must be aware that the current exposure situations will evolve in a context of climate change, which must be taken into account. In many cases, implemented WWTPs have been designed in a hydrological context, and without taking into account possible evolution of watercourse flow rates due to climate change (Zouloubis and Tolkou, 2015). Several studies have focused on the evolution of river flow regimes in various world regions (e.g. Europe, Africa), and all have illustrated a long-term flow rate decrease, especially during the summer (Bodian et al., 2018; Schneider et al., 2013). This could lead to an increase in micropollutant (e.g. pharmaceutical) concentrations in receiving watercourses (Gooré Bi et al., 2015; Schlüsener et al., 2015; Schneider et al., 2013; Zouloubis and Tolkou, 2015), due to lower dilution. Moreover, receiving water may be subjected to an increase in temperature and pH due to the decrease of their flow rate. This could enhance the toxicity of some pollutants, their biodegradation and by-product creation (Schlüsener et al., 2015), and thus ultimately influence the ecotoxicological risks.

Finally, in the present case, if one situation has been described as critical in our study compared to others (dried-up watercourse), a similar description would fit several other watercourses, which currently present a very low flow rate (e.g. N°2, 4).

Conclusion and perspectives

In the present work, an ecotoxicological risk assessment evaluation was performed for 33 urban wastewater treatment plants located on a highly urbanized territory (the 2nd most in France). Our research increased the knowledge and the methods of WWTP effluent ecotoxicological risk assessment, at a territorial scale, combining two approaches to assess the individual (classic ERA) and “cocktail” effects of pollutants (mixture ERA), better evaluating the real risk for receiving

watercourses. The classical single substance approach highlighted a significant risk for many of the WWTPs. However, the cumulative risk approach showed its usefulness in evaluating the mixture of micropollutants in treated effluents, indicating a higher risk for some receiving watercourses. A map view of the theoretical risk of multiple WWTP effluents was produced, ranking the WWTPs' risks, on the various watercourses of an urbanized area for better territorial management.

Nevertheless, from this work and previous studies (Brus and Perrodin, 2017), four limits have been identified and should be overcome in further research to achieve a completely realistic ERA of the different types of urban liquid effluents on receiving aquatic ecosystems. Thus, the following trends should be adopted:

(1) The use of experimental physico-chemical data at the various WWTPs of the urbanized territory during different seasons (climatic influence).

(2) The consideration of organism exposure parameters (e.g. half-life time and bioaccumulation factors) for a more realistic risk assessment.

(3) The consideration of other types of potentially impacted ecosystems, such as lakes or groundwater through soil and sedimentation contamination and transfer.

(4) The inclusion of other types of urban effluents (stormwater and combined sewer overflow) that may be a significant hazard for aquatic ecosystems, with a significant, highly variable, and partially similar pollution (Gosset et al., 2017).

(5) The use of new analytical chemistry methods, such as "non-target screening" (Blum et al., 2017) or "suspect-screening" (Pinasseau et al., 2019) to overcome the fact that we find only what we are looking for.

In conclusion, the present research opens up perspectives in terms of territorial management for public and private partners. Applying the proposed methodology to real effluents would allow the ranking of WWTPs in a territory in terms of ecotoxicological risk and link ecotoxic pollution to socio-economic characteristics of each WWTP territory, with the goal of reducing emissions at the source.

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N°	Coordinates of the WWTP (Latitude / Longitude)		Number of drained municipalities	WWTP population equivalent (PE)	Design flow rate of the WWTP (m ³ /day)	Main treatment process	Name of the receiving watercourse
1	45° 30' 4.59"	4° 45' 5.859"	1	113	52	Bacterial bed*	Ruisseau de bassemont
2	45° 36' 9.743"	4° 47' 0.69"	1	3724	1430	Activated sludges	Ruisseau du Bullion
3	45° 41' 11.55"	4° 36' 55.026"	1	13	6	Sand filters	L'Artilla
4	45° 41' 52.728"	4° 41' 50.612"	3	8599	6812	Activated sludges	Le Garon
5	45° 43' 33.943"	4° 44' 58.303"	1	30	12	Vertical flow constructed wetland	Yzeron
6	45° 36' 9.743"	4° 31' 42.058"	1	157	68	Vertical flow constructed wetland	La Coise
7	45° 36' 21.428"	4° 30' 43.53"	1	137	102	Natural lagoon	La Coise
8	45° 36' 44.548"	4° 33' 8.579"	1	27	11	Vertical flow constructed wetland	La Coise
9	45° 49' 4.08"	4° 36' 24.753"	2	5192	1736	Activated sludges	La Brévenne
10	45° 49' 10.9"	4° 59' 39.062"	3	36200	2160	Activated sludges	Sereine
11	45° 53' 27.563"	4° 43' 48.45"	1	1120	785	Activated sludges	L'Azergues
12	45° 54' 1.949"	4° 43' 32.624"	1	1789	1566	Activated sludges	L'Azergues
13	45° 52' 19.34"	4° 38' 57.819"	8	6824	4000	Activated sludges	L'Azergues
14	45° 33' 15.365"	4° 57' 42.224"	2	3735	195	Bacterial bed	La Véga
15	45° 39' 30.904"	5° 9' 24.302"	4	5101	960	Activated sludges	Le Catelan
16	45° 52' 30.546"	4° 43' 21.733"	2	1419	791	Activated sludges	L'Azergues
17	45° 52' 18.044"	4° 42' 55.162"	2	2059	1573	Activated sludges	L'Azergues
18	45° 39' 31.97"	5° 8' 14.835"	9	109292	29540	Activated sludges	La Bourbre
19	45° 42' 21.266"	5° 11' 23.189"	1	676	90	Natural lagoon	La Bourbre
20	45° 48' 57.842"	5° 4' 12.349"	6	17477	9020	Activated sludges	Canal de Miribel
21	45° 49' 53.882"	4° 51' 9.84"	12	47250	9670	Biofilter	La Saône
22	45° 52' 2.215"	4° 50' 18.915"	4	30892	18000	Activated sludges	La Saône
23	45° 55' 2.726"	4° 46' 25.863"	1	2262	1000	Activated sludges	La Saône
24	45° 53' 22.628"	4° 48' 18.451"	2	3665	900	Activated sludges	La Saône
25	45° 54' 34.818"	4° 48' 46.271"	4	13624	5904	Activated sludges	La Saône
26	45° 29' 21.474"	4° 49' 29.85"	22	77834	18000	Activated sludges	Rhône
27	45° 41' 33.04"	4° 50' 5.364"	34	524325	300000	Activated sludges	Rhône
28	45° 41' 28.235"	4° 50' 55.267"	20	622800	554000	Activated sludges	Rhône
29	45° 34' 22.504"	4° 47' 59.939"	18	80232	17845	Biofilter	Rhône
30	45° 34' 8.344"	4° 48' 59.773"	3	27216	3000	Activated sludges	Rhône
31	45° 47' 43.464"	5° 2' 12.133"	7	21800	9900	Activated sludges	Rhône - Canal de Jonage
32	45° 47' 28.366"	4° 59' 44.352"	2	25732	8730	Biofilter	Rhône - Canal de Jonage
33	45° 47' 15.042"	4° 53' 21.753"	10	179772	91000	Activated sludges	Rhône
34	45° 33' 56.953"	4° 44' 52.98"	1	72	18	Sand filters	Soil
35	45° 36' 19.286"	5° 6' 29.502"	1	280	60	Vertical flow constructed wetland	Soil
36	45° 36' 8.389"	5° 7' 2.35"	1	638	120	Vertical flow constructed wetland	Soil
37	45° 40' 15.965"	5° 5' 10.447"	1	808	255	Natural lagoon	Soil
38	45° 44' 35.439"	5° 6' 14.384"	1	54	15	Natural lagoon	Soil
39	45° 34' 27.378"	4° 38' 13.881"	1	33	15	Vertical flow constructed wetland	Soil
40	45° 35' 8.114"	4° 34' 21.425"	1	3	15	Vertical flow constructed wetland	Soil
41	45° 32' 9.405"	4° 41' 9.883"	1	5	2	Sand filters	Soil

633 Table 1: Characteristics of the WWTPs considered on the territory.

634 *microorganisms fixed on a solid support

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Table 2: Ecotoxicological risk ranking based on the risk quotient (RQ) values.

Risk Quotient (RQ) value	Ranking categories
$RQ < 1$	Negligible risk
$1 < RQ < 10$	Low risk
$10 < RQ < 100$	Medium risk
$100 < RQ < 1000$	High risk
$RQ > 1000$	Very high risk

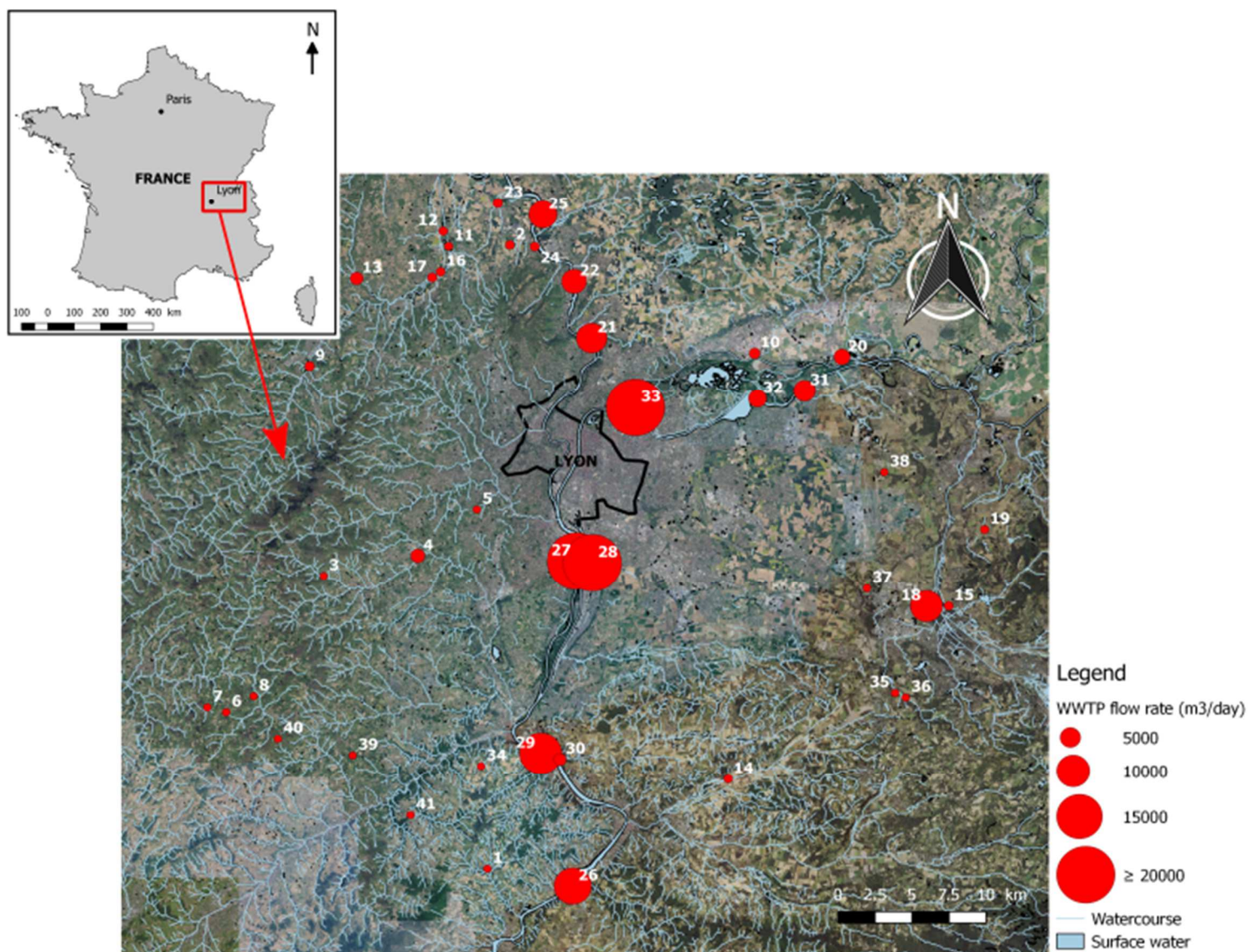


Figure 1: Mapping representation of the WWTP's location and discharge flow rates.

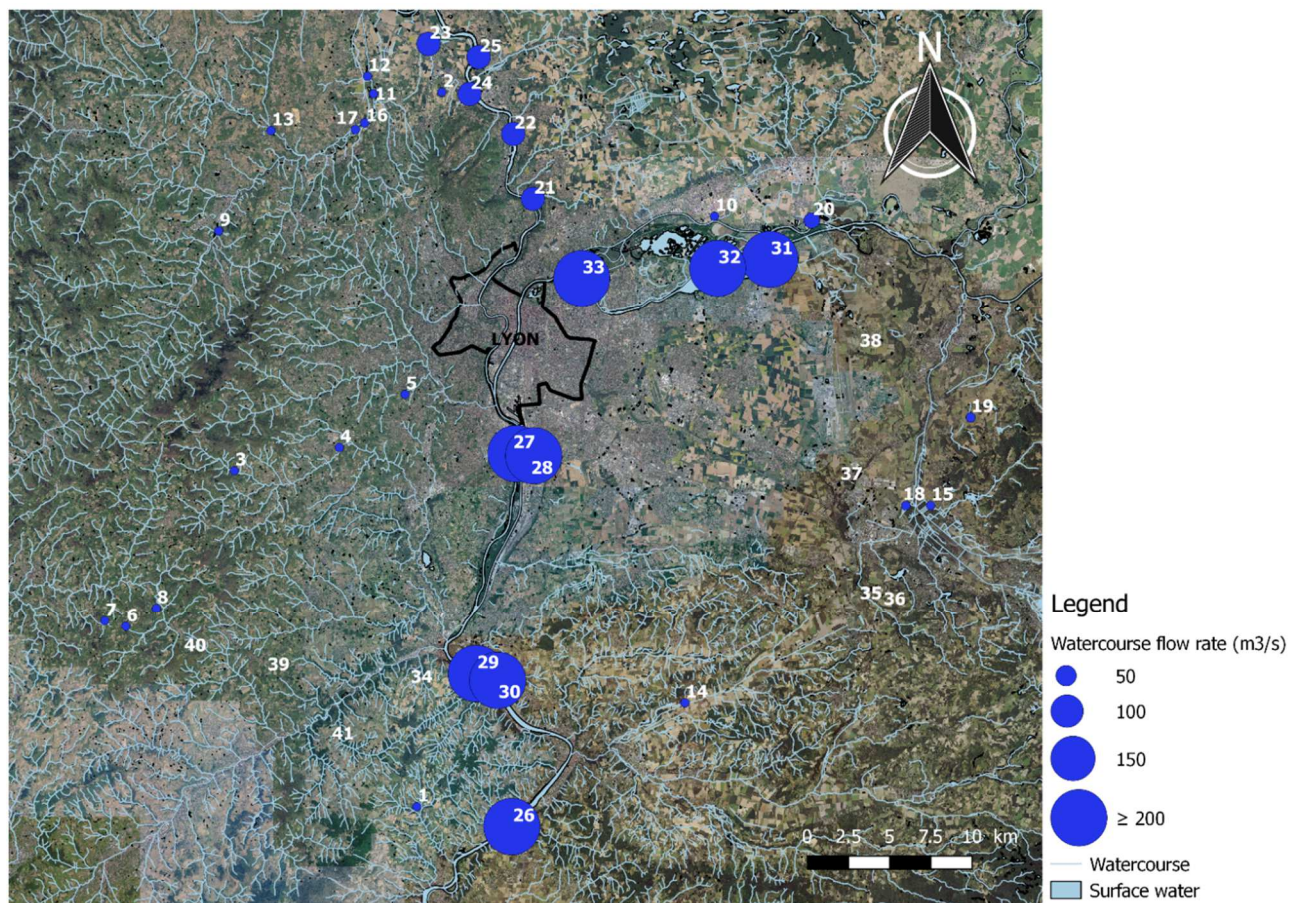


Figure 2: Mapping representation of the WWTP's receiving watercourse flow rates.

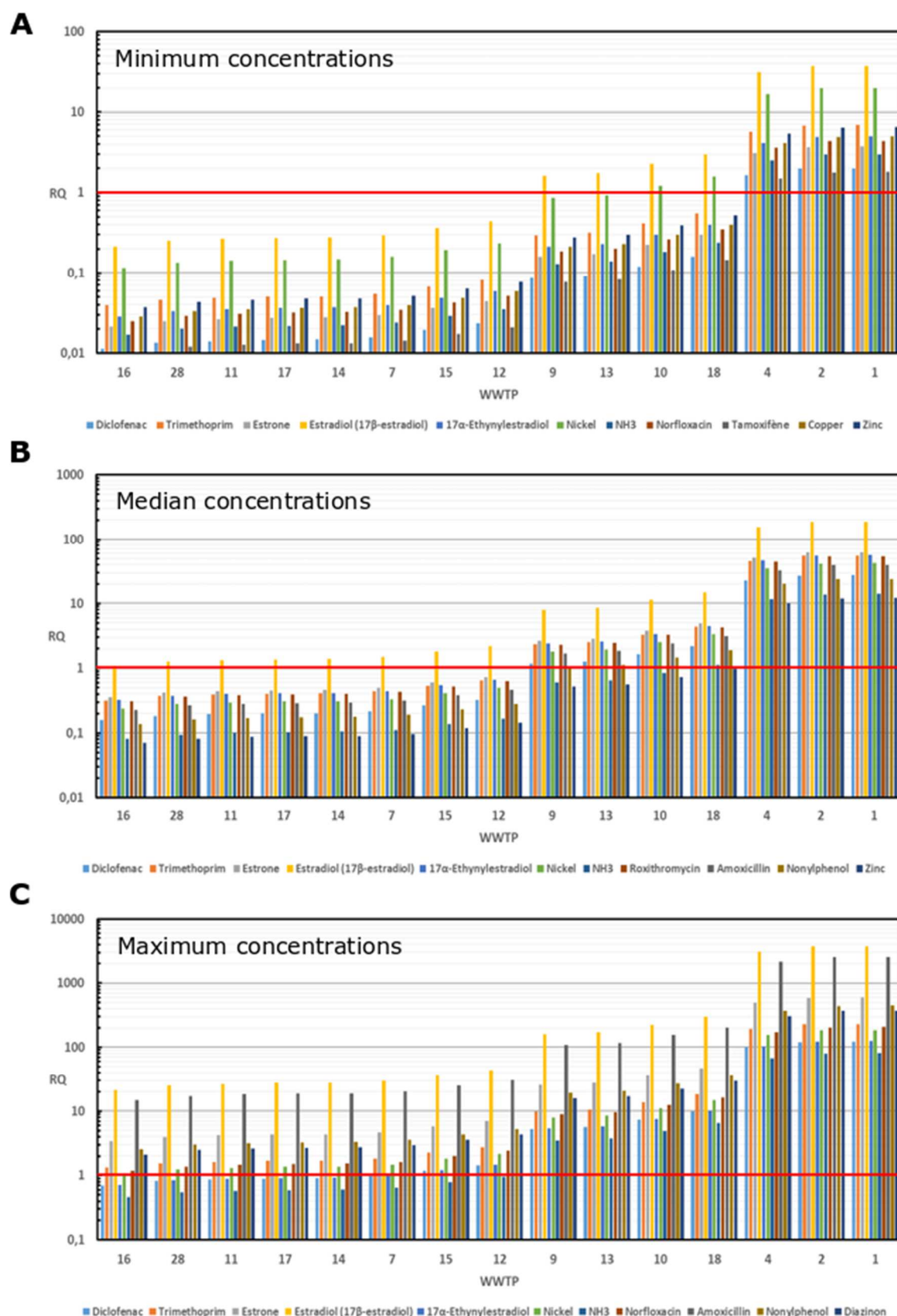


Figure 3: Ecotoxicological risk quotients (RQ) calculated for 11 main pollutants involved in the 15 riskiest WWTPs identified of the Lyon territory : (A) considering minimal pollutant concentration ; (B) median pollutant concentrations and (C) maximum pollutant concentrations. Red lines represent the significant risk schershold (RQ = 1).

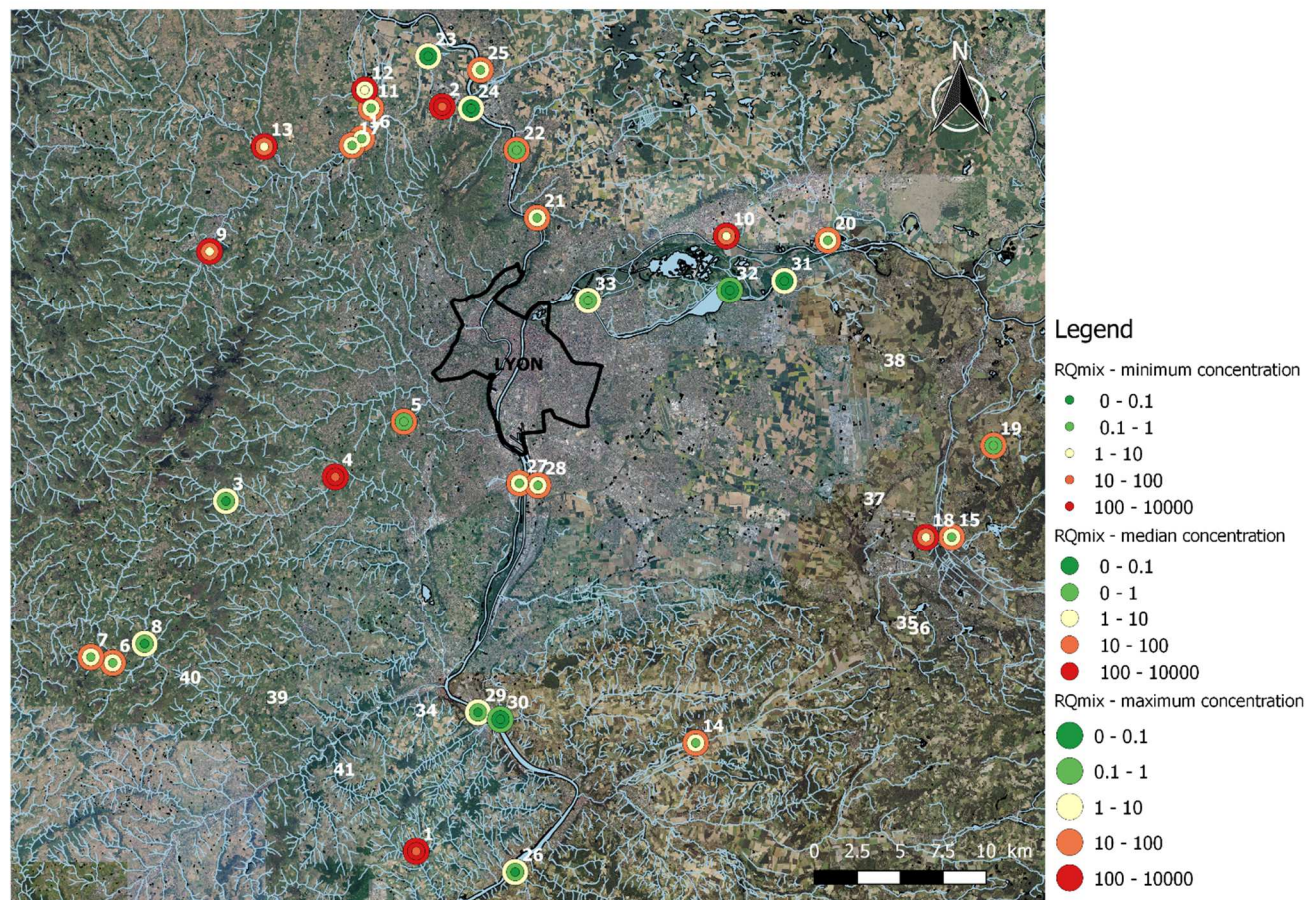


Figure 4: Ecotoxicological Risk Assessment (ERA) of WWTP effluent pollutant mixture for receiving watercourses. Little circles correspond to the mixture risk quotient (RQmix) for the minimal concentrations, medium circles for median concentrations and biggest circles for maximal pollutant concentrations.

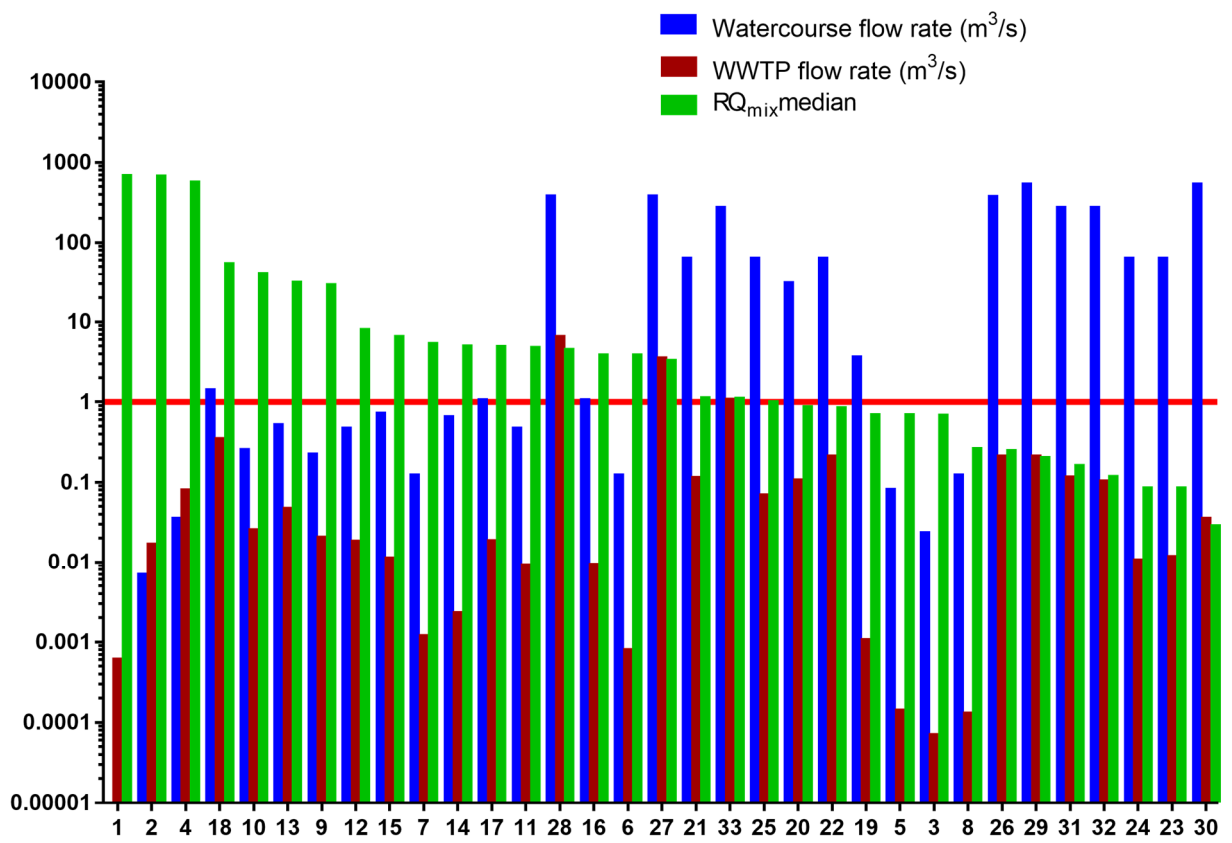


Figure 5: RQ_{mix} for median pollutant concentrations in relation to each corresponding WWTP and watercourse flow rate.