Occurrence and Toxicity of Antibiotics in the Aquatic Environment: A Review

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Abstract

In recent years, antibiotics have been used for human and animal disease treatment, growth promotion, and prophylaxis, and their consumption is rising worldwide. Antibiotics are often not fully metabolized by the body and are released into the aquatic environment, where they may have negative effects on the non-target species. This review examines the recent researches on eight representative antibiotics (erythromycin, trimethoprim, sulfamethoxazole, tetracycline, oxytetracycline, ofloxacin, ciprofloxacin, and amoxicillin). A detailed overview of their concentrations in surface waters, groundwater, and effluents is provided, supported by recent global human consumption and veterinary use data. Furthermore, we review the ecotoxicity of these antibiotics towards different groups of organisms, and assessment of the environmental risks to aquatic organisms. This review discusses and compares the suitability of currently used ecotoxicological bioassays, and identifies the knowledge gaps and future challenges. The risk data indicate that selected antibiotics may pose a threat to aquatic environments. Cyanobacteria were the most sensitive organisms when using standard ecotoxicological bioassays. Further studies on their chronic effects to aquatic organisms and the toxicity of antibiotic mixtures are necessary to fully understand the hazards these antibiotics present.

Keywords: Antibiotic, ecotoxicity, environmental concentration, human consumption, veterinary use

Abbreviations: ARB: Antibiotic Resistance Bacteria, ARGs: Antibiotic Resistance Genes, ASA: Ascorbic Acid, BRICS: Brazil, Russia, India, China, and South Africa, EMEA: European Medicines Evaluation Agency, ERA: Environmental Risk Assessment, ESVAC: European Surveillance of Veterinary Antimicrobial Consumption, GSH: Glutathione, PPCPs:

Pharmaceuticals and Personal Care Products, ROS: Reactive Oxygen Species, RQs: Risk Quotients, USA: United States of America, WWTP: Wastewater Treatment Plant

45 **Contents**

57

1. Introduction

Pharmaceuticals and personal care products (PPCPs) have received growing attention in recent years as emerging aquatic contaminants due to their possible threats to human population and to aquatic ecosystems. PPCPs include numerous chemical classes including pharmaceuticals such as antibiotics, anti-inflammatory drugs, β-blockers, lipid regulators, antiepileptics, X-ray contrast media, as well as personal care product ingredients such as antimicrobials, synthetic musks, insect repellents, preservatives or sunscreen UV filters, together with their metabolites or transformation products (Liu and Wong, 2013; Rasheed et al., 2019). Among the pharmaceuticals, antibiotics (or antimicrobials or antibacterial agents) are one of the most widely used categories, with human and veterinary applications including livestock and aquaculture growth promotion and prophylaxis (Yang et al., 2008; Binh et al., 2018; Yi et al., 2019) and prevention of crop damage induced by bacteria (Gonzalez-Pleiter et al., 2013).

Antibiotics are natural, synthetic or semi-synthetic compounds, which are able to kill or inhibit growth or metabolic acitvity of microorganims. These compounds are biologically active molecules with antibacterial, antifungal, and antiparasitic activities deliberatery designed as a medicine that treat bacterial infections in both people and animals, and as feed additives or disease prevention in animal husbandry. The first antibiotics were of natural origin, e.g., penicillin derived from *Penicillium* fungi. Currently, antibiotics are obtained by chemical synthesis or by chemical modification of natural compounds (Kummerer, 2009a, b). Antibiotics may be divided into different groups by either their mechanism of action, including bactericidal (causing bacteria to die) and bacteriostatic (inhibiting bacterial growth), or by their chemical structures such as β-lactams, quinolones, tetracyclines, macrolides, sulfonamides, and others. Presently, there are more than 250 different registered antibiotic drugs (Kumar et al., 2012).

Global antibiotic consumption has been increasing because of two main reasons. The first is the worldwide increase in human population that increased the consumption. Furthermore, the increasing prosperity coupled with an easy access to medicines also enhanced the use of antibiotics. The second reason is upward demand of animal protein, which intensifies food production requiring a greater use of growth promoters and antibiotics (Van Boeckel et al., 2015; Zhao et al., 2019).

Substantial amounts of the antibiotics used by humans and for livestock eventually find their way into the environment, where they may have a negative impact on non-target organisms in the aquatic ecosystems including freshwater algae, microphytes, macrophytes, zooplankton and fishes (Kar and Roy, 2012; Larsson, 2014; Minguez et al., 2016; Straub, 2016; Kumar et al., 2019).

Antibiotics are continuously discharged into the aquatic environment, where they may be found in the range of ng/L-µg/L. At these concentrations, they are unlikely to elicit acute toxicity (Jjemba, 2006; Yang et al., 2008; Liu and Wong, 2013; Geiger et al., 2016). However, because aquatic organisms are exposed to waterborne contaminants during their entire life cycle, antibiotics may induce chronic effects, such as changes in behavior, reproduction, and growth. There is currently little data on the non-therapeutic (low-concentration) effects of antibiotics, and most reported data are from the ecotoxicological assessment of the acute toxicity of high doses (Janecko et al., 2016). Primary producers and decomposers appear to be particularly susceptible to the adverse effects of antibiotics, leading potential disruption to the aquatic environment.

Long-term alteration of the bacterial community composition may lead to variation in biogeochemical cycling and aquatic ecosystems. For example, anoxic environments promote harmful algal blooms (Ding and He, 2010; van der Grinten et al., 2010; Janecko et al., 2016; Roose-Amsaleg and Laverman, 2016; Xiong et al., 2019). Antibiotics may have bactericide

and bacteriostatic effect with the consequent disappearance of some microbial populations and their ecological functioning. Microbial biodiversity is important for maintaining biological processes in water and soil, including biogeochemical cycles. The effects of antibiotics on ecological functions may cause change in nitrogen transformation, methanogenesis, sulfate reduction, nutrient cycling, and organic matter degradation (Grenni et al., 2018). Furthermore, antibiotic residues could accelerate the evolution of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in the environment (Qiao et al., 2017). Therefore, antibiotics present a public health concern as persistent exposure to antibiotics that result in antibacterial drug resistance (Lorenzo et al., 2018; Rousham et al., 2018; Hendriksen et al., 2019; Subirats et al., 2019; Zhang et al., 2019). The risks associated with the environmental antibiotic resistome refer to the transmission of environmental ARB and ARGs to humans (Liu and Wong, 2013; Bondarczuk et al., 2016; Hocquet et al., 2016; Garner et al., 2018; Le et al., 2018; Ben et al., 2019). The World Heath Organization (WHO) has recognized the occurrence of ARB and ARGs as one of the most important public health concerns of this century. ARGs are being recognized as an emerging environmental pollutant (Ben et al., 2019; Zarei-Baygi et al., 2019).

The present review focuses on eight representative antibiotics from six different classes, including erythromycin (a macrolide), amoxicillin (a β-lactam), tetracycline and oxytetracycline (both tetracyclines), ofloxacin and ciprofloxacin (both fluoroquinolones), sulfamethoxazole (a sulfonamide) and trimethoprim (a diaminopyrimidine). These are the key antibiotics of great concern, based on their common use in human or veterinary medicine, relatively common detection in surface waters around the world, and ecotoxicity. They were selected based on a preliminary literature review that combined detection frequency in waters and data on the toxicity of 30 pharmaceuticals. In the following sections, detailed information on the use and consumption of these antibiotics, their occurrence in the

aquatic environment, their ecotoxicity, and the assessment of risks towards aquatic organisms of different trophic levels are discussed.

2. Antibiotic consumption and release

2.1 Human consumption

Global trends in using antibiotics are of a great importance because they provide information about their potential to develop ARB to specific antibiotics. Ideally, certain antibiotics would be reserved only for certain human uses in order to minimize likely development of ARB (Laxminarayan et al., 2013). There have been few attempts to assess antibiotic consumption globally; however, reported data are from before 2010 (Kunin et al., 1990; Hogberg et al., 2014; Van Boeckel et al., 2014). Based on data from 76 countries, total global antibiotic consumption rate grew by 39% between 2000 and 2015 to 42.3 billion defined daily doses (DDDs) (Klein et al., 2018). Antibiotic use per capita was generally higher in high-income countries, but the greatest increase in antibiotic use was in low- and middle-income countries such as India, China, and Brazil (CDDEP, 2015; Zhang et al., 2018). In low- and middle-income countries, antibiotic consumption increased 77%, from 7.6 to 13.5 DDDs per 1,000 inhabitants per day between 2000 and 2015 (Klein et al., 2018).

The largest consumer of antibiotics in 2010 was India, followed by China and the US (Van Boeckel et al., 2014). The highest increase in consumption of antibiotic was observed from 2000 to 2010 in five countries with major emerging economies, i.e., Brazil, Russia, India, China, and South Africa (a group known as BRICS) (CDDEP, 2015). These countries showed an increase in antibiotic consumption of 68%, 19%, 66%, 37%, and 219% between 2000 and 2010, respectively. Although about 75% of the total increase in global consumption occurred in these BRICS countries, the overall per capita consumption in these countries was still lower than in the US (CDDEP, 2015). In 2000, the high income countries (France, New

158 Zealand, Spain, Hong Kong, and the US) had the highest consumption rate in DDDs per
159 1,000 inhabitants per day. In 2015, four of six countries with the highest consumtion rates
160 were low- and middle- income countries (Turkey, Tunisia, Spain, Greece, Algeria, and
161 Romania) (Klein et al., 2018). Consuption data from Africa are represented by data from four
162 countries: Burkina Faso, Burundi, Côte d'Ivoire and the United Republic of Tanzania. The
163 total antibiotic consumption ranged from 27.3 to 4.4 DDD per 1000 inhabitants per day in
164 2015. Penicillins accounted for nearly 40% of all consumption, followed by sulphonamides
165 and trimethoprim in Burkina Faso (WHO, 2018).

166 In most of the high-income countries, antibiotic consumption is decreasing or has
167 remained at approximately the same level since 2000 (CDDEP, 2015). European countries
168 showed no significant increasing trend during 2013-2017, and eight countries (i.e.,
169 Netherlands, Sweden, Germany, Norway, Finland, United Kingdom, Italy, and Luxembourg)
170 showed a significant decreasing trend (ECDC, 2018). Furthermore, statistically significant
171 decreasing trend was observed for tetracyclines, sulphonamides and trimethoprim
172 consumption (ECDC, 2018). The prescription rate decreased in the United States (US) by 5%
173 from 1999 to 2012, down to 0.9 prescriptions per capita outpatient annually, which is lower
174 than that in many Southern European nations but higher than that in Scandinavia and the
175 Netherlands (CDDEP, 2015). Nevertheless, consumption in BRICS countries is expected to
176 double by 2030, assuming no policy changes, as their population increases (Van Boeckel et
177 al., 2015; Klein et al., 2018). Reduction of global consumption is necessary for reducing the
178 threat of antibiotic resistance (Klein et al., 2018). In general, about 80% of the total
179 worldwide antibiotic consumption occurs in the community, outside the hospital setting. In
180 Europe in 2015, only 10% of antibiotics were used in hospitals (Van Boeckel et al., 2015).
181 About half of community use was for conditions that could not be treated with antibiotics,

such as colds, which contributes to the burden of antibiotic resistance (CDDEP, 2015; Valitalo et al., 2017).

The most frequently used antibiotics were broad-spectrum penicillins (39% of total DDDs in 2015) (Klein et al., 2018). In European countries is the consumption ranging from 36% (Germany) to 71% (Slovenia), followed by macrolides from 5% (Sweden) to 25% (Slovakia), β-lactams from 0.2% (Denmark) to 22% (Germany), and quinolones from 2% (United Kingdom) to 16% (Hungary) (ECDC, 2016, 2018). Penicillins were also the most prescribed antibiotics in the USA in 2010 (38%), followed by β-lactams (16%), tetracyclines (15%), macrolides (12%), quinolones (9%), and trimethoprim (10%) (Van Boeckel et al., 2014). However, in India, penicillins were the third most commonly prescribed antibiotics in 2008 (28%), after quinolones (34%) and cephalosporins (32%), followed by macrolides (14%) and tetracyclines (6%) (Kotwani and Holloway, 2011). A similar trend was also observed in China and Thailand (Van Boeckel et al., 2014).

Following antibiotic use by humans, the antibiotics are eliminated from the body mainly through the renal system (urine) and/or biliary system (feces), either as an unchanged parent compound, as its metabolites, or as conjugates of glucuronic and sulphuric acid (Gros et al., 2010; Milic et al., 2013; Tran et al., 2018). Pharmaceuticals vary widely in the extent to which they are metabolized before excretion, from less than 10% to more than 90%. However, when the total use of a particular antibiotic is high, even if the compound is highly metabolized, there may still be significant wastewater contamination by the parent compound (Kummerer, 2009c). Previous research showed that approximately 70%–80% of antibiotics enter sewage systems as the unchanged forms (Dinh et al., 2017). Human pharmaceuticals predominantly enter the environment through household effluents, hospital wastewaters, and industry effluents, and to a minor extent through emissions from manufacturing sites and incorrect disposal of medications (Fig. 1) (Tuc et al., 2017; Lorenzo et al., 2018; Emara et al.,

2019; Hendriksen et al., 2019). Wastewater treatment plants (WWTP) are not capable to completely remove most antibiotics (Halling-Sorensen, 2000; Homem and Santos, 2011; Nie et al., 2013; Rodriguez-Mozaz et al., 2015), which remain in the WWTP effluent and can reach surface waters, groundwater and sediments (Jjemba, 2006). Minor sources of antibiotics include leaching from landfills, septic systems, and sewer lines along with reuse of water for irrigation (Liu and Wong, 2013).

[Fig. 1 near here]

The dominant source of antibiotics in municipal sewage is households (about 75% in Europe and the US), followed by hospitals (5%–20%) (Kummerer, 2009a; Ashfaq et al., 2017). Most hospitals do not have on-site WWTPs and are connected to urban sewage systems (Kummerer, 2009c; der Beek et al., 2016). Although WWTPs are considered as the main source of antibiotics for surface waters, the current regulations in the EU and the US do not set limits for antibiotic concentrations in treatment plant effluents (Grenni et al., 2018). Antibiotics can be removed in WWTPs by biodegradation and adsorption by active sludge. However, antibiotics are usually poorly biodegradable and active sludge secondary sedimentation in most WWTPs seem to be inefficient, leading to antibiotic discharge to the receiving water bodies (Jones et al., 2002; Jiang, 2015). The erythromycin removal rate in wastewater treatment was lower than 5% (Zuccato et al., 2010). Several studies reported that the removal rates of tetracyclines, sulfonamides, and fluoroquinolones varied between 30%-80% (Watkinson et al., 2009; Gros et al., 2010; Zuccato et al., 2010). However, it may be difficult to compare the efficiency of water treatments because of different wastewater compositions, a wide variety of WWTPs types and treatment regimes (Janecko et al., 2016). Therefore, for the treatment of these antibiotics, advanced technologies such as chlorination, ozonation (Ikehata et al., 2006; Sharma, 2008), activated carbon filtration (Cong et al., 2013), membrane processes, advanced oxidation processes (AOPs) (Ikehata et al., 2006; Magureanu

et al., 2015), use of nanomaterials (Khin et al., 2012) and ferrate treatment (Sharma et al., 2008; Sharma et al., 2016) have been introduced. Degradation and removal methods were reviewed elsewhere (Homem and Santos, 2011; Rivera-Utrilla et al., 2013; Gadipelly et al., 2014; Manzetti and Ghisi, 2014).

The absence of sewerage systems or treatment technologies in lower income countries and rural areas can affect exposure pathways. It is a common practice in many low- and middle-income countries to discharge untreated sewage into rivers and other water bodies, and then apply the sewage-affected waters for the purpose of irrigation (Kookana et al., 2014; Binh et al., 2018). In regions where septic systems are used, contamination of groundwater may occur due to septic tank leakage (Carvalho and Santos, 2016).

Antibiotic production facilities can be a relevant source of pollution, particularly, if wastewater treatment facilities are inefficient, or if unauthorized discharges occur due to inadequate regulatory enforcement (Kookana et al., 2014). About 95% of antibiotics administered to food-producing animals have been found unmetabolized or in the form of antibiotic residues in urine and feces as well as in waste feed and water (FDA, 2016; Pulicharla et al., 2017). Manure may be subsequently spread on agricultural fields, and runoff of water from these fields may also introduce antibiotics to surface and ground waters (Isidori et al., 2005). In many countries, manure is usually stored in manure lagoons. Heavy rainfall or lagoon wall ruptures may also cause antibiotics to enter the aquatic environment (Obimakinde et al., 2017). Incidental spills, disposal of unused drugs, and atmospheric dispersal of feed and manure dust containing antibiotics are expected to be minimal sources of antibiotics to the environment in comparison to the previously mentioned sources (Carvalho and Santos, 2016).

2.2 Veterinary use

Antibiotic consumption continues to grow globally as the world´s population and its wealth increases along with demand of animal protein (Van Boeckel et al., 2015; Klein et al., 2018). Global antibiotic consumption in the livestock activity has been estimated at 63,200 tons in 2010, likely to be more than all human consumption (Van Boeckel et al., 2015; Pulicharla et al., 2017). According to a 2016 US FDA report, the total volume of antimicrobials sold for use in food-producing animals in the US was approximately 15,600 tons as an increase of 24% from 2009 to 2015 (FDA, 2016), which is about 80% of all antibiotics consumed in the US (CDDEP, 2015). The medically important antimicrobials used also in human health accounted for 62% of overall antibiotic sales for use in animals produced for food (FDA, 2016). Among the medically important antimicrobials used in the USA in 2015, 71% was tetracyclines (6,880 tons in 2015), 10% was penicillins, 6% was macrolides, 4% was sulfonamides, 4% was aminoglycosides, 2% was lincosamides, and groups representing less than 1% each included fluoroquinolones, cephalosporins, and amphenicols (FDA, 2016). And 74% of them was administered in the feed (unchanged from 2009) and 21% was administered by water (an increase from 19% in 2009). A total of 5% were administered by injection or oral, intramammary or topical application (FDA, 2016). In addition, in European countries, antibiotics were administered mainly in the form of mass treatment (premixes, oral powders, and solutions), followed by injection and intramammary preparations with 91.6%, 7.6%, and 0.5%, respectively. Pharmaceuticals for treating individual animals constitute 12% of sales in Europe (Elliott, 2015; ESVAC, 2016). About 75% of feedlots in the USA administered at least one antibiotic for promoting growth or preventing disease in 2011 (CDDEP, 2015). The 2016 European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) report (ESVAC, 2016) presents data on the sales of veterinary antimicrobials from 29 European countries in 2014, and changes in consumption for the years of 2011-2014. The overall sales in 2014 were about 9,000 tons of active

282 ingredients, of which 99.2% was used in food-producing animals, and the remaining 0.8%
283 was used for companion animals. The antibiotics used included tetracyclines (33.4%),
284 penicillins (25.5%), sulfonamides (11.0 %), macrolides (7.5%), fluoroquinolones (1.9%), and
285 cephalosporins (0.2%). Among these 29 European countries during 2011-2014, the highest
286 sales were in Spain, followed by Italy and Germany, but with an overall decrease in antibiotic
287 sales. This decrease is due to the implementation of European Union guidelines on the use of
288 antimicrobials in veterinary animals (EC, 2015), increased awareness of the problems with
289 antimicrobial resistance, and restrictions in use and changes in animal demographics
290 (ESVAC, 2016).

291 In China, a total of 150,000 to 200,000 tons of antibiotics are used every year, which
292 is approximately ten times the amount used in the US (Larson, 2015). 46% or approximately
293 97,000 tons of these antibiotics is used for the veterinary treatment and the growth promotion
294 (Liu and Wong, 2013). In 2010, China used the highest amount of antibiotics in livestock
295 globally (23%), followed by the US (13%), Brazil (9%), Germany (3%), and India (3%) (Van
296 Boeckel et al., 2015). It is expected that China, Brazil, India, US, and Indonesia will be the
297 largest antibiotic users in livestock in 2030 (CDDEP, 2015). Chickens and pigs consume
298 most of the antibiotics used in food animals globally, along with beef cattle raised in the US,
299 Brazil, and Argentina (Kookana et al., 2014).

300 In aquaculture, including the farming of aquatic organisms such as fish, mollusks,
301 crustaceans and aquatic plants, antibiotics are dosed directly into the water, primarily for
302 therapeutic purposes and prophylaxis (Kummerer, 2009c). The importance of aquaculture as
303 a source of antibiotic contamination has been thoroughly discussed in previous studies
304 (Cabello, 2006; Rico et al., 2012; He et al., 2016). According to the United Nations Food and
305 Agriculture Organization (FAO, 2015), 90% of the total global aquaculture production comes
306 from Asia (Kookana et al., 2014). The majority of this production comes from China, that

meets 80% to 90% of the world's shrimp and carnivorous fish demand (Marshall and Levy, 2011). Chile is a major producer of salmon in Americas, and it is often raised with a mixture of many antibiotics that are used also in human medicine. These antibiotics may promote the emergence of resistant bacteria in the farmed fish, and also transmit resistance to wild fish populations and the broader environment (Marshall and Levy, 2011; CDDEP, 2015).

3. Data collection methods

Data on the environmental concentrations of eight antibiotics were compiled using the Web of Science database. These eight antibiotics represent six different classes, including amoxicillin (a β-lactam), erythromycin (a macrolide), tetracycline and oxytetracycline (both tetracyclines), ofloxacin and ciprofloxacin (both fluoroquinolones), sulfamethoxazole (a sulfonamide) and trimethoprim (a diaminopyrimidine). They were selected based on a preliminary literature review that combined detection frequency in waters and data on the ecotoxicity of 30 PPCPs, including estrogens, anti-inflammatory drugs, β-blockers, lipid regulators, analgesics, and antiepileptics.

The data of erythromycin include data of its metabolite, erythromycin-H_2O, because most studies did not distinguish between these two compounds. Keywords such as "antibiotic occurrence surface water" or "effluent" or "groundwater" were used to identify publications of interest, sorted by relevance. Publications from the year 2010 to 2018 were preferred. Review articles were also used for data collection; however, primary sources were traced, and stated values were verified using the original publications. When data obtained by the primary search were not adequate, ie. Only a small dataset was obtained for several

antibiotics in specific locations and/or types of water; additional searches were conducted using more specific keywords (such as amoxicillin, China, Africa).

Mean, median, and maximal concentration values were collected, where possible, with detection frequencies. When those data were not given in the publication, all individual values were listed. Only maximum and mean values were used in Figs. 2-6. For the references containing more than one value due to the examined spatial or temporal differences, a median value was selected for groups containing measurements under the detection limit, or an average value was calculated to make sure that each study had equal weight in the graph. Studies also often contain values under the limit of detection or limit of quantification. These non-detect values were included in the calculations as one-half of the limit of detection or one-half of the limit of quantification of the corresponding study.

Data on the environmental concentrations of selected antibiotics were divided into several categories based on the sampling matrix, country, and type of data. The sampling matrix was further divided into effluent water, surface water, and groundwater. It should be noted that the groundwater data in Table S1 also includes well water data. The term "effluent water" refers to data from WWTP effluents, hospital effluents, and water from urban canals. The surface water data in Table S1 include river water, lake water, and water from aquaculture. Neither seawater nor coastal (brackish) waters were included in this analysis.

To further characterize global concentrations of antibiotics in various water matrices, a subset of the data in Table S1 were analyzed and presented graphically using 226 mean values (159 for surface water and 107 for WWTP effluents), and 382 maximum values (210 values for surface waters, and 172 for WWTP effluents). Antibiotic concentrations in groundwater are not presented graphically, due to the limited number of non-zero values. It should be noted that the effluent data include only WWTP effluents in the graphs, and excludes the data for both hospital effluents and urban canals. The surface water data in the

graphs exclude water from aquaculture, as this could contain higher levels of antibiotics and introduce bias.

Data regarding the toxicity of eight selected antibiotics were collected using the Web of Science database, with the keywords including "antibiotic ecotoxicity" and "aquatic organism". If needed, more specific searches were conducted using additional keywords (such as tetracycline, duckweed, daphnia). Table S2 presents a summary of the data collected from original research publications that used standardized ecotoxicological bioassays. For each antibiotic, the EC_{50}/LC_{50} values were compiled for six groups of organisms, i.e., green algae, cyanobacteria, aquatic plants, crustaceans, fish, and bacteria.

The toxicity data (Table S2) were used to characterize the overall toxicity of selected antibiotics towards multiple trophic groups of organisms. This was accomplished by calculating the average toxicity value for different categories of organisms (e.g., green algae and fish), and classifying antibiotics based on the average toxicity values for each group. Only values from short-term toxicity tests were used. As such, values from prolonged and chronic tests such as 21 d *D. magna* and 24 h *V. fischeri* tests and LOEC and NOEC values were excluded.

According to EU Directive 93/67/EEC (TGD, 2003), the EC50 values from the 72h algae test, the 48 h daphnid test, or the 96 h fish assay are used to classify substances according to toxicity. Substances are classified as very toxic (EC50 < 1 mg/L), toxic (EC50 = 1-10 mg/L), or harmful to the aquatic environment (EC50 = 10-100 mg/L). Compounds with EC50 > 100 mg/L and substances with NOEC > 1 mg/L in prolonged daphnid or fish assays are not classified as harmful for the aquatic environment (Magdaleno et al., 2015). According to EU Directive 93/67/EEC (TGD, 2003), the EC50 values from the 72h algae test, the 48 h daphnid test, or the 96 h fish assay are used to classify substances according to toxicity. Substances are classified as very toxic (EC50 < 1 mg/L), toxic (EC50 = 1-10 mg/L), or

harmful to the aquatic environment (EC50 = 10-100 mg/L). Compounds with EC50 > 100 mg/L and substances with NOEC > 1 mg/L in prolonged daphnid or fish assays are not classified as harmful for the aquatic environment (Magdaleno et al., 2015).

Selected examples of environmental risk assessment data obtained from publications identified in the Web of Science using keywords such as “antibiotic environmental risk assessment", sorted by relevance, and risk assessments from publications used for preparation of Tables S1 and S2 are shown in Table S3. The physicochemical properties of the selected antibiotics are presented in Table S4.

4. Concentrations in the Aquatic Environment

The concentrations of eight selected antibiotics in various aqueous media are presented in Table S1. Antibiotic concentrations in WWTP effluents, surface water, and groundwater were compiled and separated by country. There is tremendous variability in the concentrations found at different regions. In general, the concentrations of antibiotics in Asian developing countries tend to be higher than those reported in European and North American countries (Tran et al., 2018). For example, in the Patancheru industrial area near Hyderabad, India, enormously high levels of ciprofloxacin were detected in WWTP effluents (up to 14,000 µg/L) and in lakes (2,500-6,500 µg/L), and elevated levels were found also in groundwater (0.044-14 µg/L) (Fick et al., 2009; der Beek et al., 2016), compared to ng/L levels in surface and groundwaters of the US and the European Union (Andreozzi et al., 2003; Santos et al., 2010; Zuccato et al., 2010). Furthermore, ofloxacin was detected up to 160 µg/L, and trimethoprim up to 4.4 µg/L (Larsson et al., 2007; Fick et al., 2009) (Table S1). These values in India were among the highest levels ever recorded, and the concentrations of ciprofloxacin and cetirizine in surface water exceeded the human therapeutic blood plasma concentrations (Fick et al., 2009). Similar reports of high

406 concentrations of antibiotics in environmental media are available from other countries such
407 as China (Jiang et al., 2014), South Korea (Sim et al., 2011), and Pakistan (Fick et al., 2009).
408 In surface waters, oxytetracycline was detected in high concentrations of 361.1 µg/L and 56,1
409 µg/L in northern China and Colorado, USA, respectively (Karthikeyan and Meyer, 2006;
410 Jiang et al., 2014). Furthermore, the highest concentration of tetracycline, i.e., 15 µg/L, was
411 reported in northern Portugal and trimethoprim was found in concentrations up to 13.6 µg/L
412 in Laizhou Bay, China (der Beek et al., 2016) (Table S1). The groundwater samples were
413 generally collected from wells for municipal or agricultural supply. The maximal measured
414 concentrations across the regions were in units of micrograms per liter. (Stackelberg et al.,
415 2007; Finnegan et al., 2010; Jiang et al., 2014; Wang et al., 2017; Yang et al., 2018).

416 Of the eight antibiotics included in Table S1, the dominant antibiotics varied between
417 regions. For example, in the WWTP effluents in European countries, ofloxacin,
418 ciprofloxacin, sulfamethoxazole, and trimethoprim were commonly detected. In contrast,
419 erythromycin, sulfamethoxazole, and trimethoprim were relatively common in certain Asian
420 countries (South Korea, Vietnam, Indonesia, Philippines, Taiwan, Malaysia, Japan and
421 India). For China, data regarding antibiotic concentrations in effluent water were limited only
422 to tetracycline and amoxicillin; however, in surface waters, all studied antibiotics except
423 amoxicillin were detected. Erythromycin, sulfamethoxazole, and trimethoprim were the most
424 frequently detected antibiotics in the North American WWTP effluents. However, these data
425 may be biased by the preference for the determination of certain substances in different
426 regions. Therefore, the absence of certain antibiotics in the table does not necessarily mean
427 that they do not occur in the aquatic environment of that region.

428 The global occurrence data cannot be directly compared to consumption or sales data
429 as consumption and sales data are available only for whole classes of antibiotics. India,
430 China, and the US had the highest per capita human antibiotic consumption rates in 2010

(Van Boeckel et al., 2014). For animal use, high antibiotic consumption occurs in southeast China, the south coast of India, the Midwestern and Southern states in the US, and the Red River delta in Vietnam (Van Boeckel et al., 2015). Some of the highest measured concentrations of antibiotics are associated with these locations (Fick et al., 2009; Shimizu et al., 2013; der Beek et al., 2016) (Table S1).

Variability in the mean concentrations and composition of the detected antibiotics in effluents and surface waters was observed in each region. When the global data were combined and more than 20 mean antibiotic concentrations were analyzed for each antibiotic, there was no extreme difference between the concentrations of eight selected antibiotics in surface waters compared to those in WWTP effluents (Fig. 2). This suggests that, besides WWTPs, other sources such as animal feeding operations and runoff from soils fertilized by manure may also contribute to surface water pollution with certain antibiotics (Riaz et al., 2018). It is possible that this distribution was influenced by sample bias in the dataset, in which the articles showing high concentrations in surface waters were more likely to be published than those showing low contaminations.

[Fig.2 near here]

The mean and maximum concentrations of eight selected antibiotics in surface water and WWTP effluents are presented in Figs. 3 and 4, respectively. In surface waters, the 50^{th} percentile values of the mean values of these antibiotics were in the range of 10 ng/L, while the 50^{th} percentile values of the maximum concentrations were in the range of 10-100 ng/L (Fig. 3). For amoxicillin and ciprofloxacin, the 50^{th} percentile values of the mean and maximum values were similar, while for other antibiotics such as tetracycline and oxytetracycline, the values were at least an order of magnitude different. The highest maximum concentrations, i.e., 560 µg/L and 2,500 µg/L, were observed for oxytetracycline and ciprofloxacin, respectively (Fig. 3). Fig. 4 presents the concentrations of these antibiotics

456 in WWTP effluent globally. Most of the 50th percentile values of the mean and maximum
457 concentrations for each antibiotic were within the range of one order of magnitude, and the
458 highest maximum values were seen for ciprofloxacin, followed by ofloxacin and
459 trimethoprim.

460 [Figs. 3 and 4 near here]

461 Fig. 5 shows the distribution of the mean concentrations of antibiotics in surface
462 waters from multiple independent studies in Asia and Europe. Due to the scarcity of data
463 from Australia, the US and Canada, data for these countries were not included in Fig. 5 but
464 are shown in Table S1. Erythromycin, sulfamethoxazole and trimethoprim were often
465 detected in surface waters in both regions (Table S1). As seen in Fig. 5, there was a greater
466 variability in the mean values of antibiotics in Asia. For several antibiotics including
467 tetracycline, sulfamethoxazole, trimethoprim, and ofloxacin, the 50th percentile values of the
468 means were higher in Europe than in Asia. However, for tetracycline, oxytetracycline, and
469 ciprofloxacin, the 75th percentile values and the highest detected concentrations were found
470 in Asia. The 50th percentile of the mean concentrations for all eight antibiotics in both regions
471 was below 100 ng/L.

472 The distribution of the mean concentrations of eight antibiotics in WWTP effluents in
473 Asia and Europe are presented in Fig. 6. Due to the small data sets, data from the US, Canada
474 and Australia were not included in this graph. Overall, the 50th percentile values of the mean
475 antibiotic concentrations in WWTP effluents were higher in Asia than in Europe. This is
476 contrast with the data in Fig. 5, which shows that the 50th percentile values of the mean
477 antibiotic concentrations in surface water were higher for a number of antibiotics in Europe
478 than in Asia.

479 [Figs. 5 and 6 near here]

480

Only small quantities of data were available for certain antibiotics in specific geographic areas. For example, relatively little data were available for ofloxacin in water of the US, while it was more commonly detected in effluent and surface waters of Europe and Asia. The relatively small dataset for amoxicillin is not surprising. Amoxicillin belongs to β-lactam class of antibiotics that are structurally characterized by the β-lactam ring (see Table S4). Although β-lactams are the most commonly prescribed antibiotic class around the world, amoxicillin is usually not detected in surface waters as it degrades easily and is mostly removed during the wastewater treatment process. β-lactams are susceptible to degradation when exposed to light, heat, extreme pH, and solvents like water and methanol. Therefore, β-lactam antibiotics hydrolyze easily under environmental conditions and only low levels are usually detected in the water despite their high consumption (Milic et al., 2013; Tran et al., 2018). Nonetheless, it has been detected in some European and Australian surface waters. The highest concentration of amoxicillin identified for this review was 1.67 µg/L (see Table S1), which was detected in effluent water entering Victoria Harbor in Hong Kong, China (Minh et al., 2009).

Tetracyclines are also known for their relatively low environmental stability (Halling-Sorensen et al., 2002; Werner et al., 2006). Tetracycline and oxytetracycline were rarely detected in surface water and WWTP effluents in some regions. For example, concentrations of tetracycline in surface waters of North American were below the limit of detection in at least four studies (Hirsch et al., 1999; Haggard et al., 2006; Lissemore et al., 2006; Finnegan et al., 2010) and only one mean and one maximum concentration higher than limit of detection of 0.11 µg/L and 0.30 µg/L were collected in this review (Kolpin et al., 2002; Kolpin et al., 2004). In both studies, tetracycline was detected among 1.2% and 6.7% of the samples, respectively. Oxytetracycline was detected only in two studies of river waters in North American (Lindsey et al., 2001; Kolpin et al., 2002), while the concentrations were

lower than limit of detection for other cases (Kolpin et al., 2002; Haggard et al., 2006; Finnegan et al., 2010).

Photodegradation is considered an important fate for most antibiotics found in the aquatic ecosystem (Baran et al., 2006; Fick et al., 2009, Trovo et al., 2009; Yan and Song, 2014; Yun et al., 2018). Ciprofloxacin half-life is dependent on pH, which was explained by its amphoteric nature (Torniainen et al., 1996). The main degradation product of photolysis is a compound that replaces the entire piperazinyl ring with an amino group. Sulfomethoxazole half-life ranges from 10 h to more than 100 h; presence of dissolved organic matter, especially humic acid, accelerates the degradation (Straub, 2016). Based on their Koc values, sulfonamides and trimethoprim are expected to have high mobility, whereas erythromycin, tetracyclines, and fluoroquinolones are expected to have low mobility and to adsorb to suspended solids and sediment in the water (Table S4).

5. Ecotoxicity to aquatic organisms

5.1. Toxicity of Individual Antibiotics

Algae and cyanobacteria, as primary producers, play an important role as the base of the food chain in aquatic ecosystems (Yang et al., 2013). Their roles also include oxygen production and nitrogen fixation. Any alteration to the community of photoautotrophic organisms may result in severe bottom-up effects on other organisms at higher trophic levels (Nie et al., 2013; Valitalo et al., 2017; Binh et al., 2018). Therefore, determination of the toxicity to non-target species is crucial to understand the ecosystem effects of antibiotics. Blue-green algae (cyanobacteria) are prokaryotes and are therefore considered sensitive to antibiotics due to their close relationship to pathogenic bacteria (Jones et al., 2002; Gonzalez-Pleiter et al., 2013). The individual modes of action of antibiotics towards bacteria (prokaryotes) are well known and may explain some effects on cyanobacteria. Although

green algae are eukaryotes and the mechanism of toxicity to green algae is different (Gonzalez-Pleiter et al., 2013), antibiotics may still cause adverse effects to green algae due to the prokaryotic origin of semi-autonomous organelles such as chloroplasts and mitochondria (Nie et al., 2013). Thus, the toxic effects of antibiotics to green algae are related to the inhibition of chloroplast metabolisms such as protein synthesis and photosynthesis, affecting cell growth (Halling-Sorensen, 2000; Liu et al., 2011; Nie et al., 2013; Wan et al., 2015).

The toxicity data available in the literature are summarized in Table S2. The effective concentrations vary depending on the test method and organism, suggesting that antibiotic toxicity should be assessed with multiple bioassays for a more comprehensive analysis (Valitalo et al., 2017). The data show that green algae are more sensitive to these eight antibiotics than crustaceans and fish, and overall, cyanobacteria are more sensitive than green algae (Fig. 7). For example, the cyanobacterium *Microcystis aeruginosa* (*M. aeruginosa*) is two to three orders of magnitude more sensitive to fluoroquinolones than green alga *Pseudokirchneriella subcapitata* (*P. subcapicata*) (Robinson et al., 2005). The lowest reported EC_{50} values for ciprofloxacin were 0.005 mg/L (Jiang et al., 2014) and 1.1 mg/L (Yang et al., 2008) for *M. aeruginosa* and *P. subcapicata*, respectively. *M. aeruginosa* was also found to be more sensitive than *P. subcapitata* to erythromycin and oxytetracycline, but not tetracycline (Fig. 7). It has been proposed that cyanobacteria should be used as a sensitive screening tool for identifying antibiotic toxicity in the environment (Xiong et al., 2019). For example, the European Medicines Evaluation Agency (EMEA) explicitly recommends the use of cyanobacteria for testing of antimicrobials (EMEA, 2006). However, cyanobacteria grow more slowly than green algae, thus necessitating a prolonged growth period of up to 7 days. As such, this is not a rapid screening technique. Additionally, the prolonged exposure may influence toxicity, with the lower EC_{50} values seen following longer exposure time

556 (Robinson et al., 2005). It may be possible to reduce the exposure time to 24 h with
557 maintaining high sensitivity using cyanobacteria to evaluate the change in photosynthetic
558 activity rather than growth inhibition (van der Grinten et al., 2010).

559 Studies have shown that green algae are not susceptible to all antibiotics. Most studies
560 have found that β-lactam antibiotics such as amoxicillin do not affect green algae, with EC_{50}
561 values greater than 1 g/L (Gonzalez-Pleiter et al., 2013; Magdaleno et al., 2015). This is
562 likely to be due to the fact that the mode of action of β-lactam antibiotics is inhibition of
563 bacterial cell wall synthesis (Gonzalez-Pleiter et al., 2013). Algae and aquatic plants such as
564 duckweeds (*Lemna sp*.) showed a similar level of sensitivity to sulfamethoxazole,
565 tetracycline, and oxytetracycline, and in some cases, the aquatic plants may be more
566 sensitive. For example, the EC_{50} values for ofloxacin and ciprofloxacin have been found to be
567 between 0.1 mg/L and 0.7 mg/L for *Lemna minor* (Robinson et al., 2005; Brain et al., 2008).
568 These values are one order of magnitude lower than the lowest measured EC_{50} values for *P.*
569 *subcapitata,* which were 1.1 mg/L and 1.4 mg/L for ciprofloxacin and ofloxacin, respectively
570 (Isidori et al., 2005; Yang et al., 2008). Care must be taken in comparing toxicity values for
571 algae and plants, as assays often use different testing conditions, including different media,
572 incubation times, and measured endpoints. Duckweed (*L. minor* and *L. gibba*) toxicity tests
573 usually determine growth inhibition by frond number counts after 7 d of exposure, eventually
574 supplemented with dry or wet biomass weight. In contrast, algal toxicity tests evaluate the
575 growth rate by cell counts, usually as absorbance or chlorophyll fluorescence measurements
576 after 72 h. Moreover, algal and duckweed bioassays are performed at different pH
577 (approximately 7.0 for algae and 5.5 for duckweed assays). This can also affect the toxic
578 potential of antibiotics, based on their p*K*a values (see Table S4). For example,
579 sulfamethoxazole produced greater growth inhibition of *P. subspicata* at a lower pH (Bialk-
580 Bielinska et al., 2011), as its pKa_2 value is 5.7. The effect of pH on the antibiotic toxicity is

581 thoroughly described elsewhere (Lutzhoft et al., 1999) and therefore it is not explained here.
582 Since experimental conditions may influence the results of ecotoxicological bioassays,
583 detailed test conditions (such as pH, temperature, lighting conditions, and duration) should be
584 listed to allow comparison of the results, both within species, and between species.

585 It has been reported that many antibiotics are photosynthesis inhibitors as they can
586 block the photosystem II electron transport chain (Nie et al., 2013). Furthermore, excited
587 chlorophyll molecules can induce the formation of reactive oxygen species (ROS) and cause
588 oxidative stress. ROS removal is regulated by enzymatic antioxidants such as catalase,
589 superoxide dismutase, and glutathione (GSH)-specific peroxidase and enzymes involved in
590 the ascorbate-GSH cycle as well as non-enzymatic antioxidants, such as ascorbate and GSH
591 (Nie et al., 2013). Although the mode of action of antibiotics is well known in bacteria,
592 information about induction of oxidative stress in algae is limited. The most detailed study of
593 antibiotic toxicity to the algal antioxidant system was conducted by Nie and colleagues (Nie
594 et al., 2013), who studied the toxic effects of erythromycin, ciprofloxacin, and
595 sulfamethoxazole in green algae. One study found that erythromycin was the most toxic to
596 the antioxidant system of *P. subcapitata*, causing a significant decrease in ascorbic acid
597 (ASA) and GSH content (Nie et al., 2013). ASA and GSH are able to eliminate ROS through
598 multiple mechanisms and are also responsible for regulation of redox homeostasis. Nie and
599 colleagues (Nie et al., 2013) presumed that erythromycin interfered with ASA and GSH
600 biosynthesis, leading to oxidative stress. In contrast, algal cells exposed to ciprofloxacin and
601 sulfamethoxazole showed the increased levels of ASA and GSH, so toxicity to *P. subcapitata*
602 was much lower following exposure to ciprofloxacin or sulfamethoxazole than erythromycin
603 exposure (Nie et al., 2013). Similar effects were also found in *Chlorella vulgaris* following
604 exposure to ciprofloxacin, which affected GSH, GST, and catalase content dependent on the
605 exposure dose (Nie et al., 2008). Similarly, amoxicillin evoked antioxidant responses via

606 generation of excessive ROS and inhibited the the synthesis of GSH and GST in *M.*
607 *aeruginosa* (Liu et al., 2015).

608 Since antibiotics target bacteria, it might be expected that bacterial bioassays would
609 be a great tool for assessing the toxicity of antibiotics in the environment. However, the
610 Microtox assay showed low sensitivity to antibiotics (Ferrari et al., 2004; Isidori et al., 2005).
611 In contrast to the relatively high toxicity of antibiotics to algae and aquatic plants, antibiotics
612 produced the low acute toxicity in short-term toxicity tests against the luminescent marine
613 bacterium *Vibrio fischeri* (*V. fisheri*). The EC_{50} value was greater than 20 mg/L for
614 sulfamethoxazole and oxytetracycline and greater than 100 mg/L for erythromycin and
615 ofloxacin in 30 min of assay, and greater than 100 mg/L for ciprofloxacin and amoxicillin in
616 15 min of assay (Isidori et al., 2005; Christensen et al., 2006; de Garcia et al., 2014; Borecka
617 et al., 2016) (see Table S2). The insensitivity of *V. fisheri* to antibiotics is caused most likely
618 by short exposure time, during which the mechanism of action of antibiotics will not be
619 demonstrated. However, in a 24 h assay, the toxic effect was detected following exposure of
620 *V. fischeri* to 81 μg/L oxytetracycline or 0.014 μg/L ofloxacin, which are environmentally
621 relevant concentrations (Ioele et al., 2016). As such, the 15 min or prolonged 30 min
622 Microtox test and the 30 s Microtox Flash test appeared to be unsuitable for evaluating the
623 toxicity of some of these antibiotics. Longer exposure times should be used for assessing
624 antibiotic toxicity to *V. fisheri* in order to get more reliable effect concentrations.

625 Antibiotics induced relatively low acute toxicity in invertebrates such as cnidaria
626 (*Hydra attenuata*) and crustaceans (*Artemia salina, Daphnia magna,* and *Ceriodaphnia*
627 *dubia*). Seven-day chronic toxicity assays with *C. dubia* showed high toxicity of
628 erythromycin, sulfamethoxazole, and oxytetracycline with EC_{50} values < 1 mg/L (Table S2).
629 Only a few studies have evaluated the chronic toxicity of antibiotics to sediment-dwelling
630 organisms (Ferrari et al., 2004; Isidori et al., 2005; Rhee et al., 2013). Many studies showed

631 that antibiotics are unlikely to affect vertebrates at environmentally relevant concentrations
632 (Crane et al., 2006). Acute toxicity to fish was found only at high concentrations, and in some
633 cases, no toxicity to fish was observed (Robinson et al., 2005; Santos et al., 2010; Brausch et
634 al., 2012; Minguez et al., 2016) (Table S2).

635 Trimethoprim showed relatively low toxicity toward all tested organisms, while the
636 other antibiotics in this review were classified as "very toxic" for at least one class of
637 organisms (see Fig. 7). Crustaceans, fish, and the bacterium *V. fischeri* were not found to be
638 very sensitive to antibiotics discussed in this review at the concentrations tested. It is not
639 possible to make a final determination about the potential harm posed by these antibiotics to
640 the less sensitive organisms, as data are often reported as EC_{50} greater than a highest tested
641 concentration under these test conditions, implying low toxicity. The hazard classification of
642 antibiotics for this review used the highest tested concentrations reported as the EC_{50} values
643 to calculate means; therefore, these values may be underestimated. Erythromycin,
644 tetracycline, and oxytetracycline are ranked as "toxic" to algae and aquatic plants, and "very
645 toxic" to cyanobacteria. Tetracycline´s lower toxicity to cyanobacteria may be affected by the
646 small number of data points, as only two EC_{50} values were reported: the 7 d EC_{50} value of
647 0.09 mg/L for *M. aeruginosa,* and the 72 h EC_{50} value of 6.2 mg/L for *Anabaena sp*. It should
648 be noted that 7 d assays with *M. aeruginosa* were not excluded from the evaluation. The
649 reason is that prolonged tests are usually necessary to achieve test validity according to ISO
650 and OECD standards (ISO, 1989; OECD, 2011) to meet the criteria of at least a 16-fold
651 increase in cell numbers for controls (Halling-Sorensen, 2000; Robinson et al., 2005; Yang et
652 al., 2008).

653 Two fluoroquinolones, i.e., ciprofloxacin and ofloxacin, were found to exhibit the
654 highest hazard for the aquatic environment, with the highest toxicity among cyanobacteria,
655 lesser toxicity to aquatic plants, and the lowest toxicity to algae. Sulfamethoxazole can also

656 be classified as very toxic to photosynthetic organisms, with aquatic plants being the most
657 sensitive class, followed by cyanobacteria and algae. Cyanobacteria were found to show the
658 greatest susceptibility to fluoroquinolones and amoxicillin, with the mean EC_{50} values in the
659 range of µg/L (Halling-Sorensen, 2000; Robinson et al., 2005; Brain et al., 2008; Guo et al.,
660 2015). This is close to the environmentally relevant concentrations of amoxicillin, ofloxacin,
661 and ciprofloxacin (Fick et al., 2009; Sim et al., 2011; Leung et al., 2012; Petrie et al., 2015;
662 der Beek et al., 2016) (Tables SM-1 and SM-2).
663
664 *5.2 Environmental risk assessment (ERA)*
665 Guidelines for safe water quality concentrations of most PPCPs are generally lacking.
666 In the Europe, the EU Water Framework Directive was adopted in 2000 (Directive
667 2000/60/EC) to accomplish high water quality. The chemical status of waters is evaluated
668 based on environmental quality standards, that have been set for 45 priority substances.
669 Recently, the first watchlist of substances to be monitored in the field was launched and
670 include also PPCPs, inclusive of three macrolide antibiotics – erythromycin, clarithomycin,
671 and azithromycin (Loos et al., 2015). Both the US and European regulatory guidances require
672 ERA of new pharmaceuticals using standard acute toxicity tests, if the measured or predicted
673 environmental concentration (MEC or PEC) of the active ingredient is higher than 0.01 µg/L
674 or 1 µg/L for the European (EMEA) and US (FDA) legislation, respectively (FDA, 2003;
675 EMEA, 2006). Based on the important ecological function of natural microbial communities,
676 ERA should use more endpoints targeting bacteria (Grenni et al., 2018).
677 Risk quotients (RQs) are used for estimating adverse effects to non-target organisms,
678 based on given environmental levels and description of potential ecological risk, RQs identify
679 potential hazardous substances and their estimated concentrations in a specific environment
680 (i.e., exposure assessment) and their health effects (i.e., toxicity) (Jjemba, 2006). RQs are

681 calculated as the ratio between PEC (or MEC) and predicted no-effect concentration (PNEC).
682 PNECs are usually calculated by dividing toxicological dose descriptors by an assessment
683 factor. When only short-term toxicity data are available, an assessment factor of 1000 will
684 be applied on the lowest EC50 available. Assessment factor of 100 applies if a single long-
685 term NOEC data are available, and factor of 10 when the long-term toxicity NOECs are
686 available from at least three species from different trophic levels. Assessment factor 1 – 5 is
687 used when data are obtained by species sensitivity distribution method (TGD, 2003). The risk
688 is classified into three levels, i.e., low risk (RQs = 0.01-0.1) medium risk (RQs = 0.1-1) and
689 high risk with RQs > 1 (Jiang et al., 2014). If the ratio is equal or higher than 1, it suggests
690 that the assessed substance could cause potential adverse ecological effects and an additional
691 (Tier B) assessment using terrestrial tests is required to obtain more data for risk evaluation
692 (Gros et al., 2010). The detailed description of pharmaceutical risk assessment process is
693 described elsewhere (de Garcia et al., 2014; Kuster and Adler, 2014; Straub, 2016; Zhao et
694 al., 2016; Wang et al., 2017; Yao et al., 2017). Table S3 presents RQs determined for eight
695 selected antibiotics in various types of water and with organisms. Except for trimethoprim, all
696 studied antibiotics showed a high risk to the aquatic environment in at least one of the
697 presented ERAs. All RQs > 1 were calculated for algae or cyanobacteria in both surface
698 water and effluents, and in one case for the bacterium *P. putida* in surface water. In several
699 cases, values of RQ greater than 10 were shown (Jones et al., 2002; Ferrari et al., 2004; Gros
700 et al., 2010; Waiser et al., 2011; Guo et al., 2015).
701
702 *5.3. Ecotoxicity of antibiotic mixtures*
703 Various classes of antibiotics and other PPCPs have been detected simultaneously in
704 aquatic ecosystems (Kolpin et al., 2004; Gothwal and Shashidhar, 2015; Barbosa et al., 2016;
705 der Beek et al., 2016). Therefore, aquatic organisms may be exposed to mixtures of

706 pharmaceuticals, which should be taken into account during ERA strategies by evaluating the
707 individual effect and joint behavior (Magdaleno et al., 2015; Valitalo et al., 2017). While the
708 concentrations of individual antibiotics in aquatic environments may be too low to show an
709 effect, the combined effect could result in significant toxicity to aquatic species even at the
710 concentrations below the individual NOECs (Backhaus et al., 2011; Geiger et al., 2016). This
711 may severely underestimate the risks associated with antibiotic mixtures and their mixtures
712 with other pharmaceuticals or anthropogenic contaminants (Gonzalez-Pleiter et al., 2013).
713 Especially, two and more antibiotics are sometimes administered simultaneously as a
714 combined drug, such as sulfamethoxazole and trimethoprim. This suggested the necessity to
715 evaluate the mixture toxicity.

716 Several authors have called for mixture toxicity testing as a part of the pharmaceutical
717 risk assessment, as PPCPs are likely to be found in combinations in the environment (Cizmas
718 et al., 2015; Prosser and Sibley, 2015; Backhaus, 2016; Watanabe et al., 2016). However,
719 despite the obvious importance of understanding the effects of chemical mixtures in the
720 environment, there seems little justification for treating pharmaceuticals differently to other
721 industrial and plant protection substances, which may also be found in environmental
722 mixtures (Crane et al., 2006). The effects of mixtures of PPCPs in algae have been studied
723 (Yang et al., 2008; Gonzalez-Pleiter et al., 2013; Magdaleno et al., 2015) and some research
724 is done in the toxicity of antibiotics in combination with other groups of pollutants
725 (Backhaus, 2016; Geiger et al., 2016; Watanabe et al., 2016). In most cases, the joint effects
726 of antibiotics to green algae revealed additive toxicities (Cleuvers, 2004; DeLorenzo and
727 Fleming, 2008; Backhaus et al., 2011).

728

729 **6. Conclusions and future perspectives**

Among pharmaceuticals, antibiotics are one of the most widely used classes of drugs, both for human and veterinary use. Total global antibiotic use is increasing and is expected to further grow due to the increasing world population and the need for greater food production. After their use, significant amounts of the antibiotics eventually find their way into the environment. Although there are a relatively large number of data regarding antibiotic occurrence in the aquatic environment of North American and European countries, data from five BRICS countries that have major emerging economies (Brazil, Russia, India, China, and South Africa) are limited to China and India. Data on antibiotic concentrations from Brazil (and the rest of South America), Russia, and Africa are not presented in this review, due to the limited data from these areas. As the consumption of antibiotics is expected to grow, more studies are needed to assess the occurrence of antibiotics in different types of water in these countries.

Asian countries have relatively high mean concentrations of most of the antibiotics discussed in this review (see Figs. 5 and 6), and China and India are important contributors to the antibiotic load in this region (Table S1). These countries have a limited regulation of antibiotic use for growth promotion and they do not require a veterinary prescription for their use in food animals (Leung et al., 2012). This is likely to lead to antibiotic overuse, resulting in higher loads to the aquatic environment, which can contribute to the emergence of antibiotic resistance and disruptions in the aquatic environment.

More than 20% of the total world pharmaceutical production is originated from China. The areas with production facilities are important locations for studying the long-term impact of antibiotics and their mixtures on the ecosystems, including bacterial resistance development due to a lack of adequate treatment facilities, and the occurrence of unauthorized discharges as a result of inadequate regulatory enforcement (Liu and Wong,

754 2013; He et al., 2016). Moreover, current regulatory systems on pharmaceutical pollution do
755 not account antibiotic resistance (Kuppusamy et al., 2018).

756 Many low- and middle-income countries are substantive exporters of food animals
757 and food products. The use of antibiotics is in large quantities in agriculture, industry and
758 household products for reasons largely unrelated to human health (Limmathurotsakul et al.,
759 2019). In low- and middle-income countries and especially in rural areas, there is a lack of
760 skilled medical workers. Furthermore, majority of these counties have minimal or no
761 programs to monitor antibiotic use and their occurrence in food products and in the
762 environment (Founou et al., 2016). However, the issue of antibiotic overuse is not limited to
763 developing countries. In many high- and middle-income countries, prophylactic antibiotics
764 are used extensively in routine medical procedures (Chokshi et al., 2019). International health
765 organizations encourage all countries to reduce their use of antibiotics in both humans and
766 animals to a minimum, but the ease of availability of antibiotics and limited public
767 understanding of antibiotic resistance are likely the major barriers to decrease inappropriate
768 antibiotic use. Any reduction in antibiotic consumption would lead to proportional reduction
769 in antibiotics released into wastewater (Chokshi et al., 2019; Limmathurotsakul et al., 2019;
770 Singer et al., 2019).

771 The WHO published the global action plan on antimicrobial resistance in 2015 that
772 aims to reduce antibiotic use and misuse in human, animal and agriculture. The main
773 objectives are to educate the prescribers and users about the prudent use of antibiotics, to
774 strengthen the knowledge via surveillance and research, to develop policies that focus on
775 lowering the use of antibiotics in the veterinary sector, and to develop new sorts of antibiotics
776 and preventing the transmission of resistant microorganisms (van Rijn et al., 2019). As each
777 country has a different health care and regulatory system, all solutions must start with
778 changes in local practices and then be implemented globally (Kuppusamy et al., 2018). The

779 manufacturing process, quality, availability, and use of antibiotics need to be further
780 controlled in low- and middle-income countires, whilst hospital-based interventions and
781 antibiotic use in food-producing animals should be regulated as a priority (Chokshi et al.,
782 2019).

783 The effects of antibiotics on aquatic organisms are usually tested by standardized
784 ecotoxicological bioassays used to determine acute toxicity. Overall, algae and cyanobacteria
785 are relatively sensitive organisms, with EC_{50} values in the range of μg/L-mg/L (Fig. 7 and
786 Table S2). These values are relatively high, as antibiotics in surface waters are usually
787 detected in the range of tens to hundreds of ng/L. However, there are several cases of risk
788 assessment, usually for WWTP effluents, showing that adverse effects could occur (Ferrari et
789 al., 2004; Robinson et al., 2005; Gros et al., 2010; Waiser et al., 2011; Guo et al., 2015; Chen
790 et al., 2018). Moreover, lifelong exposure of aquatic organisms to antibiotics may produce
791 chronic health effects, which is not considered when evaluating hazards for the environment.
792 The mixture effects of antibiotics among themselves and with other contaminants might also
793 influence the toxicity and should be further studied.

794 Cyanobacteria were proved as the most sensitive organisms and have been proposed
795 as the suitable organisms for testing pharmaceutical toxicity. The disadvantage is that the
796 cyanobacterial assay requires a longer exposure time due to the slower growth of
797 cyanobacteria (usually 5 to 7 days) compared to green algae (72 h assay). For toxicity
798 screening assays, it was proposed to change the endpoint to 24 h photosynthetic activity
799 instead of cell growth. Another argument for the use of cyanobacteria instead of green algae
800 for antibiotic toxicity testing is that algae are not sensitive to all types of antibiotics. For
801 example, β-lactam antibiotics inhibit bacterial cell wall synthesis, however they may still
802 cause adverse effects to green algae due to the prokaryotic origin of organelles such as

803 chloroplasts and mitochondria. Although the mode of action of antibiotics is well known in
804 bacteria, the mechanism of toxicity to algae needs further research.

805 The standardized Microtox toxicity test does not appear to be suitable for antibiotic
806 testing. It uses the bacterium *V. fischeri* which is not sensitive to antibiotics in the typical 15-
807 30 min of testing timeframe, and inhibition of bioluminescence is not related to the mode of
808 action of most antibiotics. Therefore, prolonged assays with a 24 h exposure period should be
809 used, or there are several other standardized bacterial toxicity tests, including the Activated
810 Sludge Respiration Inhibition test OECD 209 (OECD, 2010), Toxicity Test for Assessing the
811 Inhibition of Nitrification of Activated Sludge Microorganisms ISO 9509, and the
812 *Pseudomonas putida* Growth Test ISO 10712 (ISO, 1995), which might give more reliable
813 effect concentrations. Since the experimental conditions may influence the results of
814 bioassays, we recommend that publications include standardized detailed test conditions
815 (such as pH, temperature, lighting conditions, and duration) to allow comparison of the
816 results. Data are scarce regarding the toxicity of the non-therapeutic (low-concentration)
817 effects of antibiotics towards non-target species as well as the lifetime exposure assays with
818 aquatic organisms (Wollenberger et al., 2000; Crane et al., 2006; Carvalho and Santos, 2016;
819 Watanabe et al., 2016). Those effects are not taken into account when evaluating hazards for
820 the environment. The presence of antibiotic mixtures may also influence the toxicity, and
821 further studies are required to cover these important knowledge gaps.
822

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828

829 **Disclosure statement**

830 No potential conflict of interest was reported by the authors.

831

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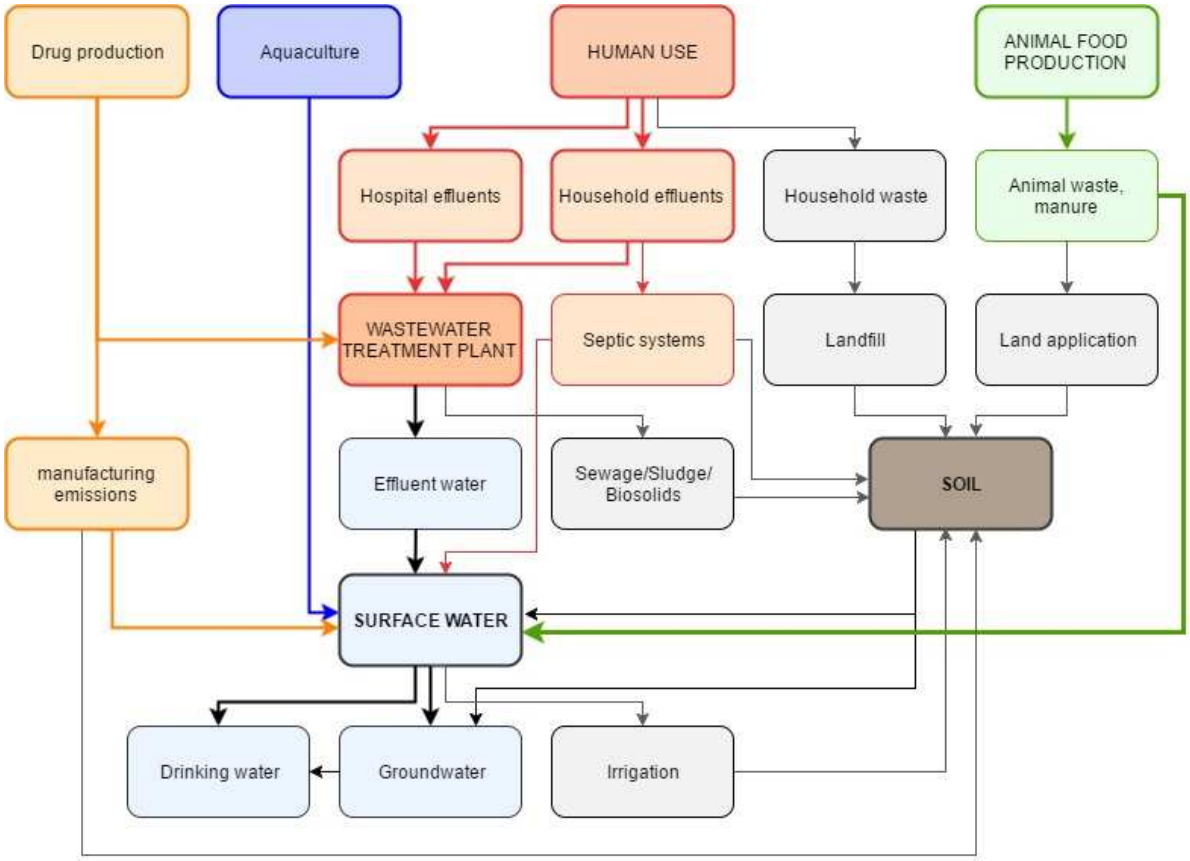


1348

1349 **Fig. 1.** Fate of the antibiotics in the environment. Transport of antibiotics intended for human
1350 consumption, animal food industry, aquaculture, and manufacturing to the surface waters are represented
1351 by red, green, blue and orange arrows, respectively. Grey arrows show transport to the terrestrial
1352 environment, black arrows inside the aquatic environment.

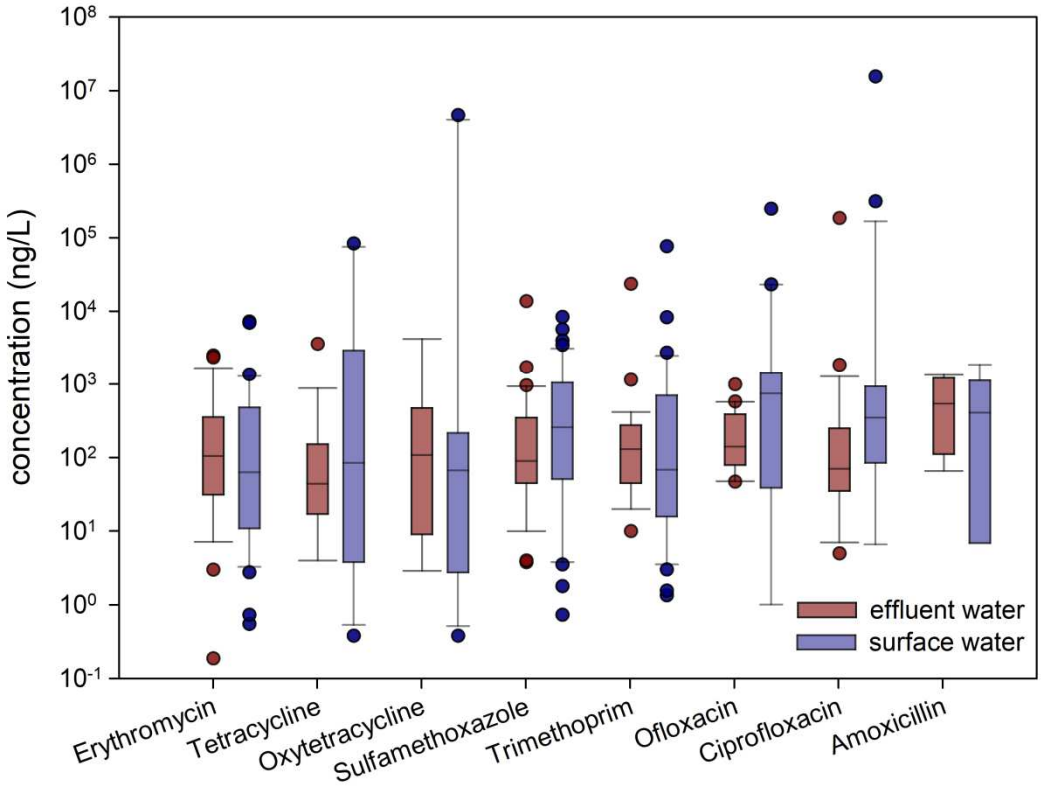


Fig. 2. Comparison of mean global concentrations of eight antibiotics (erythromycin, tetracycline, oxytetracycline, sulfamethoxazole, trimethoprim, ofloxacin, ciprofloxacin, and amoxicillin) detected in multiple independent studies of wastewater treatment plant effluents and surface waters. This presents the distribution of 266 mean antibiotic concentrations shown in Table 1, including 159 values for surface water and 107 values for wastewater treatment plant effluents. The boxes present the 25th, 50th and 75th percentile values, while the whiskers present the 10th and 90th percentiles. The values presented by dots outside the whiskers are considered outliers. Note that for erythromycin, trimethoprim, ofloxacin, and amoxicillin, the 50th percentile concentrations in effluent water are higher than in surface water. In contrast, for tetracycline and oxytetracycline the concentrations in surface waters are higher than in wastewater treatment plant effluent, suggesting that sources other than wastewater treatment plants may contribute to surface water concentrations of these antibiotics. However, it should be noted that the wastewater and surface water samples were not necessarily collected from locations near each other, so that the values may not be directly comparable. In addition, there may have been sampling bias, leading to the preferential sampling of more highly contaminated areas. The highest mean values (shown in Table S1 and presented here as outliers) were seen for oxytetracycline and ciprofloxacin in surface water.

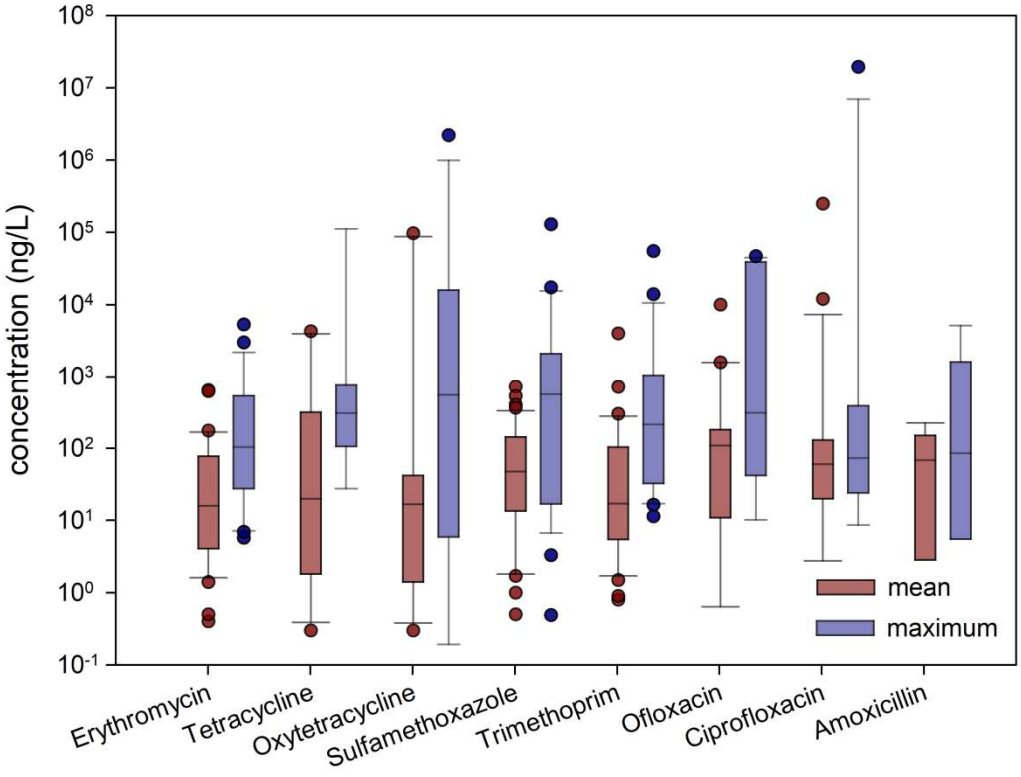


Fig. 3. Global mean and maximum concentrations of eight antibiotics detected in surface waters in multiple independent studies. These box plots present the distribution of 159 mean antibiotic concentrations and 210 maximum antibiotic concentrations in surface water (data also shown in Table 1). The boxes present the 25th, 50th and 75th percentile values, while the whiskers present the 10th and 90th percentiles. The values represented by dots outside the whiskers represent outliers. For all reviewed antibiotics, the differences between the 50th percentile of the maximum concentration values and the 50th percentile of the mean concentrations were relatively small. The highest concentrations were seen for oxytetracycline and ciprofloxacin in both maximum and mean concentrations.

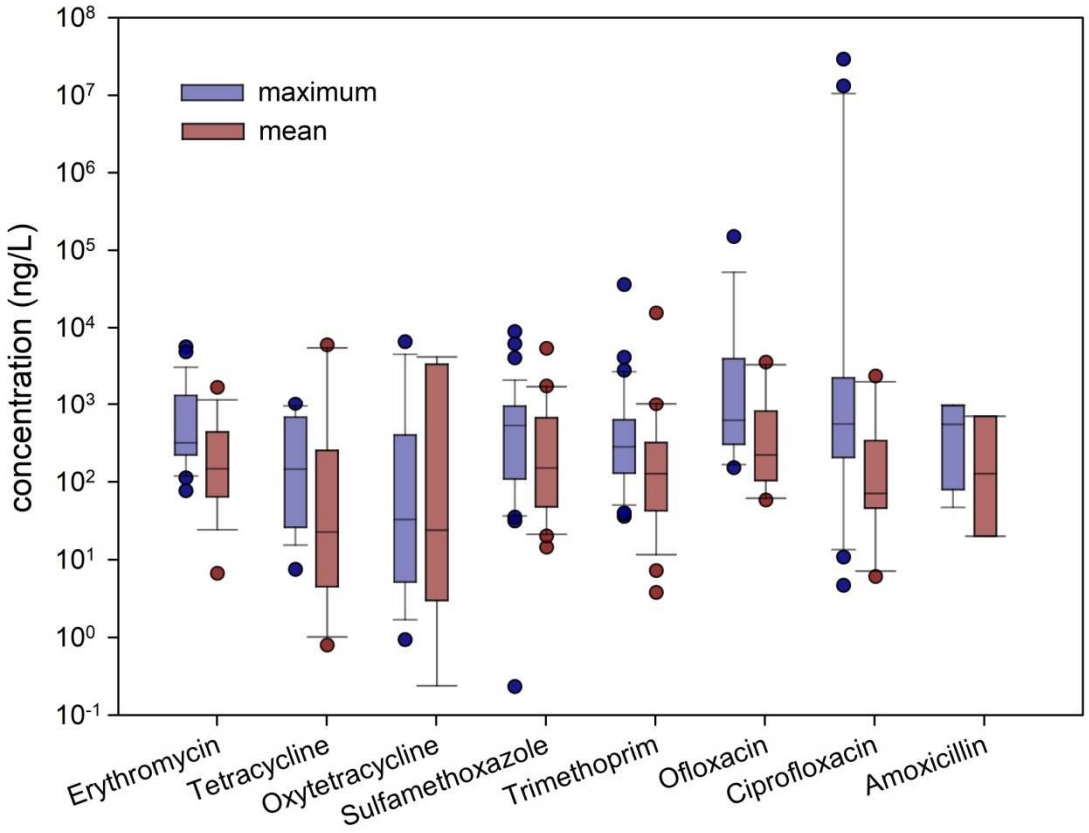


Fig. 4. Global mean and maximum concentrations of selected antibiotics in wastewater treatment plant effluents in multiple independent studies. Due to the insufficient dataset, data from North America (the US and Canada) and Australia were not shown in graphs. These box plots present the distribution of 107 mean antibiotic concentrations and 172 maximum antibiotic concentrations in wastewater treatment plant effluents (data also shown in Table S1). The boxes present the 25th, 50th and 75th percentile values, while the whiskers present the 10th and 90th percentiles. The values represented by dots outside the whiskers represent outliers. For oxytetracycline, the 50th percentile value was similar for the mean and maximum values, while for other antibiotics such as tetracycline and ciprofloxacin, the mean and maximum values showed greater variability. The highest maximum values were seen for ciprofloxacin, followed by ofloxacin and trimethoprim.

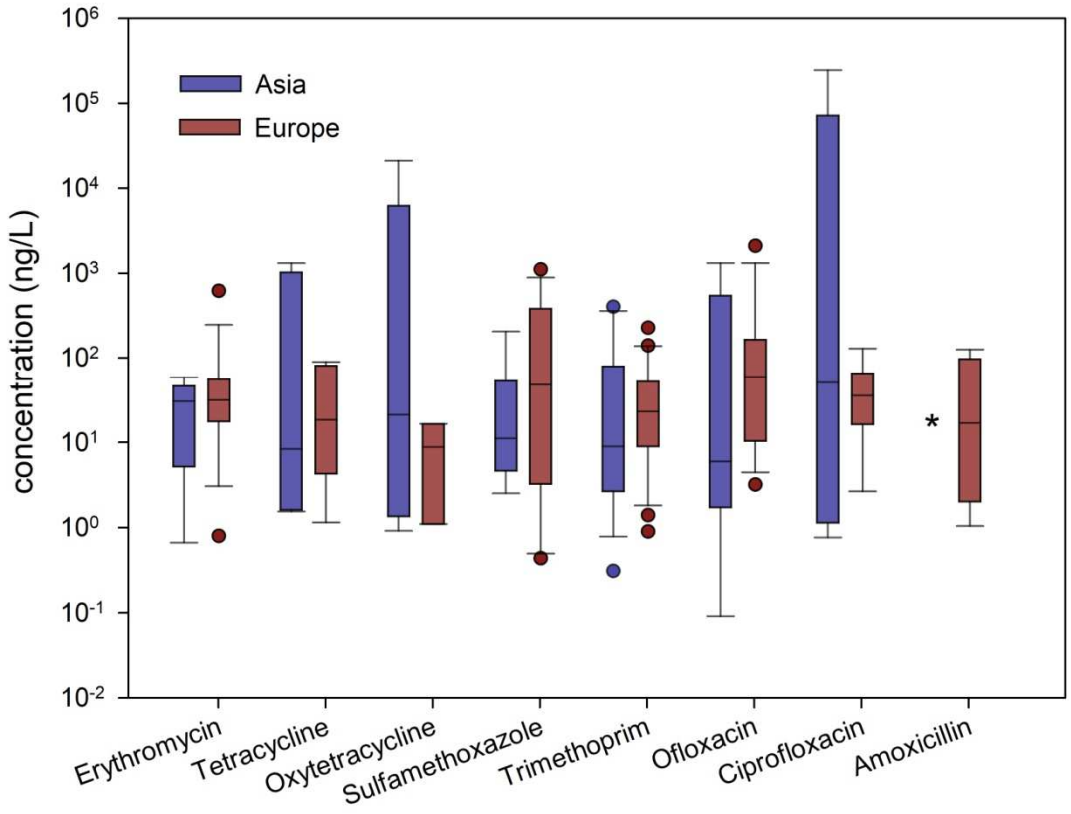
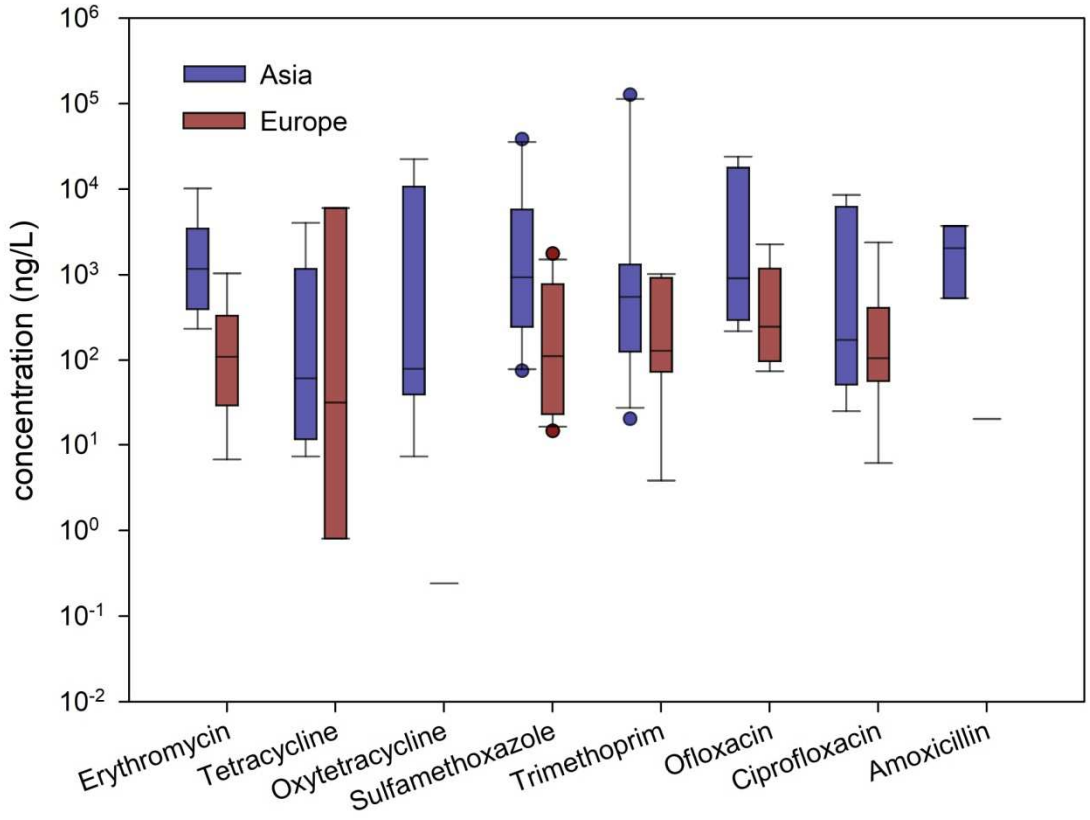


Fig. 5. Mean concentrations of selected antibiotics detected in surface waters in Asia (data from China, South Korea, India, Vietnam, and Malaysia) and Europe (data from United Kingdom, Spain, France, Germany, Italy, Portugal, and Poland). Due to an insufficient dataset, data from North America (the US and Canada) and Australia are not included. It should also be noted that no data were available for amoxicillin concentrations in surface waters in Asia, so this bar is not included in the graph. The range of mean concentrations was notably larger in Asia for tetracycline, oxytetracycline, trimethoprim, ofloxacin, and ciprofloxacin, indicating greater variability in the concentrations of these contaminants in surface waters across the region. It should be noted that there may have been sampling bias, leading to the preferential sampling of more highly contaminated areas, such as rivers and lakes near pharmaceutical production plants or animal feedlots, bringing extreme concentration values to the graph (see Table S1). Note that for a number of the compounds shown here, the 50th percentile values were similar in Europe and Asia, or lower in Asia.

* Note: for amoxicillin, no data were available for surface waters in Asia.



1404

1405 **Fig. 6.** Distribution of the mean concentrations of selected antibiotics in wastewater treatment plant
1406 effluents in Asia (data from China, South Korea, Vietnam, Indonesia, Philippines, India, and Malaysia)
1407 and Europe (data from United Kingdom, Spain, France, Italy, Portugal, Germany, Greece, Croatia, and
1408 Switzerland). It should be noted that there was only one mean concentration value available for
1409 oxytetracycline and amoxicillin in European waste water treatment plant effluents. For all reviewed
1410 antibiotics where multiple values were available in Europe, the 50th percentile values were higher in Asia
1411 than in Europe. However, there may have been sampling bias, leading to the preferential sampling of more
1412 highly contaminated effluents, so that the values may not be representative of the concentrations in all
1413 wastewater treatment plant effluents (see Table S1).

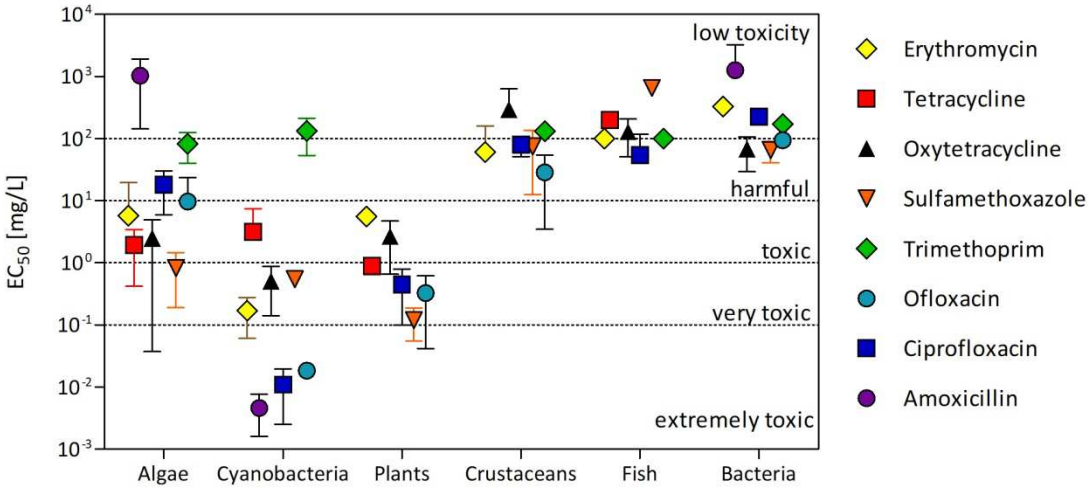


Fig. 7. Ecotoxicity of selected antibiotics towards different groups of organisms as assessed in multiple independent studies. The EC_{50} values are mean concentrations expressed in mg/L and error bars represent standard deviation. The algae are represented by several green algae strains, *i.e. Pseudokirchneriella sp*., *Chlorella sp*., and *Scenedesmus sp*.). Cyanobacteria are represented by *Microcystis sp*., *Synechococcus sp*., and *Anabaena sp*. strains. Note that overall, cyanobacteria are the most sensitive organisms to ofloxacin, ciprofloxacin, and amoxicillin, followed by aquatic plants (represented by species from the duckweed family) and algae. On the other hand, bacteria (*V. fisheri*), fish, and crustaceans (*D. magna, C. dubia*, and *A. salina*) are relatively resistant to the effects of antibiotics in standard acute ecotoxicological bioassays. Trimethoprim shows to be relatively non-toxic to all groups of organisms.