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International Water Quality: Global Patterns of Water Pollutants and Pathogens

Leslie Lange

A thesis submitted to the faculty of Brigham Young University in partial fulfillment of the requirements for the degree of

Master of Science

Benjamin W. Abbott, Chair Marynes Montiel Zachary Aanderud

Department of Plant and Wildlife Sciences

Brigham Young University

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ABSTRACT

International Water Quality: Global Patterns of Water Pollutants and Pathogens

Leslie Lange Department of Plant and Wildlife Sciences, BYU Master of Science

Water quality is an essential component of vibrant societies and ecosystems. For decades, researchers, managers, and policymakers around the world have struggled to accelerate societal progress while preserving and enhancing water quality and human health. This thesis consists of two studies that I hope will contribute to better understanding, policy, and management. In the first study, I evaluated spatial and temporal patterns in global water quality and their relationship to gross domestic product (GDP) per capita, as a metric of socioeconomic development status. Using global water quality datasets containing over 2.7 million observations, I tested the Environmental Kuznet Curve (EKC) hypothesis, which predicts that environmental degradation is highest at intermediate levels of socioeconomic development. I found that 46% of pollutants persisted at elevated concentrations despite GDP per capita. Because of this, high income countries experience a false sense of water security as water regulation violations are common on a global scale. In the second study, I measured waterborne pathogens in Guayaquil, the largest city in Ecuador. With a population of over 3 million and distinct hydrology from monsoonal rains and estuarine flooding, the Guayaquil metropolitan area faces drinking water and sanitation challenges similar to much of the developing world. I found that 100% of the samples we collected had unsafe total coliform counts. Water pollution is widespread and is a result of careless action. Moving forward, chronic pollution can be prevented with proper legislation that holds governments, companies, and individuals accountable.

Keywords: water quality, GDP, Environmental Kuznet Curve, waterborne pathogens, monsoonal season, population density, water security

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CHAPTER 1

How does Water Quality Relate to Socioeconomic Development at a Global Scale?

Leslie Lange, Mitchell Greenhalgh, Raymond M. Lee, Margaret Hancock, Isabella M. Errigo, Sophie Hill, Zach Eliason, David M. Hannah, Stefan Krause, Adam Ward, Iseult Lynch, Christa Kelleher, Kieran Khamis, Benjamin W. Abbott

Department of Plant and Wildlife Sciences, Brigham Young University, Provo, UT Master of Science

ABSTRACT

The Environmental Kuznets Curve (EKC) predicts that the relationship between environmental degradation and per capita gross domestic product (GDP) follows a bell-shaped curve with the worst conditions at intermediate levels of GDP. The EKC hypothesis has been influential in environmental policy analysis, but it remains largely untested for global water pollution. Here, we combined 23 global and national hydrochemical datasets, containing over 2.7 million observations, to test this hypothesis for a variety of water pollutants, including human pathogens, sediment, nutrients, heavy metals, pharmaceuticals, and plastics. Our analysis of data from 100 countries revealed that the EKC hypothesis was supported in only 7 of the 25 pollutants tested (28%). Overall, water quality was not clearly related to GDP, but residuals in pollutant-GDP relationships showed 44% of analytes correlated with closed anocracies and 28% of analytes correlated with continents to be important factors contributing to above-average pollution concentrations creating pollution clusters. Assuming the EKC will be followed without considering local conditions may contribute to high income countries experiencing a false sense of security regarding safety of drinking water and ecological status of aquatic ecosystems.

INTRODUCTION

The Environmental Kuznets Curve (EKC) posits that the most severe pollution occurs in rapidly developing, middle-income countries and becomes less intense in lower and upperincome countries, resulting in a pollution versus development relationship that follows a bellshaped curve (Grossman & Krueger, 1995). The EKC has been validated in some circumstances with empirical data on country and continental scales for air pollutants(Apergis & Ozturk, 2015; Shahbaz et al., 2013; Torras & Boyce, 1998) but other investigations have not found support for the EKC on similar scales (Akbostanci et al., 2009; Dogan & Turkekul, 2016; Roca et al., 2001). Others critique the statistical robustness (Andreoni & Levinson, 2001; Wagner, 2015) or the theoretical soundness (Carson, 2010; Kaika & Zervas, 2013) of the EKC because global wealth may not be normally distributed and data from poor countries is often unrepresented.

In this context, we compared the relationship between socioeconomic development and water pollution with a global dataset of diverse water quality parameters. We aim to advance this ongoing debate by gauging the validity of the EKC via global analysis of six classes of water pollutants vs annual gross domestic product (GDP; U.S. \$/capita) vs time. We hypothesize that low income, middle income, and high-income countries, as classified by The World Bank (Hamadeh et al., 2021), will indeed see different types and intensities of water pollutants. However, because the type of predominant pollution changes with socioeconomic development, we predict that the overall water quality will not be consistently correlated with GDP. We have previously shown that water pollutants can be grouped into three main "waves" correlating with different stages of economic development (Hannah et al., 2022):

- Wave 1 pollutants consist of suspended sediment loads and human pathogens associated with high population density and little wastewater treatment infrastructure in low-income countries.
- Wave 2 pollutants involve higher rates of nutrient and heavy metal pollution resulting from more intensive agricultural practices and industrial development in developing economies and middle-income countries.
- Wave 3 pollutants include emerging pollutants such as microplastic and pharmaceutical pollution. Highly industrialized countries regulate wave 1 and 2 pollutants but emerging pollutants such as microplastics and pharmaceuticals from industrial and medical advances remain largely untreated.

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In this study, we tested this "evolving chemical cocktail" hypothesis of pollution based on the three waves of pollution and hypothesized that the overlapping waves could obscure an EKC pattern for overall water quality. We tested for nonlinearities in the relationship between water pollutants and GDP. We also evaluated what factors besides GDP influence water quality, including government type, climate, and characteristics of the economy.

MATERIALS AND METHODS

Data collection

To analyze the global EKC and water quality trends, we collected data from the GEMStat database and other publication sources (Table 1-1). We averaged data by country and by decade (2000-2009 and 2010-2019), except for data from 1970-1999, which were combined due to limited data and small sample sizes. We extracted and combined microplastic data from six publications (Fok & Cheung, 2015; Koelmans et al., 2019; Kosuth et al., 2018; Novotna et al., 2019; Statista, n.d.; Yonkos et al., 2014) because microplastic data was not available through GEMStat. Microplastics were measured as particle numbers per liter (#/L); data ranged from 2010-2019 as the study of microplastics in freshwater is a relatively new field. Microplastic data was only considered from sources with somewhat comparable sampling methods and units because unit conversions are not possible.

Data Analysis

To test our evolving chemical cocktail hypotheses, we conducted three analyses: (i) smoothed conditional mean analysis with Maximum Contaminant Levels (MCL); (ii) changepoint analysis; and (iii) residual analysis calculated from the smoothed conditional means

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and grouped by country characteristic data. All data analysis and visualizations were performed in the R environment (R Core Team, 2020).

Smoothed conditional means

To assess the EKC with global data and test the evolving chemical cocktail hypothesis, we calculated the smooth conditional means for the 26 pollutant concentrations vs GDP (Table 1-1). This provided a non-parametric way of visualizing the relationship between GDP and water quality. To determine pharmaceutical pollution impacts on river biodiversity, we used alpha taxonomic diversity metrics—pharmaceutical richness, Shannon's Equitability Index (SHEI), and Shannon's Diversity Index (SHDI) (Shannon, 1948). We integrated MCL according to the EPA as a water quality index to characterize pollution concentrations as 'safe' or 'unsafe' for each analyte respectively (Srebotnjak et al., 2012; US EPA, 2014, 2015). MCL give greater clarification to pollution concentrations and trends. For example, not all concentrations of Total Nitrogen are considered harmful, therefore, adding a horizontal threshold as the MCL clarifies which countries have negative impacts on aquatic and human health.

Changepoint Analysis

To quantify nonlinearity in the relationships between pollutants and GDP, we used a nonparametric changepoint analysis: Pruned Exact Linear Time algorithm (Haynes et al., 2016). For pollutants that follow the EKC, this analysis could reveal the threshold level of wealth where the change in direction occurs. Assuming only a single changepoint in the pollutant-GDP relationship exists, there are nine generic relationships possible (Figure 1-1; Moatar et al., 2017). The changepoint relationships generalize contaminant trends before and after the changepoints or thresholds. This method of changepoint detection was used because many other methods have parametric assumptions that are not met by the water quality data in this study (Davis et al.,

2006; Killick et al., 2012; Lavielle, 2005). The analysis was performed using the *changepoint.np* package in R (Haynes et al., 2021; Table 1-2).

We ranked and transformed the data using the natural logarithm in R. The changepoint estimates were identical to the untransformed data; therefore, only the results from the untransformed data were reported.

Residual Analysis

To evaluate how other factors influence the relationship between water pollution and GDP, we conducted a residual analysis. We calculated the difference between the individual country's pollutant concentration and the smoothed conditional mean for that level of GDP described above. We then grouped each class of pollutants by each international factor data described in the section below. Analyte concentrations grouped by these identifiers showed country overperformance (e.g., above-average residuals indicating high pollution concentration) or country underperformance (e.g., below-average residuals indicating low pollution concentration).

Country Characteristic Data

To characterize relationships between water quality and country characteristics, we downloaded a suite of country-level data from Our World in Data. International data included continent, primary and secondary land use (% agriculture, forest land, cropland, or meadows and pastures), urban land use (%), country area (km²), population, and population density (# per km²). We also collected climate data categorized by the Köppen climate classification which divides climate into five groups: tropical, dry, temperate, continental, and polar or alpine climates. We collected data describing Economic Freedom using an approach developed by the Fraser Institute. Economic freedom was standardized on a scale of 0-10, where 10 represented maximum economic freedom to choose, trade, cooperate, and compete with others. We used the Economic Complexity Index to assess national industry and economy. This data used information on national exports to reduce the economic system of a country into two dimensions: (i) the sum of exported products, and (ii) the quality of exported products. An economic complexity ranking score of one corresponds to the country with the most complex economy as ranked by the Observatory of Economic Complexity each year. We used a Democracy Index to determine national political regimes and used a scale from -10 (full autocracy) to 10 (full democracy). Autocracy is defined as a governmental system with an individual having complete power while anocracy is defined as a political regime combining democracy and dictatorship, which score between -5 and 5. Anocracy is defined as a form of government which is a mix of democracy and dictatorship. We also used agricultural variables which included total agricultural withdrawals (m³/year), pesticide and insecticide use (tons/y), and fertilizer use (kg/hectare of arable land). Total agricultural water withdrawals were defined as the annual quantity of selfsupplied water withdrawn for irrigation, livestock, and aquaculture purposes. Industrial variables consisted of plastic generation (tons/year), and ore and metal exports. Ore and metal exports were defined as a percentage of all merchandise exports made of ores and metals.

RESULTS

Relationships between GDP and pollutants

The changepoint analysis indicated that 4 out of 12 trends followed the EKC. Notably, pharmaceuticals showed a frequency of 100% of pharmaceutical observations followed the EKC and a frequency of 57% of nutrient observations (Figure 1-2). In total, a frequency of 35% of global trends validated the EKC. Conversely, a frequency of 46% of global trends following a

"no relationship" trend. A frequency of 100% of microplastic observations and 100% of sediment observations produced a "no relationship" trend between concentration and GDP. Human pathogens also showed a high frequency calculated at 75% of the "no relationship" trend between GDP and pathogen concentration.

Wave 1 Pollutants: Human Pathogens and Sediment

We expected Wave 1 pollutants to decrease with GDP because of advances in water treatment technology. Contrary to this hypothesis, the trend showed that wave 1 pollutants persisted in high-income countries (Figure 1-3). This shows that increasing GDP is not always effective at remediating wave 1 pollution. However, not all wave 1 pollutants pose the same degree of risk to humans (e.g., not all coliforms are considered harmful to humans so "total coliforms" may overestimate scale of risk). The MCL showed that 87% of total coliform, 81% of *Streptococcus*, 79% of fecal coliforms, and 76% of *E. coli* observations remained above recommended freshwater criteria in high-income countries despite high GDP. Total Coliforms, *E. coli*, and fecal *Streptococcus* observations showed "no relationship" and no changepoints across time and space, indicating that human pathogen concentrations persist as GDP increases.

The changepoint for fecal coliforms shows low-income countries had high levels of fecal contamination with only 5% of low-income countries experiencing little to no fecal detection. However, the trend pre-changepoint for fecal coliforms indicates increasing concentrations for low-income countries while the post-changepoint trends indicate decreasing concentrations in middle- and high-income countries.

Higher income countries had relatively acceptable levels of sediment pollution apart from two outliers. Only 32% of countries show unsafe concentrations of sediment pollution. Unlike human pathogen pollution, global sediment pollution does not persist above MCL across GDP. Turbidity data suggest that when residuals were grouped by continent and climate types, South American, African, and Asian countries with temperate climates show above average sediment pollution from 2010-2019. No significant global effects were found for human pathogen residuals.

Wave 2 Pollutants: Nutrients and Heavy Metals

Concentrations of wave 2 pollutants were highest in low-income countries and exhibited the largest variation of pollutant concentrations (Figure 1-4). 18% of detected changepoints occurred in low GDP (\$) countries, 55% in middle GDP (\$), and 27% in high GDP (\$). The dollar threshold of changepoints can be indicative of pollutant responses associated with economic factors. Generally, our hypothesis was supported, with the data indicating that middle-income countries are more burdened by wave 2 contaminants than low or high GDP countries. As predicted, high income countries are experiencing comparatively lower levels of pollution from heavy metals and nutrients as compared to middle income countries. However, the water quality index reveals that, although high-income countries sustain lower contamination concentrations, these concentrations are not always below acceptable water quality (e.g., EPA) standards or MCL, as is the case for Cadmium (Figure 1-4 Panel 1C), Manganese (Figure 1-4 Panel 1I), Total Phosphorus (TP) (Figure 1-4 Panel 2A), and Total Nitrogen (TN) (Figure 1-4 Panel 2D) on a global level.

We found that closed anocracies experienced significant increases of Nitrate Nitrogen (NO₃-N), TN, and Dissolved Reactive Phosphorus (DRP) from 2010-2019, TP from 2000-2009, and Arsenic, Cadmium, Cobalt, Chromium, Copper, Iron, Manganese, and Nickel from 2000-2009. When residuals were grouped by continent, African countries showed above-average residual results for TP (2000-2009), TN (2010-2019), NO₃-N (2000-2019), and DRP (2010-

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2019). South American countries showed evidence of above-average Lead concentrations from 2010-2019. Additionally, countries with high population and population density (3rd quartiles) experienced high concentrations of DRP pollution (2010-2019). Finally, continental climates showed high TN concentrations (2010-2019) and dry climate countries showed high Cobalt concentrations (2000-2009).

Wave 3 Pollutants: Plastics and Pharmaceuticals

The change-point analysis for wave 3 pollutants detected thresholds of GDP (\$) only in high-income levels for pharmaceutical observations. As hypothesized, this analysis confirms that freshwater pharmaceutical pollution is an emerging problem mainly afflicting high income countries (Figure 1-5, panel A-C). Notably, the smallest and wealthiest country (Luxembourg) is experiencing minimal pharmaceutical contamination. Though microplastics data were more difficult to analyze (due to limited data), initial analysis showed little evidence of a relationship with economic indicators. Note: MCL for wave 3 contaminants are not established due to various pharmaceuticals observed with different MCL values and MCL for microplastics having not yet been determined.

Few significant international effects were found for wave 3 contaminants. The least populated countries (1st quartile), dry climate, and the continents of North America and Oceania show evidence of below average microplastic pollution, due to a combination of observer bias (whereby sampling has focused on developing countries) and "waste colonialism." No significant residual results were found for pharmaceutical observations.

DISCUSSION

In this study we analyzed the global validity of the EKC hypothesis through the evolving chemical cocktail hypothesis for 6 types of global freshwater pollutants and 26 classes of pollutants. Though we hypothesized that low, middle, and high GDP countries face distinct waves of pollution, we found that: (i) water pollution is widespread across economic development levels; (ii) water regulation violations occur frequently in high GDP countries; and (iii) data from low-income countries is underrepresented but is vital for accurate global analysis and policy making. Wave 1 (human pathogens and sediment) pollutant behavior is consistent with these points.

An uphill battle for low GDP countries

Waste trading is popular among high GDP countries to low GDP countries which have lax environmental regulations (Kellenberg, 2012). In 2020, the European Union exported 38.4 million metric tons of recyclable raw materials to non-European Union countries (*EU-27*, 2020) with 196 million metric tons of plastic were traded globally in 2018 (UNCTAD, 2021). Due to lax environmental regulations and inadequate monitoring systems, data from low GDP countries is largely unavailable and our data may be inaccurate contributing to bias in our observations since high GDP countries are more likely to self-report pollution than low GDP countries. We note that this is likely to be related to the dominant trend of high GDP countries sending their plastic waste to lower GDP countries for disposal, which has been termed 'waste colonialism' (Cornea et al., 2021; Hannah et al., 2022; Michaelson, 2021).

Actions to mitigate water pollution in low GDP countries include: (i) Leveraging long term and novel data sources (Cooley et al., 2017): what is needed is higher resolution data in low GDP countries. Further research with a global monitoring and reporting program, such as via the UN Intergovernmental Panel on Chemicals and Waste, would be a vital next step (IISD's SDG Knowledge, 2020; Mehta, 2022) to provide more comprehensive temporal and spatial data to give a clearer picture of global water quality trends. Emerging technologies could help overcome data constraints such as remote sensing, machine learning, and blockchain (Damania et al., 2019). (ii) Share knowledge: low GDP countries may benefit from experiences in high GDP countries as well as from technological and legal advances resulting in faster progress on solutions with less environmental degradation to generate a leapfrog effect for human wellbeing (Hannah et al., 2022). Policy on water quality must be based on law and regulation, rely heavily upon technology, be subjected to continuous evaluation, be backed by strong enforcement, and incorporate the polluter-pays principle (Landrigan et al., 2018). These principles can be used as models and adapted to countries at every level of income (Landrigan et al., 2018). (iii) To reach the MDGs to provide improved sanitation and safe drinking water for about 2 billion people, concerted efforts to develop and implement cost-effective sanitation systems in the growing megacities in areas with water stress are of highest priority (Schwarzenbach et al., 2010). False sense of water security and solution to persistent pollutants in high GDP countries

In just one year (ca. 2015), some 21 million Americans were exposed to unsafe drinking water in the form of *E. coli* and *Legionella* bacteria, disinfection by-products, Lead, and toxic algal blooms (Allaire et al., 2018). 100% of rivers in England, Germany, Belgium, and Sweden failed to meet "good" chemical status standards, and less than one-third of rivers met comparable ratings used in the USA (Kristensen et al., 2018). This supports our results and the notion that water quality violations are widespread and irrespective of GDP. Water violations are more likely to occur when water treatment systems are managed by the public sector. Sectors that are controlled by governments are less likely to be penalized for violations and more incentivized to

violate water regulations (Konisky & Teodoro, 2016; Rahman et al., 2010; Rubin, 2013). Therefore, generating higher GDP is not an automatic solution for poor management, weak regulation, and ineffective policy decision making by the public sector (Arrow et al., 1995; Fuller et al., 2022a).

Specific actions to illuminate persistent pollution in high income countries include: (i) identify specific places, times and conditions that degrade water quality and work to redress stoichiometric imbalances and water quality violations (Damania et al., 2019; Peters et al., 2008); and (ii) wastewater treatment plants must play a bigger role in removing plastics and pharmaceuticals from rivers (Damania et al., 2019). Therefore, point sources need increased attention and investments over the next decades (Schwarzenbach et al., 2010). Higher income countries can better insulate their citizens from the effects of environmental water pollution, whereas middle- and low-income countries cannot— the same level of pollution is much more damaging to human health on the poorer end of the development spectrum.

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FIGURES



Figure 1-1. The 9 possible water pollution- annual gross domestic product (GDP;U.S. \$/capita) relationships. (A) Postive-Postive relationship between GDP (x) and pollution concentrations (y); (B) Positive-Stable relationship between x and y; (C) Positive-Negative relationship between x and y; (D) Stable-Positive relationship between x and y; (E) No relationship between x and y; (F) Stable-Negative relationship between x and y; (G) Negative-Positive relationship between x and y; (H) Negative-Stable relationship between x and y; (I) Negative-Negative relationship between x and y. Panel C represents the general pattern we would expect to see if pollutants followed the EKC.



Figure 1-2. Relationships found between pollutant concentrations and GDP organized by most frequent trends to least frequent trends. Trends found were calculated from changepoint analysis results. A) relationships found between heavy metals concentrations and GDP; B. relationships found between nutrient concentrations and GDP; C. relationship found between pharmaceutical concentrations and GDP; D. relationships found between human pathogens and GDP; E. relationship found between microplastics and GDP; relationship found between sediment pollution and GDP.



Figure 1-3. Wave 1 pollutants. A) Relationship between fecal coliform concentrations and GDP; B) relationship between total coliform concentrations and GDP; C) relationship between fecal streptococcus concentrations and GDP; D) relationship between *Escherichia Coli (E. coli)* concentrations and GDP; E) relationship between turbidity and GDP. Vertical dotted line represents changepoint threshold. Horizontal dotted line represents Maximum Contaminant Level (MCL).


Figure 1-4. Wave 2 pollutants. Panel 1) Heavy metals (A-K) and their relationship to GDP. A) Relationship between aluminum and GDP; B) relationship between arsenic and GDP; C) relationship between cadmium and GDP; D) relationship between cobalt and GDP; E) relationship between chromium and GDP; F) relationship between copper and GDP; G) relationship between Iron and GDP; H) relationship between mercury and GDP; I) relationship between manganese and GDP; J) relationship between nickel and GDP; K) relationship between lead and GDP. Panel 2) Nutrients (A-G) and their relationship between GDP. A) Relationship between total phosphorus and GDP; B) relationship between total inorganic phosphorus and GDP; C) relationship between dissolved reactive phosphorus and GDP; D) relationship between nitrate and GDP; G) relationship between dissolved organic carbon and GDP. Red vertical dashed line represents changepoint threshold. Horizontal dotted line represents Maximum Contaminant Level (MCL).



Figure 1-5. Wave 3 pollutants. A) Relationship between pharmaceutical analyte richness and GDP per capita; B) relationship between Shannon's Diversity Index to measure pharmaceutical diversity and GDP per capita; C) relationship between Shannon's Equitability Index for pharmaceuticals and GDP per capita: D) relationship between microplastics and GDP per capita. Red vertical dashed line represents changepoint threshold. Horizontal dotted line represents Maximum Contaminant Level (MCL).

TABLES

Table 1-1. Metals and plastics parameters used multiple studies to combine global data for greater data richness. Most parameters are comprised of several indicator samples or observation types, each measured and analyzed in their respective units. Data sources included 11,248 GEMStat monitoring stations for nutrients, 5,124 stations for sediment, and 6,839 stations for pathogens.

Parameter	and data information Database/Publication	Observation	No. of	No. of	Mean No. of
		Types and Units	Countries	Observations	Observations
Human	GEMStat	Fecal	46	134,664	164
Pathogens		Coliforms,	_	-)	_
		Total			
		Coliforms,			
		Fecal			
		Streptococcus,			
		E. coli (CFU)			
Sediment	GEMStat	Turbidity	21	236,228	39
		(NTU)			
Nutrients	GEMStat	DOC, DRP,	75	2,240,449	466
		NH3N, NO3N,			
		TIP, TN, TP			
		(mg/L)			
Heavy Metals	Kumar et al., 2019;	As, Hg, Pb, Co,	43	657	513
	Zhou et al., 2020	Cu, Ni, Zn, Cd,			
		Cr, Al, Mn, Fe			
		(ug/L)			
Pharmaceuticals	Lehmphul, 2016	Antibiotics,	71	178,706	132
		Hormones,			
		Antidepressants,			
		and various			
		other drugs			
		(ug/L)			
Microplastics	Fok & Cheung, 2015;	Microplastics in	25	276	25
	Koelmans et al., 2019;	surface water,			
	Kosuth et al., 2018;	drinking water,			
	Novotna et al., 2019;	tap water etc.			
	Yonkos et al., 2014	(#/L)			

Table 1-2. Our data did not meet any of the established assumptions of changepoint detection algorithms. NP-Pelt allowed for non-parametric data and could detect multiple changepoints.

Assumptions of changepoint detection algorithms							
Detection Method	Parametric	Multiple Changepoint	A priori knowledge of (how many) changepoints required	Independence			
Sequential Change Detection (Hypothesis testing, <i>cpm</i>)	√(Yes and No)	✓ (Yes and No)	X	~			
Piecewise Regression (segmented)	\checkmark	\checkmark	\checkmark	\checkmark			
PELT	\checkmark	Х	Х				
NP-PELT (<i>changepoint.np</i>)		\checkmark					

CHAPTER 2

Mapping Waterborne Pathogens in Guayaquil, Ecuador to Improve Human Quality of Life

Leslie Lange, Marynes Montiel, Isabella M. Errigo, Joseph Berruz, Thalía Castillo, Teresa Gomez, Sara Sayedi, Justin Lemke, Zach Eliason, Mitchell Greenhalgh, Benjamin W. Abbott

Department of Plant and Wildlife Sciences, Brigham Young University, Provo, UT Master of Science

ABSTRACT

Despite centuries of effort, waterborne pathogens remain a serious global health threat, especially in developing countries. High rates of waterborne disease are affecting the nations and regions that often lack adequate monitoring and treatment, exacerbating cycles of disease and poverty. Identifying and mitigating sources of waterborne pathogens are complicated in areas that experience monsoonal flooding periods, which can overwhelm treatment infrastructure. Here, we analyzed 6 human pathogen species in the dry and wet season of Ecuador's largest city, Guayaquil. We compared pathogen abundance between the seasons and related pathogen abundance with watershed characteristics such as population size. Unexpectedly, we found significant rises in only one out of six pathogens during the monsoonal season. However, all 6 pathogen concentrations far exceeded environmental health standards during both the dry and monsoonal season, with *E. coli* exceeding standards by up to three orders of magnitude. Residents have been compelled to boil water or adopt chlorine treatment practices to purify their drinking water since Guayaquil water managers face substantial political barriers to progression. While some pollution cannot be prevented, excessive pollution is a result of careless action. Chronic pollution can be prevented with proper legislation that holds governments, companies, and individuals accountable.

INTRODUCTION

Chronic water pollution is pervasive in almost all countries (reference), but developing countries often experience the highest concentrations of human pathogen pollution. Human pathogen-contaminated water causes almost 1 million deaths annually (Magana-Arachchi & Wanigatunge, 2020; Romero-Sandoval et al., 2019; WHO, 2015). Additionally, diseases from unsafe water and sanitation often stymie human development creating lifelong handicaps such as blindness, decreased cognitive function, and other debilitating disease (Wright et al., 2007; Fuller et al., 2022a). Though it is impossible to adequately quantify the burden of loss and suffering associated with preventable waterborne disease, the economic damage is estimated at \$150 billion USD in low and low-middle income countries alone (Landrigan et al., 2018). To break this vicious cycle of poverty and disease in developing countries such as Ecuador, reducing the prevalence of waterborne pathogens is critical for improving water quality.

Ecuador is the 5th most impoverished country in South America (Sen Nag, 2019) with 10% of the population only having access to unreliable sources of clean water even across varying socioeconomic status (Swyngedouw, 1995; Wingfield et al., 2021). Because of its location on the equator, Ecuador has a monsoonal weather pattern with a dry season (May/June to October) and wet or monsoonal season (November to May). Flooding during the monsoonal season is severe due to its estuarine location around the Guayas River and many smaller tributaries (Figure 2-1). Monsoonal flooding in Guayaquil allows contaminated water from streams and rivers to reach residential areas, inundate streets and homes, and cause direct human exposure and illness from human pathogens (Altizer et al., 2006; Ivers & Ryan, 2006; Pascual & Dobson, 2005; Suwanpakdee et al., 2015).

In this context, we mapped pathogen concentrations in Guayaquil, Ecuador across seasonality and population gradients. To better understand how population density and seasonal changes impact pathogen concentrations, we map and analyze pathogen concentrations in Guayaquil, Ecuador using a mixed model system. First, we hypothesize that pathogen abundance is heightened because of pathogen suspension during flooding disturbances. Furthermore, if pathogen sources are distributed throughout the landscape (i.e., nonpoint sources), we expect to find uniform pathogen abundance across all study sites, but if a few, discrete pathogen sources are dominant, we predict highly clumped pathogen abundance. If decreased concentrations are detected during the monsoonal season, pathogens could be more diluted and widespread. We predict spatially stable physiochemical parameters correlating with pathogen concentration abundance as pathogen concentrations are spatially constant but heavily loaded. (Abbott et al., 2018; Dupas et al., 2019). Second, we hypothesize that high urban density correlates with elevated pathogen concentrations because of direct disposal of human waste in these poor, dense neighborhoods. We also predict that downstream neighborhoods bear the largest influx of pathogen concentrations during both wet and dry seasons. Abundant pathogen communities are associated with poor water quality. Coupling pathogen concentrations with physiochemical parameters aids in determining patterns of pathogen abundance and dynamics in flooding events and when negatively influenced by cumbersome population footprints.

METHODS AND MATERIALS

Study Area

Guayaquil is_the capital of Ecuador and is its most populated city. Located amid an estuarine system, Guayaquil is prone to urban flooding during the monsoonal season (January-April). The study area was divided into four zones: North, Center, South, and West. Each zone had five sample sites. In total, the 20 sampling sites were representative of urbanization gradients throughout the estuarine city. Each sample site was visited twice during the monsoonal season (January/February) and twice during the dry season (May/June). At each site, an IDEXX sample was collected to measure *E. coli* and Total Coliforms and a filtered water sample was collected for laboratory chemical analysis.

Experimental Design

To map pathogen abundance, we focused on quantifying 6 indicative pathogens of water quality. The pathogen measured were *Escherichia coli, Enterococcus Faecalis, Pseudomonas Aeruginosa, Vibrio Cholerae*- Green, *Vibrio Cholerae*- Yellow, and Total Coliforms (TC). However, here we focus primarily on quantifying *E. coli* due to its universal use as an indicator species of other pathogenic bacteria from human waste (Crosby et al., 2019; Edberg et al., 2000; Turck et al., 1969). *E. coli* is a gram negative, rod-shaped, facultative anaerobe. Though not all *E. coli* are harmful to humans, elevated concentrations of *E. coli* pose threats to human health. Health regulations require United States Environmental Protection Agency (EPA) standards for *E. coli* in recreational freshwater which are 126 Colony Forming Units (CFUs) per 100 mL of water. Likewise, standards for TC are 200 CFUs per 100 mL of water (*Recreational Water Quality Criteria*, n.d.). Survival rates of *E. coli* are dependent on several factors such as temperature, seasonality, salinity, and the presence of oxygen (Crosby et al., 2019). Furthermore, *E. coli* can also attach itself to particulate matter and become resuspended and transported in water during disturbance events (Mallin et al., 2000).

To determine *E. coli* concentrations, IDEXX Rota-corona-k99 Ag tests were used. We followed IDEXX protocol, mixing-the collected samples with the reagent provided and pouring the product into individual IDEXX quantification trays. Each tray was sealed with a household iron and incubated at 35°C for a 24-hour period. After the incubation period, the trays were analyzed using Most Probable Number (MPN) calculations to quantify TC and *E. coli*. Dilution methods of 1:10, 1:100, and 1:1000 were applied to samples with large values. Data were adjusted according to their dilution method using the MPN method. However, some IDEXX samples still showed all positive values after dilutions were used. MPN reaches to 2419.6 CFUs/100 mL. Fully positive samples could have any value above 2419.6 CFUs. To address this limitation, a 10% increase was added to the maximum threshold value of applicable samples. This created less bias than randomly assigning samples values in any distribution as they could create differences between the dry and monsoonal season.

To determine the relationship between *E. coli* and other bacterial pathogen concentrations, we quantified *Enterococcus Faecalis*, *Pseudomonas Aeruginosa*, and both Green and Yellow *Vibrio Cholerae*. These bacteria are opportunistic pathogens that are indicative of fecal waste and poor water quality (Gundry et al., 2004; Harwood et al., 2004; Mena & Gerba, 2009; Noble et al., 2003). Quantifying these bacteria gave a more nuanced view of overall water quality of Guayaquil and presented an overview of other harmful bacteria that are present. To quantify these bacteria, we used agar quantification methodology. Azide dextrose broth and KF agars were used to quantify Fecal Enterococci with the MPN. EPA regulation for Enterococcus is 33 CFUs/100 mL for recreational freshwater (*Recreational* *Water Quality Criteria*, n.d.). *Pseudomonas Aeruginosa* were quantified using the plate method on Cetrimide Agar. The plates were incubated at 37 °C for 24-48 hours, then counted to determine CFUs. EPA recreational water criteria for The EPA does not federally regulate *Pseudomonas* concentrations but the commonly used threshold level is 500 CFUs/100 mL (Development, n.d.). The plate method was also used to quantify *Vibrio Cholerae* on TCBS agar. The plates were incubated for 24-48 hours at 37°C and counted to determine CFUs. Yellow colonies indicated fermented *Vibrio* and are referred to as yellow *Vibrio* while nonfermented vibrio appeared as green colonies referred to as green *Vibrio*.

To determine pathogen-physiochemical relationships we used a HANNA multiparameter instrument (Model: HI 98194) to record pH, dissolved oxygen (%), dissolved oxygen (mg/L), oxidation reduction potential (ORP), conductivity (µS), hydraulic pressure (mmHg), turbidity (NTU), salinity (PSU), and temperature (°C) at each site. Anion and cation solute data were extracted from water samples using an Ion Chromatography analyzer to add to pathogen-physiochemical relationships such as Fluoride, Chloride, Nitrite, Sulfate, Bromide, Nitrate, Phosphate, Lithium, Ammonium, Potassium, Magnesium, Calcium, Strontium, and Sodium.

Data Analysis

To test pathogen responses to flood disturbance we used a Mann Whitney U test or Wilcoxon rank sum test for nonparametric data (Mann & Whitney, 1947) using the measured CFU values of all pathogens and compared across the dry and monsoonal season and also pathogen concentrations grouped by species. We created visual boxplots to analyze pathogen concentration relationships. To test the pathogen-physiochemical response, we compared pathogen concentrations and physicochemical water quality parameters during and after the monsoonal season using a correlation table. All mentioned analyses used R-statistical software.

To visualize spatial abundance and temporal shifts in pathogen concentrations and their relationship to population density and land use throughout the study area we mapped pathogens using ArcGIS. This required us to analyze the demographic density arrangement of Guayaquil using population density measurements for area described in number of people/km². Population demographics were categorized in ten quantiles to provide further categorical analysis as heat characteristic on each map. A statistical linear regression model was used to assess concentrations changes across population gradients using the numerical population data extracted from ArcGIS. Maps were used to determine spatial and temporal pathogen abundance to aid in visualizing pathogen hot spot locations.

RESULTS

Pathogen concentrations vs seasonality

We predicted increased pathogen concentration values in the monsoonal season compared to the dry season. During the monsoonal season, CFU concentrations increased at 65% of sites for *E. coli*, 55% of sites for *Pseudomonas*, 55% of sites for TC, 45% of sites for *Enterococcus*, 25% of sites for Yellow *Vibrio*, and 25% of sites for Green *Vibrio*. Likewise, the proportion of sites that decreased in CFU concentrations during the monsoonal season was 75% of sites for Yellow *Vibrio*, 60% of sites for Green *Vibrio*, 55% of sites for *Enterococcus*, 40% of sites for *Pseudomonas*, 35% of sites for *E. coli* and 20% of sites for TC. Overall, pathogen concentrations trends were calculated an increase of 45% during the monsoonal season with a 47.5% decrease during the monsoonal season. 7.5% of sites witnessed no change across the wet and dry seasons.

The non-parametric t.test indicated that pathogen concentrations in the dry season were not statistically different from concentrations in the monsoonal season with a p-value of 0.9447. Likewise, pathogen concentrations were not statically different for *Pseudomonas, Enterococcus, E. coli*, Green *Vibrio*, and Yellow *Vibrio* with p-values of 0.5427, 0.5417, 0.4404, 0.1051, and 0.07097 respectively across seasonality. However, t.tests indicated that TC concentrations were statistically different across seasonality with a p-value of 0.02631. Though not all pathogen concentration levels are worrisome (Figure 2-2). 100% of samples detected unsafe levels of TC according to EPA recreational water criteria. Furthermore, *Enterococcus* and *E. coli* experienced 97.5% and 96% of respective samples above EPA criteria safe levels. *Pseudomonas* detected 60% of samples above safe limits. Yellow and Green Vibrio had positive detection for 80% and 78.75% of samples respectively.

Pathogen concentrations vs population density

To understand the relationship between waterborne pathogen concentrations and their spatial spread in Guayaquil, Ecuador, we mapped each pathogen in the dry season and the wet season across population gradients (Figure 2-3). Highly concentrated clusters of *E. coli* and *Enterococcus* appear in red zones indicating high population density. Figure 2-4 shows a regression of population density and pathogen concentrations. The regression indicates that *E. coli* concentrations show the strongest positive correlations with population. *Pseudomonas* and *Enterococcus* concentrations show positive correlations with increasing population density but

have weaker correlations. Yellow *Vibrio*, Green *Vibrio* and Total Coliforms do not show any correlation with increasing population density.

Geographic location may influence pathogen concentrations as well due to land use and urbanization. The west cluster of study site have decreased population density, rural populations, and agriculture land uses. Figure 2-5 shows pathogen concentrations grouped by location. 4/6 average pathogen concentrations decreased in western study sites, namely *E. coli*, *Enterococcus*, *Pseudomonas*, and TC. Center, North, and South study sites do not show any significant differences across pathogen concentrations. However, Central study sites bear the highest average for *E. coli* concentrations indicating a hot spot location of pathogens and human health risk zone.

Multiparameter correlation

Physiochemical parameter correlations add understanding to pathogen concentration trends. Phosphate was highly positively correlated with *Enterococcus* and TC concentrations while *E. coli* showed slight inverse relationships with phosphate (Figure 2-6). Increases in phosphate are also highly correlated with increases with other parameters such as nitrate, turbidity, salinity, fluoride, dissolved oxygen, and pressure. Phosphate and nitrate concentration correlations are indicative of nutrient contaminations from human excrement present in the water. Human pathogens have no significant correlations with other physiochemical parameters measured.

DISCUSSION

In this study we mapped waterborne pathogens and analyzed the relationship between their concentrations and population and seasonality. Though we hypothesized that pathogen abundance would be heightened and statistically different during the monsoonal season from the dry season, we found that (i) concentrations of pathogens are not statistically different between the monsoonal and dry season; (ii) pathogen concentrations are dangerously high during both seasons; and (iii) *E. coli* concentrations are proportional to population density.

Political and geographical barriers inhibiting progress

In 2001, water and sanitation facilities were privatized when Interagua won a multimillion-dollar contract funded by the World Bank and the Inter-American Development Bank (Whitehead, 2016). However, in 2008, the new Ecuadorian constitution was ratified and gave greater State participation in the economy (Ecuador: 2008 Constitution in English). It is now the responsibility of the State to provide public services such as drinking water and sanitation (art. 314). The newly ratified constitution therefore prohibited the privatization of water in Ecuador (Martínez-Moscoso et al., 2022). Because Guayaquil's water treatment was privatized in 2001, it is an exception to the constitution. The privatization of water in Guayaquil may not necessarily be the cause of high concentrations of waterborne pathogens in Guayaquil. Pressures on Interagua water managers to underperform have arisen from the government because of struggles for power and control. Interagua is waiting for the building of new infrastructure which is the responsibility of the local government. Furthermore, the French company Veolia, is the largest shareholder of Interagua. Veolia has a record of fraud, failure to manage and treat water, and fails to take responsibility as is shown in the Flint Michigan Water Crisis (Democracy Now!, 2016; Klayman, 2016; Pauli, 2020) which has led to speculation as they refuse to take responsibility for water management in Guayaquil as well (Whitehead, 2016).

The geographical context of Guayaquil intensifies human pathogen persistence. Guayaquil is located downstream from the Andes Mountain basin which flows out to the ocean and receives water from upstream communities with poor water treatment practices. Quito and Cuenca, the second and third largest cities in Ecuador respectively, are managed by the State but are located at the headwaters of the Andes Mountains where water is cleaner to their advantage. *Socioeconomic factors*

For the purpose of our study, we divided the city into four zones: north, south, west, and center. Center and south zones observed the highest contamination levels. In addition to higher population density in the center and south zones, the south zone experiences low economic status. Much of the housing located in the south zone consists of small, closely built homes and stilt-slums built over water. Few to none of these homes have water treatment, ejecting their wastewater directly into the closest river. Conversely, in the west zone are several gated communities and agricultural land where waterborne pathogen concentrations are low.

High concentrations of pollution in areas with greater economic disparity compared to low concentrations of pollution in affluent areas is an example of environmental injustice. High, long-term exposure to pathogens exacerbates persistent public health issues, feeding into the poverty cycle (Schleifer & Otto, 2019). Illness from water-borne pathogens prevents attendance to school and work and requires that residents of impoverished communities (especially women and children) to spend more time locating and traveling to clean water sources. Additionally, higher rates of health care are administered in areas with high levels of contamination that many residents cannot afford.

Study limitations

Seasonal variability has carried an onslaught of disease for decades in developing countries (Ashbolt, 2004; Masters et al., 2011). When reassigning concentration values due to insufficient dilution methods, there may have been differences in concentrations between the dry

and monsoonal season that may have been unaccounted. However, reassigned data does not impact the number of values that were detected above safe limit criteria. According to EPA criteria, 100% of TC and 96% of *E. coli* detected unsafe limits for humans. Therefore, elevated and unsafe levels of pathogens can always be expected in recreational water due to poor infrastructure even though pathogen concentrations may increase slightly and insignificantly in the monsoonal season (Pachepsky et al., 2018). This suggests that residents are at an elevated risk of infection when encountering the water of Guayaquil at any point in time. Increased rates of infections during the monsoonal season may not indicate that there are increased abundances of pathogens but that residents are more likely to encounter the constantly elevated loads of pathogens during the monsoonal season while streets are flooded. In the dry season loads are similar, but residents may be less likely to encounter the contaminated water.

Though we only have one year of pathogen concentration data, the data gathered may be indicative of multi-year pathogen levels in Guayaquil, Ecuador. No abnormal outside events or influences occurred such as abnormal amounts of waste dumped into streams. Further temporal data could provide higher data resolution by monitoring or sampling streams on a monthly timescale for multiple years. This would allow more confidence in the data. Guayaquil residents should be informed about recreational water risks, especially during monsoonal seasons because of the highly elevated pathogen loads detected. Additionally, this study approach can be adapted to other geographic locations in Ecuador and along the equator where monsoonal weather patterns exist.

Moving forward to improve quality of life

More than 1/7 of the world population does not have access to clean water (Mara, 2003). Recreational water quality reflects drinking water quality and human health on a national scale. Furthermore, climate change exacerbates pathogenic vectors and human illness (Williamson et al., 2017) and nutrient overloads are predicted to worsen in much of the global south if coordinated action is not taken (Seitzinger et al., 2010). Therefore, it is vital to integrate sustainable solutions that will effectively improve the quality of life in Guayaquil without putting more of the burden on the low-income and underrepresented communities.

The Ecuadorian Constitution declares access to water as a human right (*Ecuador: 2008 Constitution in English*). The Ecuadorian government is striving to provide access to clean water for all residents, however, barriers remain to water treatment and sufficient infrastructure as unsafe concentrations pollution persist in Guayaquil. While there are solutions such as boiling water prior to consumption (Majuru et al., 2016) or chlorine treatment (Bhavnani et al., 2012), previous studies have highlighted that secondary storage practices often reintroduce bacteria to water (Levy et al., 2014). As a result, contamination should be addressed prior to arriving in the home, eliminating the need for secondary water storage (Wingfield et al., 2021).

While some pollution cannot be prevented, excessive pollution is a result of careless action, not the inevitable consequence of economic development (Hannah et al., 2021). As a result, chronic pollution can be prevented with proper legislation that holds governments, companies, and individuals accountable. Such legislation is based on the "polluter-pays principle" creating consequences for failures of wastewater treatment plants (Landrigan et al., 2018). Such solutions incentivize new, more efficient methods and technologies, force industries to acknowledge and account for the externalized cost of pollution and generate funds to help offset the cost of pollution exposure for low-income communities (Landrigan et al., 2018). These types of programs are proven to be effective if they are clear, transparent, and impartially enforced.

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FIGURES



Figure 2-1. Map of study area in Guayaquil, Ecuador.



Figure 2-2. *E. coli, Enterococcus Faecalis*, Green *Vibrio Cholerae*, *Pseudomonas Aeruginosa*, Total Coliforms, and Yellow *Vibrio Cholerae* concentrations measured in CFUs/100 mL in the dry and monsoonal season.



Figure 2-3. Map of pathogen concentrations during the dry and monsoonal season across population gradients. Red indicates high urban density while green indicates rural land use and low population density. A1 and A2: Map of *E. coli* concentrations during the dry season; A3 and A4: Map of *E. coli* during the monsoonal season; B1 and B2: Map of *Enterococcus* concentrations during the dry season; B3 and B4: Map of *Enterococcus* concentrations during the dry season; C1 and C2: Map of Total Coliform concentrations during the dry season; C3 and C4: Map of Total Coliform concentrations during the monsoonal season; D1 and D2: Map of Green *Vibrio* concentrations during the dry season; E1 and E2: Map of Yellow *Vibrio* concentrations during the monsoonal season; F1 and F2: Map of Yellow *Vibrio* concentrations during the monsoonal season; F1 and F2: Map of *Pseudomonas* concentrations during the dry season; F3 and F4: Map of *Pseudomonas* concentrations during the monsoonal season.



Figure 2-4. Regression of pathogen concentrations grouped by species vs population density measured in people/Km².



Figure 2-5. Pathogen concentrations measured in CFUs/100mL grouped by location or sampling areas: Center, North, South, and West.



Figure 2-6. Correlation table of physiochemical parameters and pathogen concentrations. Correlation value of 1 indicates cis-relationships while -1 indicates trans or inverse relationships.