Supporting Online Material for:

Pharmaceutical Pollution of the World's Rivers

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Note: Excel documents containing the Supplemental datasets accompanies this material.

List of Abbreviations:

AMR - Antimicrobial Resistance
APIs - Active Pharmaceutical Ingredients
BMI - Body Mass Index
CEC- Critical Environmental Concentration
DALYs - Disability-Adjusted Life Years
dbRDA - Distance-based redundancy analysis
DISTLM - Distance Based Linear Modelling
GDP - Gross Domestic Product
GNI - Gross National Income
LOD- Limit of Detection
LOQ - Limit of Quantification
ND - Not Detected
PNEC - Predicted No Effect Concentration
PPP - Purchasing Power Parity
USD - United States Dollar

Quality control and assurance:

Although the sample collection protocol was previously cross-laboratory validated on an international-level with the United States Geological Survey (1), field blanks provided quality control over potential field-derived interference for 35% of the sampling campaigns (n=47 field blanks). Field blanks consisted of liquid chromatography tandem mass spectrometry (LCMS)-grade analytical water subjected to the same collection methods as the environmental samples (1). The number of field blanks collected in this work were similar or greater than other large-scale monitoring campaigns [e.g., 2, 3]. During analytical runs, a QC spiked sample followed by an instrument blank was run after every 10 injections to ensure accuracy throughout analysis. Spiked QC samples consisted of Liquid Chromatography/ Mass Spectrometry-grade ultrapure water fortified with all target APIs and internal standards at 400 ng/L each (80 ng/L of internal standards) and instrument blanks were LCMS-grade water spiked with internal standards only. Prior to each analytical run, the chromatography column was eqilibrated with a series of 20 injections consisting of an equal composite of all samples included in the upcoming run. Sampling materials and methods were the same across all sampling campaigns

and analysis of the samples occurred using one method and in one laboratory. Furthermore, an extensive analysis was conducted (1) to ensure no significant sample loss occurred during shipment under various environmental conditions and transit durations. The rate of sample loss due to breakage during transit was 1.7% (18 samples arrived broken and were not included in this work). No quantifiable concentration of the target APIs was identified in the field and analytical blanks. Identification of all analytes was confirmed both *in silico* via Thermo Scientific TraceFinder 4.1 Software and by visual inspection of the chromatograms. Transition ion ratio tolerance was determined for each run as the API-specific range observed over respective calibrations.

A quality control analysis was conducted to determine the effectiveness of using grab samples as a proxy of typical pharmaceutical concentrations on a national scale. Here, the deviation of the mean cumulative concentration of APIs determined in each sampling campaign of respective countries where more than one was conducted (n=51 campaigns representing 366 sampling sites across 17 countries sampled over all 4 seasons collectively) was compared to the mean national API concentration on both temporal and spatial scales (Table S8). This analysis compiled a dataset representative section (38%) of the total dataset. A difference of 1 order of magnitude from the national mean was determined acceptable based on that used recently by long term and catchment-wide modelling of pharmaceutical concentration evaluations (e.g., 4). Analysis revealed that only 5.9% of the calculated cumulative API concentrations (i.e., 3 of 51 tested) deviated from their respective national mean values by more than one order of magnitude (Fig S3). This indicates that grab samples can be justifiably used to represent typical pharmaceutical concentrations on a national and temporal scale when collected using the criteria set out in this work.

Statistical analysis of socioeconomic variables and API concentrations:

To determine the relationship between specific socioeconomic variables and API pollution, distance-based linear modelling (DISTLM) and distance-based redundancy analysis (dbRDA) were used. Relevant data of the socio-economic factors were extracted from four different open databases, including The CIA World Factbook (5), The FAO Aquastat (6), The World Bank Open Dataset (7) and The World Health Organisation Global Health Observatory (8). As complete datasets of socioeconomic indicators were not available for some countries, the initial analysis started with fewer countries but a maximum number of socioeconomic factors. The analysis was then repeated with fewer socioeconomic factors that were identified to be significant in the initial analysis and an increased number of countries. In total, 31 indicators and 84 countries were evaluated (Table S9-11).

Prior to the DISTLM analysis, cumulative concentrations of each of the eight classes of pharmaceuticals collected from multiple rivers in the same country were first averaged. The average pharmaceutical concentrations were fourth-root-transformed to minimize the influence of extreme values and formulated on an Euclidean distance resemblance. Socioeconomic factors were log(x+1)-transformed and standardized by their individual mean and standard deviation. During the analysis, socioeconomic factors were used as independent variables, while pharmaceutical concentrations were used as dependent variables. Different combinations of the socioeconomic factors were screened using Primer with PERMANOVA+ (v7.0.17, Primer-e). The best combination of socioeconomic factors with the smallest modified Akaike Information Criterion (AICc) and largest r2 was identified using the embedded “BEST” function in DISTLM. A sequential test was also performed with the “FORWARD” function to illustrate the sequential combination of the identified factors, starting from the most significant. A dbRDA diagram was also plotted using the identified factors.

Supplemental Image:

Image S1. Miniaturised sampling kit used for all sampling campaigns in this global study (1)

Supplemental Figures:

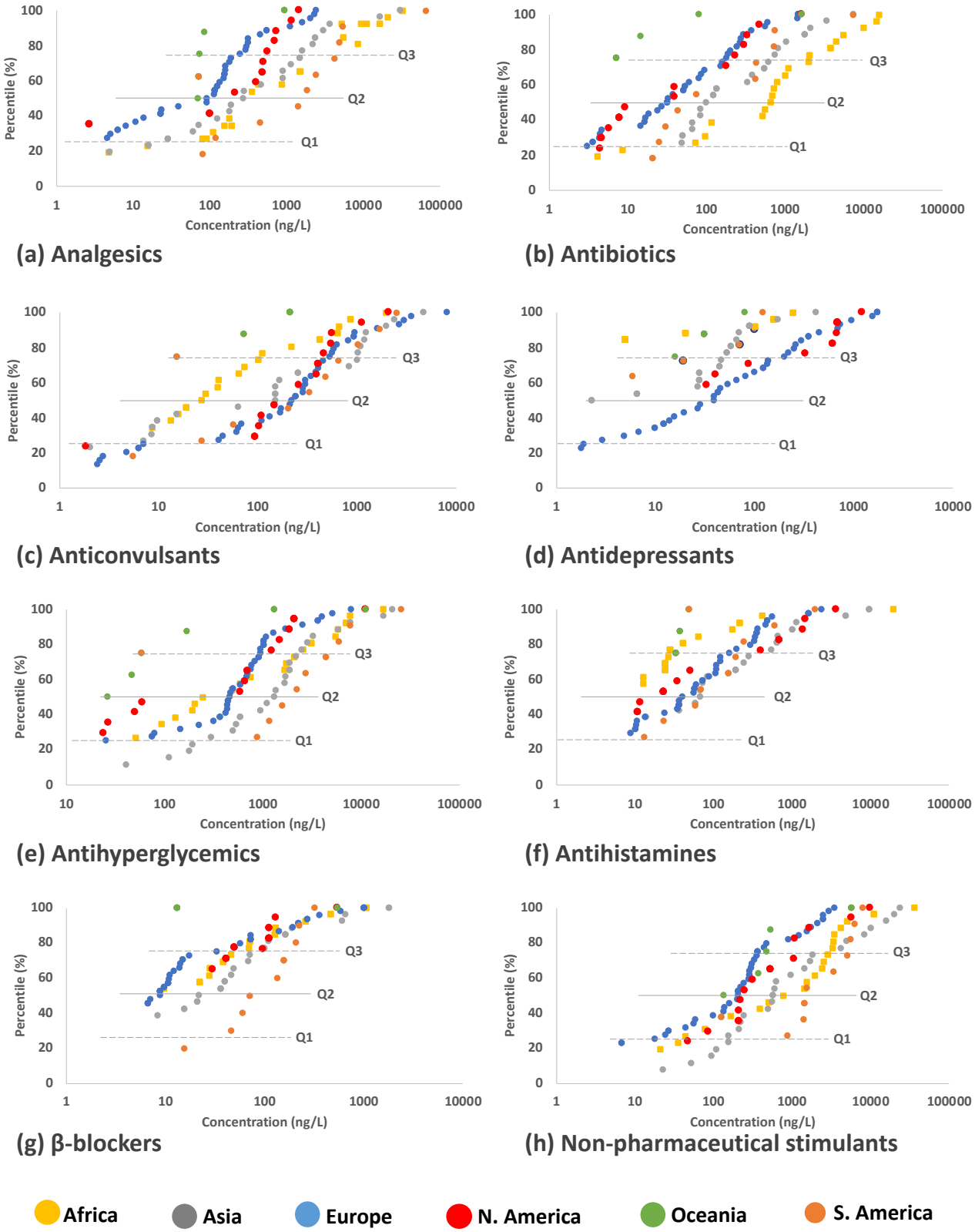


Figure S1. Distribution of cumulative concentrations of eight therapeutic classes of pharmaceuticals across Africa (n=26 sampling campaigns), Asia (n=26 sampling campaigns), Europe (n=44 sampling campaigns), North America (n=17 sampling campaigns), Oceania (n=8 sampling campaigns) and South America (n=11 sampling campaigns). Datapoints are only plotted for therapeutic classes where at least one representative API was found above the limit of quantification (LOQ). Concentrations are

presented on a Log scale, hence, the plotted distributions begin at a percentile representing API concentrations >1ng/L.

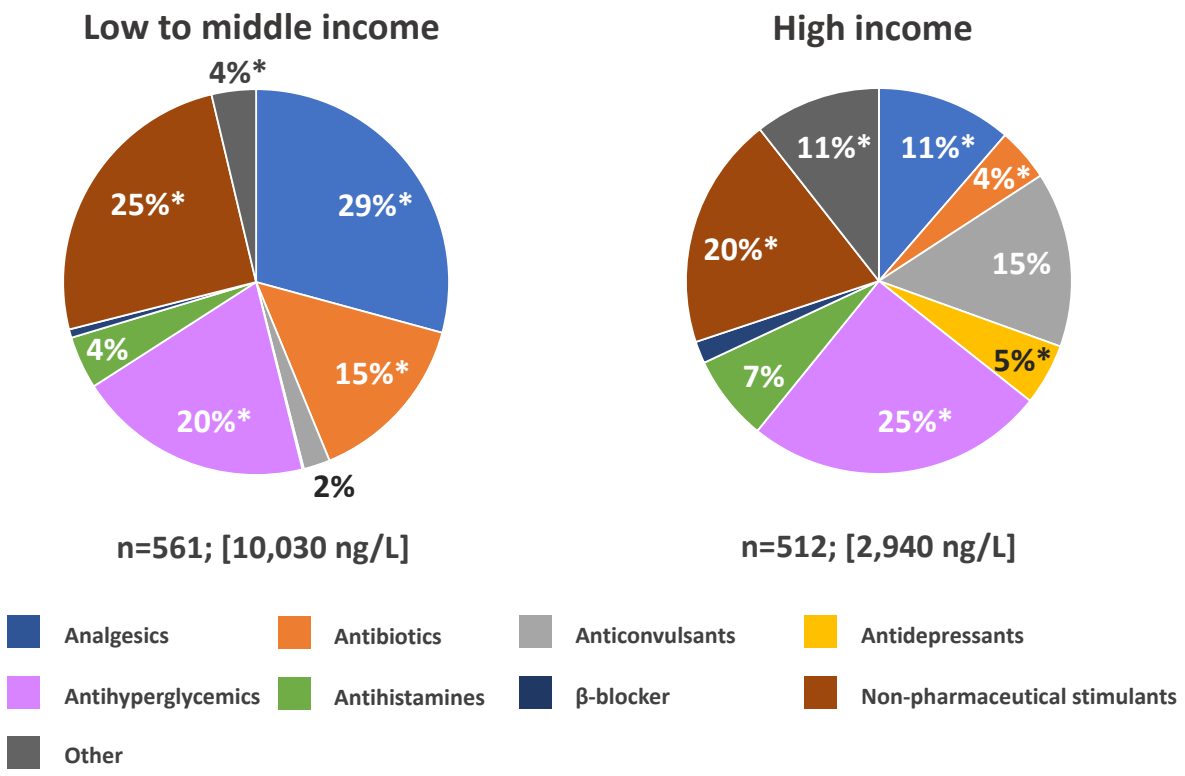
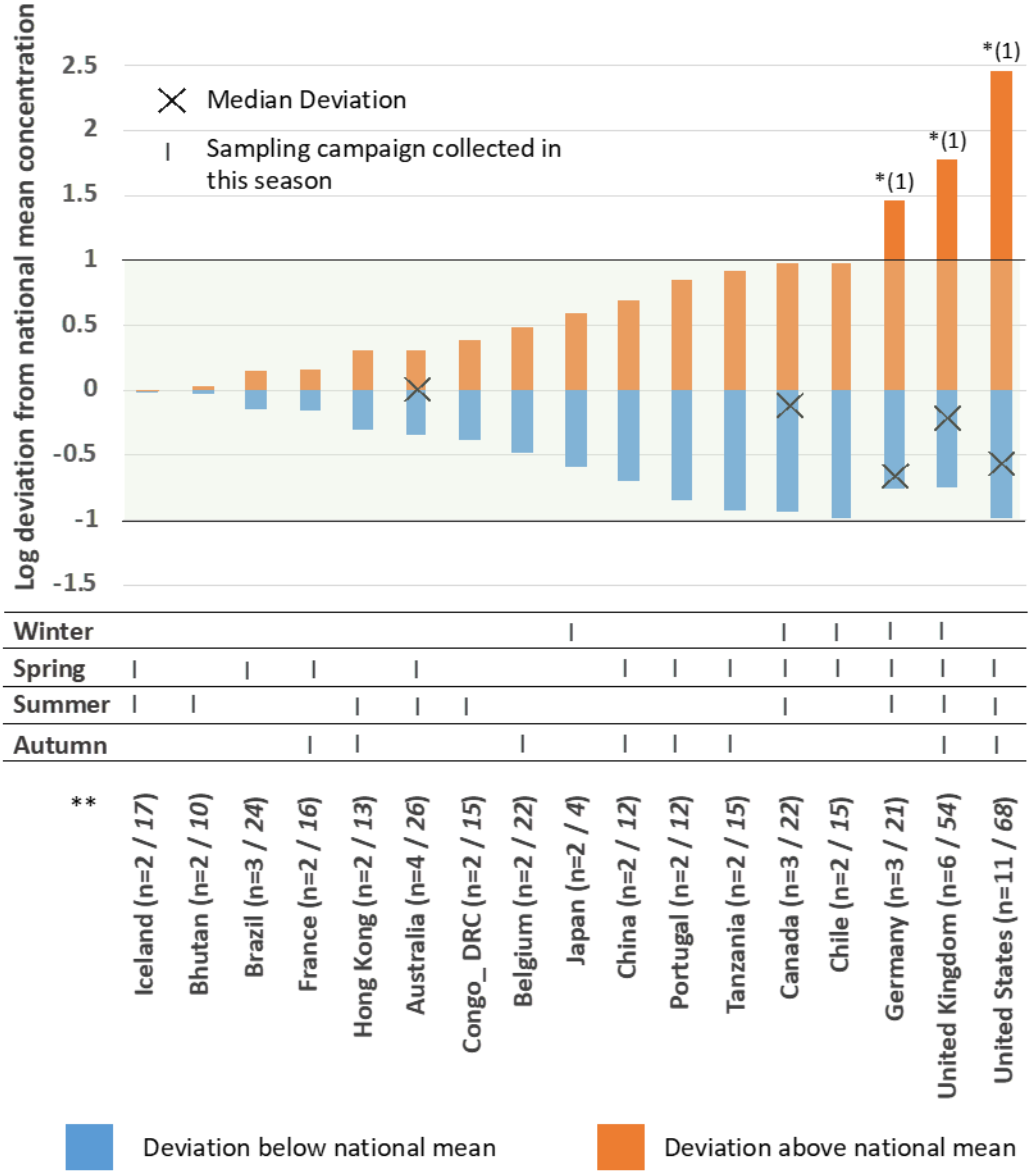


Figure S2. Mean composition by therapeutic class of the cumulative pharmaceutical concentration across all low-to-middle and high-income countries with significant differences (p defined as <0.05 in one-way ANOVA test followed by Tukey's Post Hoc test) between composition of respective therapeutic classes marked by (*). Mean cumulative pharmaceutical concentration is provided in brackets below the respective pie charts. Note: the Antarctic samples were excluded from this analysis due to a lack of appropriate Gross National Income- index data.



*Number of sampling campaigns (of 51 total in this analysis) deviating from the national mean concentration by > 1 order of magnitude

** 'n-value' refers to the (number of sampling campaigns conducted in respective countries / *number of sampling sites nation wide*)

Figure S3. Analysis of sampling campaign mean cumulative pharmaceutical concentration deviation from respective national mean concentrations across 51 sampling campaigns (n=366 sampling sites) representing 6 continents. The green zone represents acceptable deviation of 1 log unit.

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Acknowledgments:

The authors would like to thank the Centre of Excellence in Mass Spectrometry at the University of York where the mass spectrometer that performed this work is located.

The project was partly supported by the Medical Research Council (Project: MR/R014876/1), the British Council Institutional Links STREAM program (Project Number 277947262), the Instituto Antartico Chileno Regular Funding Projects (REF: INACH_RT_12_17), ANID PIA Anillo INACH ACT192057 and ANID FONDECYT 1210946. The Slovenian water sample collection was financed by the Slovenian Research Agency (ARRS), Research Programme P4-0092.

The authors would like to thank Amanda Wong and Katherine Wong for their help by collecting water samples in Calgary, Canada as well as Prof. M. M. Pathmalal for help in the collection of samples in Sri Lanka.

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