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Global occurrence of anti-infectives in contaminated surface waters: Impact of income inequality between countries

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ABSTRACT

The presence anti-infectives in environmental waters is of interest because of their potential role in the dissemination of anti-infective resistance in bacteria and other harmful effects on non-target species such as algae and shellfish. Since no information on global trends regarding the contamination caused by these bioactive substances is yet available, we decided to investigate the impact of income inequality between countries on the occurrence of anti-infectives in surface waters. In order to perform such study, we gathered concentration values reported in the peer-reviewed literature between 1998 and 2014 and built a database. To fill the gap of knowledge on occurrence of anti-infectives in African countries, we also collected 61 surface water samples from Ghana, Kenya, Mozambique and South Africa, and measured concentrations of 19 anti-infectives. A mixed one-way analysis of covariance (ANCOVA) model, followed by Turkey–Kramer post hoc tests was used to identify potential differences in anti-infective occurrence between countries grouped by income level (high, upper-middle and lower-middle and low income) according to the classification by the World Bank. Comparison of occurrence of anti-infectives according to income level revealed that concentrations of these substances in contaminated surface waters were significantly higher in low and lower–middle income countries ($p = 0.0001$) but not in upper–middle income countries ($p = 0.0515$) compared to high-income countries. We explained these results as the consequence of the absence of or limited sewage treatment performed in lower income countries. Furthermore, comparison of concentrations of low cost anti-infectives (sulfonamides and trimethoprim) and the more expensive macrolides between income groups suggest that the cost of these substances may have an impact on their environmental occurrence in lower income countries. Since wastewaters are the most important source of contamination of anti-infectives and other contaminants of emerging concern in the environment, it is expected that deleterious effects to the aquatic biota caused by these substances will be more pronounced in countries with inadequate wastewater and collection infrastructure. With the information currently available, we could not evaluate either the role of the receiving environment or the importance of regulatory frameworks on the occurrence of anti-infectives in surface waters. Future studies should focus on these two factors in order to better evaluate risks to aquatic ecosystems in LM&LICs. We propose that CECs such as anti-infectives could be used as a new class of environmental degradation indicators that could be helpful to assess the state of development of wastewater collection and treatment infrastructure around the world.

1. Introduction

The effect of income on pollution has been extensively discussed in the literature (Boyce, 1994; Dinda, 2004; Grossman and Krueger,

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1995) and a recurring topic is the Environmental Kuznets Curve (EKC) hypothesis. According to this hypothesis, the relationship between an environmental stressor as a function of gross domestic product (GDP) per capita has the shape of an inverted-U, i.e. pollution worsens as the economy of countries starts to grow and then it improves when countries reach a higher stage of economic growth. EKCs have been observed for various types of pollutants or environmental stressors such as mononitrogen oxides and suspended particulate matter in air (Selden and Song, 1994) and nitrates and chemical oxygen demand in rivers (Grossman and Krueger, 1995). However, EKC is not the only type of relationship observed between pollution and GDP per capita. For some pollutants, N-shaped (cubic polynomial) curves and monotonically decreasing relationships have been also observed for total coliforms and lead in rivers (Grossman and Krueger, 1995), respectively. Additionally, for other pollutants such as mercury, GDP per capita seems to have no effect (Grossman and Krueger, 1995). While the validity of the EKC hypothesis has been much debated (Dinda, 2004; Torras and Boyce, 1998) it has been argued that the relationship observed between GDP per capita and pollution is not causal, i.e. household income is only an indicator of various economic, social, political and technological factors that play a decisive role in the extent of pollution as GDP increases (Dinda, 2004; McConnell, 1997; Selden and Song, 1994; Torras and Boyce, 1998).

Contrary to priority pollutants, contaminants of emerging concern (CECs) are a relatively new class of unregulated environmental contaminants and their environmental fate and effects are still poorly understood. The presence of traces of CECs (usually <1000 ng L^{-1}) in the environment has attracted the attention of the scientific community, especially because of the potential environmental hazards caused by the exposure of sensitive species to these biological active substances. Among the most widely reported CECs in the literature are pharmaceuticals, personal care products, nanomaterials and additives such as plasticizers and flame retardants (Farré et al., 2011; Monteiro and Boxall, 2010). To our knowledge, a quantitative study on the relationship between CECs and income level of countries has not been published yet; for the majority of CECs, most occurrence studies have been performed mainly in high-income countries.

Anti-infectives, also broadly known as antibiotics, are a particularly interesting sub-class of CECs to study in the context of quantitative comparative studies since they were first reported in surface waters as early as 1983 (Watts et al., 1983). We chose anti-infectives for this study since their occurrence in environmental waters is of concern because of their potential contribution to the spreading of anti-infective resistance in microorganisms (Martinez, 2009), which is directly linked to public health. There are still some controversy (Kummerer, 2009) and conflicting results (Gao et al., 2012; Oberlé et al., 2012) on the correlation between anti-infective concentration and antibiotic resistance genes (ARGs) in urban wastewaters. However, studies on the impact of wastewaters from anti-infective production facilities seem to point to a contribution to the dissemination of ARGs (Li et al., 2010). Other studies have also shown that non-target species such as algae (Wilson et al., 2003) and mussels (Gust et al., 2012) could be negatively affected by traces of anti-infectives in surface waters. In addition, a large amount of information on their occurrence is available in the literature and in the last few years, several studies on the occurrence of anti-infectives in lower-middle and low-income countries have been published (Hoa et al., 2011; Shimizu et al., 2013; Takasu et al., 2011). These data together with the results of a sampling campaign in surface waters of Ghana, Kenya, Mozambique and South Africa, that we conducted and present here, provide the information required for meaningful income group comparisons.

The purpose of this study was to determine how global differences, in terms of gross national income per capita, affect the occurrence of anti-infectives in surface waters. The effect of income inequality on the occurrence of anti-infectives in surface waters is difficult to predict since different variables can act at the same time. For example, compared to high-income countries, lower income countries have: i) a higher rate of occurrence of infectious diseases (World Health Organization, 2011), ii) generally higher rates of over-the-counter self-medication (Kamat and Nichter, 1998) and iii) lower access to wastewater collection and treatment infrastructures (Sato et al., 2013; WHO/UNICEF Joint Program for Water Supply and Sanitation, 2014). On the other hand, low- and middle-income countries consume less by value of the world's medicines compared to high income countries (World Health Organization, 2004). The strategy used here was thus based on the compilation and comparison of worldwide data reported in the peer-reviewed literature in order to identify potential correlation trends between income and occurrence of anti-infectives in surface waters. As pointed out by Hughes et al. (2012) published studies on the occurrence of anti-infectives are spatially biased (e.g. samples usually collected near outfalls of wastewater treatment plants of big cities, not all regions in one country are sampled, etc.) and the information available at this point does not reflect the actual state of contamination of a region or a country. However, our objective is to determine significant trends between reported concentrations of anti-infectives in contaminated surface waters and income inequalities between countries.

2. Methods

2.1. Sample collection, preparation and analysis of surface water samples from Ghana, Kenya, Mozambique and South Africa

Samples of surface water were collected with a stainless steel bucket in rural, urban or suburb areas in different locations in Ghana, Kenya, Mozambique and South Africa, over the months of September 2010, July and September 2011, September 2012 and September 2013. Exact coordinates as well as air temperature, water temperature, pH and electrical conductivity of water are shown in the Supplemental material, Tables S1 and S2. After collection, surface water samples were stored in 1-L amber glass bottles and transported cooled to a laboratory, where they were filtered immediately through previously baked GF/F glass fiber filters from Whatman. Then, a volume (50 mL–200 mL) of each sample filtrate was introduced in 6-mL solid-phase extraction cartridges (200 mg Oasis HLB resin, Waters) previously conditioned with methanol, 0.1% formic acid in methanol and water. After cartridges were completely loaded with the samples, they were wrapped in aluminum foil, placed in plastic bags and transported to Japan in a container packed with dry ice. Once the cartridges arrived at the laboratory, they were stored in a freezer at −30 °C until analysis. Details about the sample elution procedure from the cartridges and liquid chromatography–mass spectrometry analysis are found in Shimizu et al. (2013). Briefly, cartridges were thawed, washed with H_2O and eluted with 0.1% formic acid in methanol. Internal standards were added to the eluate and then evaporated to dryness and reconstituted in an appropriate volume (0.5 mL–40 mL) of a solution of 0.1% formic acid in H_2O/acetonitrile (94:6 v/v). Samples were then injected into a liquid chromatograph (Accela, Thermo Scientific) coupled to a triple quadrupole mass spectrometer (Quantum Access, Thermo Scientific). The target anti-infectives were five macrolides: azithromycin (AZI), clarithromycin (CLA), dehydroerythromycin (ERY-H2O), roxithromycin (ROX), tylosin (TYL); seven sulfonamides: sulfadimethoxine (SDX), sulfamerazine (SMR), sulfadimidine (SDI), sulfamethizole (SMZ), sulfamethoxazole (SMX), sulfapyridine (SPY), sulfathiazole (STZ); four tetracyclines: chlortetracycline (CTC), doxycycline (DOX), minocycline (MC), oxytetracycline (OTC), tetracycline (TC); one diaminopyrimidine: trimethoprim (TRI) and one lincosamide: lincomycin (LIN). They were separated by reversed phase chromatography, ionized by electrospray in the positive mode and detected using two selected reaction monitoring (SRM) transitions. For all the African locations we did not observe any big factories of pharmaceuticals near our sampling locations. Coprostanol, a sewage marker, was also measured for the suspended solid samples trapped on the filter. Analytical procedure of coprostanol was described in detail in our previous paper (Isobe et al., 2002).

2.2. Quality control and analytical performance

Analytes were identified by the area ratios of two SRM transitions (within ±20%) and average retention time (within ± 0.3 min). Calibration curves (1, 3, 5, 10, 20, 30, 40, and 50 μg L^{-1}) with $R^2 > 0.99$ were used to quantify the analytes. Measured concentrations were corrected for recovery of internal standards (Tables S3, S4, S5 and S6 Supplemental material). Solvent blanks were used to calculate limits of detection (signal-to-noise ≥ 3) and procedural (extracted) blanks were employed to determine limits of quantification (LOQ; 10 times the procedural blank value or signal-to-noise ≥ 10 in case no peak on the chromatogram). In addition, when the LOQ was below the lowest concentration of the linearity range of the calibration curve (0.1 μg L^{-1} for all the anti-infectives except for tetracyclines and tylosin for which the lowest standard was 1 μg L^{-1}), the concentration of the lowest standard was used to calculate LOQ. Quality control and quality assurance of the procedure was also evaluated by reproducibility of the analysis and recovery of the spiked standards based on replicate analyses ($n = 4$) of wastewater effluents. Results are summarized in Tables S7 and S8 of the Supplemental material.

2.3. Data collection of reported anti-infective concentrations in surface waters

We updated a database having values of concentration of anti-infectives in environmental waters, which was analyzed and discussed in a previous publication (Segura et al., 2009). Values from 32 additional papers that were published between mid-2007 and 2014 were added to the database. The data extraction rules used in the earlier publication (Segura et al., 2009) were also applied here in order to ensure the quality of the data retained for the analysis. These rules are: i) only values expressed in numerical form were extracted (values presented in figures were rejected); ii) only concentration values higher than the limits of quantification were used; and iii) only values for which the country of origin was named and the sample clearly identified as non-spiked surface water (river water, lake water, estuary, etc.) were selected. Additionally, three new rules were put in place for the current study. First, anti-infective concentrations reported in agricultural wastewaters (aquaculture, livestock farms, etc.) or in surface waters from regions heavily impacted by pharmaceutical industry waste (Fick et al., 2009; Li et al., 2008) were rejected. This rule was necessary because agricultural wastewaters and surface waters near industrial outfalls usually contain extremely high concentrations of anti-infectives and are not representative of contaminated surface waters of a whole income group. Additionally, in order to perform correct statistical comparisons, only concentration values reported either as averages or single values were considered, therefore anti-infective concentrations expressed as medians or ranges (minimum and maximum values) were discarded. Secondly, to eliminate a possible bias due to the class of anti-infective reported, only values corresponding to macrolides, quinolones, sulfonamides, tetracyclines and trimethoprim, common to all income countries, were used. Therefore, anti-infectives from other classes such as β-lactams and azoles, reported in high- and upper-middle but not in lower income countries, were rejected. Countries were grouped by gross national income per capita according to the classification used by The World Bank Group (2013a): high-income (HICs): >$12,476; upper-middle income (UMICs): $4036–$12,475; lower-middle income (LMICs): $1026–$4035 and low income (LICs): <$1025.

2.4. Statistical analysis of reported anti-infective concentrations in surface waters

To investigate differences in the mean of the reported concentrations of anti-infectives in surface waters between income groups, we used a mixed one-way analysis of covariance (ANCOVA) model, where the dependent variable was defined as the concentration of anti-infectives in nanograms per liter regardless of the class of anti-infective. Year of sampling was introduced as a covariate in the model. Given that sampling varied among the various publications (e.g. measures at different distances from the same wastewater treatment plant) we cannot assume that those values are statistically independent. For that reason, for each reviewed publication, all values for the same anti-infective were averaged, and only one concentration value per anti-infective per publication was used, to avoid bias from pseudoreplication (for papers reporting concentrations in more than one country, one averaged value per country was used). The mixed model approach in the ANCOVA allows us to take into account the correlation between within-country observations due possibly to the fact that policies and economic factors specific to each country (e.g. resources allocated to water treatment, access to anti-infectives, etc.) may have an effect on the concentration values detected in the same country.

Consequently we adjusted standard errors for such clustering effect due to country, by modeling the possible correlation between concentration values within country, assuming a compound symmetry (equal correlation) structure for this correlation (Littell et al., 2006). The assumptions of normality and equal variances of errors, as well as the presence of possible outliers, were explored with analysis of residuals. After a preliminary analysis of residuals with the raw data, concentration values were natural-log-transformed to stabilize the variance and normalize the distribution of errors. Where warranted, post-hoc pairwise comparisons of means were performed and p-values were adjusted for multiple testing using the Tukey–Kramer method. Degrees of freedom were adjusted using the Kenward–Roger correction (Kenward and Roger, 1997) because the groups were unbalanced. In order to compare mean concentrations in surface waters between income groups by type of anti-infective we used the same mixed-model ANCOVA approach including anti-infective class (macrolide, sulfonamide, quinolone, tetracycline and trimethoprim) as a factor in the model. Final results were back-transformed to concentration units (nanograms-per-liter) and reported as geometric means. All hypothesis tests were two-sided and were performed at the 0.05 level of significance. Statistical analysis was performed with SAS version 9.3 (SAS Institute, Cary, NC).

3. Results and discussion

3.1. Anti-infective concentrations in surface waters of Ghana, Kenya, Mozambique and South Africa

Results of the quantification of the target anti-infectives in the three African countries are shown in Fig. 1 and in more detail in Tables S3, S4, S5 and S6 (Supplemental material). Our data shows that at least one target compound was found in 92% of the samples and, on average, ≈4 target anti-infectives were identified per sample. Only 5 anti-infectives (sulfamerazine, sulfadimethoxine, roxythromycin, tylosin and doxycycline) were not detected in any sample. Three anti-infectives were detected in more than 50% of the samples: sulfamethoxazole (87%), trimethoprim (74%) and dehydroerythromycin (72%). In some of the samples collected near urban and suburban areas, sulfamethoxazole concentrations were superior to the highest reported concentrations to date (Batt et al., 2006; Hoa et al., 2011). In many of the sampled areas, no sewage treatment facilities are installed and in other cases such as in Durban (South Africa), sewage treatment facilities serve only part of the city, suggesting that untreated sewage is an important route of contamination of anti-infectives in surface waters. In contrast

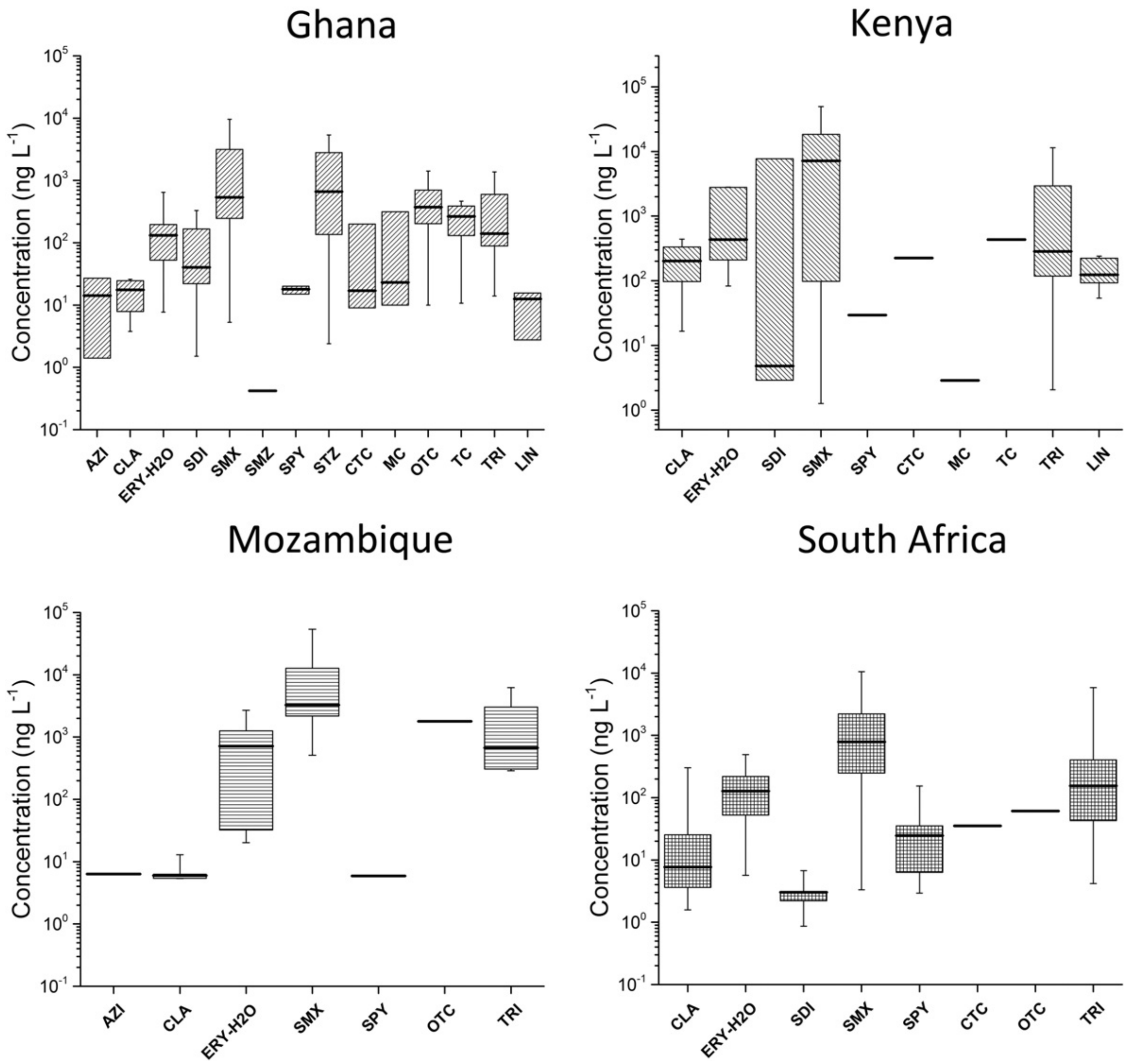

Fig. 1. Concentrations of the target anti-infectives in surface water samples from Ghana, Kenya, Mozambique and South Africa. Distance between the opposite sides of the whiskers denotes the concentration range between maximum and minimum values and length of the box is the interquartile range. The horizontal line represents the median.

to the high concentrations observed in urban and suburban areas, concentrations of sulfamethoxazole in rural rivers were low (5–130 ng L^{-1}) and comparable or lower than those in other parts of the world. This might be explained by the lower input of sewage to the rivers. Veterinary anti-infectives are used for livestock animals and they may contribute to concentrations of these substances in surface waters of the African countries. To examine the contribution from veterinary use in comparison to human use (i.e., sewage-derived anti-infectives), samples from Ghana, Kenya and South Africa, were analyzed for coprostanol which is a molecular marker of sewage (Takada and Eganhouse, 1998). For all the countries examined, anti-infective concentrations had a strong positive correlation with coprostanol, as shown in Fig. 2, indicating that sewage is a major source of anti-infectives in the surface waters of these African countries and contribution from veterinary anti-infectives is minor. This is consistent with observations in Japan (Murata et al., 2011) and tropical Asian countries where livestock wastewater contributes $\approx$10% to the anti-infectives in river water (Shimizu et al., 2013). In most of the samples collected in Ghana, Kenya, Mozambique and South Africa, sulfonamides represented by far the highest percentage of the total concentration of anti-infectives. In fact, in 66% of all collected samples, sulfonamides represented $\geq$75% of the sum of all quantified anti-infectives. High concentration of sulfonamides in surface waters of these countries could be due to their lower price compared to other anti-infectives such as macrolides (Shimizu et al., 2013).

All anti-infective concentrations quantified in surface water samples in Ghana, Kenya, Mozambique and South Africa, except values for lincomycin which does not belong to the five classes of anti-infectives selected for our comparative study, were incorporated in our database of compiled literature values. The structure of this database will be discussed in the next section.

3.2. Overview of the data compiled

Application of the rules specified in Section 2.3 reduced the size of the original database used in a previous publication (Segura et al., 2009) significantly from 2176 values to 522 values. After adding new values reported in 32 papers published between 2007 and 2014 and our data from Ghana, Kenya, Mozambique and South Africa, the updated database contains a total of 3011 anti-infective concentration values in surface waters from 26 countries (13 HICs, 6 UMICs and 5 LMICs and 2 LIC). Since only two countries (Kenya and Mozambique) were in the LICs group, we pooled together LMICs and LICs. Unfortunately, we could not use values from 20 countries (Fig. 3) since they did not comply with the data extraction rules specified in Section 2.3. The data compiled (N = 3011) formed the database from which we

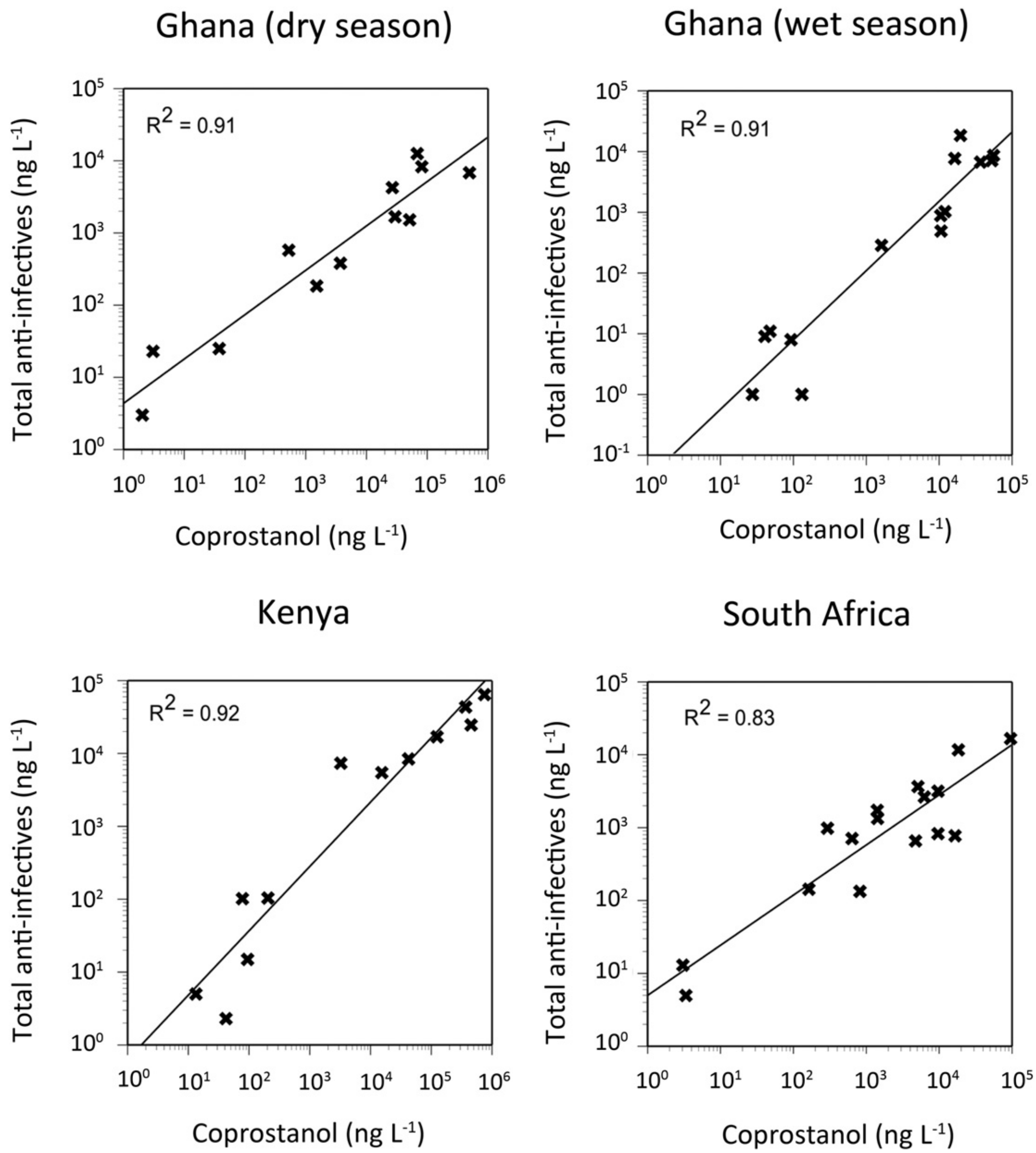

Fig. 2. Correlation of anti-infective concentrations in Ghana, Kenya and South Africa with the sewage marker.

generated a dataset by applying the natural-logarithm transformation and data averaging procedure described in Section 2.4. This dataset has a total of 420 averaged concentration values and was employed to perform comparisons between income groups. Table 1 lists the countries represented in each income group along with the number of papers compiled in each group and the resulting number of concentration values available for statistical analysis. The database and the dataset are available in the Supplementary material as an Excel file.

Plots of values from our dataset (Fig. 3) show that macrolides (ML), quinolones (QL), sulfonamides (SA), tetracyclines (TC) and trimethoprim (TRI) are usually present at concentrations between 10 and 1000 ng L^{-1} in contaminated surface waters. These results do not indicate that all surface waters in the countries listed in the database are contaminated with anti-infectives, it rather means that values reported for contaminated surface waters were in that concentration range. Therefore the term “contaminated surface waters” is more appropriate to describe the surface waters having the concentration distributions depicted in Fig. 3. Most of the concentrations compiled are inferior to the lowest observed effective concentrations (LOEC) or median effective concentrations (EC_{50}) having harmful effects on aquatic species (Santos et al., 2010), however, some studies have indicated that concentrations of anti-infectives ≤2000 ng L^{-1} can negatively impact aquatic biota. For example, Wilson et al. (2003) observed that concentrations of 12–120 ng L^{-1} of the quinolone ciprofloxacin can modify the genus composition of natural algal assemblages. Moreover, Isidori et al. (2005) showed that the median effect concentration for growth inhibition of the algae *Pseudokirchneriella subcapitata* for the macrolide clarithromycin was 2000 ng L^{-1}. A recent study also revealed that several anti-infectives (ciprofloxacin, erythromycin, oxytetracycline, sulfamethoxazole and trimethoprim) are immunotoxic to the freshwater mussel *Elliptio complanata* at concentrations between 20 and 1100 ng L^{-1} (Gust et al., 2012). Current environmental toxicology data about anti-infectives and the results compiled in our study indicate that globally the concentration of each of these substances in contaminated surface waters are usually not high enough to cause acute effects on aquatic species. However, chronic and more subtle effects on aquatic species, as Daughton and Ternes suggested 15 years ago (Daughton and Ternes, 1999), might occur and mixture effects have yet to be considered (Hughes et al., 2012; Yang et al., 2008).

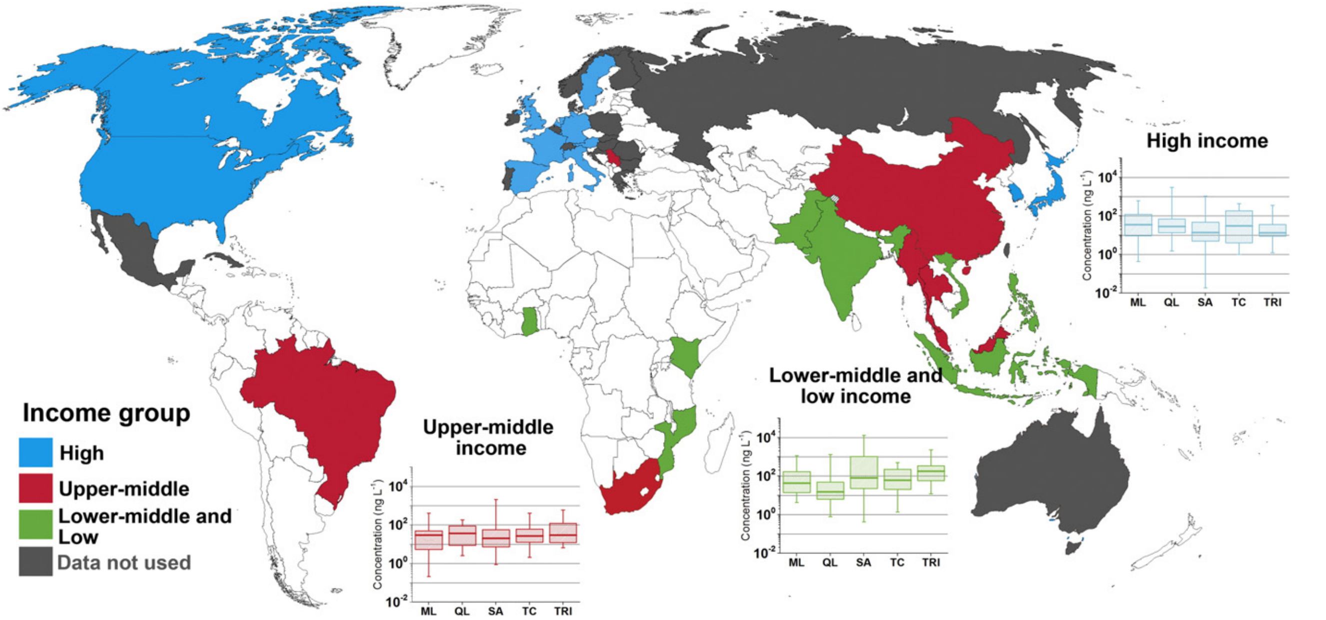

Fig. 3. Countries in which occurrence of anti-infectives in surface waters has been reported in the literature. Box plots show the concentrations of five classes of anti-infectives common to all income groups (ML = macrolides, QL = quinolones, SA = sulfonamides, TC = tetracycline and TRI = trimethoprim). In the boxplots, distance between the opposite sides of the whiskers denotes the concentration range between maximum and minimum values and length of the box is the interquartile range. The horizontal line represents the median. Countries for which data were rejected were: Australia, Belgium, Bulgaria, Croatia, Cuba, Denmark, Finland, Greece, Hungary, Indonesia, Ireland, Mexico, Norway, Poland, Portugal, Romania, Russia, Slovakia, Switzerland and Taiwan.

3.3. Link between income level and the occurrence of anti-infectives in contaminated surface waters

As indicated in the Introduction, the link between income and pollution in the environment is non-causal. However, it is an adequate parameter to categorize countries from an environmental perspective, since differences in key demographic, social, economic and technological factors that have an effect on the presence of pharmaceuticals in the environment are marked between LM&LICs and HICs (Kookana et al., 2014). Therefore income group can be used as an indicator of the numerous factors that play a decisive role on the occurrence of anti-infectives in surface waters but are difficult to use in a quantitative comparative study. The mixed-model ANCOVA with log-transformed data revealed a main effect of income group on the mean concentration of anti-infectives ($p = 0.0003$). Geometric mean concentration of anti-infectives in contaminated surface waters decrease as a function of increasing income (Table 2). In fact, after back-transforming the results, we observe that, with a 95% degree of confidence, the geometric mean concentration of anti-infectives in LM&LICs was 3.2 to 39.2 times that of HICs ($p = 0.0001$). In the case of UMICs, the geometric mean concentration was only 1.0 to 14.9 times that of HICs, and not significantly different ($p = 0.0515$). As for UMICs and LM&LICs, differences between their geometric means were not significant ($p = 0.15$).

When including class as a factor in the mixed ANCOVA model, results (Table 3) show that geometric mean concentration of sulfonamides in LM&LICs was, with 95% degree of confidence, 3.0 to 204.5 times that of HICs ($p = 0.0001$). A similar result was obtained with trimethoprim (its concentration in LM&LICs was 2.8 to 547.6 times that of HICs, $p = 0.0004$). When looking at the values obtained for macrolides, the difference was not significant ($p = 0.32$): the geometric mean of macrolides in LM&LICs was 0.6 to 46.4 times that of HICs. These results thus suggest that sulfonamides and trimethoprim are used extensively in LM&LICs compared to HICs but macrolides appear to be used to a lesser extent.

In a recent study on the potential ecological footprints of pharmaceuticals (Kookana et al., 2014), the authors enumerate six factors that determine the environmental exposure of these substances: 1) demographics (population size and age distribution), 2) access to health systems (consumption patterns, price of medication), 3) size of the manufacturing sector, 4) connectivity to sewage and sewage treatment systems 5) receiving environment and 6) availability and effectiveness of regulatory frameworks. We explored the importance of these six factors to the occurrence of anti-infectives in contaminated surface waters. Among them, size of the manufacturing sector does not have an impact on our results since we did not use values from surface waters impacted by industrial waste. As for demographics, it is expected that an aging

Table 1
List of countries represented in each income group with the number of papers compiled for each group and their corresponding numbers of concentration values. For UMICs and LM&LICs, the number of values includes our experimental data presented in this paper. Values in parenthesis indicate the number of concentration values for that country. In total, we extracted values from 62 peer-reviewed papers (a few papers reported concentrations in two income groups).

Income group	Countries	Number of papers	Number of total values
High (HICs)	Austria (4), Canada (9), France (13), Germany (25), Italy (17), Japan (20), Luxembourg (5), South Korea (6), Spain (49), Sweden (4), The Netherlands (1), UK (9), USA (48)	45	210
Upper-middle (UMICs)	Brazil (9), China (84), Malaysia (9), Serbia (2), South Africa (8), Thailand (6)	13	118
Lower-middle & Low (LM&LICs)	Ghana (13), India (9), Indonesia (8), Kenya (9), Philippines (6), Vietnam (24), Mozambique (5), Pakistan (18)	6	92

Table 2
Results of the comparison of global occurrence of anti-infectives according to income groups, $N = 420$.

Income group (number of countries)	Least squares mean ($\log_e$)	95% confidence interval ($\log_e$)	p-Value vs HICs*	Back-transformed		
				Geometric mean (ng L^{-1})	95% confidence lower limit (ng L^{-1})	95% confidence upper limit (ng L^{-1})
HICs (13)	2.4191	1.7986–3.0397	–	11.2	6.04	20.9
UMICs (6)	3.7657	2.8706–4.6607	0.0515	43.2	17.6	106
LM&LICs (8)	4.8307	4.0562–5.6051	0.0001	125	57.8	272

F test value (degrees of freedom = 2, 27.9) for income group effect was 11.16. *Tukey–Kramer adjusted p-value for differences of least squares means. Adjusted p for UMICs vs LM&LICs was 0.15.

population will use a higher amount of anti-infectives and contaminate its surface waters more, however our results do not show that trend: the average percentage of the population aged over 65 during the period from 1998–2013 in HICs is 14.6% compared to 4.2% in LM&LICs (The World Bank Group, 2015). At the present time, we do not have information to evaluate the impact of regulatory frameworks nor a quantitative value to compare the receiving environments since this information is sparse or completely absent in most of the sampled publications. Therefore in order to explain the results obtained, we examined the two remaining factors: connectivity to sewage and sewage treatment as well as access to health systems.

Major differences of connectivity to sewage and sewage treatment between HICs and LM&LICs are well documented by published data on global access to sanitation facilities and wastewater treatment. According to The World Bank Group (2013b), the percentage of the population having access to sanitation facilities (flush or pour flush toilets, ventilated improved pit latrines, pit latrines with slab and composting toilets) in 12 out of the 13 HICs surveyed is 99.9% ± 0.1%, while being only 82% ± 12% and 48% ± 23% in 5 out the 6 UMICs and 6 out of the 8 LM&LICs surveyed, respectively. In addition, only a fraction of wastewater generated is actually treated and according to Sato et al. (2013) the percentage of wastewater treated is higher in HICs (between 54.3 and 99.4% for seven countries included in our study) than in LM&LICs (for example: Ghana 7.9% and India 30.7%). A report by the Joint Monitoring Program for Water Supply and Sanitation (WHO/UNICEF Joint Program for Water Supply and Sanitation, 2014) further support this hypothesis, as it revealed that the population of countries in sub-Saharan Africa and southern Asia have the lowest access (in some cases <50%) to improved sanitation facilities of the world [i.e. "one that hygienically separates human excreta from human contact" (WHO/UNICEF Joint Program for Water Supply and Sanitation, 2014)]. Sewage treatment removes antibiotics to some extent, generally between 10–90% depending on the compound and the type of treatment, and reduces environmental burden of anti-infectives to surface waters (Göbel et al., 2007; Golet et al., 2003; Segura et al., 2007). For example, secondary treatment (activated sludge) removes ~40% of sulfonamides and ~30% of macrolides (Morimoto et al., 2011). Furthermore, in HICs some portions of treated wastewater are discharged to coastal waters via pipelines and outfalls, leading to lower concentrations of anti-infectives in surface waters. Therefore, the lack of or limited access to sewage collection and treatment in LM&LICs is very likely one of the major factors contributing to the higher concentrations of anti-infectives observed in contaminated surface waters in these countries.

As for access to health systems, we looked at the cost of anti-infectives and differences in rates of prevalence of infectious diseases. A higher rate of infectious disease in LM&LICs compared to HICs (World Health Organization, 2011) and the relative prices of the various classes of anti-infectives also play an important role in explaining the predominance of sulfonamides observed. For example, according to Shimizu et al. (2013), the price per tablet of clarithromycin in the Vietnamese market (obtained from pharmacies in town) is about 13 times higher than the price of sulfamethoxazole and trimethoprim. Our survey in Ghana confirmed the situation. That is, the price per tablet of clarithromycin (GHS 4.55–GHS 7.50) is one order of magnitude higher than that of sulfamethoxazole (GHS 0.1–GHS 0.8). In 1999 low and middle-income countries consumed <10% (by monetary value) of the world's medicines compared to high income countries (World Health Organization, 2004) and in 2008, per capita consumption of

Table 3
Results of the comparison of global occurrence of anti-infectives in contaminated surface waters among income groups with class as a factor.

Income group	Least squares mean ($\log_e$)	95% confidence interval ($\log_e$)	p-VALUE vs HICs*	Back-transformed		
				Geometric mean (ng L^{-1})	Lower limit (ng L^{-1})	Upper limit (ng L^{-1})
		Macrolides				
HICs	2.7818	2.0239–3.5397	–	16.2	7.57	34.5
UMICs	3.4900	2.4630–4.5171	1.0	32.8	11.7	91.6
LM&LICs	4.4518	3.5480–5.3556	0.32	85.8	34.7	212
		Quinolones				
HICs	3.2217	2.3258–41177	–	25.1	10.2	61.4
UMICs	3.8008	2.7229–4.8787	1.0	44.7	15.2	132
LM&LICs	4.0104	2.8067–5.2141	1.0	55.2	16.6	184
		Sulfonamides				
HICs	2.0636	1.3762–2.7509	–	7.87	3.96	15.7
UMICs	3.7309	2.7410–4.7208	0.32	41.7	15.5	112
LM&LICs	5.2807	4.3576–6.2038	0.0001	196	78.1	495
		Tetracyclines				
HICs	2.4570	1.4540–3.4600	–	11.7	4.28	31.8
UMICs	3.5055	2.3077–4.7033	0.99	33.3	10.0	110
LM&LICs	4.5222	3.3784–5.661	0.38	92.0	29.3	289
		Trimethoprim				
HICs	1.9589	1.0946–2.8231	–	7.09	2.99	16.8
UMICs	4.2494	3.0321–5.4668	0.18	70.1	20.7	237
LM&LICs	5.6240	4.4295–6.8184	0.0004	277	83.9	914

* Adjusted p-values obtained with the Tukey-Kramer method. Adjusted p-values for the comparison of UMICs and LM&LICs were 0.98, 1.0, 0.54, 1.0 and 0.95 for macrolides, quinolones, sulfonamides, tetracyclines and trimethoprim, respectively.

pharmaceuticals was five to ten times higher in HICs than in LM&LICs (World Health Organization, 2011). However, anti-infectives were more consumed by volume in low-income countries compared to other pharmaceuticals than in HICs and UMICs in 2008 (World Health Organization, 2011). These data from the WHO hint that LM&LICs consume important quantities of low cost anti-infectives compared to HICs. Our analysis of samples from Ghana, Kenya and Mozambique (LM&LICs) and South Africa (UMIC) and previous studies in tropical Asian countries (Shimizu et al., 2013) as well as our comparison of global occurrence data are in agreement with the WHO reports. Sulfonamides, especially sulfamethoxazole were predominant in Ghana, Kenya, Mozambique, Vietnam, Indonesia, The Philippines and India compared to more expensive anti-infectives such as the macrolides azithromycin and clarithromycin.

To summarize, based on the statistical analysis of our dataset, we found that differences in sewage collection and treatment as well as access to health systems in terms of cost of medication are among the main factors influencing the occurrence of pharmaceuticals in the environment. At this moment, with the amount of data presently available, it is not possible to evaluate either the role of the receiving environment on the occurrence of anti-infectives or the importance of regulatory frameworks. Future studies should focus on these two factors in order to better evaluate risks to aquatic ecosystems in LM&LICs.

4. Conclusions

A dataset of 420 averaged concentration values of macrolides, quinolones, sulfonamides, tetracyclines and trimethoprim, reported in 62 papers as well as our own first results for African countries, revealed that these substances are generally present in a range from 10 to 1000 ng L^{-1} in the contaminated surface waters of 26 countries. A mixed one-way analysis of covariance model that took into account clustering effects in our dataset due to origin and the year in which the samples were collected was used to compare global occurrence data of anti-infectives according to income group. Results showed that income level inequalities between countries had a statistically significant effect on the occurrence of these CECs in surface waters. The geometric mean concentration of anti-infectives in contaminated surface waters of HICs was significantly lower than that reported in LM&LICs ($p = 0.0001$) but not in UMICs ($p = 0.0515$). Additionally, income group comparisons using anti-infective class as an additional factor suggest that low cost anti-infectives such as sulfonamides and trimethoprim, are used more frequently than the more expensive macrolides such as azithromycin and clarithromycin in LM&LICs compared to HICs. Since it is known that the relationship between income level and contamination is not causal, these results were explained as a consequence of the lack of or inadequacy of wastewater collection and treatment systems in LM&LICs compared to HICs and high consumption of low cost anti-infectives in LM&LICs. Thus based on the data presently available, our study suggests that because of poor sewage and wastewater treatment, the aquatic ecosystems in LM&LICs, compared to those in HICs, may be more vulnerable to contamination from CECs. A global-scale analysis of freshwater threats to human water security and river diversity made by Vörösmarty et al. (2010) arrived at a similar conclusion. The authors pointed out that low technological investment in developing countries to improve water infrastructure resulted in a lower capacity to reduce threats to human water security compared to wealthy nations. Future studies should focus on both the role of the receiving environment and the impact of regulatory frameworks on the occurrence of anti-infectives in order to better evaluate risks to aquatic ecosystems in LM&LICs.

We propose that CECs such as anti-infectives could be used as a new class of environmental degradation indicators that are not related to the industrial development of a country as is the case with trace metals, sulfur dioxide and mono-nitrogen oxides. Thus, the presence of CECs in surface waters could be employed to assess the state of development of wastewater collection and treatment infrastructure around the World.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2015.04.001.

References

Batt, A.L., Bruce, I.B., Aga, D.S., 2006. Evaluating the vulnerability of surface waters to antibiotic contamination from varying wastewater treatment plant discharges. Environ. Pollut. 142, 295–302.

Boyce, J.K., 1994. Inequality as a cause of environmental degradation. Ecol. Econ. 11, 169–178.

Daughton, C.G., Ternes, T.A., 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ. Health Perspect. 107, 907–938.

Dinda, S., 2004. Environmental Kuznets curve hypothesis: a survey. Ecol. Econ. 49, 431–455.

Farré, M., Sanchís, J., Barceló, D., 2011. Analysis and assessment of the occurrence, the fate and the behavior of nanomaterials in the environment. TrAC Trends. Anal. Chem. 30, 517–527.

Fick, J., Söderström, H., Lindberg, R.H., Phan, C., Tysklind, M., Larsson, D., 2009. Contamination of surface, ground, and drinking water from pharmaceutical production. Environ. Toxicol. Chem. 28, 2522–2527.

Gao, P., Munir, M., Xagoraraki, I., 2012. Correlation of tetracycline and sulfonamide antibiotics with corresponding resistance genes and resistant bacteria in a conventional municipal wastewater treatment plant. Sci. Total Environ. 421–422, 173–183.

Göbel, A., McArdell, C.S., Joss, A., Siegrist, H., Giger, W., 2007. Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies. Sci. Total Environ. 372, 361–371.

Golet, E.M., Xifra, I., Siegrist, H., Alder, A.C., Giger, W., 2003. Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil. Environ. Sci. Technol. 37, 3243–3249.

Grossman, G.M., Krueger, A.B., 1995. Economic growth and the environment. Q. J. Econ. 110, 353–377.

Gust, M., Gélinas, M., Fortier, M., Fournier, M., Gagné, F., 2012. *In vitro* immunotoxicity of environmentally representative antibiotics to the freshwater mussel *Elliptio complanata*. Environ. Pollut. 169, 50–58.

Hoa, P.T.P., Managaki, S., Nakada, N., Takada, H., Shimizu, A., Anh, D.H., Viet, P.H., Suzuki, S., 2011. Antibiotic contamination and occurrence of antibiotic-resistant bacteria in aquatic environments of northern Vietnam. Sci. Total Environ. 409, 2894–2901.

Hughes, S.R., Kay, P., Brown, L.E., 2012. Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems. Environ. Sci. Technol. 47, 661–677.

Isidori, M., Lavorgna, M., Nardelli, A., Pascarella, L., Parrella, A., 2005. Toxic and genotoxic evaluation of six antibiotics on non-target organisms. Sci. Total Environ. 346, 87–98.

Isobe, K.O., Tarao, M., Zakaria, M.P., Chiem, N.H., Minh, L.Y., Takada, H., 2002. Quantitative application of fecal sterols using gas chromatography-mass spectrometry to investigate fecal pollution in tropical waters: Western Malaysia and Mekong Delta, Vietnam. Environ. Sci. Technol. 36, 4497–4507.

Kamat, V.R., Nichter, M., 1998. Pharmacies, self-medication and pharmaceutical marketing in Bombay, India. Soc. Sci. Med. 47, 779–794.

Kenward, M.G., Roger, J.H., 1997. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics 53, 983–997.

Kookana, R.S., Williams, M., Boxall, A.B., Larsson, D.J., Gaw, S., Choi, K., Yamamoto, H., Thatikonda, S., Zhu, Y.-G., Carriquiriborde, P., 2014. Potential ecological footprints of active pharmaceutical ingredients: an examination of risk factors in low-, middle- and high-income countries. Philos. Trans. R. Soc. B 369, 20130586.

Kummerer, K., 2009. Antibiotics in the environment — a review — part II. Chemosphere 75, 435–441.

Li, D., Yang, M., Hu, J., Ren, L., Zhang, Y., Li, K., 2008. Determination and fate of oxytetracycline and related compounds in oxytetracyline production wastewater and the receiving river. Environ. Toxicol. Chem. 27, 80–86.

Li, D., Yu, T., Zhang, Y., Yang, M., Li, Z., Liu, M., Qi, R., 2010. Antibiotic resistance characteristics of environmental bacteria from an oxytetracycline production wastewater treatment plant and the receiving river. Appl. Environ. Microbiol. 76, 3444–3451.

Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D., Schabenberger, O., 2nd ed., 2006. SAS® for Mixed Models. SAS Institute Inc., Cary, NC.

Martinez, J.L., 2009. Environmental pollution by antibiotics and by antibiotic resistance determinants. Environ. Pollut. 157, 2893–2902.

McConnell, K.E., 1997. Income and the demand for environmental quality. Environ. Dev. Econ. 2, 383–399.

Monteiro, S., Boxall, A., 2010. Occurrence and fate of human pharmaceuticals in the environment. Rev. Environ. Contam. Toxicol. 202, 53–154.

Morimoto, T., Muramatsu, Y., Takeshita, A., Shimizu, A., Murakami, M., Takada, H., 2011. Concentrations of water-soluble organic micropollutants and anthropogenic molecular markers in sewage influent and effluent. J. Water Waste 53, 635–646.

Murata, A., Takada, H., Mutoh, K., Hosoda, H., Harada, A., Nakada, N., 2011. Nationwide monitoring of selected antibiotics: distribution and sources of sulfonamides, trimethoprim, and macrolides in Japanese rivers. Sci. Total Environ. 409, 5305–5312.

Oberlé, K., Capdeville, M.J., Berthe, T., Budzinski, H., Petit, F., 2012. Evidence for a complex relationship between antibiotics and antibiotic-resistant *Escherichia coli*: from medical center patients to a receiving environment. Environ. Sci. Technol. 46, 1859–1868.

Santos, L.H., Araújo, A., Fachini, A., Pena, A., Delerue-Matos, C., Montenegro, M., 2010. Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. J. Hazard. Mater. 175, 45–95.

Sato, T., Qadir, M., Yamamoto, S., Endo, T., Zahoor, A., 2013. Global, regional, and country level need for data on wastewater generation, treatment, and use. Agric. Water Manag. 130, 1–13.

Segura, P.A., Garcia-Ac, A., Lajeunesse, A., Ghosh, D., Gagnon, C., Sauvé, S., 2007. Determination of six anti-infectives in wastewater using tandem solid-phase extraction and liquid chromatography–tandem mass spectrometry. J. Environ. Monit. 9, 307–313.

Segura, P.A., François, M., Gagnon, C., Sauvé, S., 2009. Review of the occurrence of anti-infectives in contaminated wastewaters and natural and drinking waters. Environ. Health Perspect. 117, 675–684.

Selden, T.M., Song, D., 1994. Environmental quality and development: is there a Kuznets curve for air pollution emissions? J. Environ. Econ. Manag. 27, 147–162.

Shimizu, A., Takada, H., Koike, T., Takeshita, A., Saha, M., Nakada, N., Murata, A., Suzuki, T., Suzuki, S., Chiem, N.H., 2013. Ubiquitous occurrence of sulfonamides in tropical Asian waters. Sci. Total Environ. 452, 108–115.

Takada, H., Eganhouse, R.P., 1998. Molecular markers of anthropogenic waste. In: Meyers, R.A. (Ed.), Encyclopedia of Environmental Analysis and Remediation. John Wiley & Sons, New York.

Takasu, H., Suzuki, S., Reungsang, A., Viet, P.H., 2011. Fluoroquinolone (FQ) contamination does not correlate with occurrence of FQ-resistant bacteria in aquatic environments of Vietnam and Thailand. Microbes Environ. 26, 135–143.

The World Bank Group, 2013a. How we classify countries. Available, http://data.worldbank.org/about/country-classifications (accessed 2013–12-09).

The World Bank Group, 2013b. Improved sanitation facilities (% of population with access). Available, http://data.worldbank.org/indicator/SH.STA.ACSN (accessed 2013–12-11).

The World Bank Group, 2015. Population ages 65 and above (% of total). Available, http://data.worldbank.org/indicator/SP.POP.65UP.TO.ZS (accessed 2015–03-04).

Torras, M., Boyce, J.K., 1998. Income, inequality, and pollution: a reassessment of the environmental Kuznets curve. Ecol. Econ. 25, 147–160.

Vörösmarty, C.J., McIntyre, P., Gessner, M.O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S.E., Sullivan, C.A., Liermann, C.R., 2010. Global threats to human water security and river biodiversity. Nature 467, 555–561.

Watts, C.D., Crathorne, B., Fielding, M., Steel, C.P., 1983. Identification of non-volatile organics in water using field desorption mass spectrometry and high performance liquid chromatography. In: Angeletti, G., Bjorseth, A. (Eds.), Analysis of Organic Micropollutants in Water. D. Reidel Publishing Company, Dordrecht.

WHO/UNICEF Joint Program for Water Supply and Sanitation, 2014. Progress on sanitation and drinking-water, 2014 update. Available, http://www.wssinfo.org/documents accessed 2014–05-25.

Wilson, B.A., Smith, V.H., Denoyelles, F., Larive, C.K., 2003. Effects of three pharmaceutical and personal care products on natural freshwater algal assemblages. Environ. Sci. Technol. 37, 1713–1719.

World Health Organization, 2004. World medicines situation. Available, http://apps.who.int/medicinedocs/en/d/Js6160e/ (accessed 2013–12-09).

World Health Organization, 2011. World medicines situation 2011. Available, http://www.who.int/medicines/areas/policy/world_medicines_situation/en/index.html accessed 2013–12-09.

Yang, L.H., Ying, G.G., Su, H.C., Stauber, J.L., Adams, M.S., Binet, M.T., 2008. Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga *Pseudokirchneriella subcapitata*. Environ. Toxicol. Chem. 27, 1201–1208.