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Abstract Book

Verbal Presentations

High prevalence of Multiple Antibiotic-Resistant (MAR) bacteria in riverbed sediments of the Apies River, South Africa: a possible health threat to populations living in resource poor settings

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Background In South Africa, a water-scare country, rural and peri-urban communities often rely on rivers as an alternative source of water. When river water is not treated before drinking, users are then potentially exposed to microorganisms which could lead to waterborne disease. Compounding the health issues of users is the possibility that these microorganisms might also be resistant to antibiotics due to the indiscriminate and inappropriate use of antibiotics in general. Traditionally, only the water column in the aquatic environment is sampled to identify and enumerate antimicrobial-resistant (AMR) bacteria. However, sediments have been shown to be a reservoir of microorganisms and under favourable conditions when the sediments are resuspended, bacteria move from the sediment into the water column. This movement of these organisms from the sediments include AMR bacteria as well as multiple antimicrobial resistant (MAR) strains. This study was conducted to investigate the presence of antibiotic resistant Escherichia coli in river bed sediments of the Apies River, Gauteng South Africa, in order to better inform health management decisions designed to protect users of the river. Methods Water and sediment samples were collected at 10 sites along the Apies River on a weekly basis from January to February 2014. E. coli was isolated and enumerated using the ColilertTM 18 / Quanti-Tray 2000 (IDEXX) method. Confirmation of isolates was done by streaking on Eosin Methylene Blue agar followed by the indole test using Kovac's reagent. Confirmed E. coli isolates were tested for resistance to 9 commonly used antibiotics by the Kirby Bauer disc diffusion method. Results and Discussion Water and sediments samples from the Apies River revealed a high prevalence of antibiotic-resistant E. coli. The most prevalent resistance observed was against Ampicillin (in the water column) and Nitrofurantoin (in the sediments). Over 80% of all resistant isolates (sediments and overlaying water) were resistant to 3 or more antibiotics, indicating a very high percentage of MAR. However, all strains of E. coli isolated from both the water and sediments were susceptible to Gentamicin. The abundance of E. coli in the sediments, not only adds to the evidence that sediments are a reservoir for bacteria including antibiotic resistant bacteria, but also indicates the potential that sediments have to harbour pathogens such as Vibrio cholerae. Furthermore, under the right conditions, there exists the possibility that antibiotic resistance genes could be transferred to pathogens due to the high prevalence of MAR strains of E. coli observed in the sediment. Wastewater treatment works and numerous cattle rearing farms situated along the Apies River could be contributing the MAR bacterial load observed in the sediments. Conclusions Based on the results obtained during this study, the use of untreated water from the Apies River for drinking and other household purposes poses a serious health risks for users. Firstly, the high presence of E. coli indicates faecal pollution and therefore an increased likelihood of the occurrence of pathogens. Secondly, of the E. coli strains isolated, high percentages were MAR strains. Finally, these MAR E. coli strains were also found in the sediments. During sediments resuspension events, users

are likely to be exposed to higher concentrations of these MAR strains when these strains move from the sediment into the water column.

Assessment of physico-chemical and microbiological quality of drinking water from disinfected water sources points to house hold water containers in selected communities of Akaki-kaliti sub city, Addis Ababa City Administration

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Introduction: Though ground water is much better than surface water in terms of biological quality, lack of source protection and inefficient treatment, waste management and sewerage system problem, poorly designed pit latrines and poor hygienic practice at the households affect the quality of the water. Therefore, assessment of physico-chemical and microbiological quality of drinking water from disinfected water sources points to house hold water containers in selected communities of Akaki-kaliti sub city, Addis Ababa City Administration were conducted. Methods: This study was conducted from September 2006 to January 2007. A survey of 72 triplicate water sample and sanitary surveys were conducted in 3 chlorinated, 35 pipe water and 35 randomly selected households' storage water containers. The water samples presumptive test of TTC and FS were examined using membrane filtration method. Result: Temperature at all three disinfection points were above permissible limit of 15 oC. Turbidity at CTR and FSC were meet the acceptable level of WHO and National standard limit of potability < 5 FAU and above the recommended limit at the TDR.. The pH values at all the three points were within the recommended limit (6.5 -8.5). The free chlorine residual were 0.67, 0.6, 0.68 mg/l at CTR, TDR and FSC respectively which are lass than to average value of the recommended limit of WHO (>0.8mg/l). All sample sources were contaminated with TTC and FS having cfu >1 per 100ml and this was found out to be above WHO and National standards (cfu/100ml=0). Only 1(2.9%) of pipe water samples were <15 oC whereas others above the limit of 15 oC. The average temperature of pipe water was in the range of 14.5-22.5 oC which was warmer as compared to the standard temperature (15oc). This favors the regrowth of some indicator organisms like TTC in distribution systems. Out of the examined sampling sites 34.3% of them were below the range of acceptable chlorine residual limit (0.2-0.5 mg/l) and 17.1% were above the recommended level (0.5 mg/l). In all pipe water samples, pH values were within the recommended limit. In the pipeline, only 17.1% and 31.4% of sampling sites were found acceptable based on WHO and National standard for TTC and FS counts, respectively. The overall risk-to-health classification at pipe water (N=35) were 19(54.29%) in intermediate and 16(45.7%) in low classification range for FS whereas for TTC, 19 (54.29%), 8(22.88%) and 8(22.88%) were in intermediate, high and low risk to health matrix score, respectively. For water samples at the household, only 14.3% was within recommended free chlorine residual level. 8.6% and 17.1% of sample sites (N=35) were above the recommended limit of temperature (<15oC), and turbidity (<5FAU), respectively and only 1 (2.9%) was acceptable for both TTC and FS cfu levels. The health matrix classifications for bacteriological indicators (TTC and FS) were found to be 65.7% and 20% with in the high risk and medium risk score, respectively. Conclusion and recommendation: Uncontrolled physico-chemicals parameters

such as temperature, turbidity, pH and inefficient chemical chlorine dosing, which led to low chlorine residual at distribution and household water containers were the major factors that contributed the occurrence of high bacterial numbers. Moreover, the water pipe lines and sewerage lines arrangement was also another factor that contributes for bacterial growth in the distribution system, there by compromising the quality of water at the point of use. Bacteriological load was greater at the household samples due to poor hygienic practice. Therefore, the management of water sources, appropriate treatment of the raw water sources, at home, control of phisico-chemical parameters at disinfection points, and promoting good hygienic practices are important to make the water quality acceptable in the study area. Moreover, installation and utilization of highly efficient technology is recommendable for production of high potable water. Key words: disinfection points, pipe water, house holds drinking water, physico-chemical parameters, Thermotolerant coliforms, Faecal streptococci, source protection and sanitary survey

Comparing qPCR and ddPCR microbial source tracking approaches in the complex setting of Tecolote Creek, CA

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Tecolote Creek (TC) is flowing, inland creek that flows through a steep, open-space canyon. The canyon is inhabited by a range of wildlife, and discharges into Mission Bay, a high priority recreational beach area for the County of San Diego. Mission Bay is visited by millions of beach-goers annually. There are an array of possible sources of fecal contamination in TC including that from wildlife, pets, and humans. From summer 2013 through the present, a quantitative microbial risk assessment (QMRA) study has included the quantification of fecal indicator bacteria (FIB), molecular markers for specific types of fecal contamination, and human pathogenic viruses (e.g. norovirus, and adenovirus) using state of the art qPCR and digital droplet (ddPCR) approaches. Three wet weather and 11 dry weather receiving water sampling events were conducted, along with collection of samples from specific locations to assess potential sources and extensive collections ofm scat and sediment from the creek environment. Samples were analyzed for E. coli, Enterococcus, fecal coliforms, Bacteroides-based markers of human fecal contamination, bacteriophage, adenovirus, enterovirus, and norovirus using optimized, fully validated, published microbial source tracking (MST) approaches. On a subset of samples, ddPCR was utilized to confirm findings. Initial inhibition analyses utilizing the salmon sperm DNA-based housekeeping gene assay commonly referred to as "Sketa" revealed that nearly 30% of samples were inhibited. By ddPCR some of the inhibition issues were resolved. For example, adenovirus quantification during dry weather was confirmed via qPCR on a single sample from a downstream site. Using ddPCR, seven additional samples were determined to contain measurable but low concentrations of adenovirus (ranging in concentration from 1-20 per 100 ml). Source samples (scat, sediment or water) from the creek

environment demonstrated three samples containing confirmed human fecal contamination as determined by HF183 quantification using qPCR and all were confirmed by ddPCR. The concentrations of the HF183 marker in these samples ranged from ca. 102-105 per 100 ml. One additional sample that was determined to be negative for HF183 as determined by qPCR, was strongly positive in two replicates as by ddPCR. This study will highlight multiple findings ranging in scale of impact to the water quality research community, 1) prior to the use of molecular microbial source tracking results for infrastructure modification (fixing sewage or stormwater pipes) or management decisions, it is vital to fully quantify inhibition and interference in order to prevent the decisions being made on "false negatives", 2) the use of ddPCR in confirming results from qPCR-based analyses, and 3) the continued importance of optimizing quantification of human pathogenic viruses as highly specific MST markers.

Microbial Quality of Reclaimed Water to Meet NC Type 2 Performance for Escherichia coli, Coliphage Viruses, Salmonella spp., and Clostridium perfringens

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With increased population growth and climate variability, there are growing interests in water reclamation and reuse for beneficial use purposes. The State of North Carolina recently approved tertiary treated, dual disinfected wastewater (called type 2) for both non-potable agricultural use and potable reuse, with UV radiation and free chlorine as recommended disinfectants. However, the use of reclaimed water as potable drinking water supply has sparked intense public debate over its quality and health risks. Therefore, the goal of this research was to determine if water reclamation facilities using NC type 2 reclaimed water (NCT2RW) treatment processes can produce water that is safe for potable reuse based on achieving the microbial quality levels and required log10 microbial reductions of the regulation, which are 6, 5 and 4 log10 for indictor bacteria, viruses and the protozoan surrogate. Fecal indicator and pathogenic bacteria, coliphage indicator viruses and the protozoan parasite surrogate were quantified in raw sewage and NCT2RW and log10 reductions were determined. Salmonella spp. bacteria pathogens were quantified using a culture based, multiple tube/Most Probable Number (MPN) method followed by biochemical confirmation. Escherichia coli indicator bacteria were recovered and quantified as MPN using Colilert medium in Quantitrays (Idexx). Coliphage fecal virus indicators were recovered, detected and quantified using US EPA (United States Environmental Protection Agency) Method 1602 for somatic, male-specific, and total coliphages assayed on E. coli hosts CN13, Famp, and CB390 respectively. The protozoan parasite surrogate Clostridium perfringens was quantified by standard membrane filtration techniques of US EPA and Standard Methods for the Examination of Water and Wastewater (SMEWW) using CP ChromoSelect agar medium. Log10 reduction values were calculated based on differences in microbe concentrations between raw sewage samples and reclaimed water samples treated by NCT2RW processes from 5 water treatment plants in the Research Triangle

region of NC. Initial results were 6 and 5 log10 reductions of pathogenic and indicator bacteria respectively, 5 log10 reductions in coliphage indicator viruses, and 4.5 log10 reductions in Clostridium perfringens. These observed log10 reductions indicate that dual disinfection of tertiary treated wastewater by NCT2RW processes meets the microbial quality standards of the regulation. However, additional microbial and chemical testing of reclaimed water treated by NCT2RW processes is necessary to determine if viral and protozoan pathogens are also reduced effectively and if the chemical quality is of low risk compared that of ambient surface waters allowed for use as drinking water sources in NC. The findings of this study also can be applied more broadly to other reclaimed water systems nationally where dual disinfection treatment systems are or can be used. These results can inform economic, environmental impact and health risk assessments to address the technical feasibility and performance achievement of NCT2-like reclaimed water systems compared to using other drinking water sources and more complex and costly treatment system to produce reclaimed water for potable reuse.

What would it take to comply with REC criteria during storms? Watershed-scale stormwater BMP modeling with cost-benefit optimization

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Throughout the U.S., public agencies are faced with complying with recreational water quality (REC) criteria during storm events. Stormwater runoff poses health risks to recreators including surfers but the cost of reducing those risks can be exorbitant. In the Los Angeles region, an MS4 Permit was recently issued that incentivizes stormwater agencies to quantify the type, location and cost of BMPs that would result in compliance with water quality criteria. The criteria for fecal indicator bacteria are some of the most challenging to meet of all the Clean Water Act standards. This presentation describes the outcome of advanced watershed-scale BMP modeling conducted in four major watersheds (100+ square miles) in Los Angeles County, each of which discharges near beaches. Through a linked LSPC-SUSTAIN modeling system, the networks of BMPs required to achieve REC criteria were identified including low impact development, green streets and regional BMPs. The SUSTAIN model used cost-benefit optimization to evaluate millions of potential BMP scenarios and recommend the BMPs that most effectively achieve applicable criteria. Costs for the resulting BMP networks are on the order of $5B in capital expenditure. The presentation will describe the modeling effort/results with an emphasis on lessons learned in terms of the types and extent of control measures that most efficiently control stormwater for REC criteria compliance. Furthermore, the BMP networks required to control FIB will be compared to those for controlling other pollutants including metals and legacy toxics. Finally, an overview of alternative compliance approaches will be provided including cost-optimization based on achievement of acceptable risk levels (rather than FIB criteria).

The Art of Water Reuse and Optimization in a World of Diminishing Water Supply

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Water is a unique molecule with basic properties that make it ideal for cooling water applications. It's safe, easy-to-handle, widely accessible, and inexpensive in most industrialized regions of the world. Water is a more efficient heat transfer medium than many other materials, especially compared to air, and is called the universal solvent - a property that can cause unwanted side effects for industrial applications. Water can dissolve many substances, including gases like oxygen and carbon dioxide. As a result, water can cause corrosion of metals used in cooling systems. As water concentrates in cooling systems, dissolved ions may exceed the solubility of some minerals and form scale. The life-giving properties of water can also encourage bacterial growth that can foul system surfaces. These factors are important for another reason: they have a direct effect on equipment life. These problems necessitate appropriate treatment and control to maintain the value of a cooling water system to the process it serves. Some of those problems include: ? Increased maintenance cost ? Equipment repair or replacement cost ? More frequent shutdowns for cleaning and replacement of system components ? Reduced heat-transfer efficiency leading to reduced energy efficiency of the process being cooled ? Increased energy consumption ? Potential product yield reduction or plant shutdown ? Quality control problems and escalation in product revisions ? Environmental compliance complications ? Increased greenhouse gas emissions due to higher energy use Cooling water systems are a primary part of process operations in most industries. For uninterrupted plant productivity, these systems require proper chemical treatment and preventive maintenance. In today's world of expensive energy, it is more vital than ever for heat exchange equipment to be kept free of insulating deposits that promote high energy consumption. Water recycling is emerging as a key strategy in competitiveness, corporate social responsibility, and compliance as reliability engineers have had back-up generators for power for years but are only now realizing that they need back-up water sources. It begs the question - why aren't all industries using water reuse as a best practice? And what will the future of industrial water reuse look like ten years from now? An unplanned outage or replacement of an asset during the operation and maintenance of its lifecycle can have significant negative impacts on an organization's financial statement. Typically, HVAC systems are the greatest single user of water and energy in commercial buildings. ? On average, a neglected or poorly maintained cooling tower can reduce chiller efficiency by 10% to 35%* ? 15% of all unplanned outages in commercial or industrial buildings result from water-related issues at an estimated $500,000 per outage* Water chemistry control needs to be a part of a comprehensive asset health program to deliver higher performance. This should include the inductive reasoning of a Failure Mode Effects Analysis (FMEA) with real-time data to provide early system warnings for potential critical asset failures in cooling systems. A forward looking approach to water control management including 24/7 continuous and remote monitoring and control can result in a decrease of a catastrophic system asset failure and operational risk exposure. Being sustainable is more than an environmental gesture. It makes long-term economic sense. Of the only 0.008%

of all the fresh water on this planet is readily available for potable use. The global population is predicted to double between 1989 and 2100. According to the International Food Policy Research Institute, 4.8 billion people - more than half the world's population - and approximately half of global grain production will be at risk due to water stress by 2050 if status quo continues. By wasting less, polluting less, reusing more, managing effectively and becoming more efficient in all uses of water - individual, collective, agricultural and industrial - more than 1 billion people and approximately $17 trillion of GDP could escape exposure to risks and challenges from severe water scarcity. The Solution Capture existing water sources including rainwater, greywater, stormwater, and foundation water, filter and disinfect, and reuse for cooling tower makeup, toilet flushing, irrigation, or any other nonpotable use. Cooling and process water often account for 80-90% of industrial water use. Harvested rainwater can be used for cooling in all industries and for process water in many industries. The installation of continuous water quality management and water optimization software can significantly reduce operational and capital asset risk, as well as support regulatory compliance.

Environmental Reservoirs Of Antibiotic Resistance Associated with Small Scale Poultry Farming in Northwestern Ecuador

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Small-scale poultry production that includes non-therapeutic use of antibiotics is becoming increasingly common in developing countries, contributing to an environmental reservoir of antibiotic-resistant (AR) bacteria and posing a threat to human health. We assessed the potential for transmission of AR from 'production birds' (broiler chickens and laying hens raised for sale) to the surrounding environment in Northwestern Ecuador. We sampled 300 production birds, and 455 'household birds' (raised for domestic use) from 291 households in 17 villages between 2010-2013. We also sampled drinking water, household soil and food preparation surfaces, coop surfaces and soil, and surveyed water, sanitation and antibiotic use practices. Up to three E. coli isolates per sample were tested against 12 antibiotics. We observed: 1) high levels of AR overall, particularly in production birds, which had over 60% of isolates resistant to tetracycline and sulfonamides, and significantly more AR than household birds ($p<0.01$); 2) a phenotypic resistance pattern to amoxicillin/clavulanate, cephalothin, cefotaxime and gentamicin particular to isolates from production birds and coop surfaces. The prevalence of this signature pattern of AR associated with poultry production declined with bird age and was associated with a particular purchase site. 3) Higher prevalence of resistance for all antibiotics tested in coop versus household samples ($p<0.01$). No difference in AR profiles was observed between water samples from farming versus non-farming households, or across villages with different farming intensity. These results suggest that AR strains associated with

poultry production likely originate from outside sources and are passed to the immediate environment.

Defining Best and Worst-Case E. coli Removals for a Home Water Treatment Unit

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Design and methodology: The entire range of microbial quality improvement via application of home water treatment (HWT) must be defined, not just best-case scenarios. HWT units can be used in ways the manufacturers never intended in the field. Where possible, attempts should be made to demonstrate the worst removals that could be obtained. Microbial challenge experiments were designed that examined removal of E. coli from secondary clarifier effluent from a local sewage treatment plant under best and worst-case operational scenarios by a commercially available, bio-sand type filtration unit. E. coli were quantified by IDEXX Quantitray assays and the results used to compute % and log10 removals. These removals were used to estimate disease reductions from ingestion of bacterially contaminated water per WHO guidance. Original data and results: Under worst-case experimental conditions (breakthrough sampling, continuous overproduction, un-ripened filter) an average removal of 61% of applied E. coli was documented. Worst case removals increased to 85% after filter ripening. Under the best-case, experimental conditions (non-breakthrough, intermittent normal production, ripened filter) average removals increased to over 93%. Risk calculations for Disability Adjusted Life Years (DALYs) were computed for the range of removals and then applied to a population of 100,000. Calculations showed that HWT with the TIVA unit could result in significant gains in productive days; (97,503 versus 6,533 lost days due to diarrheal illness for potable water with an initial 100 organisms/liter). Conclusion: While HWT cannot entirely eliminate waterborne disease burdens, it is often a first step for >700 million people reliant on un-improved water sources. Estimating the potential productivity increases, days people can fully engage w/o disease incapacity, is critical for communities struggling to climb the development ladder. However, realistic data must underlie these estimations to create reasonable ranges of response.

Using Human-Associated, DNA Biomarkers for Bacteroidales in a Simple, Multiparameter Model to Pinpoint Hotspots and Rank Fecal Sources in an Urban Watershed

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Human-associated qPCR biomarkers for Bacteroidales bacteria offer potent tools for the detection and control of human fecal pollution in watersheds. However, while these markers can indicate predominate sources, discrimination between the concentrations found at one site versus another, especially for markers in low concentrations, is often not statistically significant. A multiparameter approach that relates fecal loadings, fecal sources, and fecal ages, and reduces the noise about these inputs, is required to estimate the relative risk at each site. This study conducted an intensive sampling program at 19 sites in an urban watershed with sewer leaks, cross-connections, and overflows over one spring and summer. Grab samples from these sites, along with inlet domestic sewage and manhole overflows, were collected on 10 separate days, under multiple weather conditions, and analyzed for indicators of fecal load (E. coli, and a non-host specific Bacteroidales qPCR marker, AllBac), fecal source (two human-associated Bacteroidales qPCR markers, HuBac and qHF183), and fecal age (AC/TC ratio). Analysis of the resultant database found differences in the prevalence of the two human-associated markers, but when both were present their concentrations were log-linearly correlated with a slope near unity (log10 HuBac = 1.02 log10 qHF183 0.61; R2=0.90). The most prevalent, human-associated marker concentrations (HuBac) were used as an input into a new, simple, multiparameter model to calculate a Sanitary Category Value (SCV) between 0 and 3 for each location and observation. The SCV was comprised of a summation of values between 0 and 1 for each of the three indicator classes selected: 1) fecal load (E. coli categorized), 2) fecal age (AC/TC ratio), and 3) fecal source (relative strength of human-associated marker at site to that found in inlet sewage, log10HuBac/log10HuBacSewageMax). Low SCVs (<1.3) related to conditions associated with cleaner surface waters (low fecal load, little human signal, and old fecal age). Higher SCVs (>1.5) were associated with high values of fecal load, a greater than reliably detectable value for human specific qPCR markers, and a low fecal age. Common statistical analyses were done between the calculated SCVs at each site and sewage SCVs to highlight and separate leaking sewers from combined and sanitary sewer overflows. One site (D10) located along one of the creeks in the watershed was found to have SCVs indistinguishable (not significantly different by Repeat Measure ANOVA) from sewage, which was indicative of leaking sewer lines. A sanitary survey confirmed an active sewage leak impacting this site during the time of study. Sites were able to be distinguished from sewage, and ranked in terms of their SCV values from dirtiest to cleanest with some statistical significance.

Results from a Multi-Year Microbial Source Tracking Study in an Urban Watershed in Missouri

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The Little Blue River was added to the Missouri Department of Natural Resources 303(d) list for impairment due to fecal-indicator bacteria from urban runoff and storm sewers, including the reach that flows through the City of Independence, Missouri. The Little Blue River within the City receives the majority of the discharge from the Independence municipal separate storm sewer system (MS4) during storm runoff events. Probable sources of bacterial contamination in

the watershed include human sources (sanitary sewer overflows, illicit discharges to storm sewers, leaking sewer pipes, and infiltration to storm sewers, and aging or failing septic systems) and animal sources (domestic pets, livestock, and wildlife). No permitted wastewater treatment facilities discharge to streams in the Little Blue River watershed. The U.S. Geological Survey (USGS) and the City of Independence, Missouri, Water Pollution Control (WPC) Department collaborated on a 7-year study to characterize the sources of bacterial contamination to the Little Blue River. This included identifying contributions from the dominant human and animal sources in the major subbasins in the Little Blue River watershed within the city limits, as well as upstream sources entering the city limits through the Little Blue River. In addition, data were collected to identify the subbasins that were contributing the highest source loads to the main stem of the Little Blue River. From June 2008 to October 2014, 246 samples were collected at five tributaries and two main-stem sites and analyzed for concentrations of E. coli, microbial source tracking (MST) markers associated with humans, canines, and ruminants, and concentrations of wastewater indicators including caffeine. Of the 246 samples, 140 were collected during baseflow and 101 were collected during storm events. Concentrations of most constituents were generally higher in samples collected during storm events than those collected during baseflow. For E. coli specifically, which is the driver of the 303(d) listing, exceedences of the recreational water-quality standard were 32 and 85 percent for baseflow and storm-event samples, respectively. Streamflow information was collected for each sample to quantify loadings and yields of the measured constituents during baseflow and storm events, which is important for understanding patterns of water-quality impairment and for establishing appropriate mitigation strategies. A weight-of-evidence approach, based on the World Health Organization's "Annapolis protocol", was used to compensate for the uncertainty associated with any one observation or type of measurement. This approach combines information about fecal-indicator bacteria concentrations, site-specific surveys, and MST marker results and generates a prioritized list of subbasins for implementation of best management practices.

Degradation of Pathogens, General and Host-associated Fecal Indicators as Measured by qPCR and digital PCR in Freshwater

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Levels of general and host-associated fecal indicators are typically treated as proxies for total and host-associated fecal contamination, respectively. Although not frank pathogens, these indicators are monitored as surrogates for pathogens from human and non-human fecal sources. However, differential decay between fecal indicators (general or host-associated) and pathogens may render the former inadequate as pathogen surrogates. This study examined decay of these three categories of organisms as measured by qPCR and digital PCR under sunlit and shaded conditions in an urban freshwater environment. Sets of dialysis bags (6-8 kDa) containing sewage (5% w/v), cow (1% w/v) or gull (0.1% w/v) feces mixed with ambient

freshwater were suspended from PVC frames and placed in ambient freshwater in San Joaquin Marsh (Irvine, CA) for 10 days. Half of the frames were in direct sunlight, the balance were covered with shade cloth. Duplicate bags from each source and treatment were retrieved daily and the contents analyzed for general (Enterococcus, E. coli, total Bacteroidales), host-associated (e.g. for human, cow, and gull) fecal indicators, and pathogens (Campylobacter, Salmonella) by qPCR or digital PCR. A simple log linear model was used to fit the time series data, and the decay rates ranged from 0.1 to 1.8 log unit per day. While little difference was observed between decay rates of the general fecal indicators and the pathogens, the host-associated indicators decayed at significantly higher rates. Particularly, decay rates of Enterococcus and total Bacteroidales were not significantly different for any source, while the human- and cow-associated Bacteroidales decayed approximately twice ($p<0.05$) as fast as the general fecal indicators. Although sunlight generally increased decay rates for all measured targets, the increases were mostly insignificant for sewage and gull but significant for cow. Our results indicate the importance of differential decay rates when host-associated markers are used to infer general fecal load and pathogen levels from respective fecal sources.

Water Quality in an Urban Stretch of the Chattahoochee River

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The Chattahoochee River, a major Georgia waterway, is both a drinking water source for nearly 3 million people and a surface water discharge point for 100 public and private wastewater treatment plants serving metro Atlanta and the surrounding areas, as well as being a major recreation site. This project measured water quality over 6 months during two separate years at 15 sampling points along a 15-mile stretch of river that runs past the City of Atlanta. This stretch includes multiple discharge points for wastewater treatment plants serving the greater Atlanta area, and receives stormwater runoff from local communities. Water samples from the river were analyzed for human fecal indicators, including E. coli and male-specific coliphage. Water samples were collected at 1-mile intervals from the middle of the river at a depth of 6 inches. Samples were analyzed for E. coli using membrane filtration and male-specific coliphages using EPA method 1601 and two-step enrichment procedure and single agar layer. Mean E. coli concentrations across sampling sites on each sampling date ranged from 1.5-2.7 log10 CFU/100mL, with no clear trend over time. Mean E. coli concentrations at each sampling point across 2 years ranged from 1.9-2.2 log10 CFU/100mL, with no significant differences between sites. There was no significant difference in E. coli levels upstream and downstream of two wastewater treatment plant effluent discharge sites in the 15 mile stretch. All sites were positive for male-specific coliphage over the course of sampling. The presence of fecal indicator organisms in the river suggests that the waterway is vulnerable to fecal contamination from numerous sources; given the multiple uses of the river by the surrounding population, effective monitoring and watershed protection is vital for protecting water quality.

How safe are improved drinking water sources in low- and middle-income countries? Evidence from systematic reviews

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Access to safe drinking-water is a fundamental requirement for good health and is also a human right. Global access to safe drinking-water is monitored by WHO and UNICEF using as an indicator "use of an improved source," which does not account for water quality measurements. Our objectives were to determine whether water from "improved" sources is less likely to contain fecal contamination than "unimproved" sources and to assess the extent to which contamination varies by source type and setting. Studies in low- and middle-income countries that assessed drinking-water for the presence of Escherichia coli (E. coli) or thermotolerant coliforms (TTC) were included provided they associated results with a particular source type. In total 319 studies were included, reporting on 96,737 water samples. The odds of contamination within a given study were considerably lower for "improved" sources than "unimproved" sources (odds ratio [OR] = 0.15 [0.10-0.21], I2 = 80.3% [72.9-85.6]). However over a quarter of samples from improved sources contained fecal contamination in 38% of 191 studies. Water sources in low-income countries (OR = 2.37 [1.52-3.71]; p<0.001) and rural areas (OR = 2.37 [1.47-3.81] p<0.001) were more likely to be contaminated. Using predictive models developed using random effects logistic regression and selected covariates, we estimate that 1.8 billion people globally use a source of drinking water which suffers from fecal contamination, of these 1.1 billion drink water that is of at least 'moderate' risk (>10 E. coli or TTC per 100 ml). Data from nationally randomized studies suggest that 10% of improved sources may be 'high' risk, containing at least 100 E. coli or TTC per 100 ml. Drinking water is found to be more often contaminated in rural areas (41%, CI: 31%-51%) than in urban areas (12%, CI: 8-18%), and contamination is most prevalent in Africa (53%, CI: 42%-63%) and South-East Asia (35%, CI: 24%-45%). There were 22 studies that reported seasonal differences in water quality. Fecal contamination in improved drinking water sources was shown to follow a statistically significant seasonal trend of greater contamination during the wet season (p < 0.001). This trend was consistent across fecal indicator bacteria, five source types, twelve K_ppen-Geiger climate zones, and across both rural and urban areas. There were 45 studies that reported either the proportion of samples free of fecal indicator bacteria and/or individual sample bacteria counts for source and household stored water (HSW), disaggregated by supply type. A bivariate random-effects meta-analysis was used to shows that water quality deteriorated substantially between source and stored water. Mean percentage of contaminated samples (noncompliance) at the source was 46% [95% CI: 33, 60%] while mean noncompliance in HSW was 75% [95% CI: 64, 84%]. Water supply type was significantly associated with noncompliance at the source (p < .0001) and in HSW (p = 0.0275). Source water (OR = 0.2 [95% CI: 0.1, 0.5]) and HSW (OR = 0.3 [95% CI: 0.2, 0.8]) from piped supplies had significantly lower odds of contamination when compared to non-piped water, potentially due to residual disinfectant. There were 170 studies identified through a systematic review focused exclusively on packaged water. The majority of studies did not detect fecal indicator bacteria in

packaged water (78/141). Packaged water from low and lower-middle-income countries were 4.6 (2.6, 8.1) and 13.6 (7.0, 26.8) times more likely to contain fecal indicator bacteria and total coliforms, respectively, compared to packaged water from upper-middle and high-income countries. Packaged water was less likely to contain fecal indicator bacteria (OR=0.37, 95%CI [0.21, 0.63]) compared to other water sources used for consumption. In summary, access to an "improved source" provides a measure of sanitary protection but does not ensure water is free of fecal contamination nor is it consistent between source types or settings. Microbial contamination is widespread and affects all water source types, including piped supplies and packaged water. International estimates therefore greatly overstate use of safe drinking-water and do not fully reflect disparities in access. Global burden of disease estimates may have substantially understated the disease burden associated with inadequate water services. An enhanced monitoring strategy would combine indicators of sanitary protection with measures of water quality, with careful attention paid to seasonal trends in quality. Findings have been published in PLoS Medicine, Tropical Medicine & International Health, and Science of the Total Environment. http://journals.plos.org/plosmedicine/article?id=10.1371/journal.pmed.1001644 http://onlinelibrary.wiley.com/doi/10.1111/tmi.12334/abstract http://www.sciencedirect.com/science/article/pii/S0048969715000212

Systematic literature reviews and development of distribution curves for norovirus and coliphages in raw wastewater

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Results of quantitative microbial risk assessments (QMRA) and epidemiological studies over the past 30 years have shown that viruses are a predominant driver of illnesses associated with primary contact activities in recreational waters impacted by human sources. After publishing recreational water quality criteria (RWQC) for bacterial indicators in 2012, the United States (U.S.) Environmental Protection Agency (EPA) is considering developing ambient water quality criteria (AWQC) for waterborne viruses. The human enteric virus, norovirus, is responsible for the majority of gastrointestinal illnesses in the U.S. As with all human enteric pathogens, indicators are needed because pathogens are not easily or reliably detected using protocols suitable for routine water monitoring. Bacteriophage may be a useful indicator of fecal contamination, specifically occurrence of fecal-borne viruses. EPA plans to conduct a QMRA to support derivation of coliphage-based AWQC. For QMRA inputs, we developed distributions for norovirus, somatic and F-specific coliphages for three types of water (ambient, effluent, and influent). We describe the process for conducting two systematic literature reviews, developing and implementing study selection criteria, and developing and executing approaches for extracting and/or obtaining the relevant data from published literature. We then illustrate the development of the statistical models for norovirus, somatic coliphage, and F-specific coliphage densities in raw wastewater. Finally, we describe the application of two approaches for this QMRA: one approach for obtaining a "best fitting" parametric continuous distribution and one

approach for obtaining an empirical distribution function. Challenges to overcome include apparent seasonality, variability between studies with respect to detection and quantification methods, and the amount of raw quantitative data available to characterize the densities of norovirus and bacteriophage in untreated wastewater.

Use of preservative agents for increase poliovirus survival on positively charged filters

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Despite efforts to eradicate poliovirus (PV), in 2014 PV was detected in 11 countries and the WHO has declared PV spread a public health emergency of international concern. PV environmental surveillance enhances early outbreak detection and is necessary for eradication certification. Filtration of large sample volumes can increase likelihood of PV detection. However, filter processing requires specific laboratory facilities and so filters are often shipped from sampling locations to labs for processing. Expedited international shipping in cold chain is costly and so optimal viral survival conditions must be balanced with realistic budget restraints. Chemicals used for food preservation are non-toxic, economical, and inhibit microbial growth that impacts viral survival in concentrated wastewater. MS2 tracers on filters are added as a non-pathogenic quality assurance/quality control to inform sample integrity. This study examined the survival of poliovirus type 1 (PV1) and the bacteriophage MS2 on positively charged ViroCap filters when treated with food preservative agents and stored at 25_C or 4_C. Prior to PV sample filtration, ViroCap filters were pre-seeded with MS2 by filtering 175-mL volumes of PBS inoculated with ~105 PFU MS2. 10-L volumes of raw sewage seeded with ~105 PFU PV1 were then filtered through the ViroCap filters. After filtration, ViroCap filters were dosed with 175 mL 2% sodium benzoate and 0.2% calcium propionate, then this solution was pumped out. Filters were stored at temperature until elution. Filters were eluted with 175 mL of 1.5% beef extract, 0.05 M glycine, pH 9.50 for a contact time of 30 minutes. Eluate was adjusted to pH 7.0-7.5. Eluate was plated onto BGMK cells for PV1 enumeration by plaque assay. Eluate was plated into a lawn of E. coli Famp using a double agar layer method for MS2 enumeration by plaque assay. Replicates were run 4-6 times. Survival was examined on days 0, 3, and 7. Initial PV1 recovery averaged 59%. Storage in preservatives at 4_C resulted in 36% PV1 recovery after 3 days and 14% recovery after 7 days. Storage in preservatives at 25_C resulted in 45% PV1 recovery after 3 days and 18% recovery after 7 days. Comparably, storage of filters at 25_C with no preservatives resulted in 9% PV1 recovery after 3 days and 5% recovery after 7 days. Initial MS2 recovery averaged 85%. Storage in preservatives at 4_C resulted in 84% MS2 recovery after 3 days and 34% recovery after 7 days. Storage in preservatives at 25_C MS2 resulted in 49% recovery after 3 days and 12% recovery after 7 days. Preliminary investigations of filter storage at higher temperatures (30_C and 40_C) indicate low viral and bacteriophage survival. Based on these results and uncontrolled ambient temperature fluctuations in sampling

locations, filter storage at 4_C and in 2% sodium benzoate and 0.2% calcium propionate is recommended for effective viral survival during filter shipment. Under these conditions, preservative-treated filter integrity would not be compromised if the cold chain were to unexpectedly fail. Further investigations of viral and bacteriophage preservation through use of preservatives and storage at higher temperatures is recommended.

Long-term Changes in Vibrio abundance in the Neuse River Estuary of Eastern North Carolina,

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The Neuse River Estuary (NRE) in Eastern North Carolina is the site of commercial and recreational use, and since 2004, vibrios in this system have been studied as part of the NRE Modeling and Monitoring Project. Biweekly water samples, collected from near-surface and near-bottom depths, at five stations along the estuary, were analyzed for a multitude of biological and environmental parameters, including culture-based monitoring of vibrios, which include the human pathogen V. vulnificus. Utilizing multiple statistical methods, including regression, and seasonal ARIMA models, it has been determined that the abundance of vibrios in the NRE is increasing, and has the potential for further growth. Interestingly, this increase does not appear to be tied to a corresponding increase in temperature, which is widely regarded as the main driver of Vibrio concentration. While the genus as a whole is on the rise, V. vulnificus are contrastingly decreasing in recent and past years. One hypothesis for this reduction as a percentage of the total population, is the rise in salinity in the area. V. vulnificus concentrations decline in salinity greater than 25?, and recent and repeated periods of drought with simultaneous increases in salinity have been experienced in the NRE and surrounding watershed. Prior evidence suggests that vibrios that are more salt-tolerant are outcompeting the V. vulnificus, potentially leading to the increasing total Vibrio and decreasing V. vulnificus populations

Influence of Wastewater Effluent on the Proximal Microbial Community Composition in Groundwater and Sediment

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From the mid-1970's to November 2013, the Ankeny, Iowa wastewater treatment plant (WWTP) continually released treated effluent to Fourmile Creek that contained a complex mixture of pharmaceuticals and other trace organic contaminants. Because of persistent

drought conditions prior to plant shutdown, the WWTP effluent accounted for upwards of 99% of the streamflow below the WWTP discharge point for prolonged periods of time. In addition to assessing the pre- and post- shutdown chemical and hydrologic responses of the stream, bank sediment and adjacent shallow groundwater, the USGS Toxic Substances Hydrology Program's Contaminants of Emerging Concern Project is also characterizing the microbial communities of sediment and groundwater from areas that were unaffected and affected by the WWTP outfall. This project is also assessing the time frame necessary for wastewater-impacted microbial communities to return to a "native" structure, while simultaneously assessing the relationship of microbial community structure to nutrient and trace organic chemistry. WWTP effluent, bank sediment upstream and proximal downstream of the outfall discharge, and shallow groundwater samples from upstream and proximal downstream of the outfall discharge were collected during the year preceding shutdown, at shutdown, and for one year following shutdown. DNA from sediment and filtered effluent and groundwater samples was extracted using MoBio PowerSoil and PowerWater DNA Extraction Kits. Extracted DNA was used to generate amplicons from the V4 region of Bacteria 16S rRNA. Sequencing was performed using a 2x150 paired-end (PE) configuration using the Illumina MiSeq Control Software on the MiSeq instrument. Over 20 million sequences were screened for quality, aligned against the SILVA Bacteria rRNA database, filtered, checked for chimeras using the UCHIME algorithm, and then sorted into operational taxonomic units (OTUs) with a 0.97 similarity threshold using the mothur software pipeline. Preliminary results indicate that 14 phyla contributed over 94% of total sequence reads. Another 62 phyla were found to be rare contributing less than 6% of total sequence reads. Both the WWTP effluent and accompanying sediment samples had at least 50% fewer OTUs than the upstream and proximal downstream samples. Additionally, WWTP effluent had the lowest α diversity (diversity within a sample) of all samples for this study. The downstream groundwater samples were more similar to each other than the upstream samples; however, there was still significant variability among the microbial community of these samples with one sample having greater diversity than the other samples. This particular sample is a screening across a redox interface which is typically associated with increased biodegradation activities. Further analysis will evaluate the microbial response across this redox interface. Final sequencing results will be paired with accompanying chemistry and hydrologic data and will provide a more complete picture of how different factors in this system influence the sediment and groundwater microbial community composition. Knowing "who's there?" within the microbial community provides key information on what microbes are naturally present and helps gauge how this community shifts in response to the shutdown of the WWTP. Microbial community composition also provides information on natural microbe functions such as biogeochemical cycling and contaminant degradation.

Metagenomics to Unlock the Innovative Nitrogen Transformations in Aquatic Ecosystem: Denitrification Coupled to gaseous electron donors

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Nitrogen transformations in aquatic ecosystems and engineered bioreactors are important processes primarily mediated by prokaryotes. These transformations are important in engineered bioreactors as well as in natural ecosystems to provide the overall environmental sustainability. In general, the free-living dinitrogen gas fixing microorganisms provide ammonium for assimilation and nitrifying microorganisms oxidize ammonium to nitrate via nitrite as intermediate, and eventually denitrifying microbes return the oxidized nitrogen species back to dinitrogen gas. Recent discoveries of novel pathways such as those involving the anaerobic ammonium oxidation (anammox), the ammonium oxidizing crenarchaea (AOA), and the nitrite-dependent anaerobic methane oxidation (DAMO) emphasize the lack of our knowledge about the enormous biodiversity and metabolic capability of nitrogen transforming prokaryotes. Denitrifictaion is an important component of the overall nitrogen cycle because it provides the exit to the inorganic nitrogen from any aquatic and soil ecosystem. The success of denitrifictaion depends upon the presence of suitable electron donors and carbon sources. In fact, the availability of a suitable electron donor and carbon source is one of the main considerations in municipal wastewater treatment plants. In this study, a metagenomic approach was taken for examining the overall microbial diversity along with and the diversity of nitrogen cycling organisms in stream sediments and short-term laboratory scale enrichment for DAMO using nitrite and nitrate as electron acceptors and methane gas as an electron donor. For generating the metagenome, DNA was extracted and sequenced on an Ion Torrent Personal Genome Machine (PGM) (Life Technologies, USA). The raw reads generated were quality filtered, trimmed, and assembled to contigs using CLC-genomics workbench (QIAGEN, US). Open reading frame (ORF) identification and gene prediction were performed with MetaGeneMark followed by gene annotation using webMGA server. Compositional taxonomic binning of quality filtered reads was performed using tBlastX search against NCBI-NT database, and curated database for genes involved in nitrogen cycling with an E-value and similarity cut-off values of 10−5. The BLAST output file was analyzed for comparison of the hits and attribution of a taxon for each sequence, based on the Lowest Common Ancestor algorithm (LCA) using MEGAN. The results showed that on taxonomic classification using LCA, the population of prokaryotes based on microbial attributes shifted towards anaerobic conditions considering facultative and obligate anaerobes in the DAMO enrichment reactor. Further on comparative analysis of riverine sediments and enrichment reactor it was observed that there was significant (30%) increase in population of prokaryotes belonging to NC10 phylum, which harbours DAMO organisms. Bining of contigs based on coverage and %G%2BC content along with mapping of reads to a genome of recently published key DAMO organism 'Candidatus methylomirabilis oxyfera' indicated enrichment in the ongoing reactor. On comparing the two metagenomes several synergism between microbes involved in nitrogen cycling were revealed,

providing us with a snapshot of complexity involved in nitrogen transformation process at ecosystem level.

Identification of host-phage interaction network in Great Salt Lake reveals the role of bacteriophages in bacterial diversity and population

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Natural ecosystems carry a collection of bacterial communities that lives within the water and sediment. These bacteria are enormously diverse and have major functions in essentially all biogeochemical processes. In addition, the bacteria residing in water and sediment systems degrade and mineralize organic compounds and linking the dissolved organic matter to higher trophic levels. Besides bacteria, bacteriophages as the most abundant biological entities outnumber bacterial cells in many natural ecosystems and play a significant role in evolution, abundance and diversity of bacteria through facilitating horizontal gene transfer. In this study, we explore the host-phage interaction through metagenomics study of bacterial and viral samples collected from brine layer sediment. Sediment samples were collected from 27-ft deep brine layer in Great Salt Lake, Utah and stored at 4_C for further analysis. Bactriophages were extracted using 1% (w/v) potassium citrate followed by filtration through 0.22 _m filter and further purification by isopycnic centrifugation using CsCl gradient. Purified phages were DNase treated to ensure removal of any residual bacterial debris. Bacterial and viral DNA were extracted using PowerMax Soil DNA isolation kit and Norgen phage DNA isolation kit, respectively. In order to minimize any biases in our analysis, no amplification processes was carried out on the phage DNA sample. DNA samples were sequenced on Illumina MiSeq DNA sequencer with 300-cycle paired-end at HCI Core Facility, University of Utah. Raw reads were quality filtered using CLC Workbench followed by scaffold assembly with 500 bp as contig minimum length. Open reading frame (ORF) identification and gene prediction were performed with MetaGeneMark followed by gene annotation using Blast2GO. Taxonomic binning of the contigs was also performed using MEGAN to analyze the least common ancestor of the reads and generate taxonomic cladogram. The prophages in the bacterial genomes were also analyzed using PHAST online analysis tool. The results showed dsDNA phages (95%) as the major type of bacteriophage while phage metagenomes of ssDNA (0.6%), ssRNA (0.07%) and dsRNA (0.02%) were also matched in the tblastx analysis (e-value 1E-5). Morphology analysis of the phages revealed Siphoviridae (32%), Myoviridae (24%), and Podoviridae (13%) as the major families of bacteriophages. In addition, PHAST analysis of the bacterial metagenome found 33 intact prophages and 37 questionable prophages with more than 50% of the coding DNA sequence (CDS) inside the bacterial metagenome. Interestingly, the induction experiments of the sediment showed that these prophages can be induced with environmental stress factors and infects their host, which affects their diversity and population. The bacterial and phage metagenomic analysis in this study will provide us valuable information in defining the

microbial community structure, genetic diversity, and bacteriophage-host interaction in Great Salt Lake as a hypersaline model ecosystem.

Using qPCR as a Rapid Detection Method for Determining Nitrification and Data Incorporation into a Nitrification Potential Index (NPI) in a South Texas Distribution System

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Using qPCR as a Rapid Detection Method for Determining Nitrification and Data Incorporation into a Nitrification Potential Index (NPI) in a South Texas Distribution System Abstract The City of Corpus Christ, TX like many other cities across the country faces the challenge of aging infrastructure in their vast underground network of distribution lines, this coupled with prolonged residence times present a particular challenge to monochloraminated systems which are susceptible to nitrification events. The Utilities Department at the City of Corpus Christi is using molecular biology to detect nitrifying organisms in their distribution system. The disinfectant chloramine can be broken down through nitrification. Nitrification is the two-step chemical oxidation of ammonia to nitrite, then from nitrite to nitrate. Nitrifying organisms, such as bacteria and archaea, are responsible for this oxidation. Testing for chemical parameters of nitrification, such as nitrite and nitrate, is common in determining a nitrifying event. Other surrogate parameters such as heterotrophic plate count and common fecal bacterial indicators also help to determine water quality. Being able to test for the actual nitrifying organisms is ideal for determining nitrification. To test for nitrifying organisms by culture can take a few weeks as they are slow growers. Using molecular biology to test for the DNA from these organisms is a rapid method and can detect a nitrification event before water quality can degrade. After filtering 20 liters of distribution water samples through a 0.22 um filter, DNA is extracted from the filter. Afterwards, qPCR is performed using primers and probes specialized for the City of Corpus Christi distribution system. A microbial community analysis study was performed and resulted in optimized primers and probes. The data from the PCR is used in conjunction with surrogate chemical parameters, as well as the Total Coliform Rule program, to get a more complete overview of water quality. A Nitrification Potential Index (NPI) was developed using the chemical and physical parameters believed to be present during nitrification events. The NPI applies the daily Water Quality data, including the PCR data, collected from dedicated sampling sites to test for a potential nitrification event. The enhanced biological assessment of nitrifiers in the distribution system, as well as a statistical index that incorporates all of the biological and chemical data, gives the City of Corpus Christi critical tools in an early detection of a nitrification event.

Spatial distribution of various potential gastrointestinal pathogens and documentation of Shiga Toxin-producing Escherichia coli (STEC) in the far north of Cameroon.

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In developing African settings, the microbial quality of drinking water at the primary source, intermediate sources, and point of use can vary greatly. Yet less is known about how point of use quality can vary within the same urban area. This study aimed to evaluate the spatial variation of potential waterborne gastrointestinal pathogens throughout drinking water distribution systems and home drinking water storage containers in various neighborhoods within the city of Maroua, an urban center in Cameroon in which a majority of the population has access to chlorinated tap water. Drinking water samples were collected from randomly selected source (n= 28) and home storage containers (HSC) (n= 60) in four separate neighborhoods. The microbial contamination of drinking water samples was assessed and potential contamination sources identified using qPCR targeting four different potential bacterial pathogens: Campylobacter spp., Shiga toxin-producing E.coli (STEC), targeting virulence genes stx1 and stx2; Salmonella spp.; and Staphylococcus aureus; three different fecal markers: HF183 (human), Rum2Bac (ruminant) and GFD (poultry); and the tetracycline-resistance gene tetQ. Pathogens and fecal marker levels were compared statistically in aggregate and disaggregate. Mann-Whitney U tests were used to compare mean contamination levels between source and home samples and Kruskal-Wallis equality-of-populations rank analysis were used to measure the difference of mean contamination between HSC samples taken from the four study neighborhoods. Mood's median tests were used to compare median contamination values between source, HSC, and neighborhood samples. Visual geospatial analyses and map generation were performed with Quantum Geographic Information System (QGIS). With the exception of Arcobacter spp., all MST fecal markers and pathogenic bacteria/virulence genes assayed were detected in both source and HSC samples. When aggregated, both the mean and median total pathogenic bacteria/virulence gene level between HSC and source samples was highly statistically significant (both p=0.01). The mean/median values of aggregated MST fecal markers between source and HSC samples were not statistically significant. Two neighborhoods had positive detections of all or nearly all of the biological parameters assessed while the remaining two had only measurable positive frequency percentages of one or two parameters. Kruskal-Wallis analysis determined a highly statistically significant difference (p= 0.001) in the average total pathogen gene copies/100 ml between neighborhoods. Average total MST gene copies/100 ml between study neighborhoods were not significantly different. Disaggregated qPCR results determined there were only statistically significant differences between neighborhoods in terms of average total stx1 gene levels, although tetQ levels did approach statistical significance (p= 0.07). Mood's median analysis determined no neighborhood's median value for any parameter was statistically different from

the others. Geospatial analysis didn't demonstrate a clear clustering of any specific type of pathogen or fecal marker detected in HSCs or in source samples taken in any neighborhood. Spatial variation in terms of potential pathogen and MST exists between drinking water sources and HSC, and between neighborhoods. This variation should be taken into consideration during future QMRA assessments in urban Africa contexts and also should be considered during the development of drinking water quality monitoring projects.

Safe Distances between Groundwater Based Water Wells Point and Pit Latrines at Different Hydrogeological Conditions in the Ganges Atrai Flood Plains of Bangladesh

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Groundwater drawn from tubewells often becomes contaminated in Bangladesh by nearby pit latrines. This study was conducted to find out the minimum safe distance of tubewell from a pit latrine in different hydrogeological conditions of Bangladesh. Twenty monitoring wells were installed at three study sites with the vertical and horizontal distances ranging from 18-47 m and 2-15 m respectively. Water samples were collected and tested for faecal coliforms and faecal streptococci. Soil samples were analyzed for texture, bulk density and hydraulic conductivity following standard procedures. Sediment samples were collected to prepare lithological logs. Among the 3 study sites, when the shallow aquifers were overlain by 18 - 23 m thick aquitards, the groundwater of the monitoring wells was found contaminated with a lateral and vertical distances of 2 and 31 m respectively. However, where the aquitard is only 9 m thick, contamination was found up to lateral and vertical distances of 4.5 and 40.5 m respectively. The soil textures of all the sites were mainly composed of loam and sandy loam. The hydraulic conductivities in the first aquifer at Manda, Mohanpur and Bagmara were 5.2-7.3, 8.2 and 1.4-15.7 m/h respectively. The results showed that the safe distance from tubewell to pit latrine varied from site to site depending on the horizontal and vertical distances of the tubewell as well as hydrogeological conditions of a particular area.

Risk classification of Swedish Surface Source Waters

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In Sweden, approximately 70% of the drinking water is derived from surface raw waters. For example, large cities like Stockholm, Gothenburg and Malm_ take their water from lakes and rivers. Climate change is expected to affect surface waters in many aspects, including the presence, concentration fluctuations and survival of pathogenic microorganism. Such information is central for selecting appropriate water treatment techniques that ensures production of safe drinking water. Unfortunately, there is limited information on occurrence of pathogens in Swedish raw source waters and knowledge about variations and seasonality is scarce. The aim of the project is to improve information on occurrence of disease causing microorganisms in different types of Swedish surface raw waters and increase understanding about factors that cause variations and fluctuations in raw water quality. The outcome will be a microbial risk classification of the Swedish raw waters and a handbook on microbial risks directed to drinking water producers. The project will also increase the knowledge about the correlation between traditional indicators and various pathogens during events of pollution and under different climate conditions. A base-line for raw water quality is produced by examination of microbial quality in six different raw source waters by regular raw water sampling during 17 months starting in March 2014. Traditional indicators are analyzed twice a week and pathogens (Salmonella, Campylobacter, Shiga-toxin producing E. coli, Cryptosporidium, Giardia and norovirus) every second week. During events expected to affect source water quality such as heavy rain fall, drought of long duration, disappearance of thermocline, sewage overflows, snow melt etc, sampling frequency is increased. Participating water treatment plants were chosen based on a PCA-plot with nationwide historical source water data on five different parameters; rain fall, turbidity, COD, coliform bacteria and E. coli. The selection procedure also took into account that both lakes and rivers should be represented, and for practical reasons, also factors such as size and location had to be considered to ensure that sampling and transportation would work satisfactory. To improve detection of the usually very low numbers of pathogens, 60 L of source water is concentrated at the water treatment plants by ultrafiltration. Fractions of the concentrates are analyzed for Salmonella, Campylobacter and STEC using in-house developed MPN methods. Another fraction is used for analysis of norovirus. In parallel, Cryptosporidium and Giardia are concentrated and analyzed according to ISO15553 Preliminary results from the first 12 months of the study will be present. Participants: Swedish National Food Agency, Swedish National Veterinary Institute, Public Health Agency of Sweden, Stockholm Vatten VA AB, H_rn_sand Energi & Milj_ AB, Trollh_ttan Energi AB, _stersunds kommun, Bor_s Energi & Milj_ AB and Tekniska F_rvaltningen Motala. The study is financed by the Swedish Civil Contingencies Agency.

The Use of Algal Biofilms for Decentralized Wastewater Treatment

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Algal biofilms leverage the mutualistic relationship between algae and bacteria to create a diverse treatment ecology that is controlled, stable and delivers many treatment benefits. Bacterial systems are dependent upon high levels of oxygen for the oxidation of organic matter. Since the by-product of photosynthesizing algal biofilms is oxygen, the costly demand for external aeration is dramatically reduced. Through co-evolution, algae (photosynthetic microbes) and bacteria have developed an intricate relationship wherein one utilizes the waste of the other. Algae produce oxygen, consume carbon dioxide, and exude polysaccharides. Both heterotrophic and autotrophic bacteria consume oxygen, while heterotrophic microbes require organic carbon and produce carbon dioxide, an input photosynthesis. Oxygen produced by photosynthesis also greatly facilitates nitrification since algal biofilms may be 100% saturated with oxygen (Jorgensen et al 1979). This mutualistic relationship creates a highly stable and sustainable environment for both algae and bacteria to thrive. Conventional systems such as activated sludge require a population of bacteria large enough to provide consistent treatment. This bacterial population is highly dependent upon a consistent flow of BOD, sufficient aeration and management of return activated sludge (RAS) to maintain the system. In contrast, the mixed algal biofilm and bacterial system requires no sludge recycle, and provides a buffer against both shock loading and diluted flow due to the interaction between the algae and bacteria. Algal attached growth technologies are particularly applicable to decentralized wastewater treatment applications. These facilities are often seasonal and receive highly variable flows and loadings, which make it very difficult to consistently meet their permitted effluent limits.

Best methods for detecting C. perfringens in untreated and treated wastewater

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Following several of the most severe droughts on record in North Carolina, the state has passed legislation allowing for the use of a higher quality reclaimed water for agriculture, industry, and potable purposes. In order for the water to meet the higher standard it must now be tested for the fecal indicator Clostridium perfringens as a protozoan parasite surrogate. However, the best media for detection of C. perfringens in wastewater and treated wastewater samples remains unclear as previous studies of their performance and ease of use have been either in disagreement or inconclusive. This study investigated the effectiveness of three different selective agars for C. perfringens detection by conventional membrane filtration of water and wastewater samples and compared them for specificity and sensitivity by using two different confirmation tests: exposure to acid-phosphatase and stormy fermentation in iron-milk media. Clostridium perfringens selective media tested were mCp, TSC, and CP Chromoselect (CS) agars.

Unpasteurized and pasteurized samples were tested respectively for spores and vegetative clostridia and for spores only. Initial results demonstrated that CS agar provided highest C. perfringens detectability on both pasteurized and unpasteurized samples, although some false positivity occurred based on the results of confirmatory testing. TSC agar resulted in high levels of colony growth, but appeared to be least selective and produced considerable false positivity and false negativity. Colonies varied in color, leading to ambiguous identification of C. perfringens colonies. MCp agar yielded the lowest amount of colony growth (statistically significant), but was mostly accurate in its detection for C. perfringens. However, this medium was the most difficult to prepare and it required extra steps for colony detection, including the use of a caustic chemical, that made it more difficult to use. Most of the presumptive colonies detected on all three agar media confirmed as C. perfringens based on acid phosphatase and iron mike storm fermentation reactions. However, there were disagreements in the outcomes of these two confirmation tests and some presumptive colonies did not confirm by either test. Also, there were significant differences in the outcomes of the two confirmation tests for presumptive C. perfringens isolates from the three different agar media, with mCp medium giving highest confirmation rates and TSC agar giving lowest confirmation rates. After correction of C. perfringens concentrations detected on the 3 different media based on the results of confirmation tests, CS agar remained the best candidate for C. perfringens detection. In order to further confirm the identities of presumptive C. perfringens isolates they are being further characterized by MALDI TOF Mass Spectrometry. According to the results of this study, C. perfringens can be detected and quantified reliably and conveniently by the membrane filtration method using a newer selective agar medium, CS. The ability to detect and quantify C. perfringens in water and wastewater by these methods will be useful to stakeholders in the municipal wastewater and water supply community in North Carolina. Identifying the best practical method to detect C. perfringens, supports implementation of advanced reclaimed water use in North Carolina and elsewhere as a sustainable water supply that reduces the impact of future droughts.

Assessing fecal bacteria concentration and MST assay performance in gull and dog fecal samples from Wrightsville Beach, NC

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Microbial source tracking (MST) using quantitative polymerase chain reaction (qPCR) is now a commonly utilized tool for determining the sources of fecal contamination in polluted waters. Published MST assays are often used "off the shelf" and assumed to be of quality design with high specificities and sensitivities. To test the use of several published animal fecal contamination MST assays, fecal samples from six dogs and ten gulls were collected from Wrightsville Beach, North Carolina in June and July 2014. Colilert-18_ and Enterolert? were used to quantify fecal indicator bacteria (FIB) in samples from specific individual animals, and

qPCR was utilized to determine concentrations of MST markers per gram of fecal material. Assays used for MST qPCR analysis were total Enterococcus, E. faecium, E. faecalis, E. casseliflavus, fecal Bacteroides, HF183, Gull-2, and DogBac. First, fecal contamination from each individual animals exhibited extremely large variation in measured FIB concentrations, ranging from below detection limit (<100 MPN/g) to above detection limit (2.4 x 106 MPN/g). All individuals had log concentrations of Enterococcus greater than 4.5 cell equivalents (CE)/g, while some were greater than 9.0 CE/g. No dog samples and 50% of the gull samples were positive for E. casseliflavus. E. faecium, and E. faecalis concentrations were high in both types of animals. Dog samples averaged a log concentration of 10.6 copies/g of the fecal Bacteroides marker, while gull samples had a lower average log concentration of 5.8 copies/g. Half of the dog samples were positive for HF183, which is currently purported in the literature to be human specific assay, but all of the gull samples were negative. Finally, there was a large amount of cross-reactivity between the gull and dog samples for both the Gull-2 and DogBac assays. All ten gull samples were positive for DogBac marker, with an average log concentration of 9.0 copies/g. All of the dog samples were also positive for the DogBac marker, with higher average log concentrations as compared to gulls (13.6 copies/g). All of the gull samples and four of the six dog samples were strongly positive for the Gull-2 assay. Average log concentrations in the gull feces were 9.7 copies/g and in dogs were 5 orders of magnitude lower (4.3 copies/g). Assays differed in their qPCR characteristics (e.g. efficiency, limit of detection, R2) and the quality of the assay design was not equal across assays. This study demonstrates that while published qPCR assays can be a useful tool for MST studies, it is imperative that the user fully assess their qPCR assay design quality, specificity and sensitivity, preferably across a range of known fecal samples collected from the study site of interest.

Distributed metabolism in suspended and attached growth anammox bioreactors revealed through metagenomic sequencing

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Anaerobic ammonium oxidation (anammox) represents one of the most innovative biotechnologies for energy-efficient nitrogen removal from wastewater. The process represents a short cut in the overall nitrogen cycle, where ammonia is oxidized directly to nitrogen gas by bacteria using nitrite as a terminal electron acceptor. This offers significant cost savings for nitrogen removal compared to conventional processes that require energy-intensive aeration for nitrification and also consume valuable organic carbon during denitrification, which could otherwise be re-routed for biogas production. Despite its ubiquity and industrial importance, specific metabolic pathways underlying anammox bacterial metabolism remain poorly understood. Moreover, the synergy that exists between anammox bacteria and neighboring microorganisms in stable mixed communities has yet to be resolved. Elucidating these and other ill-defined reactions is essential for accurate modeling of anammox processes and for

identifying metabolic constraints that limit process performance. Metagenomic sequencing has evolved as a powerful tool for studying the interactions between microorganisms in complex ecosystems such as activated sludge. To gain further insight into the interactions between anammox bacteria and neighboring microorganisms, metagenomes were generated from biomass samples collected from two lab-scale anammox bioreactors employing suspended and attached growth modes using high-throughput Illumina sequencing. Each bioreactor was fed with partially nitrified reject water obtained from anaerobically digested sludge and maintained stable nitrogen removal efficiencies throughout the study period. Anammox sludge from the suspended growth bioreactor was used as seed for the attached growth process, which was acclimated for a period of 200 days prior to DNA extraction to facilitate the development of mature biofilms on carrier media. Resulting DNA sequence data was used to examine the microbial community structure and metabolic potential present in both suspended and attached growth bioreactors. Briefly, raw sequencing reads were assembled into contigs using Omega and subsequently binned into population genomes using MaxBin based on differential coverage and tetranucleotide frequency. This allowed for the recovery of 11 partial to near-complete genomes from metagenomic assemblies, 4 of which were estimated to be >90% complete based on single-copy gene analysis and were the focus of subsequent analyses. Phylogenetic assessment of each near-complete genome revealed that organisms recovered from both reactors were closely related to Planctomycete KSU-1 sp., Anaerolinea thermophilia, Ignavibacterium album, and Methyloversatilis universalis. Metabolic reconstruction for each of these genomes was performed to explore modes of carbon, energy, and biosynthetic metabolism and reveal potential opportunities for metabolic exchange between neighboring community members. Metabolic pathways inferred from genomic annotations indicated that bioreactor organisms were metabolically versatile and exhibited novel opportunities for metabolic-cooperation. These findings offer much needed insight on microbial interactions occurring in anammox bioreactors and will guide future efforts aimed at developing strategies for optimizing and controlling process performance.

Sampling Approaches for Detection of Salmonella in Irrigation Ponds in Southern Georgia

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BACKGROUND: Salmonella is one of the most important causes of foodborne and waterborne infectious disease in the United States and worldwide. The highest rates of salmonellosis in the US occur in the southeast, and previous studies have shown that Salmonella occurs in surface waters and irrigation ponds in southern Georgia. Irrigation water is known to be a source of contamination of fresh produce by pathogenic bacteria. The overall goal of this project was to develop knowledge to help farmers who rely on untreated surface sources of irrigation water to effectively address recently proposed FDA rules on water quality in produce production. METHODS: This project was focused on developing and evaluating sampling protocols to

accurately reflect Salmonella concentrations in irrigation ponds in southern Georgia. From March 2012 to September 2013, we carried out monthly sampling of water from five irrigation ponds at different farms to investigate the presence of Salmonella using two different sampling strategies. Strategy 1 consisted of collecting three grab samples from the bank, all in close proximity to the intake of the irrigation system. Strategy 2 consisted of collecting three grab samples from the bank distributed along the perimeter of the pond. Samples were also collected directly adjacent to the irrigation system intake, which is usually 3-6 metes from the bank and at a depth of 1-2 meters. For each strategy, a composite sample was created from the three grab samples. Water samples were analyzed for Salmonella using most probable number methods. PCR targeting the InvA gene was used to confirm the presence of Salmonella. RESULTS: Among 507 samples analyzed during the study, 217 (42.8%) were positive for Salmonella. Salmonella was found throughout the study with the highest concentrations in October and lowest in March. All five ponds were positive for Salmonella at some point, with mean concentrations ranging from 0.03 to 0.57 MPN/100mL. The mean Salmonella concentrations of the composite samples were 0.07 MPN/100mL for Strategy 1 and 0.48 MPN/100mL for Strategy 2. Overall for both sampling strategies, there was a 70.5 % match rate for Salmonella presence/absence between the intake and composite samples and this match was statistically significant. However, for individual ponds, the results were more variable, and the false negative rate ranged from 9-48%. CONCLUSIONS: These results indicate that sampling from the bank does not reliably represent water near the irrigation system intake.

Human picornavirus diversity in archival water samples in the Netherlands: 1987-2012

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Lower budgets for infectious disease surveillance would negatively influence the sample size, would limit diagnostic testing yielding less typing data for bioinformatics interpretation and yield fewer samples for further investigation. This would mean a significant loss in information about circulating pathogens, less information about the extent of an outbreak, e.g. impact of public health risks, fewer opportunities to indicate individuals at risk and also a missed opportunity of implementing intervention strategies. The main role of infectious disease surveillance is to predict, observe, and minimize the harm caused by endemic, epidemic, and pandemic situations, as well as to increase knowledge about which factors contribute to such circumstances. As environmental surveillance can cover a large population anonymously including both asymptomatic and ill individuals not attending the general practitioner and therefore unnoticed by disease or pathogen surveillance, such surveillance systems may also be desirable for pathogens other than poliovirus. Using modern molecular typing techniques, (quantitative) real-time RT-PCR but also next generation sequencing (NGS), more information will be obtained about the emerging nature of these pathogens. Elucidation of the prevalence of specific human pathogens in a specific population can aid in the application and advisory of intervention measures. To determine the diversity of circulating human picornaviruses in the

Netherlands, a retrospective study was performed in archival sewage and surface water samples originating from 1987-2000 and 2009-2012. Human enteroviruses, parechoviruses, hepatitis A viruses, Aichiviruses A, Saffold viruses and cosaviruses were detected in 97%, 80%, 33%, 93%, 43%, and 50% of the 30 samples tested, respectively. High virus diversity in virus types and variants was observed, showing the prevalent circulation of different human picornaviruses, such as enteroviruses including enterovirus 71, Aichiviruses A, Saffold viruses, cosaviruses and human parechoviruses, in the Dutch environment over the past 25 years. Frequent detection of these viruses in the tested samples might reflect endemic circulation in the human population. Several parechovirus types were detected, type 1, 2, 3, and 6. Type 1 was the most frequently detected (15/30), followed by type 6 (8/30), type 3 (6/30), type 2 was detected once. The detected sequence diversity was high, especially in the sewage samples where often multiple strains and variants were detected, but also several identical parechoviruses were detected. Also, several genotype A and B Aichi virus lineages (VP1 and 3C genomic regions) were observed over the 25-year period studied, however, considering the time-course of the viral genetic diversity, recent expansion of the genotype B population, over genotype A, could be discerned. Different genotypes of Saffold viruses in the sewage samples originating from the 1990s indicated circulation of these viruses in the Dutch population at that time. However, hepatitis A virus was predominantly detected in the first period. Approximately 30% of the identified picornavirus strains are known to be associated with severe disease, indicating that adverse health effects may occur upon exposure to these surface waters.

The detection of infectious viruses using a viability-PCR

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Human enteric viruses are major agents of food- and waterborne diseases. The detection methods for the enteric viruses are either cell culture-based or molecular-based assays. Although the advantage of cell culture methods is that they are capable to detect infectious viruses, many of the relevant enteric viruses do not have (a reliable) cell culture system. Therefore, these viruses are often detected with molecular-based assays, which is very sensitive although it cannot differentiate between the detection of infectious or inactivated viruses. To obtain information on the viability of the detected viruses is very important for environmental health microbiology and for the proper assessment of the human health risks involved. The inability of molecular assays to differentiate between infectious and inactivated viruses can lead to an overestimation of the risk to human health. Thus, molecular methods which are capable to differentiate more efficiently between infectious and inactivated viruses will give more reliable information on the possible risk to human health upon exposure to these viruses. The addition of (enzymatic) pretreatment before the RNA extraction and RT-qPCR has been described to discriminate between infectious and inactivated viruses. Propidium monoazide (PMA) can permeate only membrane-compromised micro-organisms, and after exposure to

strong visible light the dye intercalates covalently into the DNA interfering DNA amplification. The stable secondary structures of many enteric viruses may also facilitate the binding of PMA to the viral RNA. Furthermore, the addition of the nonionic surfactant Triton-X100 has been described to improve the effect of the PMA treatment on the slightly-damaged capsid. In our study we tested the applicability of a pretreatment with PMA, with the addition of Triton X-100, on viruses present in several matrices (raspberry and oyster materials) and in PBS. The spike suspension contained hepatitis A viruses and noroviruses genotypes I and II and were added to the different matrices, both as untreated as heat inactivated (5' 90?aC) virus suspensions. We also tested untreated virus suspensions, with and without PMA/Triton treatment, to determine the effect of our method on untreated viruses. No negative effect of the PMA/Triton treatment was seen on unaffected viruses. Moreover, our results show that differences were seen in the efficiency of the method between the three viruses, hepatitis A virus showed a more effective decline in detecting inactivated viruses after PMA treatment (approximately 1-2 log reduction) than the two noroviruses (approximately 0.5-1 log reduction). Although practical limitations with environmental samples have previously been shown, (enzyme) pretreatment prior to RT-qPCR could be adapted to determine the infectivity of viruses that are difficult to cultivate or those for which a cell line is not currently available. A drawback of the PMA treatment is that it depends mainly on the integrity of the viral capsid and virus inactivation may also take place without compromising capsid integrity but results in changes in capsid protein conformation (UV, storage conditions, temperature). In conclusion, assays conducted to examine the efficiency of pretreatment RT-qPCR in minimizing detection signals from thermally-inactivated viruses are promising but further research has to be done to elucidate the usefulness of such methods (in different environmental samples).

Distribution of fecal indicator bacteria around the city of Venice, Italy: temporal patterns, reservoirs and the role of tidal forcing

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The city of Venice lies at the centre of a shallow lagoon which, by covering the area of 550 km^2, is the largest in Italy and one of the largest in the Mediterranean Sea. Being founded in the fifth century, the city is one of the first examples of urban settlements in a semi-enclosed coastal area. The urban area is crossed by a 40 km-long branching network of canals, having the mean depth of 2 meters. Due to the unique location and history, the historical centre lacks adequate sewage treatment infrastructures, and only partially-treated effluents from a variety of inputs are discharged into the city canals. Being characterized by a low number of residents (ca. 65,000), the city is not among the world megacities, but experiences an annual flux of 10,000,000 tourists from worldwide, which makes the anthropogenic pressure comparable to that of larger cities. We summarize the results of a multi-year investigation to study the spatial and temporal dynamics of fecal indicator bacteria (E. coli and enterococci) around the city,

characterize their potential pathogenicity and genotypic diversity, identify reservoirs and the environmental factors driving FIB spread and persistence. Results indicate that FIB abundances in both water and sediments are elevated, and highly variable over different spatial and temporal scales. The most common macroalgae are significant reservoirs of FIB, and host replicating populations of pathogenic E. coli, thus facilitating bacterial spread and increasing concern for waterborne disease transmission. FIB abundance appears largely influenced by the tidal forcing which, with its semidiurnal regime, ensures large water exchanges with the adjacent sea. Bacterial abundance is not obviously correlated with the touristic pressure, suggesting that FIB variability depends on the complex interplay of several factors. Taken together, the results provide information about the factors influencing the variability of fecal pollution in the area, and clues useful to start a concerted, integrated management of the fecal pollution in one of the top visited cities in the world. These results have implications for environmental decisions to understand and manage the sources and fate of fecal contamination in other cities lying on coastal lagoons.

Identification of human fecal pollution indicators by deep sequencing of sewage

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Our research has focused on discovering new indicators of fecal pollution, which serve as an investigative tool to identify pollution sources and will ultimately lead to mitigation strategies that prevent adverse outcomes. We have employed deep sequencing and novel bioinformatic approaches to assess host related patterns of microbial communities. Since humans are the primary reservoir for many human pathogens, developing human-specific indicators of fecal pollution is a major emphasis of our work. A comparison of the sewage influent bacterial community from 71 US cities revealed the ubiquitous and abundant occurrence of 27 human-fecal affiliated sequence-types (i.e. oligotypes). These sequence-types represent a core set of bacteria in human stool from US populations that would make excellent targets for sensitive human fecal indicator assays. The majority of the core bacteria in US human populations were assigned to the genus Bacteroides, the focus of previous research for host-specific indicators, and the family Lachnospiraceae, a major member of the human gastrointestinal tract that also comprises up to 15% of the community in sewage. We found Lachnospiraceae had a diverse array of 16S rRNA gene sequences that demonstrated host-related patterns when comparing sewage with animal waste, including urban wildlife. In particular, strong host associated patterns emerged for sequence-types assigned to the genus Blautia. We examined the geographic relevance of the potential indicators and found both Bacteroides and Blautia were applicable to US regions, whereas in Brazil, human Bacteroides was low or absent, but Blautia was particularly relevant for detecting human fecal pollution. In our work, we have applied these indicators primarily to examine sources of fecal bacteria in urban waterways. We developed one qPCR assay targeting a human associated Blautia sequence type (Lachno2 assay)

and have consistently detected both this indicator and human Bacteroides (HF183 assay) in urban rivers and their estuaries around the Great Lakes. Quantification of these indicators also demonstrated that many stormwater outfalls in the Great Lakes region were contaminated with sewage, resulting in chronic sewage contamination of urban waterways.

Assessment of the Microbial Community in the Siem Reap River, Siem Reap, Cambodia

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Approximately 780 million people lack access to safe drinking water leading to 1.6 million deaths annually from microbial associated diarrheal disease. In Cambodia, 40% of the population lack access to safe drinking water. Biosand filters, a type of Point of Use (POU) water filtration system, have been used successfully in Cambodia, removing approximately 98% of bacteria, including diarrheal disease causing pathogens. Thousands of heavy concrete biosand filters (HBSF) have been installed in Cambodia, decreasing the levels of the indicator species Escherichia coli to a safe number. One drawback to these filters is their weight, making installation in remote villages difficult. An alternative to the HBSF is the light biosand filter (LBSF). In January, we traveled to Siem Reap, Cambodia to assess if the LBSF is as efficient at removing pathogenic bacteria as the HBSF. Water samples were collected from the Siem Reap River, a known microbiologically contaminated water source, and treated by HBSF or by LBSF. Overall, the LBSFs did not perform as well as the HBSFs, however they did decrease the amount of biological contaminants, indicating the potential for these filters to be installed in floating villages. In addition to assessing the efficiency of these filters we wanted to begin to identify the microbial population present in these contaminated drinking water sources. Currently the World Health Organization only assesses for the indicator species Escherichia coli to determine if the water is contaminated. Because of this, little is known about other bacteria present in this water source, despite the fact that many organisms can cause gastrointestinal disease. As such, a more detailed analysis of the microbial community is needed. Water samples from the Siem Reap River were collected to assess the diversity of the microorganisms found here. A 16S rRNA gene clone library was constructed with a total of 150 clones sequenced. Recovered sequences belonged to the phyla Firmicutes (56.3%) and Proteobacteria (43.7%) and were found to have a high similarity to human fecal uncultured bacterium clones indicating the presence of high concentrations of human waste. A better understanding of the microorganisms present in these waters could ultimately lead to better treatment of the gastrointestinal diseases.

Community Led Diarrhea Eradication in Lower Nyakach, Kenya

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BACKGROUND: Lower Nyakach, near Lake Victoria in western Kenya, has a population of 70,000 with most families living in extreme poverty. The absence of improved water sources requires the use of highly contaminated ponds, rivers, streams and shallow wells for drinking water, leading to a chronically high incidence of waterborne diseases. A priority for the community-based organization, Friends of the Old (FOTO), is to eliminate waterborne diseases by: 1) using practical field methods to evaluate the bacterial quality of drinking water; 2) bringing evidence-based microbiology to communities; 3) providing practical household water treatment and storage methods. MATERIALS: Water quality testing was performed using two tests specific for Escherichia coli: the Colilert_ 10 ml presence/absence test and the E. coli/Coliform Count PetrifilmTM, a quantitative test for 1 ml. Results from these tests correlated with WHO disease risk categories: low, moderate, high, or very high. Two household water treatment methods were used: providing families with a commercially available 150 ml bottle of 1.2% sodium hypochlorite that treats 850-1000 liters of water, and use of a simple solar cooker that uses sunshine to pasteurize water by heating to 65_C. RESULTS: FOTO staff worked with communities to provide evidence-based microbiology that drinking water sources posed a high/very high disease risk, and that chlorination or solar pasteurization killed the germs making water safe to drink. The visual test results dispelled the myth that clear water was safe to drink. In February, 2012, FOTO started distributing the chlorine solution to 9,600 families over two months. This quantity was increased to all 14,400 families in August, 2013. Starting in August, 2014, chlorine was also distributed to all 42 schools. Approximately 1,000 families have solar cookers. The District Hospital reported a 41% decrease in diarrhea cases from January, 2012, before chlorine distribution, to January, 2013. Comparing January-July in 2013 with 2014, a 53% decrease in diarrhea cases was reported. CONCLUSION: The strategy of involving communities in evidence-based microbiology testing of water sources and providing inexpensive treatment options has reduced the burden of waterborne diseases in Lower Nyakach, with the goal of eliminating waterborne diseases.

Quantitative Microbial Risk Assessment Case Study for Recreational Waters: Tecolote Creek, CA

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Tecolote Creek is flowing, inland creek within the limits of the City of San Diego, CA. The creek flows through a steep, open-space canyon which is inhabited by a range of wildlife, and

discharges into Mission Bay, a prominent, heavily visited, and famous beach recreational area in San Diego County. Possible sources of fecal contamination in the creek include animals, transient encampments, and sewage infrastructure. From summer 2013 through the present, a quantitative microbial risk assessment (QMRA) study has included the quantification of fecal indicator bacteria (FIB), molecular markers for specific types of fecal contamination, and human pathogenic viruses (e.g. norovirus, and adenovirus) using state of the art approaches. The QMRA study, which might be the first conducted in a flowing freshwater setting, is being adaptively managed within a stakeholder process including strong and ongoing research and policy interaction with USEPA. One goal of this study is to generate information that can inform future QMRA approaches conducted across the nation for recreational waters. This project has also included attention to characterization of this complex system using integrated hydrology, microbiology, molecular biology, and risk assessment approaches. Special attention has been paid to collection of data in a manner that reduces the problems associated with inhibition of molecular analyses, concentration/filtration of stormwater samples, and optimization of molecular quantification approaches. Three wet weather and 11 dry weather receiving water sampling events were conducted, along with collection of samples from specific locations to assess potential sources and extensive collections of scat and sediment from the creek environment. Samples were analyzed for E. coli, Enterococcus, fecal coliform, Bacteroides-based markers of human fecal contamination, bacteriophage, adenovirus, enterovirus, and norovirus. In addition, on a subset of samples, digital droplet (dd) PCR has been utilized to confirm findings observed with qPCR. The watershed has exhibited contrasting conditions in terms of molecular markers of human fecal contamination (such as HF183), with relatively few detections in the upstream portion of the canyon but relatively high concentrations at sites in the lower reaches of the creek. In addition, quantification of human specific molecular markers were confirmed in most instances through the use of both qPCR and ddPCR. Intensive source identification efforts are ongoing to address the contamination in the lower portion of the creek. Highlights of this presentation will include lessons learned from the use of tiered, comprehensive molecular analyses framework, and the complexities of risk assessment in flowing freshwater streams.

Concentration of Microorganisms by Concentrating Pipette and Wet Foam Elution

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Rapid microbiological detection systems have taken dramatic steps forward in the last two decades and today detection of even a single organism is possible in less than one hour. Unfortunately, development of rapid detection methods has far outpaced development of sample concentration techniques, which are necessary to enable detection of low microbial concentrations in water. Currently, without sample concentration, rapid detection techniques alone produce results that are hundreds to thousands of times less sensitive than the minimum

desired detection limit for microbial water contaminants. The vast majority of rapid microbiological methods are only capable of assaying between 1 _L and 200 _L. Even at an assumed detection limit of 1 organism this only equates to detection limits between 500 and 100,000 organisms per 100 mL - far exceeding appropriate actionable levels for most water types. In the near term, because of these assay volume limitations, rapid microbiological methods will only reach their full potential if reliable, efficient front-end concentration approaches are developed. For over eight years, the InnovaPrep team has been performing research and development of novel concentration approaches for microbial water contaminants. This research has culminated in the development of a novel Wet Foam Elution process, for efficient recovery of microorganisms and viruses from membrane filters, and a novel system for rapid concentration of microorganisms and viruses using a single-use membrane filter tip to eliminate cross contamination between samples. The Wet Foam Elution process uses an expanded wet foam, created from a carbonated, buffered surfactant solution, to efficiently recover microorganisms into significantly lower volumes than is possible with liquid elution processes. The Concentrating Pipette instrument (CP) and the single-use Concentrating Pipette Tip (CPT) evolved from early reusable concentration devices developed by InnovaPrep for biodefense applications. The CP can concentrate microbes directly from large water volumes into volumes that are compatible with rapid detection systems. Microorganisms are captured onto a flat or hollow fiber membrane filter through dead-end filtration and are recovered using the Wet Foam Elution process. The concentrated sample can then be delivered directly to a rapid microbiological method - bypassing enrichment entirely. The CP and CPTs can be readily combined with a variety of rapid microbiological methods and bridges the gap between today's rapid microbiological methods and the real world needs of the water industry. Processing rates, coupling requirements and efficiencies for concentration of bacteria and viruses from drinking water and environmental waters will be presented. Application to specific water industry needs for drinking water, waste water, environmental and recreational waters will be discussed.

Role of market intervention: A path to achieve sanitation improvement in rural Assam, India

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The problem of open defecation in absence of toilet facilities is found to be acute in India. 600 million people are defecating openly in India and this is posing a major threat to public health. In a poor state like Assam, situated in the north eastern part of the country 60 per cent of the total households in the rural areas do not have access to any kind toilets. Although Assam has been getting funds to eliminate open defecation and the utilization rate of these funds is better compared to other states. But still there is not much improvement on the performance in terms of decrease in number of people who defecate in the open. The State Government of Assam have failed to provide sustainable solution for this major sanitation concern. The overall

situation is gloomy, making the elimination of open defecation a distant dream for the people of the state. In this situation it is essential to supplement the state's efforts with market interventions to make the good sanitation dream a success. There are certain issues with the supply chain and the demand side expectations for construction of latrines. There should be an initiative to bridge this gap in which a market solution is proposed that will consistently deliver an attractive toilet which will provide safety, security and prestige to the household at an affordable price and at the same time, an opportunity for entrepreneur to make money. To reach this solution huge capacity and knowledge issue in the existing delivery mechanism required to be ironed out. What is required is to encourage larger businesses to enter the on-site sanitation sector and promote small scale enterprises at the village level. As of now the private capital has not given enough attention to this particular sector whereas it has a lot of potential to realise a social dividend and economic returns as well. Considering this, the state has to take initiative to pave ways for private capital to roll in the sanitation sector for achieving larger public good.

EPA's Recreational Water Quality Criteria: Tools for Developing Site-Specific Alternative Criteria

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EPA's 2012 Recreational Water Quality Criteria (RWQC) recommends fecal indicator bacteria levels below which there were no observed statistically significant increase in swimming-associated illness. The health-based recommendations reflect the illnesses reported during epidemiological investigations at coastal beaches affected by secondary treated and disinfected effluent. The RWQC also includes a summary of tools that can be used by States to develop scientifically-defensible and protective site-specific alternative criteria that reflect local environmental conditions, sources of fecal contamination and human exposure patterns. An alternative water quality criteria could involve the derivation of different numerical value(s) that are based on: (1) an alternative health relationship derived using epidemiology and/or Quantitative Microbial Risk Assessment (QMRA); (2) QMRA results to determine water quality values associated with a specific illness rate; or (3) a different indicator/method combination. EPA recommends that alternative criteria reflect a similar health goal as the RWQC (e.g., 3.2 percent or 3.6 percent NGI rate). EPA is providing additional information on these tools for developing site-specific alternative criteria in Technical Support Materials (TSM) documents. This presentation provides updated information on EPA's current thinking and status regarding the TSM documents and provides more detail on the tools than was published in the 2012 RWQC document (EPA-820-F-12-058) or the TSM Guide (EPA-820-R-14-010) published in 2014.

Interlaboratory Variability in Human-Associated Fecal Source Identification qPCR Conditions for Data Acceptance

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There is a growing interest in the application of human-associated fecal source identification quantitative real-time PCR (qPCR) technologies for water quality management. The transition from a research tool to a standardized protocol requires a high degree of confidence in data quality across laboratories. Data quality is typically determined through a series of specifications that ensure good experimental practice and the absence of bias in results due to DNA isolation and amplification interferences. However, there is currently a lack of consensus on how best to evaluate and interpret human fecal source identification qPCR experiments. This is, in part, due to the lack of information on interlaboratory variability in conditions for data acceptance. The aim of this study is to provide users and reviewers with a complete series of conditions for data acceptance derived from a multiple laboratory data set. To establish these benchmarks, data from HF183 and HumM2 human-associated qPCR methods was generated across 14 laboratories. Each laboratory followed a standardized protocol utilizing the same lot of reference DNA materials, DNA isolation kits, amplification reagents, and test samples to generate comparable data. After removing outliers, a nested analysis of variance was used to establish metrics that include lab-to-lab, replicate testing within a lab, and random error for inhibition and sample processing controls. Other conditions addressed include extraneous DNA contamination assessment (no template and extraction blank controls), calibration model performance (R2, amplification efficiency, and lower limit of quantification), and proper documentation of results. To demonstrate the new procedure, comparable data from three additional laboratories was reviewed. Overall, findings confirm the importance of quality assurance conditions to promote integrity of findings, as well as a greater consistency between laboratories.

Balancing Environmental Stewardship and Cost in the Disinfection of Wet Weather Flows in Water Resource Recovery Facilities

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Achieving the targeted level of disinfection for intermittent peak flows in water resource recovery facilities (WRRFs) poses unique challenges in that the investments made will only be used on an intermittent basis. At the same time, for a large number of impacted waterways

throughout the US, the pathogen loadings associated with wet weather flows (both sewer overflows and stormwater flows) have been identified as a primary cause of water standard targets not being met, and the need for regulatory action to rectify the observed water quality degradation. Wet weather discharges from WRRFs can consist of mixtures of primary and secondary effluents, and in some instances flows that have received solely primary treatment. Disinfection of low quality wastewater streams, such as primary effluent and combined sewer overflows, pose challenges including elevated concentrations of not only potential pathogens but also organic particulates and soluble organic and inorganics that must be accounted for in order to identify the range of potential treatment alternatives. The relatively recent advances in UV disinfection technology, rapidly acting inorganic disinfectants, such as bromine, and organic compounds such as peracetic acid and guanidine-based macro-molecules have made these options potentially viable, albeit expensive candidates for these challenging flows. Within this framework, an evaluation of disinfection alternatives for Detroit's Water and Sewerage Department (DWSD) identified and rated alternative disinfection technologies applicable to the WRRFs wet weather flows, which consisted of combinations of conventionally and chemically treated primary effluent, as well as secondary effluent. Following a Triple Bottom Line Analysis to ensure integration of DWSD's evolving vision to their public health and environmental stewardship mission, a series of bench scale tests on the facility's potential wet weather flowstream permutations was executed. Coupled with a hydraulic analysis, the testing identified an innovative process configuration that integrated high rate disinfection with in-plant flow re-routing allowing DWSD to achieve its treatment objectives with respect to disinfection of peak flows, while at that same time allowing it to make progress on one of its environmental stewardship goals - the protection of the Rouge River from wet weather related organic inputs. The ensuing plan, which included a series of risk management elements such as incorporation of chemically enhance primary treatment (CEPT) capabilities, received regulatory approval from Michigan Department of Environmental Quality (MDEQ) while averting the need for a new disinfection facility, originally priced at $90 million dollars, and replacing it with an upgrade project estimated at less than $15 million.

Effective use of Peracetic Acid to reduce effluent disinfection byproduct in Water Resource Recovery Facilities

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The need to control disinfection by-products (DBPs) in wastewater effluents has focused the industry on identifying viable alternative disinfection technologies. Among them are chloramination, UV and peracetic acid (PAA) to meet reduced Total Trihalomethane (TTHM) and brominated DBPs in particular, rather than conventional chlorination. One such example is The City of Largo in Florida which owns and operates the Largo Wastewater Reclamation Facility (WWRF) with a permitted capacity of 18.0 MGD on an Annual Average Daily Flow

(AADF) basis. The City has received a Consent Order from the Florida Department of Environmental Protection (FDEP) that requires the City to reduce its disinfection byproducts (DBPs) in the surface water discharge by nearly a half. In particular, bromodichloromethane (BDCM) must meet a maximum limit of 22 µg/L. This has spawned the City to explore alternative disinfection technologies. Full treatment and split treatment options were considered using an alternative disinfectant. For full treatment options, the alternative disinfection approach is applied to the total flow. Under the split treatment options, sodium hypochlorite would be used to disinfect part of the flow and an alternative disinfection approach would be applied to a parallel or split flow. The reason to consider a split treatment approach is that the use of sodium hypochlorite is generally the least expensive option for disinfection. Split treatment allows the coupling of the least expensive approach with a more expensive approach applied to a lesser flow. Initially, both UV and PAA were considered as alternatives but UV was ruled out due to relatively low UVT in plant effluent, averaging 55%, which would drive up the cost. Bench-scale testing and pilot testing showed that BDCM was not formed during the application of PAA and that it was effective to achieve non-detection of fecal coliform at a concentration of about 3.0 mg/L-PAA at a contact time as low as 15 minutes. The testing showed that there were no other adverse impacts of applying PAA for disinfection and there was even an added benefit from the PAA residual reacting with the chlorine residual to quench each other. Overall the testing determined that the most economical solution for the City of Largo to reduce the formation and discharge of BDCM to meet the Consent Order was a split treatment with two different disinfectants, Sodium Hypochlorite (NaOCl) and Peracetic Acid (CH3CO3H). A blended combination of 60% PAA treated effluent and 40% effluent treated by sodium hypochlorite will produce a target of 15 µg/L, which is below the limit of 22 µg/L. At times, the PAA treated effluent could be discharged to the surface water and the chlorinated water could be discharged to the Reclaimed Water Storage Tank. While operating in this way, there would be little or no BDCM in the surface water discharge. Any disinfectant residual present in the effluent will be quenched with Sodium Bisulfite, prior to discharge to the Feather Sound Outfall. Full scale design of this system has been completed and construction will commence in the coming months.

Should Water Treatment Planning for Potable Reuse Directly Address Norovirus Risk?

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Reclaimed water is domestic or municipal wastewater, which has been treated to a suitable quality for a specific use and takes the place of potable and/or raw water that would otherwise be needed from another source. Water reuse is an important component of the future water supply portfolio for many locations throughout the world to support population growth and continued economic development and to address increasingly prevalent drought conditions. Initial reclaimed water uses were primarily for irrigation of agriculture. Today, reclaimed water

is used for a wide range of beneficial purposes including power plant cooling water, commercial and municipal irrigation, river and stream flow enhancement, natural gas exploration activities, and augmentation of drinking water supplies (potable reuse). Indirect potable reuse (IPR) is the use of reclaimed water for potable purposes by discharging to a water supply source, such as a surface water or groundwater. The mixed reclaimed and natural waters then receive additional treatment before entering the drinking water distribution system. Direct potable reuse (DPR) is the introduction of reclaimed water either directly into the potable water distribution system or into the raw water supply entering a drinking water treatment plant. Whereas numerous IPR projects have been successfully implemented, DPR implementation is much less common. However, for various reasons including severe droughts, increased population, and increased confidence in water treatment technologies, many municipalities are now considering DPR. In 1980, EPA developed the first Guidelines for water reuse as a technical research report for the Office of Research and Development (ORD). EPA released the most recent update to this Guidance in 2012. However, there are significant scientific gaps regarding potable and non-potable water reuse and public health safety. These knowledge gaps are of particular interest currently because States and local agencies are considering new reuse projects that are based on widely variable standards and treatment trains. Various States, particularly in the drought-impacted Southwestern USA are using screening level risk assessments to establish treatment performance criteria for DPR applications. For example, the draft California recommendations for DPR currently suggest that 12 logs of culturable virus, 10 logs of Cryptosporidium, and 10 logs of Giardia reduction would yield finished drinking water risks of infection of less than 1 in 10,000 per year. However, these recommendations have not specifically considered Norovirus (NoV) as an etiological agent of concern, yet NoV are reported to cause the largest number of illnesses in the US among known pathogens (Mead et al., 1999; Scallon et al., 2011). Herein, we use the California framework methodology to consider and put into context the potential risks associated with NoV in DPR implementation and the log reductions needed to achieve benchmark illness levels. We also discuss known uncertainties associated with published dose-response relationships, and the limitations associated with the analytical methods used to enumerate NoV at various stages of water treatment. Note: The views expressed in this presentation are those of the authors and do not necessarily represent the views or policies of the US EPA.

Minimizing disease transmission risk in emergency settings: laboratory-based evaluation and optimization of two novel protocols for the in situ physico-chemical disinfection of pathogen-laden hospital wastewaters

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The operation of a health-care facility, such as a cholera or Ebola treatment center in an emergency setting, results in the production of pathogen-laden wastewaters that may

potentially lead to onward transmission of the disease. This presentation outlines the results of laboratory studies devised to inform the design and operation of a novel full-scale treatment protocol to disinfect pathogen-laden hospital wastewaters in situ, thereby eliminating the need for potentially hazardous road haulage and disposal of human excreta or wastewater to poorly-managed waste facilities. It is envisaged that the resulting technology will eventually provide an effective barrier to disease transmission by means of a novel but simple sanitary intervention. Given the issues of human excreta management raised during the recent Ebola outbreak, the focus of this presentation is on the ability of the approach to reduce virus levels, through the enumeration of bacteriophage species as potential surrogates of viral pathogens. The performance of two physico-chemical protocols has been carefully monitored by means of bench-scale tests performed on a 'fecal waste matrix', created by mixing various municipal wastewaters and sludges in a proportion aimed to mimic the composition of the fecal waste produced by health-care facilities in emergency settings. The two studied protocols investigated achieve coagulation/flocculation and disinfection by exposure to high or low pH environments, using thermotolerant coliforms, intestinal enterococci, and somatic coliphages as disinfection efficacy indices and several other physico-chemical parameters as indicators of treatment performance. In the high pH treatment protocol, the addition of hydrated lime results in wastewater disinfection and coagulation/flocculation of suspended solids. In the low pH treatment, disinfection (and partial colloidal destabilization followed by sedimentation) is achieved by the addition of hydrochloric acid, followed by pH neutralization. A potential further step in this second protocol is the coagulation/flocculation of suspended solids using aluminum sulfate. Removal rates achieved for the high pH treatment protocol, in terms of physico-chemical parameters, were: COD >80%; suspended solids >85%; turbidity >85%. Removal rates in terms of microbiological parameters were: thermotolerant coliforms >5 Log10 and intestinal enterococci > 2Log10. The removal of somatic coliphage to date has been significantly higher than 2 Log10, with ongoing tests suggesting that a well-designed bench-scale treatment can achieve a removal rate higher than 4 Log10. Removal rates achieved for the low-pH treatment protocol, in terms of physico-chemical and microbiological parameters, were: COD >80%; thermotolerant coliforms between 0.2 and 1.2 Log10, with a mean removal of 0.75 Log10 and > 3Log10 removal for intestinal enterococci. The removal of somatic coliphage to date has been in excess of 4 Log10. Ongoing tests suggest that a well-designed bench-scale treatment can achieve removal results close to 5 Log10. The differential removal rates observed between the two faecal indicator bacteria using the two treatment protocols is likely to reflect differences in cell composition (Gram negative vs. Gram positive) and highlights the importance of using a range of indicator organisms. Production rate and concentration of the sedimented sludge (volume sludge/volume influent, dried solid content) have also been monitored. The approach taken in this ongoing study is the first known successful attempt to disinfect wastewater in a disease outbreak setting without resorting to the alternative, untested, approach of 'super-chlorination', which, it has been suggested, may not consistently achieve adequate disinfection. In addition, a basic costs analysis has demonstrated significant savings in reagent consumption compared with super-chlorination. The approach to in situ sanitation in cholera treatment centers and other disease outbreak settings presented here offers a timely response to a UN call for onsite disinfection of wastewaters generated in such emergencies and it is noteworthy that the 'Coalition for Cholera Prevention and Control' recently highlighted our research as

meriting serious consideration and further study. Further applications of the method to other emergency settings are being actively explored by the authors in discussion with the World Health Organization, in response to the ongoing Ebola outbreak in West Africa, and with the UK-based NGO Oxfam, as a potential key component of excreta-borne disease management in the Philippines and Myanmar, in support of post-disaster incremental improvements to local sanitation chains.

The Surfer Health Study: Microbial Water Quality Measurements Supporting a Combined Wet Weather Surfer Epidemiology and QMRA Study in San Diego, CA

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Fecal indicator bacteria (FIB), e.g. Enterococcus, are commonly used to measure marine recreational beach water quality. Epidemiological studies performed at beaches with known human fecal contamination have shown that FIB are reliable predictors of swimming related illness; and current water quality standards are based on these relationships. In contrast, the correlation of illness rates with FIB counts or direct measurement of pathogens at California marine beaches impacted by storm water has not been extensively studied. Furthermore, quantitative measurement of pathogens along with FIB can enable estimation of dose and exposure necessary for a site-specific predictive modeling of health outcomes via Quantitative Microbial Risk Assessment (QMRA). We measured microbial water quality at two storm water impacted beaches with large surfer populations during a combined wet weather surfer epidemiology and QMRA study in the winter of 2014 in San Diego, CA. Surf zone water samples were collected daily from Ocean Beach and Tourmaline Surfing Park during January 2014-March 2014 and December 2014-March 2015. Storm water was collected from the main discharges at the study beaches: the San Diego River at Ocean Beach and Tourmaline Creek at Tourmaline Surfing Park during storm events in February 2014 and December 2014-March 2015. Microbial water quality was determined by quantifying FIB (total and fecal coliforms, Enterococcus) through standard cultivation techniques, F and somatic coliphage through standard cultivation techniques, human-specific and general FIB markers through digital QPCR (e.g. HF183, Enterococcus QPCR), and pathogenic bacteria (Salmonella invA, Campylobacter VS1), viruses (human Adenovirus, human Norovirus, and Enterovirus), and protists (Cryptosporidium COWP, Giardia β-giardin) through digital QPCR in storm water and beach water samples. Environmental measurements including water temperature, salinity, surf height, tide, current, wind speed, wind direction, and discharge flow were measured during daily and storm event sampling. There was a strong positive correlation between FIB and precipitation, and a positive correlation between FIB and tide, particularly during spring tide events. However water samples above the single sample Enterococcus limit (104 CFU/ml) had only a weak correlation with an increase in illness rates from wet weather ocean exposure measured in the epidemiology study. F and somatic coliphage numbers did not correlate with tide, and were not

consistently detected at the beaches; but coliphage numbers did correlate with precipitation and discharge flow. Although pathogen measurements did not perfectly co-vary with FIB measurements, quantification of pathogenic bacteria and viruses using digital QPCR allowed for estimation of pathogen load in the storm water and surfer exposure in the surf zone. This study demonstrates the power of combining sensitive molecular measurement of microbial water quality, QMRA predictive modeling, and empirical illness rates to characterize public health risk at previously uncharacterized sites or environmental conditions.

A Comparison of EPA Method B for Bacteroidales to Traditional Fecal Indicator Bacteria

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Introduction: Fecal contamination of freshwater beaches in the Great Lakes is often tested using traditional fecal indicator bacteria such as Escherichia coli (EC) and in some cases Enterococci (ENT). However, rapid molecular methods have been proposed using non-traditional fecal indicator bacteria (FIB) such as Bacteroidales. EPA Method B (EPA-B) is an assay used for general Bacteroidales that has little comparative data to traditional fecal indicators. This presentation examines relationships between EPA-B and traditional EC and ENT concentrations at three close proximity sites at a Lake Erie beach recreational area near the City of Cleveland. Methods: Three locations (two beach and one nearby river site) were sampled (n=100) to evaluate freshwater FIB contamination levels. Samples were tested for traditional indicators (EC and ENT) using both membrane filtration (MF) (EPA 1603 and 1600, respectively) and chromogenic substrate tests (CS). In addition, 100 mL samples were filtered, frozen, and later tested for general Bacteroidales using the EPA-B assay. Results: The highest average and median levels of EC, ENT (by both CS and MF), and Bacteroidales were found in the creek water as compared to the beach sites. In nearly all paired samples (94 %), the number of target sequences (TS) of Bacteroidales were higher (per 100 mL) than traditional indicators. When Bacteroidales were completely absent or below a confident limit of detection (< 4500 TS/100 mL in this study), EC (73%) and ENT (95%) were also often below threshold EPA water quality standards (235 CFU/100 mL and 35 CFU/100 mL, respectively). Bacteroidales TS showed a strong correlation with EC and ENT in the more contaminated creek samples (R2= 0.51 and 0.36, respectively) and a weak correlation with EC (R2= 0.19 and 0.17) and ENT (R2=0.17 and 0.22) at the two beach sites. Conclusion: Low concentrations of EPA-B were often predictive of EC and ENT concentrations that were below water quality standard thresholds. EPA-B showed a stronger relationship with traditional FIB in a creek site with higher contamination. The weak correlation between EPA-B and EC and ENT at the beach sites could be a predictor of diffuse sources of contamination, wind/wave-driven resuspension, or other factors. The use of EPA-B and traditional FIB could be combined as a multiple-method approach to better determine water quality impairment at these and other Lake Erie beaches.

Persistence of plasmids in antibiotic resistant stream water Escherichia coli harboring integron, conjugation, and/or mobilization genes

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The occurrence of integrons and mobilization genes that can impact persistence and dissemination of antibiotic resistance is increasingly being associated with plasmid-mediated antibiotic-resistant bacteria in the environment. Escherichia coli exhibiting resistance to up to six different antibiotics including trimethoprim and sulfamethoxazole were recovered from wastewater treatment plant effluent and receiving stream water, both upstream and at two locations downstream of effluent input in Northwest Arkansas. The objectives of this research were to determine the presence of class 1 and 2 integrase and mobilization genes in plasmids in trimethoprim/sulfamethoxazole resistant isolates; and to determine persistence of antibiotic resistant isolates with different mobilizable gene and integron profiles. Plasmids were extracted from 76 bacterial strains grown on selective media containing 4 _g/ml trimethoprim/ 80 _g/ml sulfamethoxazole followed by detection of sulfamethoxazole resistance (sulII), trimethoprim resistance (dfrA1, dfrA14, dfrA17, and dfrB3), class 1 integrase (intI1), class 2 integrase (intI2) and mob genes (MOBP11, MOBP14, MOBP51, MOBF11, MOBF12, MOBQ11, and MOBQu) using PCR amplification. The isolates were classified into four groups based on the presence/absence of integrase and mob gene features and were grown individually in synthetic wastewater media with addition of trimethoprim/sulfamethoxazole at 23_C. Viable cells were enumerated from each culture at 1, 7, 9 and 11 days after inoculation. Plasmids from 64 (84%) isolates harbored a sulII gene; 56 (73%) isolates harbored at least one tested trimethoprim resistant gene; 36 (47%) and 19 (25%) conferred a class 1 and class 2 integrase gene, respectively; and 18 (24%) isolates harbored class 1 plus 2 integrons. There were 38 (50%) isolates harboring plasmids conferring at least one mob gene with MOBF12 (43%) detected most frequently followed by MOBQu (14%). In the persistence experiment, 70 (92 %) isolates persisted at a steady level whereas six isolates [isolates 21 (MOBP51, MOBF12), 33, 39 (MOBF12, intI1 and intI2), 63 (intI1), 91 (MOBF12), 96 (intI2) decreased in concentration from day 9 to 11 when grown in the presence of trimethoprim but not did not decrease in the presence of sulfamethoxazole. Isolate 75 (MOBP51, MOBF11, MOBF12, IntI1) was the least persistent in both antibiotics and decreased in concentration by almost 3 log between 1 day after inoculation and 7 days. These results indicate that antibiotic resistant bacteria containing integrase and mobilization genes have a high potential for persistence but may exhibit different persistence of trimethoprim and sulfomethoxazole resistances.

Salmonella transport from pond water sources through irrigation systems on mixed produce farms in the southeastern United States

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Salmonella is an important cause of foodborne illness. Previous studies have shown that Salmonella is often detectable in ponds used to store water for irrigation in the southeastern United States. The goal of this project was to understand whether Salmonella moves from pond water sources through irrigation systems on mixed produce farms of the southeastern United States and whether it persists on crops until harvest. To address this goal, Salmonella was monitored in five irrigation water sources (surface water ponds and groundwater wells) and various irrigation systems (drip, center pivot, and solid-set sprinklers) between May and September 2014. A total of 96 composite samples of water and 35 composite samples of harvested produce were analyzed for generic E. coli and Salmonella using an enrichment-based most probable number (MPN) method with PCR confirmation. The Salmonella strains found in irrigation ponds were sometimes present in irrigation systems, and in a few cases Salmonella appeared to persist on the surface of some crops until harvest. The concentrations of Salmonella in irrigation water sources and irrigation systems ranged from undetectable (below 0.055 MPN/100mL) to 0.99 MPN/100mL in individual samples, while the concentrations of indicator E. coli in irrigation water sources and irrigation systems ranged from undetectable (below 1 MPN/100mL) to greater than the FDA's proposed statistical threshold value of 410 MPN/100ml in individual samples.

Modelling the fate and transport of microbial pathogens in the Sediment of the Apies River, Pretoria, South Africa: A better understanding of the human health risk associated with the use of surface water

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Background In developing countries, such as South Africa, aquatic ecosystems and water resources are often subjected to high levels of microbial pollution. Unfortunately, there are still population groups that might use surface water (e.g. rivers) for drinking purposes without prior treatment. Surface water is also often used for irrigation and recreation purposes. The dynamics of microbial pathogens in water resource sediments and the extent of accumulation of pathogens, and how this is associated with different input loads or how pathogens might be remobilised to contribute to the microbial load in the water column is not well understood. This gap in knowledge with regard to sediment microbial dynamics leaves surface water users very

vulnerable to possible adverse effects on their health and also hinders the effectiveness of water management. In this study, a three pronged approach was used to gain some insight into the microbial sediment dynamics of the Apies River, Pretoria, South Africa. This approach involved field observations in conjunction with laboratory experiments, the use of areal models and simple hypothetical models. Methods Water and sediment samples were collected for a year (during the wet and dry seasons) from 10 different sites along the Apies River. Each site had a different soil composition. Bacterial enumeration techniques were then used to isolate, identify and enumerate Escherichia coli. The use of a simple additive weighting technique and potential maps was used to determine pathogen loads from point, non-point and land use practices in the study area to compile a faecal coliform budget. An attempt at developing simple hypothetical models to understand bacterial transport along the river and movement from sediment into the water column was attempted through a complex process of theoretical interpretation of the river sediment composition values in terms of river flow conditions using the river classification guidelines, a spatial-temporal analysis of total suspended solids (TSS) and turbidity data and the turbidity data to confirm or help explain the sediment composition of the river bed at the different sampling sites, statistical modelling of possible flow rates using historical flow rate and waste water discharge data, spatial-temporal analysis of the faecal coliform and E. coli data and determination of the minimum flow conditions required for sediment particles of different sizes to be resuspended, entrained, transported and deposited using the Shields and updated Hj_lstrom diagrams. Results and Discussion E. coli was found in water and sediment samples throughout the entire sampling regimen (wet (summer) and dry (winter) season). The simple additive weighting technique (areal modelling) revealed highest potential pathogen load into the river was associated with waste water treatment works. From hypothetical modelling the following was observed, sediment on the river bed harbours pathogen cells consistently as was shown by the nearly all year-round presence of E. coli during both the wet and dry seasons and in large quantities. There was generally a relationship between turbidity, sediment composition of the sediment on the river bed and E. coli counts in the water. A critical threshold of 16% (0.02 to 0.25 mm) size sediment particles on the river bed was determined. Values above this threshold showed a rapid decline in the number of E. coli cells attached the sediment particles in this size range.

Development and performance of the Phytoxigene CyanoDTec Test: A rapid molecular assay for the routine monitoring and differentiation of toxin producing cyanobacterial blooms

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There is now a good understanding of the genetic basis for toxin production in cyanobacteria. The discovery of these toxin biosynthetic pathways has enabled the development of genetic screening tests of environmental samples so as to measure the total amount of cyanobacteria along with its capacity and ability to produce toxins or not. Molecular genetics underlying

cyanobacteria biotoxin production in aquatic environments, but more specifically, the toxin biosynthesis genes have been used to develop a multiplex quantitative PCR assay for the simultaneous detection and quantitation of cyanobacteria 16S rRNA (16S), microcystin and nodularin synthetases (mcy/nda), cylindrospermopsin synthetase (cyr) and saxitoxin synthase (sxt) genes. Along with the copy number of the relevant cyanotoxin biosynthesis gene the internal cyanobacteria-specific 16S rDNA control target can be used as a biomass reference target. _Validation and test performance data for the assay will be presented along with details on how the assay could be used in a real world setting. This multiplex quantitative PCR assay has been designed so as it can be carried out in a standard laboratory with in a 3 hour turn around, and should become a very important addition to the resources available to laboratories and authorities for better surveillance, detection, prediction and monitoring of harmful algal blooms.

The Impacts of Bacteriophage Recreational Water Quality Criteria on the Wastewater Treatment Industry

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In an effort to improve recreational water quality, the EPA plans to introduce bacteriophages as recreational water criteria elements. While some research suggests that bacteriophages may serve as improved indicator organisms for enteric viruses and that greater bacteriophage inactivation may improve recreational water quality, the impending bacteriophage recreational water quality criteria is concerning for the wastewater industry. This criteria may significantly impact the wastewater industry because wastewater treatment plants use a variety of disinfection processes, bacteriophages respond differently to the numerous disinfectants, and bacteriophage quantification remains problematic. For example, wastewater treatment plants with disinfection processes that poorly inactivate bacteriophages may require costly capital upgrades. Many wastewater treatment plants may have to shift to disinfection processes that result in discharge of effluents with higher concentrations of carcinogenic disinfection byproducts. Such a change in disinfectants could undermine the industry's recent efforts to minimize disinfection byproducts. Bacteriophage quantification also remains problematic. While novel molecular methods have resulted in improved detection abilities, many of these methods cannot differentiate between viable and inactive bacteriophages. Because the culture based quantification methods depend on both the physiology of the host and the growth medium, these culture based methods can produce variable bacteriophage population counts. To explore and clarify the potential impacts of bacteriophage regulations on the wastewater industry, Hazen and Sawyer has compiled the performance data from a variety of different disinfection processes in regard to the disinfectants' efficacy on bacteriophages and viruses. This research suggests that free chlorine, advanced oxidation processes, and UV inactivate

bacteriophages better than both monochloramines and peracetic acid. Furthermore, this work highlights some of the concerns with the current methods for quantifying bacteriophages.

The use of remote sensing and geographic information systems in microbial source tracking

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Microbial source tracking (MST) is a set of techniques used to determine the sources of fecal contaminants in environment, mostly in recreational and drinking water, which plays a very important role in identifying specific pollutant sources and protecting public health from waterborne diseases. Current MST techniques, including detection of molecular biomarkers, phenotypic and genetic typing of indicator bacteria and tracing chemical indicators, are designed to identify whether microbial sources are from particular host species such as humans, dogs or gulls. However, these techniques cannot geographically determine particular sources because they do not provide any spatial information. In this study, we explore whether remote sensing (RS) and geographic information systems (GIS) can be applied in tracking microbial sources. Remote sensing is a technique to be able to acquire land cover/land use (LCLU) information from airplanes or satellites without on-site observation and GIS is a tool to store and analyze spatial data. The combination of RS and GIS technique provide powerful tools to identify LCLU information of the earth surface, which indirectly might determine the sources of fecal contamination because the habitats and activities of different contamination sources are related to different types of LCLU. To examine the possibility of applying RS and GIS in microbial source tracking, we obtained rough estimates of microbial sources based on MST data from two geographically disparate watersheds, namely the Dungeness watershed in Washington State and the Blackstone River watershed in Massachusetts. We then obtained land cover information of the Dungeness Watershed by classifying a high resolution satellite image and land use information for the Blackstone River Watershed from a GIS dataset, and subsequently analyzed the relationship between calculated microbial sources and LCLU components . We found positive correlations between calculated human sources of fecal contamination and developed areas (including residential and commercial areas), between calculated wildlife sources and agriculture, forest and pasture areas; and between calculated bird sources and water area. In addition, we also found that the microbial source diversity was positively correlated with landscape fragmentation and diversity metrics, implying that microbial sources are more complicated in a fragmented and diverse landscape. The study demonstrates the use of remote sensing and GIS to acquire LCLU information for MST studies and offers new insight in possibilities for tracking the sources of fecal contamination in water.

Development of a rapid enrichment and real-time PCR assay for the detection of F+ DNA (Inoviradae) coliphages in water

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Introduction: Fecal indicator bacteria (FIB) are commonly used for recreational water quality monitoring; however, due to the morphological and functional characteristics of FIB, its adequacy to indicate viral or protozoan contamination remains a question. Quantitative microbial risk assessment and epidemiology studies have shown that enteric viruses are a major cause of swimming related illnesses in the United States. Therefore, testing for fecal indicator viruses, such as coliphages can enhance recreational water quality monitoring efforts. Another limitation of current recreational water quality monitoring practices for FIB is that most methods take more than 16 hours for results. As microbial water quality can change quickly, monitoring results from samples obtained a day ago can potentially expose many to unnecessary pathogen risk or in contrast unneeded beach closures, both of which result in economic loss. Rapid methods are needed for timely responses and coliphages are ideal candidates. Coliphages are viruses that infect E. coli. These viruses are good candidates for rapid detection because they can produce more than 10,000 PFU/mL of progeny phages within a few hours, which makes it possible to rapidly detect these progeny phages by molecular or biosensor assays. Currently, almost all published molecular or biosensor assays for F%2B coliphages have focused on the RNA phages (Levivirus and Allolevivirus). However, F%2B DNA coliphages are found in high proportions in municipal wastewater, bovine, swine and waterfowl wastes and should not be overlooked as candidates for the development of rapid indicator virus detection methods. This study describes the development of a rapid liquid broth enrichment culture procedure that can greatly propagate a few initial F%2B DNA coliphages (1 - 9 PFU) in 1 L of water to concentrations >10,000/L for detection by molecular assays in few hours; and to develop and use a real-time PCR assay to confirm the presence of F%2B DNA coliphages in rapid enrichment culture. Method: Five different growth media: Tryptic Soy Broth (TSB), Lauria Broth (LB) and minimal media M9 with 20% glucose, lactose or glycerol were evaluated for their ability to propagate the growth of F%2B DNA coliphages after 3-6 hours of incubation in a 37_C water bath. F%2B DNA concentrations were determined by the standard EPA Method 1602 double agar layer assay. Coliphages in liquid enrichments were further concentrated by a rapid and simple procedure and nucleic acid extracted before real-time PCR. Different coliphage concentrations and DNA extraction methods were evaluated. Currently, only three F%2B DNA strains have been completely sequenced (M13, f1 and fd). Their complete genomes were aligned using Clustal Omega and primers and probe sets were generated using Primer3Plus. Field isolates and laboratory prototype strains were used to develop the real-time PCR assay. Results: M9 with 20% glucose was the best liquid medium for the rapid growth of F%2B DNA coliphages followed by TSB then LB. All three media were able to propagate 1 - 9 PFU/L of F%2B DNA coliphages to >104 PFU/L in 3 hours of incubation in a 37_C water bath. F%2B DNA coliphages cultured in M9 with 20% lactose or glycerol did not reach 104 PFU/L until 4 hours of incubation. High genetic similarity was observed for M13, f1 and fd. The development of F%2B

DNA coliphage real-time PCR assay is ongoing. Conclusion: Liquid culture enrichment of water samples can produce enough F%2B DNA coliphages in 2-3 hours to be detected by real-time PCR. The complete procedure takes approximately 4-6 hours and provides same day results on viable and infectious viral fecal indicators. This new rapid method can potentially enhance recreational water quality assessment.

Poster Presentations

Microbial and turbidity removal by chitosan coagulation to optimize ceramic water filtration for household drinking water treatment

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Rotavirus is the leading cause of moderate-to-severe diarrhea in infants and children under age 5. While household level purification has drastically reduced diarrheal illness among users of household water treatment technology (HWT) users, the majority of technologies fail at removal of virus due to inability physically filter out viruses because of their small-size without sacrificing flow rate. Ceramic water filters (CWF) are a HWT that have been a subject of continued research and they are increasingly implemented and used around the world, but critical performance deficiencies and knowledge gaps still exist. For example, the treatment of highly turbid (cloudy) water can cause negative consequences for filters, resulting in rapid clogging and the need for frequent cleaning that shortens the lifespan of the filter and sometimes leads users to abandon them. Additionally, while most filtration technologies can significantly decrease bacterial and parasite contamination of water, they are not capable of substantially removing viruses or cause virus inactivation. No prior research has explored pre-treatment of water using chitosan as a coagulant and subsequently filtered water using a CWF use to improve effectiveness and user satisfaction. Chitosans is a natural organic polymer that is ubiquitous, inexpensive, widely commercially available, but has seen only limited use as a water coagulant. Chitosans are non-toxic and biodegradable polymer materials that are derived by simple chemical treatments from chitin, a major source of which are the leftover shells of crustacean seafood, such as shrimp, prawns, crabs and lobsters. They have not been widely or systematically investigated as a water treatment chemical to remove microbes from water or as a pre-treatment for a point-of-use water treatment filter. Our approach to integrate chitosan use with the CWFs is to add it to turbid water spiked with bacteria and virus, allow coagulation and settling out of particles to occur, and then transfer the pre-treated water (the supernatant water after coagulation and particle setting) into the CWF for further treatment by filtration. To accomplish this goal, test water was prepared by adding Escherichia coli strain K011, E.coli K011, MS2 bacteriophages as surrogate for virus detection, and kaolinite clay to mimic turbidity. The chitosans that were evaluated were selected based on previous work that systematical screened and identified the following chitosans as candidates for household use based on their virus reductions: chitosan hydrochloride, chitosan acetate, and chitosan lactate. Spread plate method was used to enumerate E. coli K011, Single Agar Layer (SAL) assay was used for MS2 detection, and Hach 2100N Turbidimeter was used to measure turbidity. Log10 values were calculated based on the reduction of E. coli K011, MS2, and turbidity on initial concentrations quantified in influent water, before filtration, as compared to effluent water, after filtration. Initial results show approximately 0.2 and 3 log10 mean reduction for virus and bacteria, respectively for water with no-chitosan pretreatment, whereas with chitosan pretreatment, mean reduction was 3 and 6 log10 reduction for virus and bacteria, respectively. Turbidity reduction with chitosan treatment consistently resulted in >1 NTU, therefore meeting turbidity standards as established by US EPA. As such, these results support the use of chitosan

to optimize water filtration processes for household drinking water treatment through significant virus reduction and decreasing clogging of filters thereby increasing their lifespan.

Antimicrobial Resistance: Environmental Pathways and Impact on Human Health

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It is well documented that there is considerable overuse, careless use, inappropriate use and unregulated availability and use of many antibiotics in both medicine and veterinary medicine, as well as extensive and largely unregulated use in animal agriculture and aquaculture, including for animal and fish growth promotion. These imprudent uses and abuses of antibiotics contribute to the extensive presence of antibiotic residues, their metabolites, multiply antibiotic resistant bacteria and their functional genes in human and animal wastes and in fecally and otherwise antibiotic-contaminated water, soil, sediments and in water-dependent food crops such as seafood and produce. Human excreta and wastewater are recognized and documented as major sources of antibiotics, their metabolites, ARB and AMR genes because of the widespread and extensive use of antibiotics by human populations. Furthermore, human wastewater and excreta are used extensively in agriculture as sources of water and plant nutrients and such use is encouraged by management practices such as ecological sanitation and municipal wastewater (re(use). However, there is public health concern that ARBs and AMR genes from human waste sources could potentially impact water sources receiving untreated or treated wastewater effluent and result in exposures of human populations that use such water as drinking water sources, for bathing, washing and other domestic sources, for primary contact recreation and as irrigation water. To better document and understand the environmental and public health risks, we conducted a systematic literature review summarizing the growing body of scientific information showing that there is growing evidence of widespread global occurrence of antibiotics, their metabolites, ARB and ARM genes in wastewater use systems, aquaculture systems, recreational water and drinking water. The widespread production and use of antibiotics has led to the extensive introduction, dissemination and occurrence of antibiotics, ARB and their genes in various environmental media to which humans are exposed that is now relatively well documented. However, this systematic review of the literature also revealed that the potential human and animal health effects and quantifiable disease risks expressed as DALYs, illnesses or deaths from exposures to these antibiotics, their metabolites, ARB pathogens and their genes via water and wastewater exposures and via foods such as produce are poorly identified, characterized and quantified at this time. Overall, there is lack of recognition, consideration and management of antibiotics, their metabolites, ARBs and their genes in the environmental media by various health-related environmental stakeholders concerned with water, sanitation and hygiene. Furthermore, the WHO Guidelines for drinking-water, recreational water and the use of wastewater and excreta in agriculture do not address and provide specific guidance on these health-related contaminants. Therefore, there is a major

gap in consideration and coverage of a class of contaminants of environmental health concern that requires more and better risk assessments, effective management approaches and systems and specific guidance for effective management.

Assessment of bacteriological quality and management practice of drinking water at their ground water sources in Akaki Kaliti sub-city, Addis Ababa City Administration

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Abstract 1 Introduction: Since water is essential to sustain life, and when supplied as drinking water to consumers, a satisfactory quality must be maintained so that, provision of water, sanitation and good hygiene services are vital for the protection and development of human resources. Though ground water is much better than surface water in terms of biological quality, lack of source protection, waste management and sewerage system problem, poorly designed pit latrines affect the quality of the water sources. Therefore, Assessment of bacteriological quality and management practice of drinking water at their ground water sources in Akaki Kaliti sub-city, Addis Ababa City Administration were conducted. Methods: This study was conducted from September 2006 to January 2007. A survey of 9 triplicate water sample and sanitary surveys were conducted in 7 raw water sources and 2 non-chlorinated water points. The water samples presumptive test of TTC and FS were examined using membrane filtration method. Result: All raw water samples were positive for TTC and FS. High bacteriological load were found in Bore hole 8 (BH8) (52.5 cfu/100ml) for Fanta spring (FS) and Fanta Spring None chlorinated (FSNC) (29 cfu/100ml) for Thermo tolerant Coli forms (TTC). TTC and Fecal Streptococci (FS) concentrations detected were 12.3 and 11.6 cfu/100ml, respectively for bore hole Emergence Point 4 (EP4). For un-treated water sources the (Kaliti geberel well(KGW), Emergence point 5(EP5) TTC counts were higher (>15 cfu per 100ml). Similarly, the FS count for KGW (>15 cfu per 100 ml) were higher than EP5 (2 CFU/100ml). The health risk matrix assessment indicated that both the EP5 and KGW for TTC were within high risk score while FS risk to health classification EP5 and KGW lie on medium and high risk score respectively. Conclusion and recommendation: Distributing water without treatment and lack of appropriate water source protection are the major factors that contributed the occurrence of high bacterial numbers. Moreover therefore, the management of water sources, appropriate treatment of the raw water sources promoting good hygienic practices are important to make the water quality acceptable in the study area. . Key words: Thermotolerant coliforms, Faecal streptococci, source protection and sanitary survey

Fecal Indicator Bacteria in Sachet Water Collected from the Streets of Ghana

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Water sachets are one of the main sources of potable drinking water for the people in West Africa. These small sealed packets of water are inexpensive to purchase, approximately twenty cents per bag, and sold on the streets of Ghana, with little information about their sources. Currently, there are no regulations in place for the water quality of these bags. The aim of this study was to identify the microbiological pollution in these commercially available bags. Overall, a total of 47 bags were collected from different brands in five locations across the country. Samples were filtered onsite and the filters were transported to JPH College of Public Health Core Laboratory. DNA was extracted from these filters and E. coli concentrations were detected using quantitative polymerase chain reaction (qPCR) method. All cell equivalent gene numbers were transformed to cells dividing by seven, with the assumption of E. coli cells have an average of seven copies of the target gene. The results showed that samples purchased from two different companies in Kintampo had E. coli concentrations ranging from <1-79 CFU /100 ml. Overall, 58% of the samples was positive for E. coli. High numbers of E. coli pose health risk and can cause acute gastrointestinal diseases. In addition, detecting this bacterium in drinking water means that there is also the potential for contamination by other pathogenic bacteria. Ghana is one of the most improved countries in West Africa with a population of 25,758,108 and GDP of $90.41 billion. Our results have shown that even in the most advanced country of West Africa, there are still a lot of problems regarding water, sanitation and hygiene. Detection of E. coli in these sachet water samples show a potential risk for public health and better policies should be implemented in this region to prevent waterborne illnesses.

Evaluation of CHROMagar Clinical Diagnostic Culture Media for Direct Detection and Enumeration of Antimicrobial Resistant E. coli and Coliforms in Sewage and Other Environmental Samples

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The emergence and spread of antimicrobial resistant bacteria (ARB) as a global health concern has sparked interest in the development of methods for improved detection and quantification of these bacteria in the environment and assessment of their associated human health risk. However, major gaps exist in the availability and documented performance of practical analytical methods for the direct detection of ARB in environmental samples. The World Health Organization and others have proposed the development of an indicator system for the detection of ARB in water, wastewater, and other environmental media. E. coli and coliforms are already widely accepted and used as bacterial indicators of water and wastewater. Therefore, the direct detection and quantification of the antimicrobial resistant members of

this fecal indicator group, and specifically those with Extended Spectrum Beta Lactamase (ESBL) resistance and Klebsiella Pneumoniae Carbapenemase (KPC) resistance have been suggested as candidates to be analyzed using standard chromogenic clinical diagnostic culture media. The goal of this research was to 1) compare a chromogenic clinical diagnostic medium for enteric bacteria, CHROMagar Orientation agar (Sigma-Aldrich), to a standard microbiological medium for E. coli and coliforms in wastewater, Rapid E. coli 2 agar Bio-Rad), and 2) to evaluate the use of CHROMagar ESBL agar for the detection of ESBL resistant bacteria and CHROMagar KPC agar for the detection of carbapenemase resistant bacteria in environmental samples, as proof-of-concept. Raw sewage samples were collected as grab samples from 5 wastewater treatment plants in the Research Triangle area of North Carolina according to Standard Methods for the Examination of Water and Wastewater (SMEWW). Bio-Rad Rapid E. coli 2 agar, CHROMagar Orientation, CHROMagar ESBL, and CHROMagar KPC were prepared according to manufacturers' instructions and were stored for no more than 5 days at 4_C. Total coliform bacteria and Escherichia coli were quantified by standard membrane filtration techniques of the United States Environmental Protection Agency (US EPA), adapted from Method 1604, with the agars listed above. Colony forming units (CFUs) of the target bacteria were recorded according to colony color guides provided by the media manufacturers. Initial results indicate that there is no statistically significant difference in detection of total coliforms between the standard microbiological medium, Bio-Rad Rapid E. coli 2 agar and the clinical medium, CHROMagar Orientation ($p>0.05$). However, there was a statistically significant difference in the detection of E. coli between these two agar media. On average 2.8 x 10^6 E. coli per 100mL were detected on Bio-Rad Rapid E. coli 2 agar compared to 8.4 x 10^6 E. coli per 100mL on CHROMagar Orientation. This difference in E. coli detection may have been a result of the relatively small numbers of E. coli detected as a fraction of total coliforms and other types of bacteria (pseudomonads, acinetobacters, staphylococci, etc.) on the CHROMagar, which could have compromised reliable visual detection of E. coli colonies. Based on the number of E. coli and total coliform colonies detected on CHROMagar Orientation, CHROMagar ESBL medium detected approximately 2.5% of the E. coli and 3.1 % of the total coliforms to be ESBL resistant. CHROMagar KPC medium detected approximately 0.09% E. coli and 0.2% of the total coliforms as being KPC resistant, out of the total colonies quantified by CHROMagar Orientation. Although these results indicate that only small percentages of the E. coli and total coliforms detected in raw sewage on these agars were ESBL and KPC resistant, the occurrence of such highly antimicrobial resistant bacteria is indicative of the presence of such bacteria in the population and their possible spread to others who may become exposed to them via environmental, food and person-to-person transmission routes. From the results of this study, using CHROMagar media for membrane filter analysis of wastewater is a potential candidate indicator system for the detection and quantification of ARB E. coli and coliforms in environmental media. The human health risks posed by such profoundly antimicrobial resistant bacteria are uncertain and need to be better quantified.

Using molecular assays and other water-quality and environmental variables to predict harmful cyanobacteria blooms in freshwater lakes

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The presence of harmful cyanobacteria blooms (cyanoHABs) and associated cyanotoxins, such as microcystin, have been identified at Ohio Lake Erie and inland lake waters. Predicting when and where a bloom may occur is important to protect the public that uses and consumes a water resource; however, because of the many factors affecting toxin production, predictions are complicated and likely site specific. Monitoring for cyanobacteria using molecular assays such as quantitative polymerase chain reaction (qPCR) or quantitative reverse transcriptase PCR (qRT-PCR) and for chlorophyll or phycocyanin using optical sensors may provide an early warning system for cyanoHABs. To test these monitoring approaches, the U.S. Geological Survey (USGS), in cooperation with the Ohio Water Development Authority, University of Toledo, and Clermont County Soil and Water Conservation District, collected samples at Ohio recreational sites during May-November in 2013 and 2014. In 2013, samples were collected monthly at eight sites to complete a general survey and aid in the selection of sites for more intensive sampling during 2014. In 2014, samples were collected approximately weekly at five sites in three lakes to better understand the links among cyanobacteria community structure, toxin production, and environmental and water-quality factors. Field crews measured physical water-quality parameters at the time of sampling. Composite samples were preserved and analyzed for dissolved and total nutrients, cyanotoxins, phytoplankton abundance, and cyanobacteria genes by qPCR or qRT-PCR. Molecular assays for cyanobacteria were applied on four levels: (1) total cyanobacteria, (2) genus-specific genes for total Microcystis and Anabaena, (3) genus-specific mcyE toxin genes (DNA) for Microcystis, Anabaena, and Planktothrix, and (4) genus-specific mcyE RNA transcripts for Microcystis, Anabaena, and Planktothrix. The genus-specific mcyE DNA assays provided data on whether or not the toxin gene was present and in what quantity; the RNA mcyE assays provided data on the quantity of the expressed toxin gene. To assess sampling and analytical variability for the molecular assays, 12 concurrent field replicates were collected and qPCR lab replicates were processed in 2013–14. Lab variability was small and statistically lower than within- or between-bottle variability for the five DNA assays but not for the two RNA assays. Sampling variability was small as compared to filtering and processing variability. The molecular assay results that were most significantly correlated to microcystin concentrations were different among the three lakes sampled--the Microcystis mcyE DNA gene at two beaches at a water-supply reservoir; the Planktothrix mcyE DNA gene at a beach and boater-swim sites at a shallow canal-lake; and the total Microcystis gene at a Lake Erie beach site. Microsystis mcyE RNA was significantly correlated to microcystin concentrations at only the Lake Erie beach. Phycocyanin, nutrient concentrations, pH, lake-level change, and turbidity were among the variables significantly related to microcystin concentrations. Although results from this study showed that the use of molecular assays, sensor measurements, and other variables to provide an early warning of cyanoHABs is promising, data need to be

collected more frequently and for consecutive days in order to develop accurate models to predict toxin concentrations at freshwater lake sites.

Best Practices in the Use of Micropipets

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The results of this study demonstrate that even minor variation in the operating technique of handheld air-displacement pipets can result in measurable errors in accuracy and precision. This study did not evaluate errors resulting from combining multiple of the discussed technique variations, although this is commonly observed in the field. Compounded errors can easily reach 12%, and are often even larger, as data from field surveys suggest. The following steps will ensure the most accurate and precise results: - Prewet tips at least three times - Use proper pipetting mode - Work at temperature equilibrium - Immerse tips to proper depth - Aspirate with pipet in vertical position - Pause after aspirating - Do not touch vessel wall during or after aspiration - Use consistent plunger speed and pressure - Minimize heat transfer from hands - Avoid tip wiping - Examine tip prior to dispensing - Use high-quality pipet tips

Culture-independent method for source determination of fecal wastes in surface and storm waters using reverse transcriptase-PCR detection of FRNA coliphage genogroup gene sequences

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A method, incorporating recently improved reverse transcriptase-PCR primer/probe assays and including controls for detecting interferences in RNA recovery and analysis, was developed for the direct, culture-independent detection of genetic markers from FRNA coliphage genogroups I, II & IV in water samples. Results were obtained from an initial evaluation of the performance of this method in analyses of waste water, ambient surface water and stormwater drain and outfall samples from predominantly urban locations. The evaluation also included a comparison of the occurrence of the FRNA genetic markers with genetic markers from general and human-related bacterial fecal indicators determined by current or pending EPA-validated qPCR methods. Strong associations were observed between the occurrence of the putatively human related FRNA genogroup II marker and the densities of the bacterial markers in the stormwater drain and outfall samples. However fewer samples were positive for FRNA coliphage compared to either the general bacterial fecal indicator or the human-related bacterial fecal indicator markers particularly for ambient water samples. Together, these methods show promise as

complementary tools for the identification of contaminated storm water drainage systems as well as the determination of human and non-human sources of contamination.

Geostatistical prediction of microbial water quality on an urbanizing inland stream network

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B. Everett Jordan Lake serves as a drinking water supply in the rapidly urbanizing Research Triangle Area of central North Carolina. Its watershed is primarily impacted by nonpoint source (NPS) pollution. Pathogen contamination, much of which derives from NPS, is the primary cause of impaired surface waters nationwide. North Carolina uses fecal coliform bacteria (FC) as a proxy for pathogen contamination, limiting the acceptable FC concentration to 200 cfu/100mL. Measuring FC is expensive and time consuming, limiting its use in managing NPS pollution, which by nature may differentially impact all portions of a water body. Additional tools are required to assess water quality for water bodies in their entirety. Geostatistical models offer a promising approach to monitoring microbial water quality under the limited sampling framework currently used. Under a geostatistical model, the space/time autocorrelation in sparse sample data is harnessed to predict water quality outcomes at unmonitored locations and times. Predictions are augmented by incorporating estimates of the associations between the water quality outcome and potential covariates, such as land use. The geostatistical approach presents a powerful tool not only to estimate contamination at unmonitored locations for monitoring purposes, but also to investigate the conditions and locations associated with elevated contamination to inform targeted remediation strategies. We fit geostatistical models to fecal coliform concentrations measured in bimonthly dry-weather samples collected over the course of one year from fifteen sites on a stream network in the Jordan Lake watershed. Additional FC measurements were collected during three storm events and from the EPA STORET database at 8 additional sites. We estimated the space/time autocorrelation from the FC observations with two approaches: as a function of Euclidian distance between observations, and as a weighted moving average based on distance along the stream network between the points, designated the "tail-up" model. The "tail-up" model accounts for the possibility of higher correlation between flow-connected points on a stream than points closer in space but not flow-connected. We used generalized least squares to estimate association between FC concentration and land cover characteristics of the upstream watershed at each sample location and the meteorological conditions (e.g., precipitation, temperature) at each sample time. All spatial, temporal, and linear predictor parameters were estimated by maximum likelihood; the covariates included in the final model were chosen through backwards selection by minimizing AIC.

The final model accounted for 59% of the total variance in FC concentration, with the greatest contribution provided by the linear predictors. The "tail-up" covariance function explained 2% of the variance, the Euclidean covariance function 1%; of the remaining 56% of variance explained, meteorological covariates expressed the strongest association with FC. Forest cover,

negatively associated with FC, was the only land cover covariate retained in the final model. Prediction at unmonitored times was weakened by limited temporal covariance range at two days. For certain sample days, however, predictions across the stream network demonstrate different trends in contamination than would be predicted by the covariates alone, suggesting the value of spatial interpolation for monitoring.

Testing Pathogen Reduction in Deployed Composting Toilets

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Pathogen transmission is a major concern when implementing sanitation systems in resource poor areas. Multiple studies have shown that ecological sanitation systems are an effective means for improving health in areas where standard systems cannot reach disparate populations. Emerging designs of ecological sanitation systems provide needed access to hygiene but they still require confirmation of their effectiveness in reducing pathogen loads in the waste and transmission of disease. A novel composting toilet design is being tested using an auger system to mix and transport feces and a cover material in an attempt to incorporate critical elements to accelerate pathogen die off. Test units have proven to be popular among recipients. This highly economical composting system has been successfully deployed into 11 different communities with a total of 98 toilets in Ecuador that previously had no improved sanitation options. A subset of active composting toilets were monitored for 2 months, taking samples at different points in the treatment line to determine the presence and quantity of total coliforms, E.coli, helminth ova and Salmonella spp. Samples were used to determine pathogen die-off through the treatment line according to time, pH, temperature and moisture content. Using linear regression extrapolation we found that total coliforms and E.coli reduction were primarily dependent on compost storage time. Treatment within the reaction chamber and other environmental factors did not significantly contribute to indicator reduction. Treated compost directly out of the reaction tube showed minimal reductions in indicator levels, and were above acceptable delineated by EPA biosolids class B and WHO guidelines for reuse in agriculture. However, significant reductions were found after being stored for varied periods of time, as recommended by system developers. Some Helminthes were also present in the treatment line and at some endpoints for some of the study's toilets. Salmonella spp. were not found in samples throughout the treatment line. Under the current deployment and usage practices, these composting toilets will require additional treatment and extended storage time prior to beneficial use of the compost as a soil amendment. Further development of the technology to improve environmental conditions in the treatment line could change pathogen survival patterns and corresponding recommendations for compost use.

Understanding the Role of Soil in Zoonotic Pathogen Transmission and Water Contamination in Cameroon using One Health Approach

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Even though both soil and water pollution are widely recognized as major environmental issues worldwide, less widely studied are interactions between pollutants in soil and water. Surface water can be contaminated with microbial pathogens chemicals, that are potential risks to human health, when these tainted contaminants come into contact with resource water by storm water, agricultural runoff, soil erosion, or human activities. Soil from agricultural land that is contaminated with pesticides, fertilizers, and animal wastes contributing to severe water pollution. This contaminated soil is a potential pollutant source of pollutants in water and has an effect on both ecological and public health. Although there is a limited understanding of the magnitude of external sources of contaminants in water, the quality of water, as it relates to soil source, is sparse. The role of soil, especially zoonotic pathogen transmission and reservoir, is hardly investigated in developing countries where human and animals are in close proximity. The objective of this study was to determine the extent of fecal and pathogen contamination of soil in rural areas in Cameroon. Soil samples (N=170) were collected from three different areas; livestock range (cattle), residential districts, and vacant areas. Molecular detection was performed to measure ruminant fecal marker, Rum2Bac; human-associated fecal markers, HF183 and <cite>gry</cite>B; tetracycline-resistance gene, <cite>tet</cite>Q; <cite>Campylobacter</cite> spp.; <cite>Salmonella</cite> spp.; <cite>Arcobacter</cite> spp.; and Shiga-like toxin producing gene, stx1 and stx2 using a real-time quantitative PCR (qPCR) assay. We detected each target gene (Rum2Bac 42.3%, HF183 4.3%, <cite>gry</cite>B 23.3%, <cite>tet</cite>Q 9.2%, <cite>Salmonella</cite> spp. 19.9%, <cite>Campylobacter</cite> 4.9%, <cite>Arcobacter</cite> 4.9%, stx1 11.7%, and stx2 6.1%) in soil. The concentrations were 2.9 x 10⁴ - 4.4 x 10⁷ (Rum2Bac), 5.8 x10 - 3.5 x 10³ (<cite>gry</cite>B), 1.1 x 10² - 2.2 x 10³ (<cite>tet</cite>Q), 2.2 x 10² - 3.4 x 10³ (<cite>Salmonella</cite> spp.), 8.4 x 10³ - 5.0 x 10⁴ (<cite>Campylobacter</cite> spp.), 8.3 x 10 - 3.1 x 10² (<cite>Arcobacter</cite> spp.), 1.3 x 10⁴ - 6.3 x 10⁵ (stx1), and 1.3 x 10⁵ - 1.9 x 10⁶ gene copy numbers/g of soil (stx2). Rum2Bac had substantially higher positive detection frequencies. This study suggests that soil plays an important role as a reservoir of pathogens and in transmission of zoonotic pathogens between animals and humans. Thus, soil contamination should be considered to protect public health and drinking water quality in developing countries where human and animals reside together. Further work, in-depth examining the relationship between soil and water quality, will be warranted to better understand their interactions and impact on water quality.

Survival and Transport of Salmonella in Soil Microcosms

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Nearly half of foodborne illnesses in the United States can be attributed to produce consumption. Produce safety studies have largely focused on harvesting and packaging processes, with fewer studies exploring the role of field conditions, even though irrigation water has been associated with large outbreaks. In agricultural settings that rely on surface water for irrigation, understanding the pathways of microbial loading into surface water is integral to the prevention of produce contamination. In our ongoing research in southern Georgia, we have regularly detected Salmonella in irrigation ponds, but the source and mechanisms of surface water contamination in this area are unclear. Studies have shown that soil moisture content has the potential to influence microbial persistence and to modify contaminant dispersion rates. It is also known that precipitation can impact surface water quality but research on the nature of this relationship has been inconclusive; some studies find a positive association between precipitation and pollution, while others find a negative association due to dilution by constant water flux. Moreover, the impact of antecedent conditions prior to heavy rainfall events is also unclear. To investigate how antecedent precipitation modifies the impact of heavy rainfall events on microbial fate and transport, we constructed soil microcosm columns using soil collected on farms near Tifton, GA. Microcosms were filled with autoclaved soil (30g/microcosm) and inoculated using Salmonella recovered from irrigation ponds in the study region. We established two treatment groups: no watering (dry) or watering reflecting precipitation conditions above the 90th percentile in Tifton (wet). Our preliminary research has demonstrated the persistence of Salmonella in microcosms for more than 50 days, as well as the potential for growth during this time. In one experiment, in microcosms inoculated with 6.44 MPN/microcosm, culture-based MPN methods of Salmonella enumeration indicated an increase of 1-2 log10 MPN/microcosm in 4 days in wet microcosms and a decrease in Salmonella concentrations to below the lower limit of detection (1.045 MPN/microcosm) in 2 days in dry microcosms. These preliminary results demonstrate that soil moisture content may influence Salmonella survival in soil. These microcosms will be employed to better calculate differences in Salmonella growth and decay depending on soil moisture conditions. Through ongoing simulations of heavy rainfall following periods of varying precipitation levels, we will determine the impact of antecedent precipitation conditions on the efficiency of Salmonella survival and transport through soil during heavy rainfall events.

Fecal indicator bacteria in riverine sediments and overlying waters before and after a storm event

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Studying the fate and environmental reservoirs of fecal indicator bacteria (FIB) in the aquatic environment is important to identify the potential transmission routes to humans, determine the sources of pollution and identify those processes able to influence bacterial persistence and spread. Storm events can mobilize enteric bacteria from either point (wastewater treatment plants) and non-point sources (animal waste) to the receiving water bodies. While the significant influence of stormwater runoff in waters is known, the potential storm effect on fecal indicator bacteria has been less studied in the sediment reservoir. In shallow areas, storms can transfer animal and human wastes to the receiving water body and, following sedimentation, also to the sedimentary environment. At the same time, runoff can determine sediment re-suspension and lead to mobilization of FIB residing in sediments toward overlying waters. We determined the abundance of Escherichia coli and enterococci, along with the main environmental variables, in surface waters and sediments prior to, and after a storm event in six freshwater sites in Brisbane, Australia. The sites were located along a putative gradient of fecal pollution, including areas upstream and downstream of a wastewater treatment plant. Samples were collected following a dry period (no rain fall in the days prior to sampling) and after a storm event. Isolates were further characterized by identifying phylogenetic groups and cryptic clades (for E. coli), and speciation (for enterococci). The results showed the existence of a diffuse sediment reservoir across the study area, with peaks up to 1.5 x 10^4 CFU g-1 for E. coli and 2.6 x 10^4 CFU g-1 for enterococci, which points to the need of considering the sedimentary compartment to reliably assess or predict fecal pollution. Storm activity caused a general increase in FIB abundance in both water and sediments, suggesting the likely existence of strong linkages between the two compartments. The results are discussed on the light of their potential implications for the management of water bodies at the catchment scale.

The Affect of Pause Time on the Efficiency of Light Biosand Filters in Siem Reap, Cambodia

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Worldwide, approximately 780 million people lack access to any improved water source, leading to 1.6 million deaths annually due to microbial associated diarrheal disease. Sadly, 90% of these deaths occur in children under five years of age. In Cambodia, 40% of the population

does not have access to safe drinking water, however it has been shown that Point of Use (POU) water filtration systems, including biosand filters are an effective way to make water safe to drink. Biosand filters have been shown to remove approximately 98% - 99% of bacteria, including pathogens that cause diarrheal disease. In Siem Reap, Cambodia, thousands of these biosand filters have been installed in remote Cambodian villages. These filters have been shown to decrease the number of Escherichia coli cells (an indicator species of fecal contamination in water) to a safe level. The filters currently being installed are made of concrete, which makes installation nearly impossible in the floating villages of Cambodia. An alternative to the Concrete Biosand Filter (CBSF) is a lighter, PVC based filter, (light biosand filter: LBSF). In January 2013, a group of researchers from Bridgewater State University in Bridgewater, Massachusetts traveled to Siem Reap, Cambodia to begin testing the efficiency of these new LBSFs. Water samples were collected from the Siem Reap River, a known microbiologically contaminated water source, and treated by HBSFs or by LBSFs. Overall, the LBSFs did not perform as well as the HBSFs, however they did decrease the amount of biological contaminants, indicating the potential for these filters to be installed in remote and floating villages, providing safe drinking water for families living there. This past June, we again traveled back to Siem Reap, Cambodia. Specifically, this year we assessed how pause time, or the time the water remains in the filter prior to being collected, affects the efficiency of these LBSFs. Again, water samples were collected from the microbiologically contaminated Siem Reap River. Water was left untreated (control), or treated by LBSFs. Water was allowed 1, 2, and 4-hour pause times. Treated water was then filtered onto cellulose acetate filters (Millipore) and the filters transferred to the coliform-selective, E. coli differential media, RAPID' E. coli 2 (BioRad). Plates were run in triplicate, incubated at 44.5_C for 22 - 25 hours, colonies counted and colony forming units (CFUs) per 100 milliliters (mls) of water calculated from each of the three treatments. Data was analyzed using the Independent t-test to assess for statistical differences between the pause times. While the HBSFs outperformed the LBSFs at the 1 and 2-hour pause time, the LBSFs performed equally as well as the HBSFs after a 4-hour pause time. Future work will entail assessing the shortest pause time that will allow LBSFs to perform as well as the HBSFs in removing biological contaminants.

Exposure to human source fecal indicators and self-reported illness among bathers at recreational beaches

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Introduction: Although indicator microorganisms have been used to indicate the presence of fecal pollution in water and assess associated health risks in recreational waters, few studies have examined microbial indicators that can distinguish human fecal contamination. Our objective was to estimate associations between human source fecal indicator organisms and self-reported illness among bathers at recreational beaches in the United States. Methods: We

used data from 20,133 beach-goers enrolled in the National Epidemiological and Environmental Assessment of Recreational Water study in 2002-2009. Participants were surveyed about beach activities, water exposure, and baseline symptoms on the day of their beach visit, and health symptoms they experienced 10-12 days after the beach visit. Up to 18 water samples per day were collected and tested for human-specific Bacteroides spp. (HF183 TaqMan, BsteriF1, Bunif2, HumM2) using quantitative polymerase chain reaction (qPCR). We classified these human indicators in several binary categories according to the proportion of samples per day in which the indicator was detected: ≥50%, ≥80%, and ≥90%. Adjusted incidence odds ratios (aIOR) and 95% confidence intervals (CI) for the indicator-illness associations among bathers who immersed their bodies to the waist or higher were estimated using logistic regression. Robust standard errors were calculated due to clustering within beach and household. Results: Human-associated Bacteroides spp. was detected in 2% to 64% of samples per day, and varied by beach and assay used. In general, the greater the proportion of samples per day in which human indicators were detected, the more positive a trend for gastrointestinal and respiratory illness in the 10-12 days after bathing. Of the four indicators, the most precise estimates across all health outcomes were for Bacteroides uniformis, followed by Bacteroides stericoris, and the least precise were for HumM2. We found plausible positive associations with diarrhea on days when ≥80% of samples indicated the presence of Bacteroides stericoris (aIOR=1.28 (1.01, 1.62)) and with respiratory illness among bathers exposed to Bacteroides uniformis (aIOR=1.28 (1.02, 1.60) or to any of the four indicators (aIOR=1.27(1.02,1.57)). These estimates were among the most precise. Conclusion: In these preliminary analyses, we found plausible evidence that exposure to human source fecal indicator organisms are positively associated with self-reported diarrhea and respiratory illness. The most precise indicator, Bacteroides uniformis, produced the strongest associations for respiratory illness, while Bacteroides stericoris produced the strongest associations for diarrhea. Future work will explore additional ways of classifying exposure and the joint effect of these human assays with conventional markers of fecal contamination. Disclaimer: This is an abstract of a proposed presentation and does not necessarily reflect Environmental Protection Agency policy.

Strengthening the Capacity of Households and Communities for Improved Water, Sanitation and Hygiene: Water Testing Experiments with School Children and Adult Household Members in Ghana

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About 1.8 billion of the world's population consumes water from highly contaminated sources. Level of fecal contamination of water differs between point-of-source (POS) and point-of-use (POU). Providing water quality information to households is known to improve water, sanitation and hygiene behaviors and reduction of diarrheal diseases. Studies in which water quality information is disseminated to randomly selected households potentially underestimate

the impacts, missing the potential learning experiences from household self-water testing and also missing the most effective channels in the delivery of such information to the treatment groups. We conducted water testing experiments in southern Ghana (Greater Accra region) in which students in public basic schools, and adult household members were randomly assigned to receive water testing kits and water quality improvement messages. Selected participants were also trained on the use of water testing kits in testing for E. coli; an indicator bacteria of fecal contamination of water. Baseline orthogonality tests are used to check the similarities and differences between the intervention groups (clustered randomized design). Difference in difference (or comparison of means) estimators are analyzed by gender and type of participants (students vs adult household members). Robustness checks and sensitivity analysis are performed by testing for heterogeneity in treatment effects in order to limit false predictions/estimates. Short-run program effects are estimated for a wide range of outcome variables including water sources, diarrheal diseases, water transportation, and handling and storage techniques, among others. Demand for water testing (measured by participation rate) was high for students' intervention group compared to adult household members group. Participation rate (used to proxy demand) was slightly higher for females compared to males.

Modeling Household's Decisions on Water Supply and Sanitation in Greater Accra Region of Ghana

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Over the past decades (more especially since 2000), access to improved water supply and sanitation services in developing countries (including Ghana) have been widely discussed in the media, and in several conferences, meetings, workshops and seminars. This situation has assumed global prominence due to the disproportionate achievement of water and sanitation-related Millennium Development Goals (MDGs) between developed and low resource countries, and also increasing morbidity and mortality as a result of poor water and sanitation environment. There is the need for additional studies in understanding household decisions and choices in improved water supply and sanitation services; both as a separate and joint-decision making options. This study is based on household survey conducted in April-May 2014 with 505 randomly selected households in two districts in the Greater Accra region of Ghana. Logit, multinomial logit, bivariate probit, and ordered probit models are used to estimate the choice probabilities across improved water supply and/or improved sanitation options. The dependent variables are based on WHO and UNICEF's Joint Monitoring Program indicators/measurements (including "drinking-water ladder" and "sanitation ladder"). The results show significant differences in choices of improved water supply and sanitation services. Moreover, the choice and use of improved water supply decreased consistently from main-drinking water to secondary general purpose-water sources. The econometric estimations indicate several socio-economic factors influence the choice and use of improved water supply and sanitation services; both as a joint and separate decision making options in Greater Accra region of Ghana.

Comparative Genomics of *Rhodococcus opacus* Strain M213 Reveals Potential for Bioremediation and Lipid-Biofuels Production

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The genome of *Rhodococcus opacus* strain M213, isolated from a fuel-oil contaminated soil, was sequenced, assembled and annotated which revealed a total genome length of 9,194,165 bp encoding 8680 putative genes, and a G+C content of 66.72%. Among the protein coding genes, 71.77% occurred as COGs (clusters of orthologous groups of proteins), and 55% of the protein coding COGs were present as paralog clusters. Furthermore, 22.5% and 20.7% of protein coding genes were connected to KEGG and MetaCyc pathways, indicating the presence of a cohort of genes that likely form new enzyme/other bioactive compounds. Moreover, strain M213 has a strong potential for both, bioremediation and lipid-biofuels production because it contains 284 genes for biosynthesis of terpenoids and polyketides and other secondary metabolites such as caffeine, flavonoids, indole, isoquinoline, and alkaloids along with 300 genes for lipid biosynthesis and metabolism, including unsaturated and saturated fatty acid, making it a lucrative candidate for the industrial production of biofuel precursors and bioactive steroids. Genome-wide comparative analysis with 43 whole genome sequences of other rhodococcii showed that strain M213 did not have a high level of synteny with other taxonomic relatives including *R. opacus* strain B4 and *R. opacus* strain PD630; rather strain M213 aligned more closely at the functional level with the catabolically versatile *Rhodococcus wratislaviensis* strain IFP 2016, *R. imtechensis* RKJ300, *Rhodococcus* sp. JVH1, *Rhodococcus* sp. DK17 and *R. jostii* RHA1, as revealed by a hierarchical clustering of orthologous groups of proteins (COGs) analysis. A total of 1361 genes, representing 16% of the genome, were found to be unique to strain M213; many of these genes possessed metabolic functions. Moreover, as many as 154 genomic islands (GEIs), many with catabolic genes, particularly for PAHs biodegradation, were found within the genome of strain M213 that were likely acquired via relatively recent acquisition, loss, and evolution of catabolic genes. Identification of clustered regularly interspaced short palindromic repeat [CRISPR] genes and high number of GEIs further provided clues on the genome plasticity of this soil isolate, brought about by alteration in the strain's genome by horizontal gene transfers (HGT), bacteriophage attacks, and genetic reshuffling during its evolutionary trajectory. Overall, we show that diverse genetic and metabolic traits possessed by strain M213 such as the cohort of plasmid-borne biodegradative genes, likely enhances its ecological fitness in a complex soil habitat where survival is mainly via exploitation of toxic hydrocarbons.

Potential for small solar water pasteurizer techniques for water disinfection in rural area of developing countries

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Abstract Limited access to safe drinking water has always been a threat for people living in rural area. Millions of children in developing countries die every year due to lack of access to safe water and sanitation. There are variety of methods and technologies to purify water. Many organizations involved to solve this issue however unstable economic, social and political situation are the main obstacles for impressive progress in rural area of developing world. Attempt on this paper is to evaluate the feasibility of application of inexpensive, single-family use of solar water pasteurizer as a reliable and worth to invest technology for social entrepreneurs.it can easily meet the demand for clean water in rural area of developing countries. The potential users for this technology are mainly those living in villages or low-density populated area of developing countries, those who live on with less than $2 a day. Demographic, economic, social factors and water supply and demands are analyzed to prove the potential need of this technology for Cambodia as a representative for this study. Keywords developing countries, Cambodia, solar water pasteurizer, water, poor people

Scientific Basis for Defining Microbiological Health of Chicago River System

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The Chicago Area Waterway System (CAWS) is an urban waterway system consisting of man-made canals and modified natural streams. The flow in the CAWS is predominated by secondary treated effluent from the three largest water reclamation plants operated by the Metropolitan Water Reclamation District of Greater Chicago (District). The District has undertaken a systematic scientific approach as a basis to define the CAWS microbial community. This approach, which includes a comprehensive water-quality monitoring program supplemented with microbial risk assessment and epidemiology studies have played a key role in water quality management and policy decisions. Further facility improvements are underway which are still changing the dynamics of water management in the Chicago metropolitan area. These improvements include effluent disinfection and storm water capture systems, aimed to protect water quality. This paper contains three parts: I. Description of the CAWS; II. Summary findings of the CAWS published public health studies; and III. CAWSMicro: Seven-year microbial source tracking project. The CAWSMicro research project was launched in collaboration with Argonne National Laboratory to characterize the microbiome of the CAWS using metagenomic

approaches. The CAWSMicro project will capture microorganism's taxonomic information to assess the 'microbial health' of the CAWS before and after effluent disinfection is implemented and after the storm water reservoir projects are operational to assess the relative impact of these improvements. The discussion will include the methods used in the study. The genomic DNA from water and sediment samples are analyzed using two approaches: 1) amplicon sequencing: the 16S rRNA genes through next generation sequencing (Illumina miseq), that gives information about the taxa present ("Who is there"); 2) shotgun metagenomics: the sequencing of the pool of genome fragments of the environmental sample that informs about the functional potential of the community ("what these organisms might be doing").

Streamlining viral concentration methods

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The primary goal is to validate a simple method that can be applied for tracking sources of human fecal contamination in places like bayous, where bacterial method may not be appropriated. For this work, we streamlined concentration methods and used pre-made solutions, thus reducing sample manipulation. The final concentrated samples should be compatible with infectivity assays and be archived for further analysis or purifications in case of high inhibitors samples. This study builds in previously published work on the use of 8 _m pore size electronegative filters for concentration of adenovirus (Wu et al J Applied Microbiology 114: 1332-1865). The nitrocellulose electronegative filters HA (0.45 _m) and SCWP (8.0 _m) were evaluated. 300ml of tap water sample was supplemented with concentrations of MgCl2 of 2.5 mM to 5.0 mM using a solution of 1M MgCl2. Filters were washed with a 0.85% NaCl solution (pH 3.00) and eluted with 10mL of 1.5% beef extract supplemented with 0.05 M of glycine and pH of 9.5. Initial testing was performed with phage MS2 and de-chlorinated tap water. For the HA (0.45 _m) filters, better recovery was observed with water samples supplemented with 35 mM of MgCl2 and recovery efficiency of 47% (n = 5) and for the SCWP (8.0 _m) with water sample supplemented with 50 mM of MgCl2 and recovery efficiency of 85.6% (n = 5). Different secondary concentration steps are being evaluated in order to further decrease the final concentrated volume to 0.5 ml. Preliminary experiments with PEG using a 2X solution (12% PEG and 0.6M NaCl) for a final concentration of 6%PEG and 0.3M NaCl yielded recovery efficiency of 12.2%. Different PEG conditions are being evaluated in order to improve recovery efficiency. The final conditions will be evaluated. Final protocol condition will be evaluated with different human viruses, such as adenovirus, enterovirus, and polyomavirus and using different types of water.

Simple, Low Cost and Ready-to-use microbiological test platform

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Introduction: Advancements and trends in food and water microbiology include easier-to-use, zero preparation, and robust methods that make it simpler for non-microbiologist and field personnel to perform quicker real time assessments of microbial hygiene and quality. Peel Plate is a new traditional microbiological test platform that uses a 47mm shallow plastic dish with adhesive cover with a self-sample-wicking medium to produce color quantifying and distinguishing colonies. The method uses a 1mL sample but can used with 47mm 0.45um mixed-acetate filters to inexpensively test 100mL sample sizes. Visual colonies are easily picked and transferred into more selective and identifying medium. Purpose: This study evaluated two Peel Plate methods, E. coli/coliform and heterotrophic plate count, with various environmental, municipal and bottled water samples. Methods: Peel Plate EC (E. coli/coliform, Charm Sciences, Inc.) produce red coliform colonies with β-galactosidase enzyme activity on Sal-gal substrate and blue/purple/black colonies with β-glucuronidase enzyme activity on X-glu substrate. Peel Plate HET (Heterotrophic count, Charm Sciences, Inc.) produce red colonies with dehydrogenase enzyme activity on triphenyl tetrazolium chloride (TTC) indicator. Various natural water samples spiked with coliform and E. coli were tested (n=10 replicates) for 24 hours at 35_C in comparison to reference methods using three different microbial levels spanning 1-2 log. Irrigation water from a mixed surface/ground source was compared to EPA Method 1604 for coliform and E. coli. Commercial bottled water and neutralized lettuce flume water was compared to FDA BAM Chapter 4, m-endo agar method for coliform. Various municipal water samples were tested for heterotrophs using Peel Plate HET, R2 agar and heterotrophic agar at room temperature for 5 days. Results: E. coli/coliform results were compared to reference methods using a paired t-Test analysis. E. coli and coliform results were not significantly different as defined by log mean difference 95% confidence intervals outside of _0.5 log. At most natural and spike concentrations mean log differences were less than 0.1 log with relative standard deviations less than 5% of count. Irrigation water samples contained some natural pink pigment producing non-coliform that could be distinguished by trained eye. These non-coliform can eliminated by rehydrating a 5 ug/mL cefsulodin reagent with sample that suppresses non-coliform growth. Heterotrophic plate counts were similar and mainly consistent with the other reference methods which also displayed some differences depending on water samples. Significance: The Peel Plate methods showed consistency and equivalence with conventional reference methods for E.coli/coliform and heterotrophic plate count. The methods could be useful as process indicators for disinfection treatment and as general sanitation/hygiene indicators in water assessment, production and management.

Biogeographic patterns of Escherichia coli, Enterococcus spp., and Bacteroidales fecal indicator bacteria at a Villa Angela and Euclid Creek Beach, Ohio

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Introduction: Fecal contamination of freshwater beaches in the Great Lakes is a public health risk and results in closure of these beach waters for swimming. Villa Angela (VA) and Euclid Beach (EUB) near Cleveland, Ohio have a history of high fecal indicator bacteria (FIB) contamination leading to beach closures during the summer. Euclid Creek (EUC), which drains through a populated residential area, is likely more influenced by human contamination and enters Lake Erie immediately north of the VA beach; it is hypothesized that EUC acts as a FIB contamination point source for both VA and EUB beaches. Methods: Five locations were selected to evaluate freshwater FIB contamination levels in the VA and EUB beaches and EUC. Due to historic inconsistency of results between different FIBs, diverse indicator analytes (beyond traditional FIB) were employed to paired samples to reveal biogeographic patterns of contamination between different sites. Traditional FIB levels of Escherichia coli (E. coli) and Enterococcus spp. and an alternative fecal indicator, Bacteroidales, were determined using corresponding United States Environmental Protection Agency (EPA) methods. Results: The highest levels of E. coli, Entercoccus spp., (by both chromogenic substrate and membrane filtration) and Bacteroidales were found in EUC with averages of 1.61 x 10^3 (EPA 1603), 1.63 x 10^3 (EPA 1600), and 1.96 x 10^5 (EPA Method B) per 100 mL, respectively. At bathing beaches, the highest average traditional FIB and Bacteroidales were observed at EUB East and VA East, respectively. Overall, beaches exceeded freshwater FIB threshold values 48.0 and 24.5% of the days sampled for E. coli and Enterococcus spp., respectively. Relationships among all indicator bacteria were examined at each location to determine if correlations existed. FIB levels were more related among beach sites but not to EUC data. Conclusion: FIB contamination at both VA and EUB beaches, and EUC showed elevated FIB concentrations for traditional indicators, often exceeding established EPA water quality standards. Bacteroidales were most prevalent in the creek water and varied among beach sampling sites. EUC data and nearby beach data were not strongly related, indicating that beach water FIB may be more influenced from diffuse contamination than the creek.

Neural Networks as a Tool in Microbial Water Quality Analyses: Two Case Studies Neural Networks as a Tool in Microbial Water Quality Analyses: Two Case Studies

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Although neural networks are a promising technique for modeling complex, non-linear problems, applications in the context of microbial water quality are still uncommon. We apply neural network-based solutions to two significant challenges in microbial water quality data analyses: (i) fitting dose-response curves; and (ii) predicting pathogen occurrence in water bodies based on measured indicator data. We assess the performance of neural network-based models in terms of statistical goodness-of-fit metrics and report the models' performance relative to conventional methods. Both case studies rely on real-world data reported in previous publications.

Artificial neural networks draw from the design of biological neural systems with system inputs and outputs connected by a complex, multi-layer web of nodes. As a data modeling technique, neural networks are essentially models that are weighted and mathematically transformed combinations of many simple processing units. While it is difficult to communicate the relative influence of input variables on the final outcome and an explicit functional form with neural networks, an advantage of the framework is that it makes no assumptions about underlying data distributions or about the covariance between predictor variables. The method is also attractive when there is no strong theoretical basis for an underlying mathematical or mechanistic model.

Our case studies cover both ends of the spectrum in terms of the presumed suitability of "black-box" methods such as neural networks. For dose-response curve fitting, neural networks appear to be an awkward choice given (i) the widely accepted theoretical basis for probabilistic processes such as mixing and infectivity and (ii) the practical advantages of generating a curve described by an explicit mathematical function for use in downstream applications such as QMRA. Despite this, we thought it would be interesting to compare the predictive power of neural networks compared to traditional methods as well as instructive to study its low dose predictive behavior. For predicting pathogen incidence in ambient water, on the other hand, the lack of an a priori mechanistic theory as well as the non-parametric distribution of predictor variables implies that neural network models may well be a sound choice.

In the first study, we used pathogenic human challenge data to design a neural network-based dose response curve. We tested single and multiple layer neural networks with both linear and sigmoidal transformative functions and chose the most appropriate network based on statistical criteria. We compare our results to those based on conventional curve fitting.

In the second study, we predict the incidence of pathogenic viruses in ambient waters using a matrix of simultaneously measured water quality indicator data measurements. We assess the performance of models based on single indicators versus multiple indicators and compare our best-fit neural network model to a linear model with the same predictors.

We conclude that neural networks are a potentially valuable tool in microbial water quality assessments that are well suited to meeting multiple emerging analytical needs. We foresee that machine learning- based methods such as neural networks will be of increasing relevance

as measured data grows rapidly and scientists and regulators require predictive models combining diverse predictors in real time.

Capture and concentration of waterborne parasites - current limitations & opportunities for risk assessment

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Several protozoan parasites of public health significance have the potential to be transmitted by the waterborne route. Understanding the importance of the waterborne route of infection, particularly of Cryptosporidium spp. and Giardia spp. requires analysis of water samples for the presence of these parasites. Robust quantitative data are required e.g. for surveillance programs, outbreak investigations, to assess performance of intervention measures and as input into risk assessments where the level of risk to a population is estimated. Due to the low infective dose, individual oocysts or cysts need to be isolated and enumerated. Generally, methods used to achieve this are represented in 3 stages: capture, concentration and detection. Typically methods need to be capable of capturing low numbers of the parasites in large volumes of water and isolate them from other organisms and debris present in the water concentrate. Standard methods, such as filtration and immunomagnetic separation (IMS), have been developed and are widely used across the globe. In recent years, numerous alternative techniques and approaches have been investigated to improve performance criteria including reproducibility, specificity, sensitivity, cost and ease of use. However, a common limiting factor with the majority of methods is poor recovery rates. These have been reported to be as low as 1 - 10% for raw water and 9 - 59% for artificially inoculated water samples. These low and inconsistent recovery rates have significant impact on the ability to accurately quantify levels of these agents in waters tested and this could lead to a gross underestimation of risk. In order to improve recovery rates, various groups have been modifying existing methods and developing new methods for both capture and concentration. This poster reviews various existing and developing methods for the capture and concentration of Cryptosporidium spp. and Giardia spp. from source waters and treated drinking water. These include filtration and non-filtration approaches, immunomagnetic separation, flow cytometry, selective immunocapture and microfluidic devices. Advantages and limitations of each for exposure/ risk assessment are discussed, together with a comparison of other characteristics typically used for selection in different applications.

An assessment of the risk of infection by Cryptosporidium parvum and Giardia lamblia from exposure to livestock waste and tubular digester effluent in Costa Rica

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Livestock manure contains recoverable nutrients for use in agriculture, but also can contain high concentrations of organic matter and pathogens. Small-scale tubular anaerobic digesters are promoted globally for treatment of livestock manure, because they treat the livestock manure, produce a renewable energy source from biogas and the effluent can be used as a fertilizer. Unfortunately tubular digesters do not significantly reduce concentrations of pathogens, specifically, Cryptosporidium parvum and Giardia lamblia. This study investigated the risk of infection from exposure to raw livestock manure and tubular digester effluents in two rural communities in Costa Rica using static quantitative microbial risk assessment (QMRA). QMRA provides an opportunity to quantify the impact of manure pathogens on public health, which can assist in developing region-specific risk management guidelines. The risk of infection from Cryptosporidium parvum and Giardia lamblia was assessed for occupational and public exposure pathways, fomite and soil contamination and crop contamination from runoff. Raw dairy cattle and swine manure was collected from 13 farms (22 pigs and 326 cattle) and sampled for the presence and concentration of Cryptosporidium sp. oocysts and Giardia sp. cysts. Since none of the pigs or cattle tested positive for Cryptosporidium sp. or Giardia sp. during the study period, reported concentrations of (oo)cysts in raw dairy cattle and swine waste from the literature were used to determine the risk of infection. Modeled effluent (oo)cysts concentrations from tubular digesters were also used to determine the risk of infection. There were three main contributing factors to the one-time risk of infection from both parasites at the different exposure pathways. The first was the difference in the concentration of (oo)cysts in the raw cattle and pig manure. At the three exposure pathways, the risks related to cattle manure were greater than swine due to the higher concentration of pathogens (3 - 7 orders of magnitude) typically in raw cattle manure compared to pig manure. Animal-specific risk management guidelines should be developed to reduce exposure to manures with high pathogen loads. Second, the inactivation rates at the various exposure pathways were the main contributing factor to the risk of infection. The risk of infection at all exposure pathways decreased with increasing inactivation rates. In addition, Cryptosporidium parvum posed a greater risk than Giardia lamblia in all exposure pathways due to Cryptosporidium parvum oocysts lower inactivation rates compared to Giardia lamblia cysts. Lastly, it was noted that in the community using tubular digesters to treat livestock waste, the risk of infection from exposure to contaminated soil and crops was significantly lower compared to the community where livestock waste was applied to soil untreated. For example, 7 days after a rainfall event, the risk of infection from consumption of food contaminated with Giardia lamblia was 5.19 x 10-7 when tubular digester effluent was used as a fertilizer and 7.61 x 10-1 when raw manure was used. This indicates that treatment of livestock manure in small-scale tubular digesters has the potential to significantly decrease the risk of infection below the acceptable individual annual risk of infection (10-4).

Toward a rapid enzymatic method for detection of coliform bacteria in water

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Enzymatic methods based on assays for β-D-galactosidase and β-D-glucuronidase activity have been widely used to detect coliform bacteria (total coliforms, fecal coliforms and Escherichia coli) in water and other environmental samples. However, the procedures and performance of these methods vary considerably with the choice of analytical methods and specific circumstances, such as water quality, with typical protocols requiring 18 to 24 hours to obtain results. In this study, the possibility to develop a fast, low-cost and reliable coliform bacteria detection method based on expression of enzyme activity was explored by testing a defined substrate medium, Colilert-18, under various experimental conditions, including different pH levels and temperature, lysing bacterial cells, and adding enzyme inducers. The reaction between enzymes in fecal bacteria and chromogenic substrates was found to not be detectable instantly, while cell lysis and enzyme inducers decreased the time to detection of activity. The results of this study provide information that can help users optimize enzymatic methods to detect target fecal indicator bacteria rapidly and thus tailor the procedures of candidate methods to achieve effective performance.