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 the 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 were 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 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 neither the role of the receiving environment nor 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.

Keywords: antibiotics; occurrence; sulfonamides; macrolides; tetracyclines; trimethoprim; quinolones; developing countries; rivers.

1. **Introduction**

The effect of income on pollution has been extensively discussed in the literature (Boyce 1994; Dinda 2004; Grossman and Krueger 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 mono-nitrogen 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 choose 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 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, Table 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 filtrates 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 cartridges arrived to the laboratory, they were stored in a freezer at –30 ºC 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 ± 0.3min). 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 (0.1μg L^{-1} for all the anti-infectives except for tetracyclines and tylosin for which the lowest standard was 1μg 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; iii) only values for which the country of origin and the sample was 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

178 (2013a): high-income (HICs): > $12,476; upper-middle income (UMICs): $4,036 - $12,475;
179 lower-middle income (LMICs): $1,026 - $4,035 and low income (LICs): < $1,025.

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181 *2.4. Statistical analysis of reported anti-infective concentrations in surface waters*

182 To investigate differences in mean of the reported concentrations of anti-infectives in surface
183 waters between income groups, we used a mixed one-way analysis of covariance (ANCOVA)
184 model, where the dependent variable was defined as the concentration of anti-infectives in
185 nanograms per liter regardless of the class of anti-infective. Year of sampling was introduced as a
186 covariate in the model. For each reviewed publication, all values for the same anti-infective were
187 averaged, and only one concentration value per anti-infective per publication was used, to avoid
188 bias from pseudoreplication (for papers reporting concentrations in more than one country, one
189 averaged value per country was used) . The mixed model approach in the ANCOVA allows us to
190 take into account the correlation between within-country observations due possibly to the fact
191 that policies and economic factors specific to each country (e.g. resources allocated to water
192 treatment, access to anti-infectives, etc.) may have an effect on the concentration values detected
193 in the same country.

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195 Consequently we adjusted standard errors for such clustering effect due to country, by modelling
196 the possible correlation between concentration values within country, assuming a compound
197 symmetry (equal correlation) structure for this correlation (Littell *et al.* 2006). Given that
198 sampling varied among the various publications (e.g. measures at different distances from the
199 same wastewater treatment plant) we cannot assume that those values are statistically
200 independent. For that reason for each reviewed publication, all values for the same anti-infective
201 were averaged, and only one concentration value per anti-infective per publication was used. The
202 assumptions of normality and equal variances of errors, as well as the presence of possible
203 outliers, were explored with analysis of residuals. After a preliminary analysis of residuals with
204 the raw data, concentration values were natural-log-transformed to stabilize the variance and
205 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 Figure 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, in 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 deserve only part of the city, suggesting that untreated sewage is an important route of contamination of anti-infectives in surface waters. In contrast 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 to 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-infectives concentrations had strong positive correlation with coprostanol, as shown in Figure 2, indicating that sewage is 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 ≈ 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 ≥ 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 the *Data collection* section 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 (Figure 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 generated a dataset by applying the natural-logarithms transformation and data averaging procedure described in the *Statistical analysis* section. 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 [Environ. Int. Segura 2015(data).xlsx].

Plots of values from our dataset (Figure 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 Figure 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 it 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).

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 at 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 of our results since we did not use values from surface waters impacted by industrial waste. As for demographics, it is expected that an ageing population will use a higher amount of anti-infectives and contaminate more its surface waters, 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 the 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 price of the various classes of anti-infectives play also 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 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-infective compared to HICs. Our analysis of samples from Ghana, Kenya and Mozambique (LM&LICs) and South Africa (UMIC) and previous studies in tropical Asian

380 countries (Shimizu *et al.* 2013) as well as our comparison of global occurrence data are in
381 agreement with the WHO reports. Sulfonamides, especially sulfamethoxazole were predominant
382 in Ghana, Kenya, Mozambique, Vietnam, Indonesia, The Philippines and India compared to
383 more expensive anti-infectives such as the macrolides azithromycin and clarithromycin.

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385 To summarize, based on the statistical analysis of our dataset, we found that differences in
386 sewage collection and treatment as well as access to health systems in terms of cost of
387 medication are among the main factors influencing the occurrence of pharmaceuticals in the
388 environment. At this moment, with the amount of data presently available, is not possible to
389 evaluate neither the role of the receiving environment on the occurrence of anti-infectives nor the
390 importance of regulatory frameworks. Future studies should focus on these two factors in order
391 to better evaluate risks to aquatic ecosystems in LM&LICs.

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393 ## 4. **Conclusions**

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395 A dataset of 420 averaged concentration values of macrolides, quinolones, sulfonamides,
396 tetracyclines and trimethoprim, reported in 62 papers as well as our own first results for African
397 countries, revealed that these substances are generally present in a range from 10 to 1000 ng L^{-1}
398 in the contaminated surface waters of 26 countries. A mixed one-way analysis of covariance
399 model that took into account clustering effects in our dataset due to origin and the year in which
400 the samples were collected was used to compare global occurrence data of anti-infectives
401 according to income group. Results showed that income level inequalities between countries had
402 a statistically significant effect on the occurrence of these CECs in surface waters. Geometric
403 mean concentration of anti-infectives in contaminated surface waters of HICs were significantly
404 lower than those reported in LM&LICs ($p=0.0001$) but not than those in UMICs ($p=0.0515$).
405 Additionally, income group comparisons using anti-infective class as an additional factor suggest
406 that low cost anti-infectives such as sulfonamides and trimethoprim, are used more frequently
407 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 casual, 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 to 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 it 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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Tables

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

				Back-transformed		
Income group (number of countries)	Least square mean (log_e)	95% Confidence interval (log_e)	*p* value vs HICs*	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	125.3	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.

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

				Back-transformed		
Sulfonamides						
Income group	Least Square Mean (log_e)	95% Confidence Interval (log_e)	*p* value vs HICs*	Geometric Mean (ng L^1)	Lower Limit (ng L^{-1})	Upper Limit (ng L^{-1})
Macrolides						
HICs	2.7818	2.0239-3.5397	-	16.15	7.568	34.46
UMICs	3.4900	2.4630-4.5171	1.0	32.79	11.74	91.57
LM&LICs	4.4518	3.5480-5.3556	0.32	85.78	34.74	211.8
Quinolones						
HICs	3.2217	2.3258-41177	-	25.07	10.23	61.42
UMICs	3.8008	2.7229-4.8787	1.0	44.74	15.22	131.5
LM&LICs	4.0104	2.8067-5.2141	1.0	55.17	16.56	183.8
Sulfonamides						
HICs	2.0636	1.3762-2.7509	-	7.874	3.960	15.66
UMICs	3.7309	2.7410-4.7208	0.32	41.72	15.50	112.3
LM&LICs	5.2807	4.3576-6.2038	0.0001	196.5	78.07	494.6
Tetracyclines						
HICs	2.4570	1.4540-3.4600	-	11.67	4.280	31.82
UMICs	3.5055	2.3077-4.7033	0.99	33.30	10.05	110.3
LM&LICs	4.5222	3.3784-5.661	0.38	92.04	29.32	288.9
Trimethoprim						
HICs	1.9589	1.0946-2.8231	-	7.092	2.988	16.83
UMICs	4.2494	3.0321-5.4668	0.18	70.06	20.74	236.7
LM&LICs	5.6240	4.4295-6.8184	0.0004	277.00	83.89	914.5

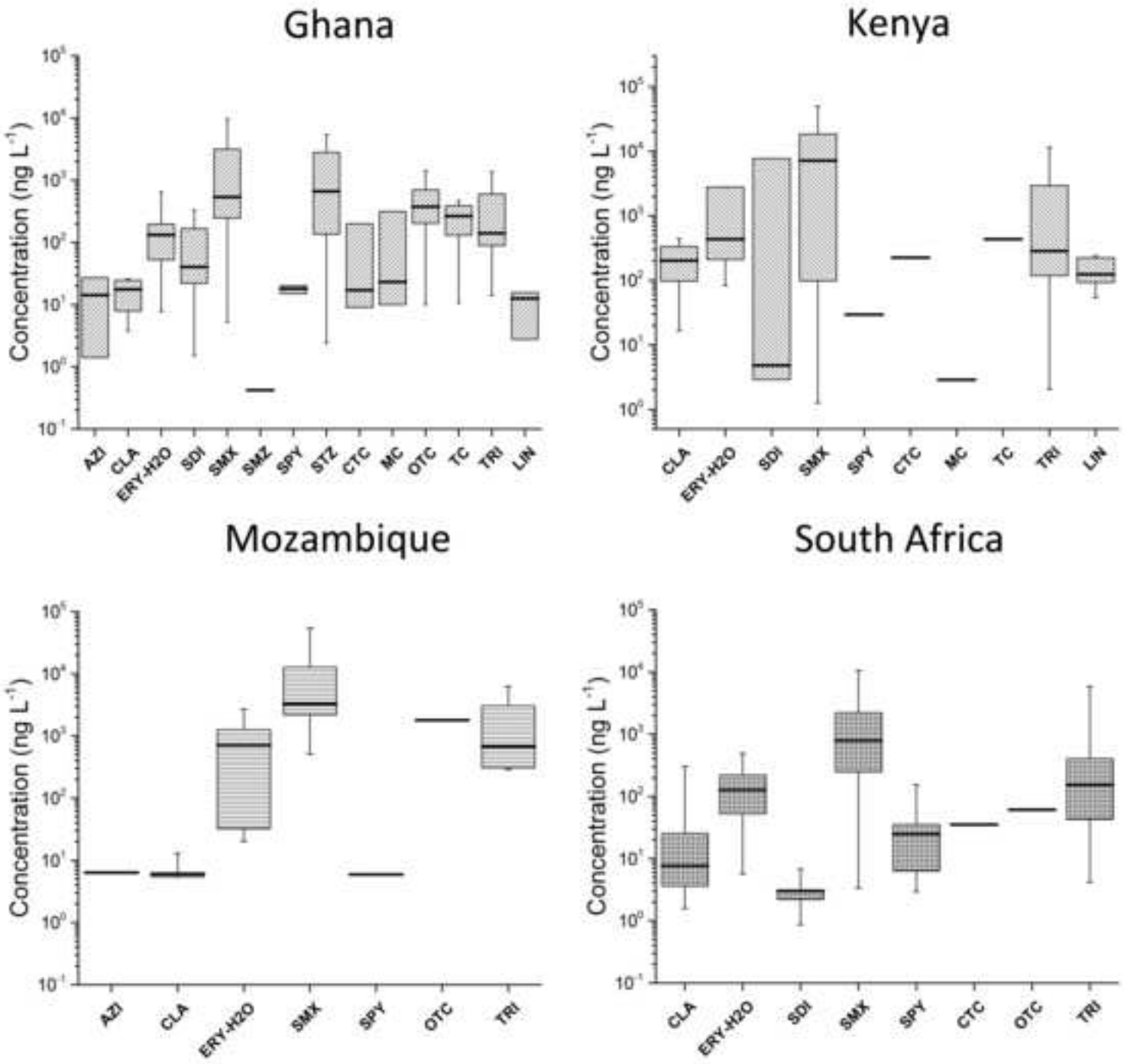
*Adjusted *p* values obtained with the Tukey-Kramer method. Adjusted *p* values for the comparison of UMICs and LM&LICs were 0.98, 1.0, 0.54, 1.0 and 0.95 for macrolides, quinolones, sulfonamides, tetracyclines and trimethoprim, respectively.

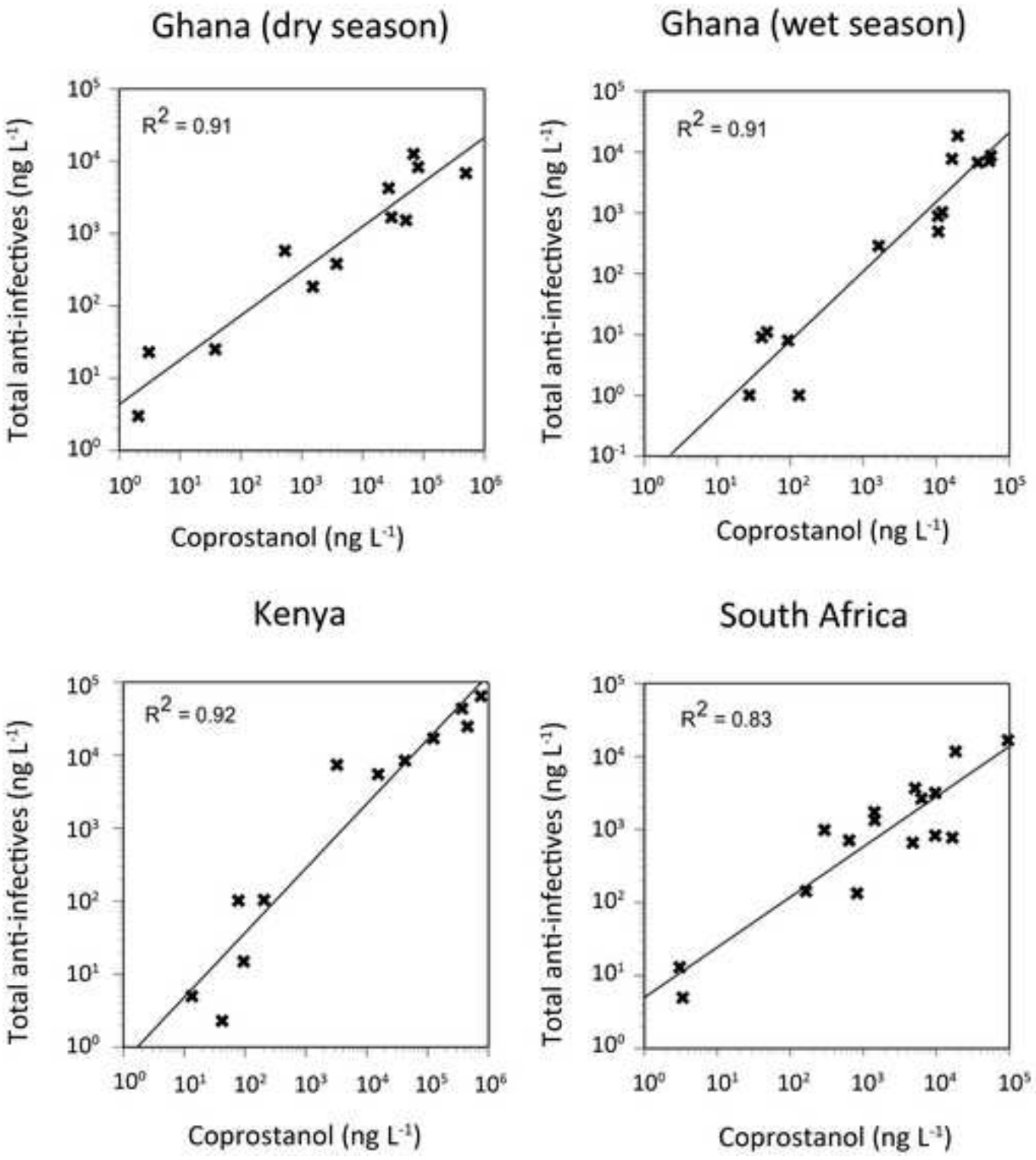
Figure Captions

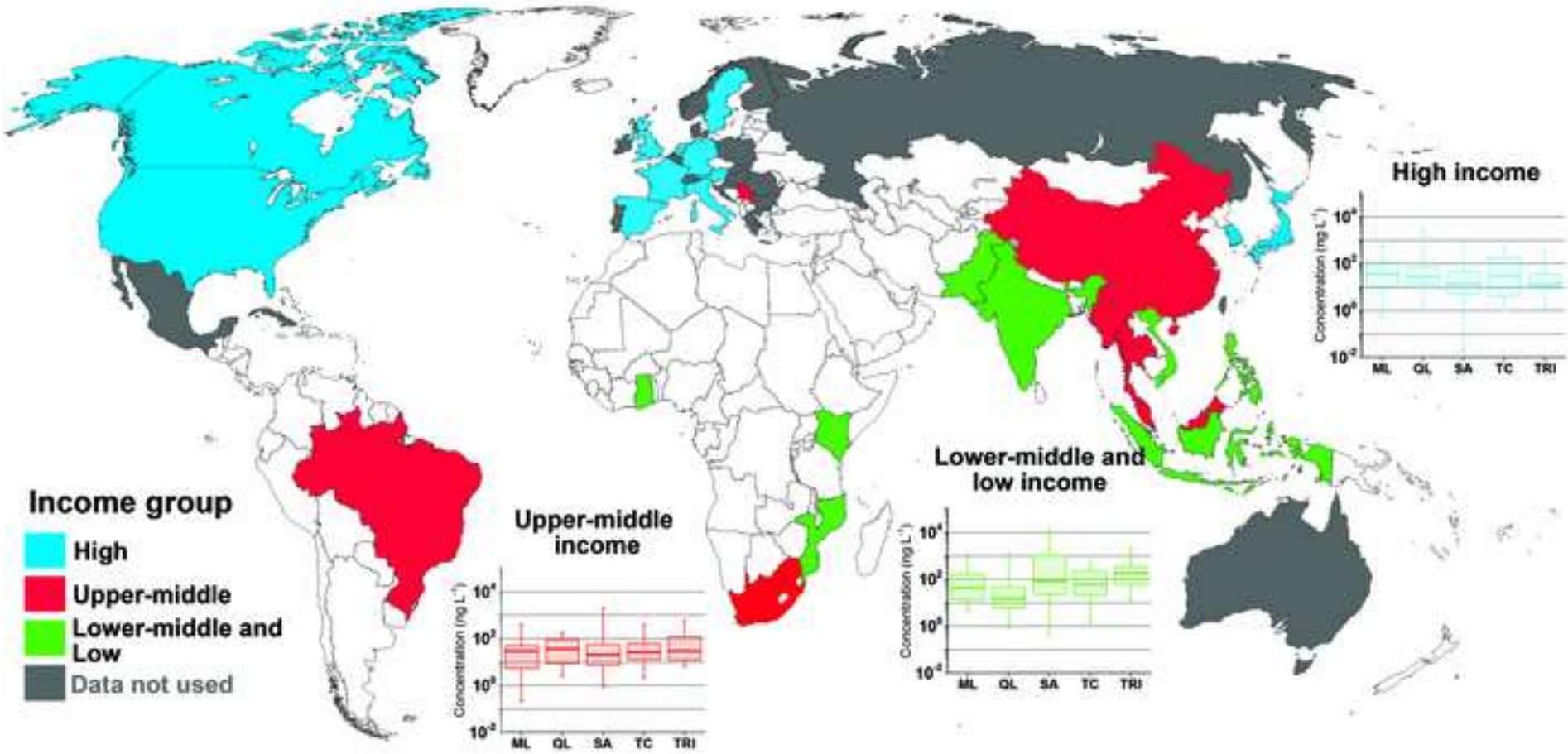
Figure 1. Concentrations of the target anti-infectives in surface water samples from Ghana, Kenya, Mozambique and South Africa. Distance between the opposite sides of the whiskers denotes the concentration range between maximum and minimum values and length of the box is the interquartile range. The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

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

Figure 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.


Ghana
Concentration (ng L-1)
AZI
CLA
ERY-H2O
SDI
SMX
SMZ
SPY
STZ
CTC
MC
OTC
TC
TRI
LIN
Kenya
Concentration (ng L-1)
CLA
ERY-H2O
SDI
SMX
SPY
CTC
MC
TC
TRI
LIN
Mozambique
Concentration (ng L-1)
AZI
CLA
ERY-H2O
SMX
SPY
OTC
TRI
South Africa
Concentration (ng L-1)
CLA
ERY-H2O
SDI
SMX
SPY
CTC
OTC
TRI


Ghana (dry season)
R^2 = 0.91
Total anti-infectives (ng L^{-1})
Coprostanol (ng L^{-1})
Ghana (wet season)
R^2 = 0.91
Total anti-infectives (ng L^{-1})
Coprostanol (ng L^{-1})
Kenya
R^2 = 0.92
Total anti-infectives (ng L^{-1})
Coprostanol (ng L^{-1})
South Africa
R^2 = 0.83
Total anti-infectives (ng L^{-1})
Coprostanol (ng L^{-1})


Income group
High
Upper-middle
Lower-middle and Low
Data not used
High income
Upper-middle income
Lower-middle and low income
Concentration (ng L⁻¹)
ML
QL
SA
TC
TRI