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Hugh Henry Wescoat Green Date

The Hazards of the "Holy Dip and Holy Sip;" A Case for Sustainable Wastewater Treatment in Varanasi, India

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Emory University
2013

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An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
In partial fulfillment of the requirement for the degree of
Master of Public Health
In Environmental Health
2014

Abstract

The Hazards of the "Holy Dip and Holy Sip;"
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By Hugh Henry Wescoat Green

Background: The inhabitants of Varanasi, India rely upon the Ganges River as a water source for drinking, bathing, and religious activities. Due to population pressure, the capacity of the sanitation system of the city of Varanasi has been exceeded, allowing untreated wastewater routinely to be discharged directly into the Ganges River. Diarrheal disease is a major threat to the public health of inhabitants of Varanasi, as it is second leading cause of death in children under five years of age and the leading cause of death in children five to fourteen years in India.

Objective: This study assessed the microbiological quality of Ganges River water at Varanasi from May 29th to June 25th 2013 to determine if concentrations of Total Coliforms and *E. coli* exceeded national standards set by the Central Pollution Control Board (CPCB). This quantification of the magnitude of fecal contamination in the river can support advocacy efforts for improved sanitation and wastewater treatment interventions.

Methods: Five sample locations on the Ganges River were selected based on bathing population size at peak bathing hours in excess of 30 people. Water samples were collected daily from highly used *ghats* (stone steps into the river), where inhabitants and pilgrims worship, bathe, and conduct everyday activities. Samples were tested for Total Coliforms and *E. coli* using the IDEXX Quanti-Tray 2000® method to assess overall water quality and fecal contamination.

Results: A total of 165 water samples were collected and analyzed. *E. coli* concentrations ranged from 2.93×10^3 to 3.97×10^5 with a geometric mean of 2.77×10^4. None of the water samples met the CPCB standards for drinking, bathing, or agriculture. Total Coliform concentrations were found to be nearly 100 times over permissible limits. Moreover, there were spatial and temporal differences in microbial concentrations between the sites.

Conclusions: Fecal contamination of the Ganges River at Varanasi presents a serious public health issue. Bathers are at risk of exposure to fecal contamination and related enteric pathogens at Varanasi's bathing ghats. While the sites varied in indicator organism concentrations both spatially and temporally, all the indicator concentrations measured in the samples were well above national and international standards for safe water. These results indicate the critical need for improved collection and treatment of municipal wastewater in Varanasi before discharging effluent to Ganges River.

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Acknowledgments

I would like to thank:

Dr. Christine Moe and the Center for Global Safe Water for their continuous support and introduction to the field of environmental microbiology and public health;

Dr. Veer Bhadra Mishra, late President of the Sankat Mochan Foundation, for his kind invitation to work with the *Swatcha Ganga Research Laboratory* (SGRL) and for his lifelong dedication to Ganga Ma, without which my work would not have been possible;

Dr. V.N. Mishra and The Sankat Mochan Foundation for hosting me at Tulsi Ghat and supporting my research efforts, and for continuing the imperative work of his father in a difficult political environment;

Dr. Bailey Green for sharing Mahant ji's vision of cleaning the Ganges River with a sustainable engineered solution, and for laying the foundation for my research in Varanasi;

Dr. June Scott for thesis guidance and pushing me to question the status quo;

Dr. Matt Strickland for statistical advice and continued support;

My parents and friends, whose love and support led me to peruse a Master of Public Health degree and research in India;

My sincere gratitude goes out to all those who helped guide me through this process and brought this document to life.

Thank you!

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Introduction

The Ganges River is the lifeblood of northern India and the holiest river in the Hindu faith. Flowing from its headwaters in the Himalayas to the Bay of Bengal, this river begins in the highest mountains and ends in the largest delta on Earth (Hugh-Jones, 2007). Along its journey to the ocean, the Ganges flows through 1560 miles (2510 kilometers) of northern India and Bangladesh (Ahmad, 2013). This mighty river has supported civilization for thousands of years and is worshiped as a goddess, *Ganga Ma* (Mother Ganges), by Hindus. However, modern population growth and failing infrastructure now threaten the health of *Ganga Ma* and those who rely on her waters for drinking, bathing, and physical and spiritual wellbeing.

The Ganges has been, and continues to be, polluted to the detriment of those who live along the river's bank. One of the places where pollution is most evident is the holy city of Varanasi (*Banaras, Kashi*) where the devout take a "holy dip and holy sip" to purify the body and soul. Yet, *Ganga Ma,* which is believed to cleanse the body and soul, has become a modern reservoir of fecal contamination and waterborne pathogens. The pollution of the Ganges River is particularly impactful at Varanasi due to the spiritual importance of the city to Hindus. Varanasi is considered to be the sacred city of Lord Shiva, creator and destroyer, one the chief deities of the Hindu pantheon (Eck, 1999). Varanasi is also a pilgrimage site and the site for many important rituals. It has great relevance for ancient and modern Hindus, who have prayed on the banks of the Ganges River for millennia. It is believed that a devout Hindu who dies and whose body is cremated on the banks of the Ganges River at Varanasi will be able to escape the cycle of death and rebirth, *Samsara* (Eck, 1999).

Accordingly, the most important feature of this holy city is the river and deity *Ganga Ma*. Traditionally, the deity *Ganga Ma* has washed away all filth and pollution, both spiritually and physically. Now, due to the sheer magnitude of modern pollution in the Ganges River, those who come to worship on her banks may be at serious health risk from exposure to contaminated water.

Disease Burden

Diarrheal disease is a very real threat to the inhabitants of Varanasi and the developing world at large. One in nine child deaths world wide are attributable to diarrheal diseases, making diarrhea the second leading cause of death among children under the age of five and accounting for 11% of child deaths overall (Liu et al., 2012). In 2010 in India, 212,000 of 1.68 million children deaths under the age of five were related to diarrheal disease (Liu et al., 2012). The state of Uttar Pradesh, where Varanasi is located, has the second highest rate of diarrheal disease mortality in India, 26.3% (Bassani et al., 2010). The Million Death Study found that diarrheal disease was the leading cause of death in India for children ages five to fourteen years, accounting for nearly 60,000 deaths in 2005 (Morris et al., 2011). In addition, the morbidity from diarrheal disease accounts for an estimated 6.4 million disability-adjusted life years (DALYs) in Uttar Pradesh (UP) alone (Mallikarjun, 2003). DALYs are a way of quantifying the burden of disease by calculating the number of healthy years of life lost due to disease, disability, or death. The DALY estimate of Mallikarjun et al. is equivalent to the loss of one month of life for every urban and peri-urban resident of Varanasi. Providing safe water, sanitation, and hygiene could prevent 88% of these deaths (Black, Morris, & Bryce, 2003).

The pollution of the Ganges River is the cause of an estimated 9 to 12% of the total disease burden within the state of Uttar Pradesh (U.P., population 200 million), where Varanasi is located (Das, 2011). The holy city of Varanasi attracts up to 60,000 religious pilgrims a day, who come to worship Hindu deities in the many temples of Varanasi and worship *Ganga Ma* along the banks of the river (Guynup, 2010). With Varanasi's urban population of 1.6 million residents and an additional 2.1million peri-urban residents, the total population of 3.7 million that may be exposed to the pollution in the Ganges River at this city alone should be cause for national concern from a public health, religious, and cultural heritage standpoint (Guynup, 2010).

While data on water quality and the incidence of waterborne diseases are sparse, there are several studies that have documented the presence of enteric pathogens in the Ganges River and potential health risks associated with contact. A Varanasi water quality study conducted between 1985 and 1987 reported the presence of entero-pathogenic bacteria in all 407 samples taken near the river's bathing *ghats* (stone steps leading down to the River) and adjacent sewage outfalls (De, Sen, & Tewari, 1993). Organisms isolated included *Vibrio cholerae* 0-1, Non 0-1 *Vibrio cholera*, *Vibrio fluvialis*, *Aeromonas* species, *Plesiomonas* species, *Salmonella* species, *Shigella* species, *Pseudomonas* species, *Proteus* species, and *E. coli.* (De et al., 1993). While this study was conducted nearly three decades ago, little has changed in Varanasi to suggest an improvement in microbiological water quality. In fact, the Sankat Mochan Foundation has been monitoring the water quality of the Ganges at Varanasi for more than two decades and has documented no marked improvement (Hamner et al., 2013). Hamner's 2013 paper presents weekly water quality

data on Biochemical Oxygen Demand (BOD) and Fecal Coliform Count (FCC) for *Tulsi Ghat* starting in 1992.

Despite the known presence of fecal contamination, there is little recent documentation of the health effects associated with ingestion of, or contact with, the Ganges River. A 2004 health survey found that residents who reported that they used Ganges River water for washing had an odds ratio of 5.05 (2.95-8.66 95% CI) for enteric disease (Hamner et al., 2006). Those who washed with water from the Ganges River, a common practice, were five times more likely to likely to show symptoms of diarrheal disease than those who did not wash with Ganges water. The same survey conducted by Hamner et al. estimated "the overall rate of enteric disease incidence [prevalence over one year recall], including acute gastrointestinal disease, cholera, dysentery, hepatitis A, and typhoid to be 66%" of the residents who relied upon Ganges River water for every day tasks (Hamner et al., 2006). Another study based in Varanasi reviewd records from five hospitals located near the river from 1996 to 1999. Multiple regression ANOVA tests were performed and an R^2 of 0.64 was calculated, indicating that 64% of variation in waterborne disease was accounted for by four variables. "The concentration of Nitrate, Chloride, Conductivity and Faecal coliforms in the Ganga water have highly significant effect (P = .00031, .004, .006 and .03, respectively)" (M. Pandey, 2005). This study found that the drinking water residents ingest was highly likely to be the source of enteric disease, either due to poor treatment, infiltration of pipes, or the use of raw Ganges water (M. Pandey, 2005).

Hazards of Recreational Water Contact

An US EPA review of the literature from 1950 to 2009 found that the weight of the evidence supports that fecal indicator bacteria are able to predict GI and respiratory illnesses from exposure to recreational waters (U.S. Environmental Protection Agency, 2009). The hazards of swimming in water where fecal contamination is present are numerous. A 2003 WHO report entitled "Guidelines for Safe Recreational Waters Environments" includes reference to studies finding association between contaminated waters and gastroenteritis, acute febrile respiratory illness (AFRI), and ear infections. Additionally, there is an apparent link between swimming in general and increased rates of eye symptoms and skin symptoms (World Health Orginization, 2003). While there are many potential adverse health outcomes associated with swimming in fecal-contaminated waters this study focused specifically on the association between contact with polluted recreational water and gastroenteritis. Past studies have documented a relationship between the presence of *E. coli,* even at relatively low concentrations 10 MPN/100mL, and incidence of gastrointestinal illnesses among swimmers (Cabelli, 1982). Moreover, children 10 years of age or younger were found to be at greater risk of acquiring GI illness swimming in recreational water polluted with sewage (Wade et al., 2008) In a pool study conducted in the US, participants 18 years or younger swallowed an average of 37mL and adults swallowed an average of 16mL of swimming pool water during 45 minutes of swimming (AP. Dufour, Evans, Behymer, & Cantu, 2006). Fecal contamination of the Ganges River represents a major public health hazard for those who come in contact with polluted waters.

Sources of Pollution

According to the 2009 State of the Environment Report of the Ministry of Environment and Forests (MoEF), who utilize a network of more than a 1,000 water quality monitoring stations throughout India:

> The water quality monitoring results obtained between 1995 to 2006 indicate that organic and bacterial contamination continues to be critical in water bodies. This is mainly due to discharge of domestic wastewater mostly in untreated form from the urban centers of the country. The municipal corporations at large are not able to treat the wastewater, increasing municipal sewage load flowing into water bodies without treatment. (Varughese, Lakshmi, Kumar, & Rana, 2009)

India's waterways are generally polluted by fecal contamination, the primary pollutant of the Ganges River at Varanasi is municipal sewage (V. B. Mishra, 2005).

Many factors contribute to the failure of the sewerage system, but the greatest factor is rapid population growth. Since its independence in 1947, the Indian government has been struggling to provide for its more than tripled population (Indian Census, 2011). India is currently the second most populous country in the world behind China, with 1.23 billion people. In 2010, 30% of the population was urban, and the projected annual growth rate from 2010 to 2015 is 2.4% (Central Intelligence Agency, 2013). According to Population Census India (2011), there were 3,682,194 people living in the city of Varanasi in 2011, with slightly more men than women (Indian Census, 2011). Perhaps more importantly, from 1991 to 2001 the population grew 25%, and between the 2001 and 2011 census the population grew 17% (Indian Census, 2011).

Population growth, coupled with a lack of public sewerage infrastructure, has greatly deteriorated the water quality of the Ganges River. When India was under British

rule (1858-1947),[1] Varanasi had a population of 200,000 people, and was served by an adequate, albeit rudimentary sewage collection system (V. B. Mishra, 2005). Without any sewage treatment plants (STP), the untreated sewage was intercepted and diverted from the *ghats* to a downstream river outfall, and thus people were not directly exposed to fecal contamination (V. B. Mishra, 2005). Since 1947, the urban population of Varanasi has increased eight-fold without effective sewerage improvements (Indian Census, 2011). Each day, the raw sewage of the city flows into the Ganges River. In the 1990s, the state engineering firm UP Jal Nigam estimated the discharge of drains at Varanasi at 309.8 million liters per day (MLD) (Uttar Pradesh Jal Nigam). Therefore, the risk of bathers' exposure to fecal contamination and subsequent disease through ingestion of and contact with the Ganges River is a major public health concern (Abraham, 2011).

While the population of Varanasi has grown rapidly, its sewage capacity has not. The three activated sludge STPs that Varanasi has do not begin to meet the total capacity of the city's effluent. The total nominal capacity of the city's STPs is approximately 100 million liters per day (MLD) (Uttar Pradesh Jal Nigam). However, the city produces between 300 MLD and 400 MLD, leaving a minimum of 200 MLD of untreated sewage, which is discharged directly into the Assi, Varuna, and Ganges rivers on a daily basis (Uttar Pradesh Jal Nigam). The Ganga Action Plan, launched over 25 years ago, has not stopped 200 to 400 MLD of raw sewage from entering the river adjacent to the bathing sites and *ghats* of

[1] The *British Raj* was a period of time of British sovereignty in India. The *raj*, or reign in Hindi, came to an end in August of 1947. Indian independence is celebrated on the 15th of August the day after Pakistan's independence day, which marks the partitioning of former India into two states. In 1971 East Pakistan now Bangladesh seceded. Both Pakistan and Bangladesh are primarily Islamic countries; where as, modern India is predominantly Hindu.

Varanasi, where pilgrims and residents alike are exposed to fecal contamination and the risk of waterborne disease transmission.

The Government attempted to address pollution of the Ganges River through the Ganga Action Plan, Phase I (GAP-I) launched in 1985 (Murty, 2000). The goal of the action plan was to facilitate the immediate reduction of the sewage pollution load on the Ganges River. However, this plan was not successfully implemented. Professor G.D. Agarwal, former chair of the Department of Civil and Environmental Engineering at the Indian Institute of Technology in Kanpur and past Member Secretary of the Central Pollution Control Board, wrote in 2007 "…the implementation of GAP-I at Varanasi has been a TOTAL FAILURE" (Agrawal, 2007). The Comptroller and Auditor General of India wrote, "The GAP, launched in 1985, with the objective of bringing water quality of river Ganga and its tributaries to bathing levels, was not able to achieve its objectives, despite a total expenditure of Rs 901.71 crore over a period of 15 years" (*crore* is ten million) (Murty, 2000). Beyond poorly allocated resources, the primary reason GAP-I failed in Varanasi was that the interventions were not appropriate to the environment. The sewage pumping stations (SPS) and the Activated Sludge STPs required more electricity than the electrical grid could supply on a continuous basis (Green, 2013).

Sewage Treatment for the Indian Condition

The Activated Sludge STPs installed in Varanasi were designed by multinational engineering firms using technologies designed for resource-rich countries. These STPs were installed without considering local climate, energy, and human capacity factors (Sankat Mochan Foundation and Oswald Green LLC, 1997). The Activated Sludge

wastewater treatment process is an aerobic decomposition process that uses mechanical aeration and mixing to promote rapid microbial degradation of organic waste. The Activated Sludge process needs three inputs to maintain functionality – a steady supply of wastewater, continuous power, and a skilled operations staff of mechanical and process technicians to operate and monitor the facility (World Bank, 2014). Without continuous power, the aerobic microbial population dies off, and the system stops working. Varanasi experiences frequent power outages that can last up to twelve hours (personal experience). Additionally, Varanasi has a monsoon season with heavy rainfall that often inundates pumping stations for several months of the year. Unable to run continuously, Varanasi's STPs fell into disrepair, and while some appeared to be partially treating wastewater, none had final disinfection (Green, 2013; National Environmental Engineering Research Institute, 1994). It should also be noted that the laboratories of the three STPs were not equipped to measure coliforms (Green, 2013). Untreated sewage was then discharged directly into the river where bathers and worshipers conducted their holy dip and sip. For example, the STP at Benaras Hindu University was neither receiving influent nor producing effluent when inspected this summer, prior to the monsoon season. Despite the fact that the STP was not treating sewage, the operators decided to maintain the operation of a splashier aerator, presumably to give the appearance of operation to the casual observer.

Regulatory Challenges

Regulation is another major issue impacting the quality of water in the Ganges River. Indian river water quality standards for Class A rivers, i.e., those used for drinking and bathing, have been relaxed from 50 most probable number (MPN) of coliforms/100ml to allow today a maximum of 5,000 MPN per 100ml, the national water quality standard

fixed by the Central Pollution Control Board (CPCB) of India (Das, 2011; Mallikarjun, 2003). In comparison, the United States Environmental Protection Agency (US EPA) and World Health Organization do not allow for the detection of any coliforms in drinking water. Additionally, the US EPA's bathing criteria does not allow geometric mean *E.* coli concentration to exceed 126 CFU (colony forming units)/100mL (U.S. Environmental Protection Agency, 2003). WHO contended, "There is inadequate evidence with which to directly derive a [recreational] water quality guide-line value for fresh water…. guideline values should be interpreted or modified in light of regional and/or local factors" (WHO, 2003).

Coliform bacteria are used as an indicator of fecal contamination as they are universally present in the feces of mammals; however, some coliforms, like *E. coli* are more fecal-specific than others (Heymann, 2004). To determine microbiological water quality, one would ideally test for specific pathogens, but monitoring technologies are limited, expensive, and time intensive. Testing for an indicator organism of fecal contamination, like *E. coli*, can provide reliable, inexpensive, fast results. While *E. coli* is a more accurate indicator organism than Total Coliforms, it is less persistent in the environment, which can be both positive in that pollution must come from a local source and negative in that die off occurs rather rapidly (Edberg, Rice, Karlin, & Allen, 2000). *E. coli* is also the preferred indicator organism as it is shed exclusively in the feces of warm-blooded animals, whereas Total Coliforms may have non-fecal sources in the environment ((Dichter; Heymann, 2004; US Environmental Protection Agency, 2012). Finally, *E. coli* is recommended as the

preferred indicator organism for fresh water fecal contamination by the US. Environmental Protection Agency (U.S. Environmental Protection Agency, 2012).

The pollution levels of the Ganga at Varanasi are many orders of magnitude above CPCB-defined safe levels, and the current level of sewage pollution of the Ganga used for drinking and bathing is unacceptable by international and national standards and guidelines (Hamner et al., 2013; U.S. Environmental Protection Agency, 2012; WHO, 2011). The MoEF reported that, "the level of fecal coliform bacteria in most rivers often exceeds WHO standards and is responsible for causing a number of gastro-intestinal ailments among the population" (Varughese et al., 2009). The Uttaranchal Environment Protection and Pollution Control Board (UEPPCB) recently classified Indian rivers into four categories ranging from A to D. The categories are based on criteria for Total Coliforms, pH, Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), and free Ammonia (as N): category "A being fit for drinking, B for bathing, C for agriculture and D is for excessive pollution level" (Environmental Protection and Pollution Control Board, 2014). The Ganges River water at Varanasi received a classification of D because of the extremely high concentrations of coliforms. As a class D river, it is deemed unfit for drinking before treatment, drinking after treatment, bathing or agricultural use (Daftuar, 2011). However, millions rely upon these polluted waters to conduct their daily activities and rituals (Environmental Protection and Pollution Control Board, 2014).

Partner Organization

The Sankat Mochan Foundation (SMF) is a Varanasi-based NGO founded in 1982 with the express purpose of protecting the Ganges River. The late founder and president of

the Sankat Mochan Foundation, Dr. Veer Bhadra Mishra (Mahant Ji), established the Swatcha Ganga Research Laboratory (SGRL) in 1993. The SMF has been formally monitoring water quality since 1992 (Hamner et al., 2013). The SMF has three objectives: 1) monitor the water quality of the Ganges river at Varanasi, 2) advocate for technological solutions to improve water quality; and, 3) conduct public outreach and education about the Ganges River pollution problems. The SGRL is the only laboratory regularly monitoring water quality and has played an important role in holding the Government of India and the U.P. State Government accountable. As previously mentioned, the laboratories of the three STPs are not equipped to measure coliforms nor do they have disinfection for their final effluent (Green, 2013). Data collection and analysis for this thesis was conducted in conjunction with the research of the SGRL but used a more sophisticated method of water quality testing. In order to affect policy change, there is a need for hard data that accurately quantifies the magnitude of pollution in the Ganges River in order to advocate for improved sewerage and wastewater treatment in Varanasi.

Purpose of the Study

The purpose of this study was to accurately assess the microbiological water quality of the Ganges River at Varanasi using indicators of fecal contamination (*E. coli* and Total Coliform). While many people in Varanasi are generally aware of the pollution of the Ganges River, solutions to this problem are complicated by politics and religion. This research brought rigorous, internationally approved quantification methods to the city of Varanasi to define accurately the magnitude of fecal contamination.

Methods

Ganges River water samples were collected daily from five bathing sites from May 27th to June 25th, 2013. Samples were then analyzed for *E. coli* and Total Coliforms using the IDEXX Quanti-Tray 2000® System (IDEXX Laboratories, Westbrook, ME).

Sampling Locations

Sampling locations were selected based on usage and proximity to the Swatcha Ganga Research Laboratory at *Tulsi Ghat.* Usage was determined based on a criterion of 30 or more people actively engaged in bathing in the water during bathing hours. Bathing hours were defined as the hours from 6:30am to 8:30am. The five locations chosen were: *Tulsi Ghat, Kedar Ghat, Raja Ghat, Ahilyabai Ghat,* and *Rajendra Prasad Ghat* (Figure 1). The distance between *Tulsi Ghat* (upstream) and *Rajendra Prasad Ghat* (downstream) was roughly 2 kilometers.

Table 1: Water Sampling Locations in Varanasi, India

Ghat Name	Coordinates (^{0}N lat, ^{0}E long)†	# Upstream Outfalls	# Samples collected	Primary Observed Activities ‡
Tulsi	25.288, 83.006	3	34	Religious bathing [A]*
Kedar	25.299, 83.008	7	28	Pilgrimage site [B]
Raja	25.303, 83.008	10	25	Bathing and laundry
Ahilyabai	25.306, 83.010	12	25	Bathing and laundry
Rajendra Prasad	25.306, 83.011	13	31	Religious bathing

* Hamner, 2013; † Google Earth; ‡ Personal Observation.

[A] Religious bathing involved full submersion of the head and body under water.

[B] Pilgrimage site offer the same religious bathing experience, but for primarily non-resident.

Figure 1: Aerial Map of the Varanasi with Labeled Sample Locations

Sampling Technique

Daily grab samples were taken from May 27th to June 25th between the hours of 6:30am and 8:30am. Grab samples, also known as catch or point samples, are samples taken at a specific time and offer a snapshot of the water quality at a specific location and a point in time. Samples were collected from surface water with a 100mL sterile Whirl-Pak® bag. Water samples were then kept on ice until they were processed in the laboratory within four hours of collection.

Sampling Processing

All samples were processed using the IDEXX Laboratory's Quanti-Tray 2000®

System, included in the *Standard Methods for the Examination of Water and Wastewater*, 22nd Edition (Rice & Bridgewater, 2012). River water samples were diluted using a pipette and pre-tested Bailey™ bottled water in a second sterile 100mL Whirl-Pak® bag. The first two days of analysis were spent testing multiple dilution factors to find the appropriate range for bacteria concentration. Dilution factors were then matched to sites *Tulsi* and *R.P. Ghats,* which required 1:200 dilutions, whereas, the concentrations at the three other sites were quantifiable using a 1:100 dilution factor. The Colilert® reagent was then added to the bag, and the sample was vigorously shaken until the reagent was fully dissolved. The resulting mixture was then poured into a Quanti-Tray 2000®. Using the IDEXX Sealer, the Quanti-Tray 2000® was heat-sealed, dividing the sample with reagent into 48 large wells and 49 small wells. The sealed tray was then incubated at 35±0.5°C for 24 hours. Initial methods also included two incubation temperatures. However, due to the ambient temperature fluctuation, the field incubator was unable to maintain a temperature of 44.5°C. Therefore, only the SGRL incubator was used and maintained at 35±0.5°C with back up generators when grid power failed.

After 24 hours, the trays were removed from the incubator and wells were counted for yellow color by daylight and blue fluorescence when viewed with an ultraviolet light (366nm) (Figure 2). Colilert® results are definitive at 24–28 hours (IDEXX Laboratories, 2014). The Total Coliform Most Probable Number (MPN) was determined by matching the observed number of yellow wells with the IDEXX MPN table. *E. coli* MPN was calculated in a similar fashion for blue fluorescent wells under an ultraviolet lamp.

Figure 2: Sample Quanti-Tray 2000®

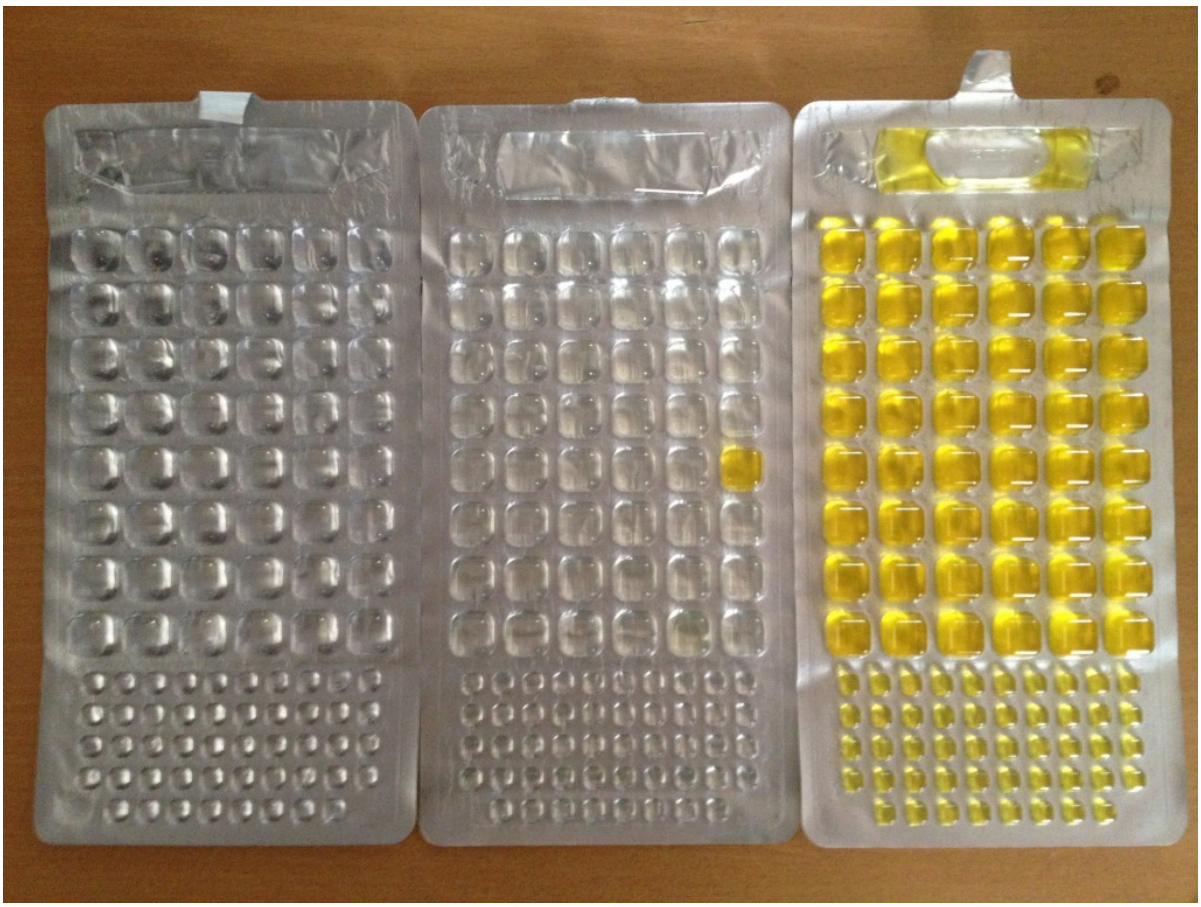


Photo credit: Hugh Green

Water Microbiology

Bacteriological analysis of samples was conducted with a defined substrate test (IDEXX Colilert®). The two substrates present were *ortho*-nitrophenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl-β-D-glucuronide (MUG). In addition to these substrates, the IDEXX reagent contains a proprietary suppression system that selects for only coliform growth. ONPG and MUG are spectrophotometric substrates that produce a characteristic color when they are metabolized. ONPG contains the chromophore, part of a molecule responsible for color, *ortho*-nitrophenyl. ONPG can be

metabolized by the enzyme *β*-galactosidase produced by coliform bacteria. *β*-galactosidase hydrolyses the ONGP molecule into galactose, which can be metabolized, and *ortho*-nitrophenyl. After this process has occurred, free *ortho*-nitrophenyl is responsible for the yellow color in the well. *β*-galactosidase is produced by most coliform bacteria, and therefore the presence of any coliform will turn the well yellow during the incubation process. However, only *E. coli* bacteria, a sub-group of coliform bacteria, have the enzyme *β*-glucuronidase. The second substrate, MUG, targets *E. coli* specifically. *β*-glucuronidase hydrolyses MUG into glucuronide and the fluorescent 4-methylumbelliferyl. The 4-methylumbelliferyl may then be detected under an ultraviolet lamp (366nm) as a fluorescent blue color. Non-coliform bacteria do not have these enzymes and are unable to grow in this defined substrate test. Only viable bacteria that were able to hydrolyze the substrates and grow on the provided sugars were represented in the concentration estimate. Damaged bacteria, mutated bacteria, and suppressed bacteria are not represented in the final MPN estimate, which may result in a slight underestimate of the bacteria concentration. However, this method offers a more accurate concentration estimate than a simple enzyme test that may include non-viable bacteria.

Most Probable Number of Bacterial Concentration

Bacterial density is estimated using most probable number (MPN) for Total Coliform and *E. coli.* The MPN is a tool that allows the researcher to estimate the number of viable bacteria in a sample without direct counting of colony forming units (CFU). This analysis relies on statistical probability rather than a direct count measurement and is therefore an estimate of the indicator concentration in the sample. The MPN of bacterial density is derived from the combination of the number of negative and positive wells,

which may then be referenced from an MPN table. Although the specific formula employed by IDEXX is proprietary, it is based on the theory established in 1915 using multiple fermentation tubes and a presence-absence test (McCrady, 1915). This test was later improved upon by using multiple serial dilutions. Thomas' formula (Figure 3), included in *Standard Methods for the Examination of Water and Wastewater*, 22nd Edition, is able to calculate an MPN approximation given number of positive wells and volume of sample that presented a negative result (Rice & Bridgewater, 2012).

Figure 3: Thomas' Formula for Estimating MPN

$$MPN/100mL = \frac{\#\ positive \times 100}{\sqrt{(negative(mL) \times total(mL)}}$$

The enumeration tool used splits the 100mL sample into many smaller sample wells, which are heat sealed to prevent mixing. The tray of wells (IDEXX Quanti-Tray 2000®) is made up 49 large wells of volume 1.86ml and 48 small wells of volume 0.186ml that effectively provide a 49 x 48 ten-fold serial dilution. The theory behind MPN assumes that organisms are randomly distributed throughout the sample and target bacteria will grow in the presence of the defined substrate if one or more organisms are present. The MPN can then be calculated using a programmed calculator or an MPN chart, which is a matrix with large positive wells as rows and small positive wells as columns. The benefits of this defined substrate test include ease of use, short preparation time, and unambiguous results. The positive wells can be easily counted and used to calculate an MPN with an MPN chart (IDEXX Laboratories, 2014). The MPN was then used to determine the indicator concentration (MPN/100mL), taking the dilution factor into account. The lower limit of detection <1 MPN/100mL to <1 MPN/0.33mL depending on dilution and the upper limit of

detection was 725,880 MPN/100mL, using a 1:300 dilution. Levels of indicator organism were present in all samples analyzed.

Data Management

Field collected data were recorded first in hard copy and then entered and cleaned in Microsoft Excel 2007 for Mac. Further analyses were conducted using SAS 9.1 (SAS Institute Inc., 2002-2003).

Statistical Analyses

Descriptive statistics, such as geometric means, were calculated using Microsoft Excel. Scatter plots and histograms were constructed in Excel to assist in data visualization. Because the MPN estimate assumes that the indicator bacteria have a Poisson distribution, data were analyzed with a Poisson Regression model in the statistical program SAS 9.1, using the 'PROC GENMOD' command. This non-parametric test was used to examine spatial and temporal differences between sampling locations (space) and pre- and post-monsoonal rain (time). The outcome variables of these two analyses were a predicted mean concentration and the predictor variables were the sampling site and date, respectively. *Ahilyabai Ghat* exhibited the lowest mean value and was therefore used as the intercept for spatial comparison. The Pre-Monsoon period was used as the intercept for temporal comparison. The maximum likelihood parameter estimates from this model were then exponentiated to determine the model-based predicted value of the mean MPN at each sampling location and time period. Statistical significance was defined as a P value < 0.05. All statistical analyses were conducted using SAS 9.1.

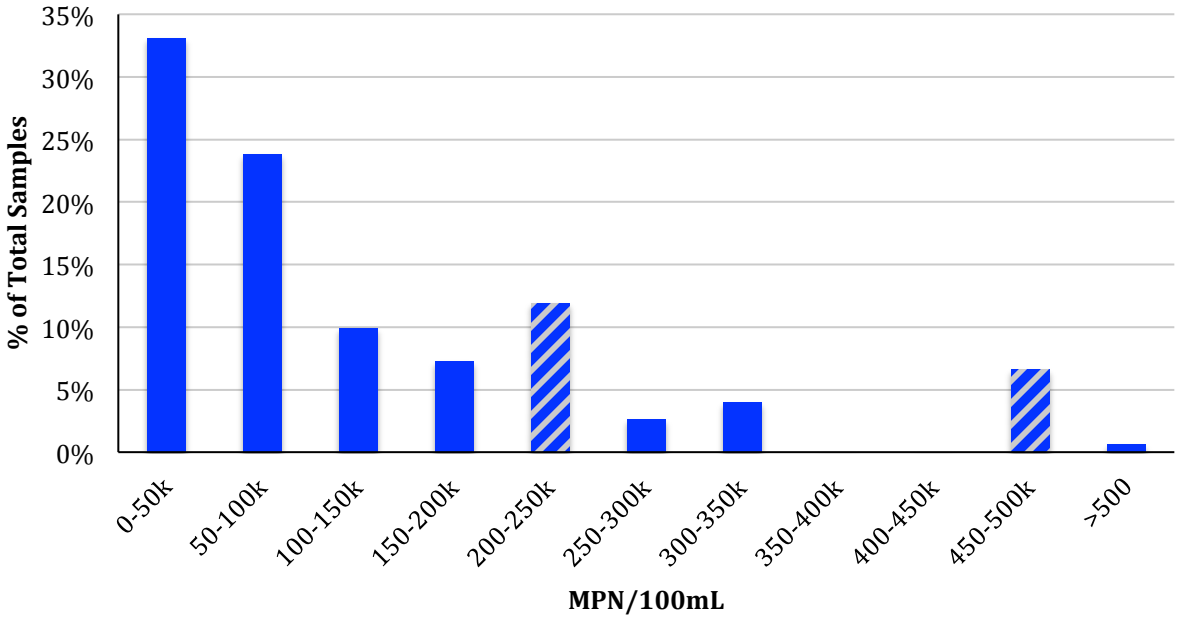
Results

A total of 165 water samples were collected from five sampling locations on the Ganges River and tested for Total Coliform and *E. coli* concentrations (MPN/100mL). The distribution of indicator bacteria concentrations in these samples was examined, and the geometric mean and variance were calculated. The data were then analyzed for spatial and temporal trends, using a Poisson Regression model.

Distribution of Microbial Concentrations

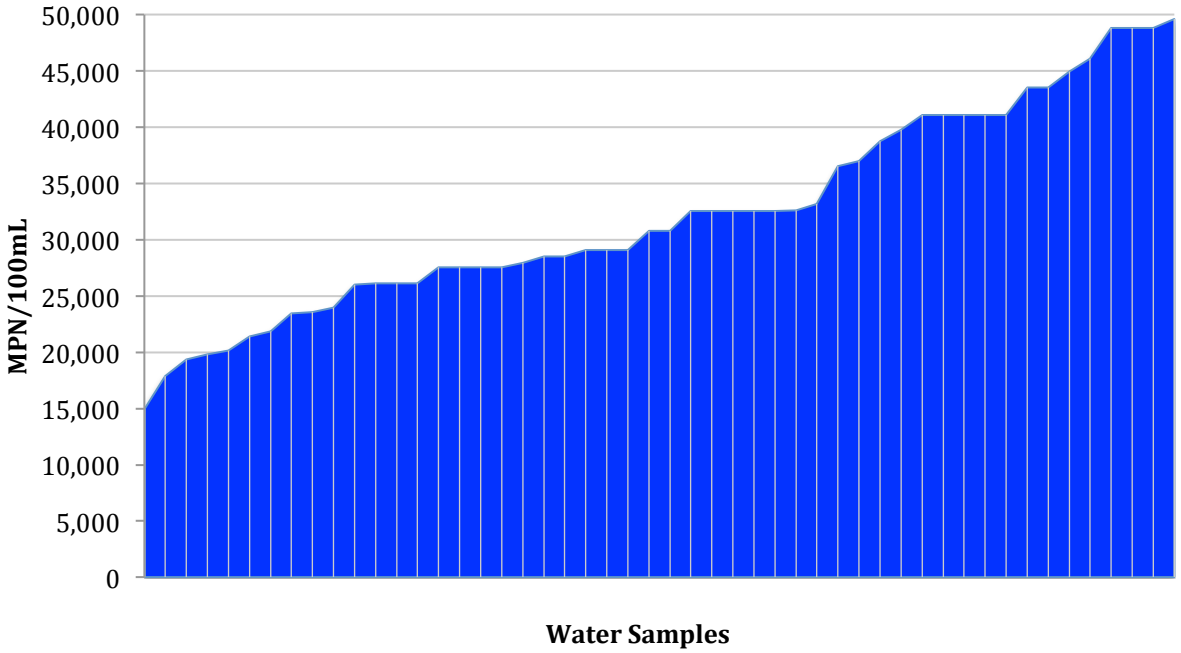
The distribution of Total Coliform concentrations was examined using data collected between May 29th and June 25th, 2013. The first two days of sampling were excluded because the sample dilution was not sufficient and led to underestimates of the indicator bacteria concentrations in the samples. For all the samples, Total Coliform concentrations ranged from 1.5 x 10^4 to 1.5 x 10^6 MPN/100mL. Most samples had high concentrations of Total Coliform, with 57% of the samples in the range of 10^4-10^5 Total Coliform MPN/100mL (Figure 4 and 5). The remaining sample concentrations were all above 10^5 Total Coliform MPN/100mL (Figure 4). The geometric mean Total Coliform concentration for all samples was 9.0 x 10^4 MPN/100mL.

Figure 4: Distribution of Total Coliform Concentrations in 151 Water Samples, Varanasi, India – May 29th to June 25th, 2013



* Diagonal stripes denote some Quanti-Tray 2000s® with 100% positive wells in the specified range

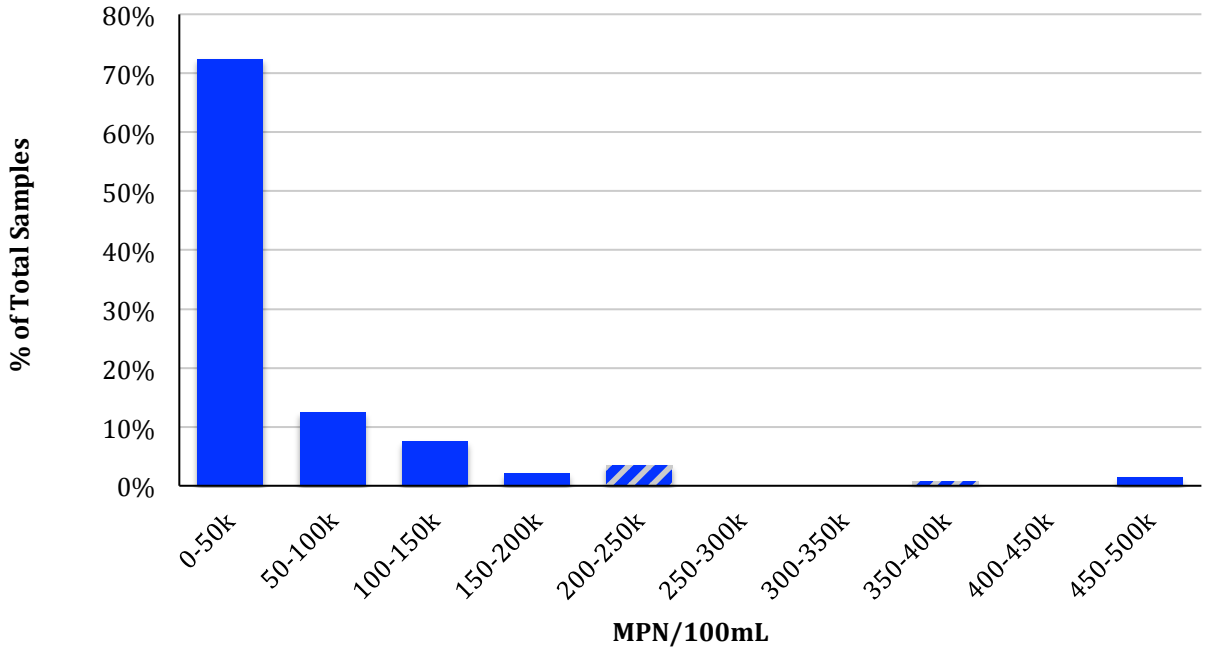
Figure 5: Water Samples with Total Coliform Concentrations under 50,000 MPN/100 mL (N=50), Varanasi, India – May 29th to June 25th, 2013



Samples estimated to have 200,000-250,000 MPN/100mL and 450,000-500,000 MPN/100mL included 10 and 8 Quanti-Trays® where all the wells were positive at the 1:100 and 1:200 dilutions, respectively (Figure 4). Thus, the MPN estimates of Total Coliform concentrations in these samples are underestimates of the actual Total Coliform concentrations**.** All of the water samples exceeded the Indian Central Pollution Control Board (CPCB) maximum standard for Total Coliform of 5,000 MPN/100mL for drinking water, bathing water, or source water for potable treatment.

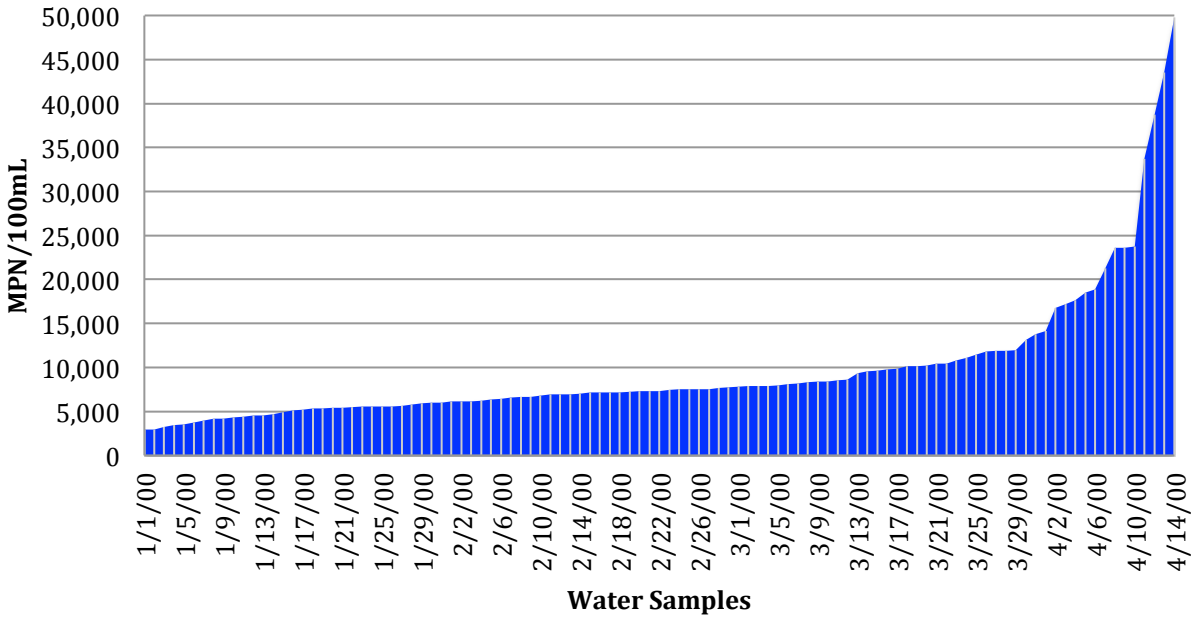
While the CPCB does not set standards for *E. coli,* the samples were also tested for *E. coli* because it is a more fecal-specific indicator than Total Coliform. Over 70% of the samples had *E. coli* concentrations between 2.9×10^3 and 5×10^4 MPN/100mL (Figure 6 and 7). The remaining sample concentrations were all above 5×10^4 MPN/100mL (Figure 6). The geometric mean *E. coli* concentration for all samples was 3.9×10^4 MPN/100mL. Samples estimated to have 200,000-250,000 MPN/100mL and 450,000-500,000 MPN/100mL included 4 and 2 Quanti-Trays® where all the wells were positive at the 1:100 and 1:200 dilutions, respectively (Figure 4).

Figure 6: Distribution of *E. coli* Concentrations in 145 Water Samples, Varanasi, India – May 29th to June 25th, 2013



* Diagonal stripes denote some Quanti-Tray 2000s® with 100% positive wells in the specified range

Figure 7: Water Samples with *E. coli* Concentrations under 50,000 MPN/100 mL (N=105), Varanasi, India – May 29th to June 25th, 2013



Spatial Analysis

To examine differences in water quality by sampling site, the geometric mean Total Coliform and *E. coli* concentrations were calculated for each location (Tables 2 and 3). The water samples from both *Tulsi* and *R.P. Ghats* had the highest geometric mean Total Coliform and *E. coli* concentrations.

Table 2: Geometric Mean Total Coliform Concentrations from Five Sampling Sites along the Ganges River, Varanasi, India – May 29th June 25th, 2013

Ghat Name	Total Coliform Geometric Mean (MPN/100mL)	Sample Size	Confidence Intervals ($\alpha = 0.05$)	Coefficient of Variance
Tulsi	2.39×10^5	27	$2.00 \times 10^5 – 2.77 \times 10^5$	0.41
Kedar	5.58×10^4	26	$3.48 \times 10^4 – 7.68 \times 10^4$	0.79
Raja	6.28×10^4	24	$3.49 \times 10^4 – 9.06 \times 10^4$	0.80
Ahilyabai	5.63×10^4	25	$4.60 \times 10^4 – 6.66 \times 10^4$	0.42
Rajendra Prasad	1.30×10^5	27	$5.66 \times 10^4 – 2.03 \times 10^5$	0.97

Table 3: Geometric Mean *E. coli* Concentrations from Five Sampling Sites along the Ganges River, Varanasi, India – May 29th June 25th, 2013

Ghat Name	*E. coli* Geometric Mean (MPN/100mL)	Sample Size	Confidence Intervals ($\alpha = 0.05$)	Coefficient of Variance
Tulsi	9.31×10^4	26	$1.11 \times 10^4 – 7.49 \times 10^5$	0.46
Kedar	7.17×10^3	25	$-1.13 \times 10^3 – 2.56 \times 10^4$	2.95
Raja	7.63×10^3	23	$4.15 \times 10^3 – 1.11 \times 10^4$	0.92
Ahilyabai	7.40×10^3	24	$6.28 \times 10^3 – 8.53 \times 10^3$	0.36
Rajendra Prasad	2.35×10^4	26	$-1.33 \times 10^4 – 6.02 \times 10^4$	1.60

The difference between the indicator concentrations at each site was tested in a Poisson Regression model that estimated the mean predicated concentration using

sampling location as a the input variable. No other covariates were tested in this model. The predicted indicator concentrations at all sites were significantly different ($P < 0.05$) from one another (Table 4 and 5). Tables 4 and 5 show the predicted mean Total Coliform concentration at each site based on the maximum likelihood parameter estimates from the Poisson regression model using *Ahilyabai Ghat* as the referent group. *Ahilyabai Ghat* was selected as the intercept because it has the lowest mean concentration (MPN/100mL) and therefore yields positive estimates for each of the other ghat sampling locations. Multiple regression analyses were conducted using each site as an intercept. In each case, the predicted mean Total Coliform and *E. coli* concentrations (MPN/100mL) were found to be statistically different by site (results not shown).

Table 4: Analysis of Maximum Likelihood Parameter Estimates for Total Coliform Concentrations (MPN/100mL) at Five Sampling Sites along the Ganges River, Varanasi, India – May 29th to June 25th, 2013

Ghat Name	Total Coliform Predicted Mean Value (MPN/100mL)	Confidence Interval ($\alpha = 0.05$)	P value
Tulsi	2.45×10^5	$2.44 \times 10^5 – 2.46 \times 10^5$	<.0001
Kedar	6.85×10^4	$6.82 \times 10^4 – 6.88 \times 10^4$	<.0001
Raja	8.39×10^4	$8.35 \times 10^4 – 8.42 \times 10^4$	<.0001
Ahilyabai *	6.18×10^4	$6.17 \times 10^4 – 6.19 \times 10^4$	<.0001
Rajendra Prasad	2.00×10^5	$2.00 \times 10^5 – 2.01 \times 10^5$	<.0001

* *Ahilyabai Ghat* was used as the referent group

Table 5: Analysis of Maximum Likelihood Parameter Estimates for *E. coli* Concentrations (MPN/100mL) at Five Sampling Sites along the Ganges River, Varanasi, India – May 29th to June 25th, 2013

Ghat Name	*E. coli* Predicted Mean Value (MPN/100mL)	Confidence Interval ($\alpha = 0.05$)	P value
Tulsi	1.03×10^5	$1.02 \times 10^5 – 1.04 \times 10^5$	<.0001
Kedar	1.60×10^4	$1.58 \times 10^4 – 1.61 \times 10^4$	<.0001
Raja	9.29×10^3	$9.19 \times 10^3 – 9.39 \times 10^3$	<.0001
Ahilyabai *	7.86×10^3	$7.82 \times 10^3 – 7.89 \times 10^3$	<.0001
Rajendra Prasad	5.97×10^4	$5.92 \times 10^4 – 6.03 \times 10^4$	<.0001

* *Ahilyabai Ghat* was used as the referent group

Temporal Analysis

Indicator bacteria concentrations varied over time during the study period (Figure 8). Data were missing on June 4th due to inability to collect samples. For some locations, such as *Tulsi* and *Ahilyabai Ghats,* both the Total Coliform and *E. coli* concentrations showed limited variability (Tables 2 and 3, and Figures 9 and 10). For other sampling locations, such as *Rajendra Prasad, Raja* and *Kedar*, the indicator bacteria concentrations were highly variable during the study period (Tables 2 and 3, and Figures 9 and 10). The data show two peaks in both Total Coliform and *E. coli* concentrations on June 5th and June 18th (Figure 8). The first peak coincided with heavy rainfall (data not shown), and the second peak coincided with anecdotal reports of heavy rainfall and flooding in an area upstream from the study area. High water levels were observed at Varanasi on June 18th.

Figure 8: Daily Total Coliform and *E. coli* Concentrations (MPN/100mL) Aggregated by Date along the Ganges River, Varanasi, India – May 29th to June 25th, 2013

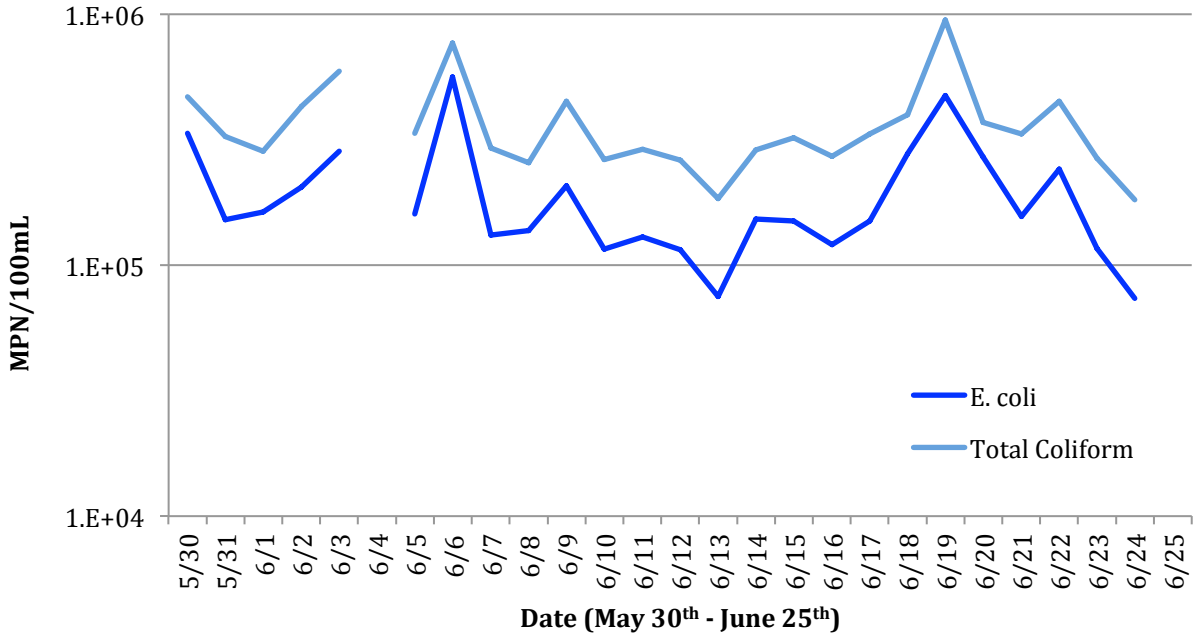


Figure 9: Daily Total Coliform Concentrations (MPN/100mL) at Five Sampling Sites along the Ganges River, Varanasi, India – May 29th to June 25th, 2013

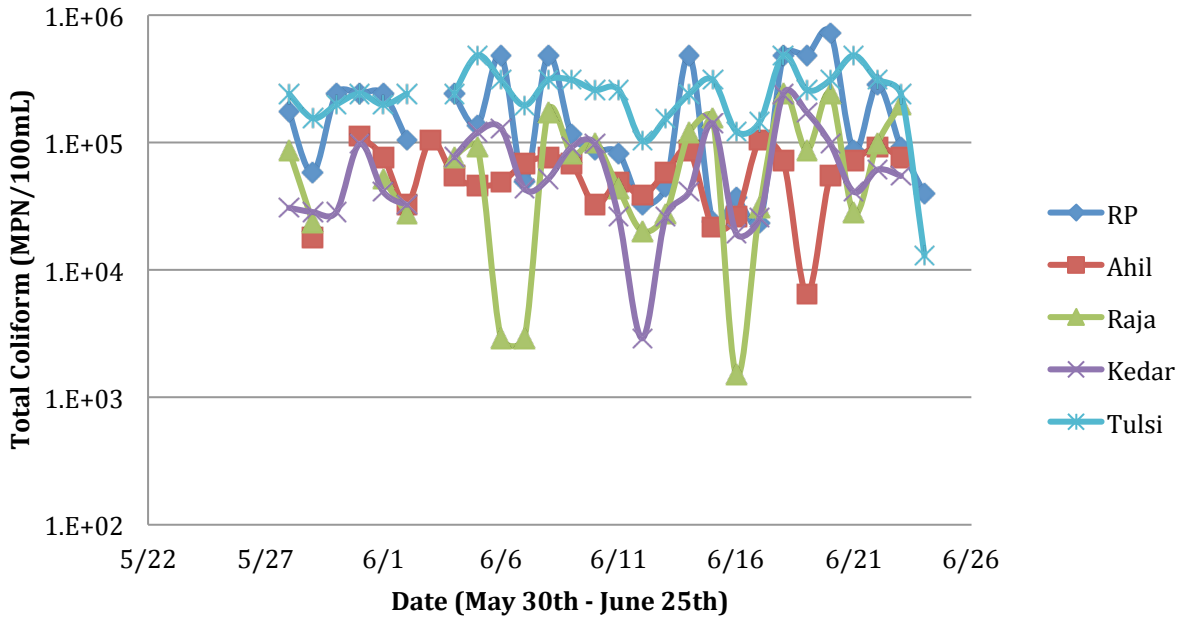
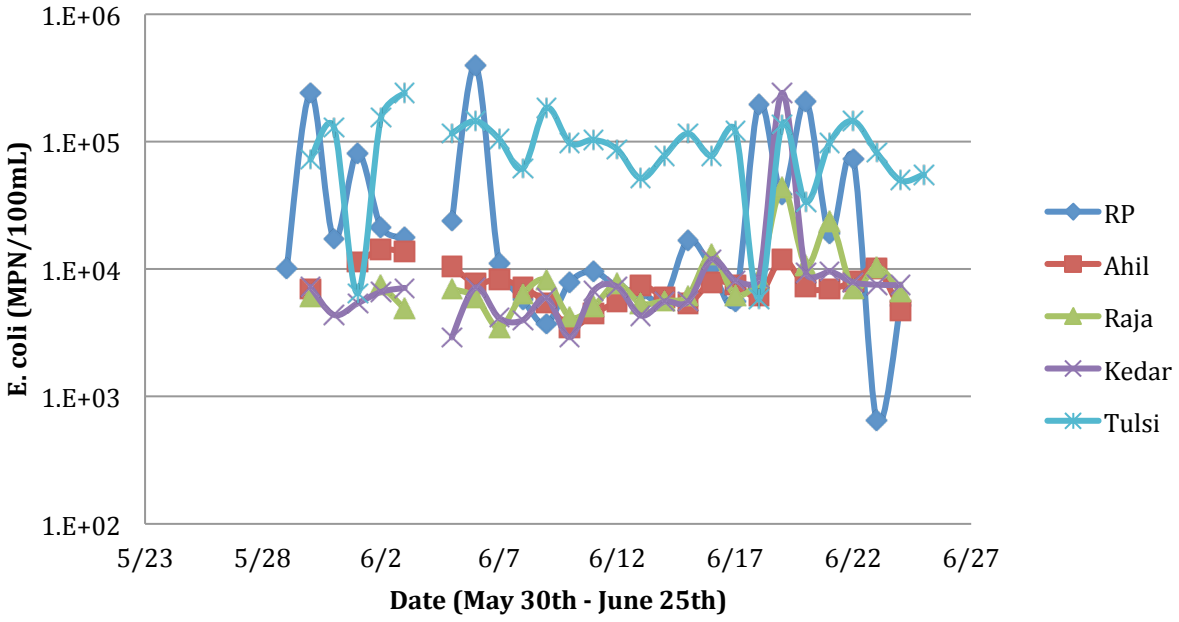


Figure 10: Daily *E. coli* Concentrations (MPN/100mL) at Five Sampling Sites along the Ganges River, Varanasi, India – May 29th to June 25th, 2013



In order to examine temporal differences in microbiological water quality more closely, we defined the sampling dates from May 29 through June 4th as the "pre-monsoon" period (n=6); the sampling dates from June 5th through June 17th as the "monsoon period" (n=13); and the samples from June 18th through 25th as the "high water level period" (n=8). Predicted Total Coliform and *E. coli* concentrations for these three periods were estimated from a Poisson Regression model that estimated the mean predicated concentration using time period as the input variable. No other covariates were tested in this model. Findings suggest that Total Coliform concentrations increased during the monsoon period and then again during the high water level period (Table 6). Conversely, *E.* coli concentrations increased during monsoon period, but then decreased during the high water level period (Table 7).

Table 6: Total Coliform Concentrations (MPN/100mL) Predictive Model Stratified on Weather conditions, Varanasi, India – May 29th to June 25th, 2013

Weather	Total Coliform Predicted Value (MPN/100mL)	Confidence Interval ($\alpha = 0.05$)	P value
Pre-Monsoon	1.080×10^5	$1.079 \times 10^5 – 1.082 \times 10^5$	<.0001
Monsoon	1.200×10^5	$1.195 \times 10^5 – 1.202 \times 10^5$	<.0001
High Water	1.681×10^5	$1.677 \times 10^5 – 1.685 \times 10^3$	<.0001

* The Pre-Monsoon period was used as the referent group

Table 7: *E. coli* Concentrations (MPN/100mL) Predictive Model Stratified on Weather Conditions, Varanasi, India – May 29th to June 25th, 2013

Weather	*E. coli* Predicted Value (MPN/100mL)	Confidence Interval ($\alpha = 0.05$)	P value
Pre-Monsoon	4.579×10^4	$4.570 \times 10^4 – 4.589 \times 10^4$	<.0001
Monsoon	4.699×10^4	$4.678 \times 10^4 – 4.719 \times 10^4$	<.0001
High Water	4.530×10^4	$4.512 \times 10^4 – 4.547 \times 10^4$	<.0001

* The Pre-Monsoon period was used as the referent group

Discussion

Introduction to the Problem

The Ganges River at Varanasi is used for drinking, bathing, and conducting religious activities, yet our water quality data indicates that the river has high levels of fecal contamination and suggests that bathers are likely exposed to a host of enteric pathogens. A 1998 study conducted in Bhagalpur, a city with similar discharges of untreated sewage into the Ganges River, found high concentrations of fecal streptococci (up to 4.45×10^3 MPN/100 ml), *E. coli* (up to 3.15×10^5 MPN/100 ml) and *Clostridium perfringens* (up to 3.5×10^4 MPN/100 ml), as well as species of *Salmonella* and *Shigella* (Bilgrami & Kumar, 1998). Additionally, a 2007 study isolated potentially pathogenic *E. coli* O157:H7 from the river and found evidence of cholera in hospital surveys associated with washing and bathing in the Ganges River (Hamner et al., 2006). When ingested in sufficient numbers, these enteric pathogens can cause diarrheal disease in a susceptible host. Some pathogens require large doses while others require only a few organisms to cause infection and possibly disease (Heymann, 2004).

The primary source of microbiological pollution in the Ganges River at Varanasi is untreated and partially treated sewage (V. B. Mishra, 2005). This sewage is discharged into the river and then comes into contact with bathers. In Varanasi, many bathers will take a holy dip and holy sip to purify the body and soul. Both the dip and sip provide a route of exposure, allowing contaminated water to be ingested. Varanasi is a city of 3.7 million people reliant on a polluted waterway, and each day thousands of people are exposed to fecal contamination and potentially to waterborne enteric pathogens, either during the

course of conducting their daily life or seeking religious purification.

Approach and Limitations

Ideally, this study would measure every pathogen in the Ganges River at Varanasi; however, due to limited time and resources, the study design enumerated indicator organisms commonly used to assess the quality of drinking water and recreational water. While useful for determining the presence and magnitude of fecal contamination, indicator organisms do not provide data on the presence of specific pathogens. Not identifying specific pathogens and their concentrations in the Ganges River at Varanasi is a limitation of this study. However, previous studies have demonstrated the presence of pathogens in the Ganges River (Bilgrami & Kumar, 1998; Hamner et al., 2007).

The magnitude of concentrations in the samples collected was so high that there was some initial difficulty remaining in the detection range of the test. Some (12% of Total Coliform and 4% of *E. coli*) water samples had Quanti-Trays 2000® that were positive in every well and were therefore known to be underestimates of the indicator concentration in the water sample. Also due to the magnitude of concentration, samples were heavily diluted leading to potential measurement error. These sources of measurement error are non-differential, and the indicator concentrations in the samples were still orders of magnitude above national and international standards. Therefore, the sources of potential measurement error have relatively little impact on the findings of this study.

While anecdotal and observational data were collected, there were no quantitative data on rainfall that could be compared to spikes in bacterial concentration. Therefore, this potential variable is missing from the Poisson Regression model, along with hydraulic data

on river flow, temperature, number, location, and volume of sewage outfalls, and other potential drivers of fecal indicator concentrations. Finally, the one-month sampling period was relatively short.

Findings

The most basic finding of this study reaffirms the past findings of SMF and the SGRL that the Ganges River at Varanasi is polluted beyond national standards and international guidelines for microbial water quality. The lowest sample concentration collected from the Ganges during the one-month sampling period was 1.5×10^4 MPN/100mL and 2.9×10^3 MPN/100mL for Total Coliform and *E. coli,* respectively. SGRL has collected a 20-year weekly dataset of Fecal Coliform Counts (FCC) and recently published their findings (Hamner et al., 2013).

> It is likely that the pollution load from raw sewage entering the Ganga in Varanasi has increased over time due to the cumulative effects of continued discharges linked with population increase and deterioration of the sewerage network. (Hamner et al., 2013)

This study found that concentrations of indicator organisms have increased over time, for a comparison of fecal indicator concentrations from the same sampling locations see Table 8.

Table 8: Comparison of Fecal Coliform Counts, *E. coli* MPN, and Total Coliform MPN Concentrations from 2010 and 2013 at Varanasi, India

Ghat Name	2010 - Hamner **Fecal Coliform Average*** (FCC/100mL) (n)	2013 - Green ***E. coli* Average*** (MPN/100mL) (n)	2013 - Green **Total Coliform Average*** (MPN/100mL) (n)
Tulsi	2.91×10^4 (51) †	1.03×10^5 (26) ‡	2.49×10^5 (27) ‡
Rajendra Prasad	7.33×10^4 (20) †	5.97×10^4 (26) ‡	2.00×10^5 (27) ‡

* Arithmetic Mean
† Sampling conducted throughout 2010, weekly for *Tulsi Ghat* and biweekly for *R.P. Ghat*
‡ Sampling conducted May 30 – June 25, daily for both *Tulsi* and *R.P. Ghats*

Of these three indicators of water quality, *E. coli* is the most specific for fecal contamination. Our findings of greater average concentrations of *E. coli* in 2013 than the previously reported average Fecal Coliform concentrations in 2010 at Tulsi Ghat suggests that the fecal contamination in the river at this point has increased during the past three years. The fact that *E. coli* concentrations were slightly lower at *Rajendra Prasad Ghat* in 2013 than the Fecal Coliform concentrations measured in 2010 does not mean that the fecal contamination was of a lesser magnitude, as *E. coli* is a more fecal-specific sub-group of the Fecal Coliforms.

The Central Pollution Control Board (CPCB) of the Government of India (Table 9) requires Total Coliform concentrations to be less than 50 MPN/100mL for drinking water, less than 500 MPN/100mL for outdoor bathing, and less than 5,000 MPN/100mL for source water for potable treatment. *Tulsi Ghat* is directly upstream of the drinking water intake for the City of Varanasi (approximately 100 meters); the average Total Coliform concentration was nearly fifty times the standard permissible for a drinking water source after potable treatment. Moreover, the same average was five hundred times greater than

the allowable limit for outdoor bathing, and five thousand times greater than the allowable limit for drinking water without treatment. According to the data collected in this study and the national standards for water quality, the Ganges River at Varanasi is unfit for drinking, bathing, or for use as source water for potable treatment. Moreover, the CPCB standards are relatively relaxed by international standards. WHO guidelines for drinking water are <1 MPN/100mL for Total Coliform and *E. coli (WHO, 2011).* The US Environmental Protection Agency's bathing criteria does not allow geometric mean *E.* coli concentration to exceed 126 CFU (colony forming units)/100mL (U.S. Environmental Protection Agency, 2003). By comparison the CPCB standards are fifty times less stringent for drinking water and four times less stringent for bathing. The geometric mean *E. coli* concentration from May 29 – June 25, measured in this study (3.9×10^4) is over 300 times greater than the US EPA criteria for recreational water.

Table 9: Government of India Central Pollution Control Board Water Quality Standards

Class of Water	Criteria	Designated-Best-Use
A	Total Coliforms Organism MPN/100ml shall be 50 or less pH between 6.5 and 8.5 DO 6mg/l or more BOD 5 days 20°C 2mg/l or less	Drinking Water Source without conventional treatment but after disinfection
B	Total Coliforms Organism MPN/100ml shall be 500 or less pH between 6.5 and 8.5 DO 5mg/l or more BOD 5 days 20°C 3mg/l or less	Outdoor bathing Organized)
C	Total Coliforms Organism MPN/100ml shall be 5000 or less pH between 6 to 9 DO 4mg/l or more BOD 5 days 20°C 3mg/l or less	Drinking water source after conventional treatment and disinfection
D	pH between 6.5 to 8.5 DO 4mg/l or more Free Ammonia (as N) 1.2 mg/l or less	Propagation of Wildlife and Fisheries
E	pH between 6.0 to 8.5 Electrical Conductivity at 25°C micro mhos/cm Max.2250 Sodium absorption Ratio Max. 26 Boron Max. 2mg/l	Irrigation, Industrial Cooling, Controlled Waste disposal
Below-E	Not Meeting A, B, C, D & E Criteria	

Adapted from: (Environmental Protection and Pollution Control Board, 2014)
DO: Dissolved Oxygen
BOD: Biochemical Oxygen Demand

The second major finding of this research is that Total Coliform and *E. coli* concentrations at each of the five sites were significantly different from each other. The spatial differences in indicator concentrations between sites have many possible explanations such as the proximity and magnitude of sewage discharges, river characteristics, and climatic factors. The differing concentrations observed in this study are

likely explained by a combination of variables. A previous study using discriminant analysis found "nine parameters (pH, temperature, alkalinity, Calcium-hardness, DO, BOD, chloride, sulfate and TKN) to afford 91% right assignations in spatial analysis of three different regions in the basin" in the Gomti River, a tributary of the Ganges River (Singh, Malik, Mohan, & Sinha, 2004). Total Kjeldhal Nitrogen (TKN) is the sum of organic nitrogen and ammonia in a water body and generally denotes sewage contamination. Discriminate analysis is an alternative to regression analysis. In this case, 91% of the variance in the model can be assigned to the aforementioned variables. These results pertain to natural seasonal variation as wastewater discharge was found to be a source of pollution throughout the year. Therefore, additionally variation between sites is likely related to magnitude and composition of sewage discharged.

The final finding suggests that concentrations of Total Coliform appeared to increase with monsoon and upstream flooding; whereas, *E. coli* concentrations appeared to increase with monsoon, but decreased with upstream flooding. These results from the temporal analysis are likely due to the differences between the indicators and are partially supported by a study of urban watersheds in Raleigh, NC (Hathaway & Hunt, 2011). Total Coliform can survive and grow in water and can occur in both sewage and natural waters. The increase in concentration with increase in volume suggests that large numbers of bacteria are washed into the river during the monsoon.

The *E. coli* data present a slightly more nuanced result, as *E. coli* are not found in the environment without first originating from a fecal source. The initial increase may be due to a 'first flush' effect, whereby most of the fecal matter in the streets and *nallas* (open drains) flows in to the Ganges River. Moreover, the initial spike in concentrations occurred

just a few days after the first rains of monsoon, and the second spike in concentration occurred on the day that upstream floodwaters caused the river level in Varanasi to rise dramatically.

The first flush phenomenon is well documented in the literature for many types of pollutants being washed into a receiving water body during the beginning of a storm event (Bertrand-Krajewski, Chebbo, & Saget, 1998; Chow, Yusop, & Toriman, 2013; H. Lee, Lau, Kayhanian, & Stenstrom, 2004; J. H. Lee, Bang, Ketchum Jr, Choe, & Yu, 2002). However, there is a debate about how to best quantify and define the first flush phenomenon (Bach, McCarthy, & Deletic, 2010; McCarthy, 2009). In his study of the first flush phenomenon for *E. coli* in four urban catchment in Melbourne, Australia, McCarthy found that *E. coli* might not follow the traditional first flush models, which compare concentration levels at the beginning and end of a storm event, because *E. coli* might be present in larger numbers at the end of the event (McCarthy, 2009). A 2011 study investigating *E. coli*, fecal coliform, enterococci, and total suspended solids (TSS) over 20 storm events, found evidence of a first flush effect for Total Coliform, but not for *E.* coli, demonstrating the effect is weak and sometimes not present (Hathaway & Hunt, 2011). Clearly, there is need for further research into the first flush phenomenon in relation to fecal indicators, as most studies of the first flush phenomenon are related to storm water management in developed countries, where stormwater and sewage are often separated. Because Varanasi has a failing sewerage system, as well as a rudimentary stormwater system, there are significant differences in comparing first flush effects in Varanasi with those in more modern cities such as Melbourne, Australia; Los Angeles, California; and, Raleigh, North Carolina in which the first flush phenomenon has been studied (Bach et al., 2010; Chow et al., 2013; H. Lee et

al., 2004; McCarthy, 2009). Fecal contamination can concentrate in the "antecedent dry weather period" as described by McCarthy, especially in *nallas* and open storm drains that are present in Varanasi and also receive sewage outfalls, but are not present in the more "developed" urban context (McCarthy, 2009). Therefore, the concept of the first flush phenomenon as it is currently described may need to be modified to fit the context of a low-resource, densely populated urban setting like Varanasi.

Strengths of the Study

The increasing magnitude of fecal contamination in the Ganges River at Varanasi is cause for serious concern. This study collected daily river water samples during the beginning of the monsoon season, which is a historically difficult time to sample as boat traffic is illegal once flooding begins and the Ganges River begins to rise. Previous studies have reported a contamination dilution effect from monsoon in August/September (Hamner et al., 2013), but this study in June 2013 observed an initial increase in the concentrations of indicator organisms at the beginning of the monsoon period. The Sankat Mochan Foundation and its Swatcha Ganga Research Laboratory have collected 20 years of Ganga water quality data, including weekly fecal coliform concentration, which demonstrated seasonality with the monsoon (Hamner et al., 2013). Moreover, two of the five sampling sites in the current study were included in previous SGRL water quality research. Because of the daily sample collection and measurement of *E. coli*, this study offers a more in-depth analysis of fecal contamination during this pivotal moment in seasonality, the beginning of monsoon.

Recommendations for Future Research

In order to build a more informative microbial risk assessment model, the following data should be gathered both upriver of Varanasi and in Varanasi: 1) sewage discharge flow and location, 2) daily precipitation data, 3) hydrologic data, such as river flow, depth, mixing, and sediment transport, and 4) physiochemical water quality parameters, such as DO and BOD. This study was also limited to one summer month. The dry season lasts typically 10 months of the year, and future research should include the water quality measurement during both dry weather and wet weather flows of the Ganges River. Ideally, the Swatcha Ganga Research Laboratory would be able to incorporate these methods of *E. coli* detection into their weekly sampling.

The results of this study suggest 'number of upstream outfalls' is not as important a predictor for bacterial concentration, as the discharge volume of the outfalls. However, more data is needed to test this hypothesis. *Tulsi Ghat*, the ghat with the fewest upstream discharges, had consistently the second highest indicator concentrations behind *Rajendra Prasad* (R.P.) *Ghat*, the ghat with the most upstream discharges. The middle three ghat sampling locations also displayed no trend. *Tulsi Ghat* is downstream of the Assi-Ganga confluence, and the pollution of the Assi River and Nagwa Drain may be a major determinant of the water quality at the Tulsi Ghat. Hamner found an average Fecal Coliform concentration of 3.84×10^6 FCC/100mL at the Nagwa Drain (Assi River) in 2010 (Hamner et al., 2013). The Nagwa Drain is a large sewage discharge of approximately 67 MLD, according to SMF staff (R. K. Mishra, 2013) (Figure 11).

Figure 11: Assi River / Nagwa Drain Varanasi, India

Photo Credit: Hugh Green

Fecal contamination can either come from the Ganges River and its upriver tributaries, runoff from streets and *nallas*, or from the sewerage system, whose operation is intermittent and which can be rendered inoperable due to flooding. These three sources of contamination warrant further study, and research should be conducted on monsoon floodwater to test for coliform growth in the water itself. Data should be collected on these three potential sources of contamination to inform a more sophisticated model.

In addition to further research into spatial variability, there should be further inquiry into the temporal variability associated with the monsoon. Water quality metrics

should be gathered on the floodwater upstream of Varanasi to determine if increased contamination is originating from the city or not. Precipitation and hydraulic data would add important predictor variables to the model and would potentially describe a portion of the variance observed.

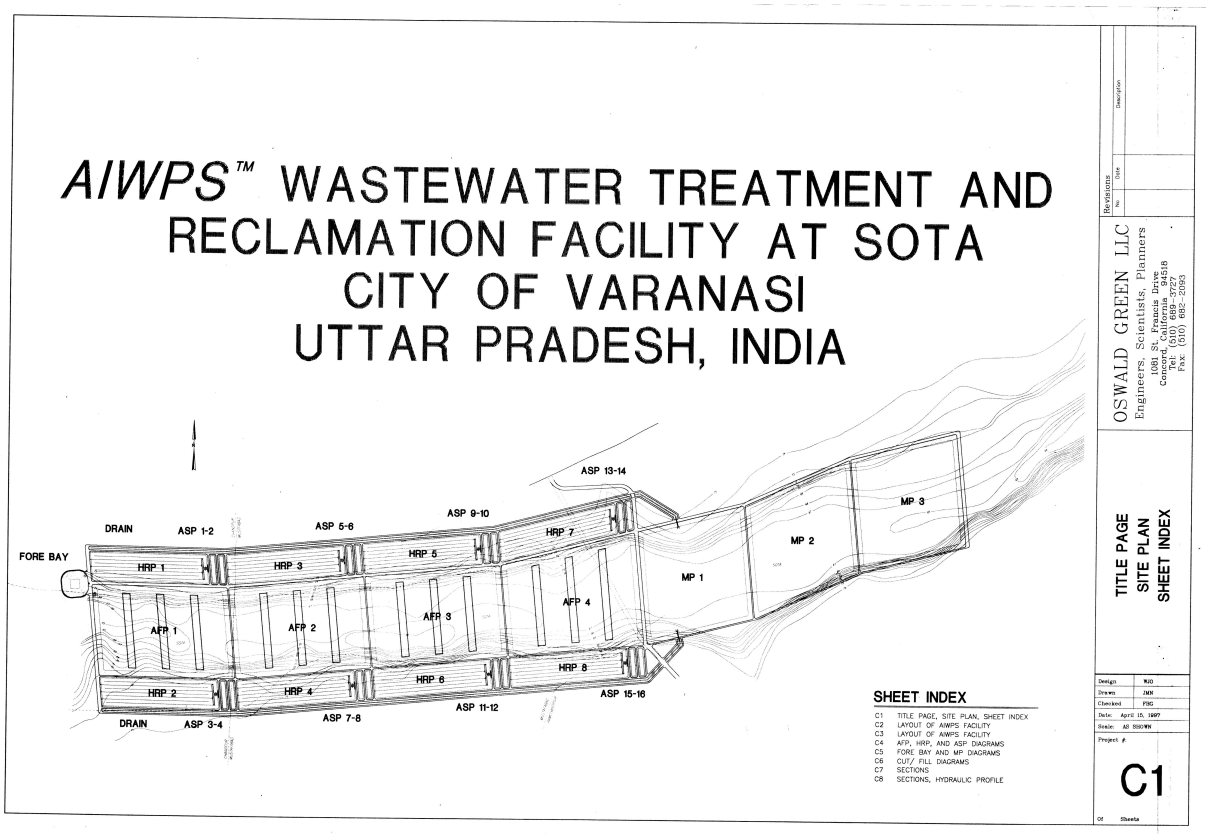
International collaboration between the Sankat Mochan Foundation (SMF), the Indian Institute of Technology at the Benaras Hindu University (IIT BHU), and Emory University's Rollins School of Public Health would build the research capacity of the Swatcha Ganga Research Laboratory. Improved research capacity of SGRL would also strengthen SMF's advocacy campaign to protect and restore Ganga Ma and to improve Varanasi's sewerage system in order to address a major public health risk in the City of Varanasi.

Recommendations for Action

The Sankat Mochan Foundation has been advocating for a sustainable sewerage treatment plan for decades. In 1997, at the invitation of the Ministry of Environment and Forests, SMF and Oswald Green, LLC prepared, and the City of Varanasi Municipal Corporation (Nagar Nigam) approved, a sewerage facility plan or *Preliminary Feasibility Report* for a gravity riverfront sewage interceptor from the Assi-Ganga confluence to the downstream oxbow channel (known as the "Sota") where a terminal sewage pumping and an Advanced Integrated Wastewater Pond System® (AIWPS®) Sewage Treatment Plant (Figure 12) could be located (Sankat Mochan Foundation and Oswald Green LLC, 1997). The AIWPS® STP, combined with a sewage interceptor pipe running underneath the bathing ghats, would intercept, divert, convey, treat and reclaim all of Varanasi's municipal

sewage prior to: 1) beneficial irrigation reuse during the dry period, and 2) safe discharge into the Ganges River during the monsoon period. The Government of Uttar Pradesh owns sufficient land at the Sota site, seven kilometers downstream of Varanasi and the Varuna-Ganga confluence, where the AIWPS® STP could be constructed without requiring the Government of UP to purchase additional private land through eminent domain and the displacement of farmers. This downstream location would eliminate the need for riverfront sewage pumping stations by using a gravity-fed interceptor pipeline to a terminal sewage pumping station at Sota and the AIWPS STP.

Figure 12: Plan Drawing of Proposed AIWPS STP downstream of Varanasi, India



Reprinted with permission of Oswald Green Technologies, Inc., formerly Oswald Green LLC (Sankat Mochan Foundation and Oswald Green LLC, 1997).

In the interim, before a complete solution is reached, minor sewage outfalls could be modified to provide passive aeration prior to discharge. The Main Trunk Sewer Outfall, which was constructed during the British Colonial Period (Figure 13), utilized a cascading design that passively oxygenates the effluent as it is discharged to the Ganges River. This Main Trunk Sewer Outfall is located downstream of the Varuna-Ganga confluence. Small, inexpensive interventions like this stepped design could increase the dissolved oxygen concentration of the sewage and potentially improve the microbiological water quality of the Ganges River at Varanasi.

Figure 13: Main Trunk Sewer Outfall Varanasi, India

Photo credit: Pushkar Mishra

Conclusions

One in twelve people on the planet rely upon the Ganges River Basin. Nearly 65% of Indians live with unimproved sanitation facilities (Central Intelligence Agency, 2013; UNICEF and WHO, 2012). The pollution of the Ganges River has wide reaching public health impacts, especially in relation to the persistence of diarrheal disease. According to a recent World Bank report, the annual cost of poor sanitation in India estimated in US dollars is approximately $53.8 billion primarily due to adverse health impacts (Tyagi, 2012). The majority of the burden of waterborne disease could be prevented through WASH interventions (Black et al., 2003). This research has found that the water quality of the Ganges River is unfit for drinking, bathing, or potable treatment. Past studies from Varanasi have presented no evidence that the situation is improving (Hamner et al., 2013). The findings of this research support the assertion made by the SMF, G.D. Agrawal, and Hamner et al. that fecal contamination of the Ganges River has continued to increase and that the Ganga Action Plan has not improved the water quality of the Ganges at Varanasi (Agrawal, 2007; Hamner et al., 2013; V. B. Mishra, 2005).

The city of Varanasi, other cities located along the Ganges River, and other river cities in India should and must treat their wastewater before discharging it into the river. At the very least, sewage discharges should be located down stream of the bathing population and drinking water intake, thereby mitigating the health risks associated with fecal contamination of the river. The status quo of discharging untreated and partially treated wastewater exposes large populations to fecal contamination, enteric pathogens, and risk of disease (Bilgrami & Kumar, 1998; Hamner et al., 2007; Hamner et al., 2013; Hamner et al., 2006; Uttar Pradesh Jal Nigam).

In 2009, the World Bank loaned the Government of India US$1 billion dollars to clean the Ganges River (Connors, 2013). Technical solutions exist and have been approved at the municipal level (Green, 2013). The Ganges at Varanasi is increasingly polluted by municipal sewage, but does not have to be. There currently exists a desire, funds, and a solution to address the pollution of the Ganges River at Varanasi. The ancient and modern City of Varanasi could become a model for other river cities throughout India and South Asia; but until it does, the water that Hindus believe washes away all physical and spiritual impurities will continue to transmit waterborne disease. The fecal contamination of the Ganges River endangers the health of *Ganga Ma* and those she supports including the inhabitants, pilgrims, and visitors of this most ancient city on the banks of Mother Ganga.

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