DRINKING WATER QUALITY MONITORING

by

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ABSTRACT

This dissertation involves two different studies. The first concerns the real-time detection of microbial contamination in drinking water using intrinsic fluorescence of the microorganisms. The prototype, “Blinky”, uses LEDs that emit light at 365nm, 590nm, and 635nm for ultraviolet, amber, and red light, respectively. At 365 nm, the cellular components excited include reduced pyridine nucleotides (RPNs), flavins, and cytochromes to distinguish viable bacteria; at 590 nm, the cellular components excited include cytochromes for non-viable bacteria; at 635 nm, the cellular components excited include calcium dipicolinic acid (DPA) for spores. By using these three different wavelengths, the prototype can differentiate between viable and non-viable organisms and also has the potential to detect spores. The aim of this study was to improve the detection limit by modifying the design of the instrument and to establish the detection limit of viable and non-viable bacteria and spores.

The instrument was modified by replacing existing LEDs with LEDs that had 50% more intensity. Two additional LEDs were added for amber and red light, bringing the total to four LEDs for each. The LEDs were also positioned closer to the photomultiplier tube so as to increase sensitivity. For UV, only two LEDs were used as previous.

The detection limit of the viable bacteria was ~50 live bacteria/L. No change in the intrinsic fluorescence below the concentration of ~10^8 dead bacteria/L was observed. The results for spore measurements suggested that most of the spores had germinated

before or during the measurements and could not be detected. The instrument was successful in detection of viable bacteria and also differentiating viable and non-viable bacteria. The instrument was not successful in detection of spores.

The second study was designed to assess the water quality of well construction in southeastern Tanzania. Three designs were tested: Msabi rope pump (lined borehole and covered), an open well converted into a closed well (uncovered well into a covered and lined well), and an open well (uncovered and may or may not be lined). The study looked at the microbial and chemical water quality, as well as turbidity. The survey included 97 water collection points, 94 wells and three rivers. For microbial analysis, heterotrophic plate count (HPC), total coliforms and *E. coli* tests were performed. Fifteen of these wells were further analyzed for microflora and diversity for wells comparison purposes, using culture methods, followed by polymerase chain reaction (PCR) and genome sequencing. Ten wells out of the fifteen were analyzed for calcium (water hardiness), potassium, nitrates, nitrites, chloride, fluoride, bromide, sulfate, iron, and arsenic. Two water collection points were also selected for organic compound analysis (gasoline components).

All samples tested positive for coliforms. Two samples tested positive for *Escherichia coli* for the lined borehole (Msabi rope pump) and four samples from closed wells. All open wells tested positive for *E. coli*. There was more microbial diversity in open wells than the closed wells and Msabi rope pumps. Potential bacterial pathogens were detected

in seven wells out of the fifteen examined. The wells that tested positive were one Msabi rope pump, one closed well; the rest were from open water sources. Open wells had high turbidity followed by closed wells. Msabi rope pumps had low turbidity comparing to the two wells designs. No traces of gasoline components were detected in any of the water sources. One well out of ten had high amounts of nitrates-nitrogen (> 10 mg/L). The results of this study showed that the Msabi rope pumps performed comparably to the closed wells in terms of microbial quality but performed better with regard to turbidity. The open wells performed poorly in terms of microbial water quality as well and turbidity. There was a statistical difference in HPC, total coliforms, *E.coli* numbers and turbidity between open wells, closed wells and the Msabi rope pumps. However, there was no statistical difference in HPC, total coliforms and *E.coli* numbers between the closed wells and Msabi rope pumps. Msabi rope pumps performed better in turbidity.

INTRODUCTION

Problem definition

Water is the most important resources for sustaining an ecosystem and to provide life support. However, contaminated water is one of the major causes of illnesses in the world (Snow, 1849). Since John Snow, the father of epidemiology, discovered disease-causing microorganisms in drinking water, scientists have been trying to either eradicate diseases caused by microorganisms or to monitor so as to ensure public safety and prevent disease outbreaks. In 1993, over 70 years later, one of the largest waterborne outbreaks occurred in the United States, the Milwaukee cryptosporidiosis outbreak (MacKenzie et al., 1994), which led to additional requirements for drinking water treatment. Waterborne disease outbreaks (WBDOs) associated with drinking water contamination has caused epidemics throughout history (Snow, 1849; MacKenzie et al., 1994; Hrudey et al., 2003). For an outbreak to be considered a WBDO, two or more people must experience a similar illness after exposure to water and there must be epidemiological evidence linked to the time and location of exposure to the water (Gunther et al., 2010). In the US, the Centers for Disease Control and Prevention (CDC) and the Environmental Protection Agency (EPA) have maintained a collaborative surveillance system to monitor for WBDOs.

Public water supplies involve the distribution system, consisting of millions of miles of interconnected pipes and storage facilities. From 1971-2006, 656 deficiencies occurred under the jurisdiction of public water supplies. These deficiencies included inadequate water treatment, cross connections, and cross contamination resulting in 610 outbreaks associated with acute gastrointestinal illnesses (Gunther et al., 2010). The size of the

pipes and the materials and other components used in distribution systems varies. As a result, corrosion, erosion, and pressure may occur at any point along the distribution system. These breaches may cause floods and mobilize potential pathogens into the distribution water system or may cause reduced pressure which causes backflow events (USEPA, 2010). Deficiencies such as cross connections and the backflow of contaminated water, faults in storage facility design, operation and maintenance, insufficient installation, repair, or rehabilitation practices, intrusion due to pressure conditions and physical gaps in distribution system infrastructure, and the formation of biofilms have all been associated with adverse health effects (USEPA, 2010; USEPA, 2002; Public Health Risk from Distribution System Contamination, 2006).

The reporting of these outbreaks is voluntarily and hence the true numbers are thought to be higher than the number actually reported (Levine et al., 1990; Blackburn et al., 2004; Craun et al., 2000). Between 1971 and 2006, the causes of outbreaks associated with drinking water distribution systems deficiencies still remained relatively unchanged (Gunther, 2010). Between 2007 and 2008, gastroenteritis illness was the dominant illness type, related to 61% of drinking water associated outbreaks. During this period, a total of 24% were a result of treatment deficiencies and distribution system failures (CDC, 2011).

Given the challenges that we still face today, more reliable technologies and timely responses are needed to ensure public safety. Hence, fast, rapid, low cost methods are needed to monitor drinking water in our distribution systems in-real time so as to alert the

public when drinking water has been impaired. Current methods do not allow us to make assessments quickly enough to ensure an immediate response when an outbreak occurs.

In the developing world, outbreaks occur frequently and are not properly documented, leading to a further underestimation of the true numbers and extent of the problem. Most of the causes of these outbreaks are related to poor distribution water system infrastructure, water treatment, and monitoring. Most people rely on streams, hand dug wells, and other sources of water that may be available to them. The lack of safe water availability in the developing world transcends health issues, and is also a strong indicator of human poverty and a lack of economic resources.

Prior to the millennium development goals (MDG), by 1990 51% of the global population lacked access to clean water. To date, there are 784 million people who do not have access to improved water sources and 2.4 billion people do not have adequate sanitation (WHO/UNICEF, 2010). An improved water source is defined by the WHO/UNICEF Joint Monitoring Program to be water piped to the dwelling or protected sources such as a dug well or protected spring. Across the globe, 1.8 million children die every year due to a lack of safe drinking water and adequate sanitation (UNDP, 2006). In Africa alone, 345 million people lack access to clean and safe water, causing 3.4 million to die each year. The 2012 report on the progress on drinking water and sanitation concluded that even though the MGD goals will have been met for half of the population without access to sustainable access to safe drinking water by 2015, over 784 million people will still be without access to improved drinking water sources (WHO/UNICEF,

2012). As for sanitation, the MGD progress has not been on task, with 2.4 billion people still lacking improved sanitation. If the current trend continues, it is estimated that by 2015, 605 million people will be left without access to improved sources of drinking water and 2.4 billion people will lack improved sanitation facilities (WHO/UNICEF, 2012).

Even though there is strong support from developed countries to help and try to close the gap by providing clean and safe water, this task has been moving slowly due to many obstacles and challenges. One of the challenges is a lack of infrastructure. Hence, the easiest and most practical thing to do is to provide a borehole or hand dug well. While this eliminates the need to travel long distances to fetch water, it does not always ensure water safety, especially since these water sources are often not monitored. Another challenge is the lack of water policies and guidelines in these regions. There are no clear guidelines or plans to be followed or built upon using collected knowledge from those on ground. Collective efforts and knowledge such as pilot studies for appropriate well designs, water monitoring protocols, and building capacity at the local and national level may help these regions move forward in fulfilling the MDG goals. Though a lot of money has been poured by the MDG to improve access to water sources in these regions, due to the disconnected efforts, it is unclear how local agencies, communities, and government can participate in order to help achieve these goals.

Having different organizations implement their own models introduces additional challenges for project oversight and monitoring. These obstacles and other economic

difficulties have further contributed to more difficulties in providing clean drinking water at a national level. In cases where protected water sources are provided, no screening for water quality is performed due to a lack of resources to perform any type of water quality screening.

Literature Review

1. Relevance and limitations of current water monitoring methods

It is difficult to detect microbial pathogens in water whether they are bacteria, viruses or protozoa. The detection of individual pathogens would be an expensive approach. Consequently, this approach would not result in a quick turnaround time that would help to ensure water safety and to make timely decisions. Therefore, indicator microorganisms such as total coliforms or fecal coliforms (e.g., *Escherichia coli*) are used. However, the microorganism has to meet certain criteria to be considered an indicator microorganism. Ideally, indicator microorganisms should be easy to detect and should not be found in natural water environments; hence their presence should be an indication of contaminated water. They should also be able to survive longer than enteric pathogens under similar conditions (Thomann and Mueller, 1987; Grabow, 1996; Hurst et al., 2002). The roles indicator microorganisms have played in assessing microbial water contamination are changing. Studies have shown that the presence of these indicators does not always correlate with the presence or absence of pathogens; these indicators also tend to be present in the environment (Winfield and Groisman, 2003; Savichtcheva and Okabe, 2006). Hence, none of the current indicator organisms meet all the ideal criteria (Cabelli et al., 1982).

Indicator microorganisms are detected using both cultural and molecular methods. The most common culture based methods include most probable number (MPN), membrane filtration (MF), and designed substrate technology (DST) methods. The most common

molecular method employed is polymerase chain reaction (PCR) assays such as Real-time PCR (qPCR). All these methods tend to be time and labor intensive, expensive, and generally only allow for the testing of a small volume of water relative to the total sample volume.

1.1 Cultural methods – MPN, MF and DST

The most probable number methods, membrane filtration, and designed substrate technologies are culturable methods used to detect and quantify indicator bacteria. These methods rely on certain metabolic activities specific to certain groups of bacteria. Cultural methods are well understood and have been used for a long time. Additionally, they have a demonstrated correlation with health risks (Cabelli, 1983; Cheung, 1990; Wade 2003) aside from protozoa and viruses (Shwartzbrod, 1991; Leclerc et al., 2000; Harwood et al., 2005). However, culture methods rely solely on the ability of the microorganisms to multiply (metabolic activities). The type of media and the temperature and incubation time determines the types of microorganisms that are able to grow. Culture media preparation and enumeration methods make the process labor intensive. Most of these methods also require confirmation steps. MPN tests involve the fermentation of lactose with the production of gas, while the MF test is based on the fermentation of lactose. Both methods take up to 48 hours excluding the confirmation tests.

Enzyme bases technologies such as Colilert® simultaneously detect coliforms and *E. coli* using two key nutrient indicators; ortho-nitrophenyl (ONPG) and 4-methyl umbelliferyl

(MUG). In the presence of coliforms, the solution turns yellow. The media turns yellow and fluoresces under UV light in the presence of *E. coli* (IDEXX Laboratories, 2013). By using a QuantiTray®, the numbers of coliforms and *E. coli* can be quantified. Even though the Colilert® method has a low detection limit, it still requires incubation for 24 hours to obtain a result (IDEXX Laboratories, 2013).

1.2 Molecular methods- PCR

Molecular methods rely on specific DNA and RNA sequences for the identification of the microorganism. One of the most common methods is PCR. This technique is an enzymatic reaction that amplifies copies of the targeted DNA sequence through a series of temperature cycles. The most commonly targeted sequence is 16S rRNA, which is a highly conserved region and is present in all bacteria. This enables the identification of the total community DNA extracted from the environment without culturing (Maier et al., 2009). Samples from the environment can be analyzed by amplifying the 16S rRNA, the PCR products are cloned, and the microorganisms are identified through sequencing and comparison to a DNA library database. PCR is faster than cultural methods and is very sensitive compared to most of the other methods currently being used. However, PCR suffers from a number of limitations which includes: 1) there is sometimes inhibition of DNA amplification caused by substances in environmental water samples, 2) only small volumes can be assayed (10 to 100 microliters), 3) it requires specific primers for each organism or groups of organisms, 4) it does not determine the viability of the organism, and 5) the recovery and purification of DNA from low numbers of microorganisms is problematic and difficult to quantify (Toze, 1999; Ashbolt, 2001).

Some steps may be undertaken to address these limitations. These include nested PCR or semi-nested PCR, multiplex PCR, and real-time PCR. In nested PCR, a second PCR reaction using additional primers and the original PCR product as template is performed. This improves the sensitivity of the detection (Toze, 1999). Multiplex PCR is able to detect more than one target in a single PCR reaction by the use of multiple sets of DNA primers (Pepper et al., 1997; Picone et al., 1997). Real-Time PCR uses fluorescent molecules which bind to amplicons as they are being synthesized during the PCR process. These fluorescent amplicons are used to quantify the amount of PCR product as it accumulates. Real-time PCR allows for greater accuracy and sensitivity for analyses of DNA and RNA from even a single molecule. It is quantitative, and the process itself takes less time (about 30 minutes vs. about 3-4 hours for regular PCR) (Maier et al., 2009). However, the quality of the data depends on the sample preparation and the quality of the standards. Since the PCR product increases exponentially, variation increases with the cycle number. With an overlap of emission spectra, there can be false positives generated (Klein, 2002). Also, since this process still involves sample collection and preparation, it does not actually allow for real-time water monitoring.

2. Review of Biosensors for Drinking Water Monitoring

2.1 Overview

This is a relatively new approach for the detection of microorganisms. Most of these devices are still in the developmental stages. Even though there is a wide range of biosensors, they all can be narrowed down and defined by their intended function - an analytical device composed by a biological recognition element. This recognition element produces signals which can be detected and correlated to quantify the concentration of the microorganisms (Sethi, 1994; Rogers and Gerald, 1996). The incorporation of a biological recognition element within a device that converts a physical quantity into an electrical signal (transducer) allows for the quantification of the signals proportional to the concentration (Mohanty and Kougianos, 2006). Recognition elements may include enzymes, microorganisms, and organelles (Sethi, 1994; Rogers et al., 1996; Lei et al., 2006; Mohanty et al., 2006). To date, biosensors that are currently in development rely on biochemical recognition (e.g. ELISA based biosensor), fluorescence through binding with a dye such as with flow cytometry (Gunasekera et al., 2000), enzyme based (D'souza, 2001), or intrinsic fluorescence of the microbe themselves through the excitation of cellular components (Kim et al., 2004).

In order to convert the biochemical signals into a physical signal that can be quantified, the recognition element is integrated with the transducer through adsorption, encapsulation, entrapment, covalent binding, or through cross-crosslinking (Sethi, 1994; D'Souza, 2001a; D'Souza, 2001b; Lei et al., 2006). Biosensors which use these methods have additional problems that may need to be resolved. For instance, biosensors that use

adsorption may suffer limitations due to desorption of the microbe; those that employ encapsulation may have lower sensitivity due to the interference of the encapsulation materials (Su et al., 2011). Biosensors that use the microbial intrinsic fluorescence are not limited by these issues. An example of this type of biosensor is one that use light pulses at specific wavelengths that excite targeted cellular components. The analog signal is then converted into digital signals and quantified (Kim et al., 2004). Biosensors that quantify intrinsic fluorescence may offer a better solution for monitoring drinking water in real-time and *in situ* since they do not require any additional steps.

2.2 Advantages and disadvantages of biosensors

Biosensors have the potential to provide solutions for how we can efficiently monitor drinking water and detect microorganisms in the environment. They offer many advantages over cultural methods and PCR. However, most biosensors still need to resolve several issues, particularly interferences from dissolved and particulate matter in water, non-specific response to substrates, and poor selectivity and reproducibility (Su et al., 2011). Biosensors have the potential to enable rapid real-time detection without sample contact while analyzing large volumes of water. Even though different biosensors have different capabilities and limitations that may need to be overcome, once the development costs are recovered, these devices are potentially cost effective.

3. *Emerging Biosensors for Water Monitoring*

There are several biosensors currently being evaluated for microbial water quality monitoring. The JMar BioSentry Technology (JMar, 2011) sensor uses multi-angle light scattering technology (MALS). The unique light scattering property generates patterns when small particles such as microorganisms pass through the beam of light. The system uses three different channels to distinguish and classify bacteria based on their bio-optical MAL signatures. However, this system does not determine organism viability and cannot differentiate between dead and live microorganisms (USEPA, 2010; JMar, 2011). Another system is the Hach Event Monitor Guardian Blue (Hach) UV-VIS s::can spectrolyser. This sensor uses ultraviolet–visible absorption spectroscopy in the range of 250-750 nm. This instrument can be selected to detect total suspended solids (TSS), turbidity, NO_3-N, chemical oxygen demand (COD), biological oxygen demand (BOD), total organic carbon (TOC), direct oxygen demand (DOC), temperature, and pressure (Weingartner and Hofstädter , 2006; S::CAN, n.d). This probe can be used in a wide range of liquids. Hence, this instrument can be used to monitor overall changes and fluctuations in the composition of both drinking water and surface waters. However, the instrument is subject to fouling of the flow cell and flow velocity determines the sediment buildup (USEPA, 2010; JMar 2011). In some cases, the detected change cannot be directly correlated to a known substance (Van den Broeke, 2006). Surface enhanced Raman spectroscopy is another application for some type of sensors that are being evaluated. Raman spectroscopy is based on introducing a light source which interacts with molecules and results in their excitation. Raman spectroscopy provides specific molecular identity of molecules. Hence, different molecules have their own specific

Raman signature (Atanu et al., 2006; Chan et al., 2008). Surface enhanced Raman is a more advanced technique to spontaneous Raman spectroscopy, where Raman signals can be enhanced by the incorporation of metallic nanostructure (Liao et al., 1981; Jackson and Halas, 2004; Atanu et al., 2006). Signals can be enhanced as much as six to seven times orders of magnitude compared to spontaneous Raman spectroscopy (Kneipp et al., 2006). One of the limitations of this technology is its sensitivity to metabolic and environmental factors which results in spectral deviations (Storey et al., 2011). Other real-time monitoring technologies that are currently on the market include TOXcontrol (microloan), ToxProtect, Algae toximeter (BBE), and the Daphia toximeter, all of which are based on biological toxicity detection in environmental samples (Storey et al., 2011).Others may not be as common, but hold promise for future including Censar, which provides early warnings based on algae blooms and also collects simultaneous measurements for color, turbidity and temperature, and YSI Sonde, which provides warning based on the detection of a combination of chlorophyll activities and blue green algae. This also collects additional data on other physical parameters such as conductivity, salinity, temperature, turbidity, and dissolved oxygen (USEPA, 2010; Storey et al., 2011; Atkinson and Mabe, 2006). Other parameters of water quality such as chlorine, pH, temperature, flow, and turbidity are also monitored in real-time.

Some of these biosensors have been studied and evaluated and are thus better understood than others. For instance, a study performed by the United States Environmental Protection Agency (USEPA) looked at the performance of different sensors including the JMAR BioSentry and S::CAN Spectroanalyser. JMAR BioSentry had a lower detection

limit than the S::CAN Spectroanalyser (600 CFU/mL vs. $2.5x10^4$ CFU/mL, respectively) (USEPA, 2010). Another study that evaluated the JMAR BioSentry, the S::CAN Spectroanalyser, and the Hach Event Monitor concluded that the JMAR BioSentry was the only sensor with the potential to detect microbial contaminants. However, turbidity studies showed that the JMAR BioSentry reported results higher than the actual bacterial concentration (Miles et al., 2011).

One of the advantages of using the above mentioned real-time monitoring technologies is the fact that they tend to be non-invasive and do not require reagents, have a quick turnaround since the results can be obtained in real-time, and are not subjected to grab-sample limitations (i.e., large volume of water can be scanned). Among the common problems that most of these sensors face is the fact that the detection of microorganisms is possible only at higher concentrations (lack of sensitivity). Even though BioSentry can detect as few as 600 CFU/ mL, this detection limit may not be adequate to ensure drinking water safety. Also, the method does not determine viability and therefore may result in an overestimate of bacterial numbers. On the other hand, technologies that use the more sensitive Raman spectroscopy may suffer due to a spectral deviation caused by environmental factors (e.g., dissolved substrates, particulate matter) and metabolic factors (Atanu et al., 2006; Hoogh et al., 2006; McKenna et al., 2008; Mons, 2008; Van der Gaag and Volz, 2008) which may interfere with the accuracy of the results, at times resulting in false alarms.

Even for instruments as highly promising as the JMAR BioSentry, a multiple test approach may be necessary in the quest to switch from culturally dependent methods into real-time, reagentless, onsite monitoring techniques. For instance, event detection algorithms (algorithms that determine the point at which sample behavior change occurs) could be used to lower the detection limits (Atanu et al., 2006) and molecular identification could be used to further confirm the types of microorganisms when an alarm occurs, together with physical water parameters such as turbidity and total organic carbon (TOC). Even though some of these sensors may overestimate the bacterial concentration due to high turbidity, a sudden change of clean water (particulate free water) to turbid waters in the distribution system may indicate an intrusion event. Most likely, the perfect sensor will need a combination of these different technologies to monitor various water quality parameters simultaneously, so as to provide an early warning.

4. *Current State: Drinking Water and Sanitation in Developing World*

Even though the world has made significant advances in science and technology in the treatment of water to prevent disease, one-third of the world depends on open water sources such as rivers, streams, and hand dug wells. Due to a lack of infrastructure, very few have improved water sources such as piped drinking water. Even where such infrastructure is available, water is not properly monitored to ensure public safety. Where people use open water sources, these sources are often contaminated. Contaminated waters transport communicable diseases such as cholera, dysentery, and typhoid. In 2008 alone, 2.5 million people died of diarrheal diseases worldwide (WHO, 2011a). In 2011,

approximately 589,854 cases of cholera were reported, an 85% increase from 2010 (WHO, 2012). It is estimated that 3.4 million people die each year from water related diseases and 99% of these deaths occur in developing countries (WHO, 2008). In Africa, approximately one million children die every year from sanitation, hygiene, and drinking water related diseases (Graham and Polizotto, 2013). In addition to diarrhea cases, there are other water related diseases of concern such as schistosomiasis. The World Health Organization (WHO) estimates that 200 million people are affected by schistosomiasis yearly and approximately 800 million are at risk (Steinmann et al., 2006). Often times, the actual numbers of these diseases are underestimated since they are not reported or properly diagnosed.

The millennium development goals (MGD) program has resulted in some progress. As of 2011, 89% of the world population has access to drinking water. However, in some places, the number of people with access to clean drinking water sources stayed relatively the same or had decreased. It is estimated that one-third of Africa still lacks access to clean water (Graham and Polizotto, 2013). This number may be underestimated due to the lack of data or data accuracy (Rosen and Vincent, 1999)

Most agencies, foreign and domestic, may provide boreholes (wells) to reduce the distance needed to be travelled to obtain water. Hence, where water availability is a concern, providing a lined borehole alone may be sufficient to declare victory and may be counted as access to “safe drinking water”. In most African countries the obstacle is still water availability itself, whether safe or not. Hence, testing for water quality and

monitoring is not a priority since it is not seen as a necessity. However, as defined by the World Health Organization (WHO), “Safe drinking water, is water with microbial, chemical, and physical characteristics that meet WHO guidelines or national standards on drinking water quality” (WHO, 2013). Hence providing a borehole alone may not be sufficient to declare victory in the quest of providing access to safe drinking water. The major water sources used in these regions includes boreholes, protected dug wells, protected springs, and rainwater. By protecting these sources, water is assumed to be safe. However, without conducting any type of assessment, one cannot assume the safety of these waters, especially since most developing countries still use pit latrines; therefore, there is a possibility for water that was presumed safe to be contaminated. There have been limited studies of pit latrines and groundwater impairment. One study found that ground water was microbiologically impacted up to 25 m by pit latrines (Graham and Polizotto, 2013). Of course, such contamination depends on the soil type, the depth of the latrine, the borehole, and the water table.

4.1 Lack of Governance in Providing Safe Drinking Water in Tanzania

In 1990 in Tanzania, the percentage of people using improved drinking water sources was 55%, compared to 53% in 2012 (United Nation site for millennium development indicators , n.d). Obstacles to implement the millennium development goals in Tanzania may not be unique, but may be faced in most African nations. One of the major obstacles hindering the progress is proper governance and water management. First, institutions need to be put in place to better manage water resources, both locally and at a national level. Second, water is not seen as an investment; hence, its protection and management

have not been given priority until very recently. From the 1970's until the early 1990's, water was supplied as a free basic good. This practice was not sustainable, and as a result, collapsed (Nkongo, 2009).

The current state of improving water availability in both urban and rural areas involves mobile independent local providers who sell water directly to households. It is estimated that 180,000 households are served by such local providers (Mariuki and Schwartz, 2005). In the capital city of Dar es Salaam, around 1,000 boreholes are drilled each year, registered and unregistered, extracting 70,000 m^3 per day (Bauman and Ball, 2005). In rural areas, groundwater is one of the major sources of water. Most of the technologies to drill boreholes were started by foreign donors to introduce water sustainability to the country. Different organizations had different models such as community participation (Mariuki and Schwartz, 2005). These models were not successful. As a result, the models to provide safe drinking water were geared towards water privatization. As of 2012, the millennium development data still shows over 53% of the population without access to safe water (United Nation site for millennium development indicators, n.d).

Future goals in providing access to clean water in the region should involve sustainable practices. Efforts to provide safe and clean water should also involve some type of monitoring appropriate for the condition of the region. One way of building local capacity and to ensure long term sustainable practices is to provide maintenance of water sources (e.g., well or boreholes) and monitoring through education and community participation. This can be done by implementing models that involve fee recovery to

generate revenue to support these practices. This may also provide local employment. These models would have to be supported at the local level, with oversight at the national level where entities such as government and other stakeholders can provide guidance and assist with resources. This will not only help improve the quality of the water that is being delivered, but will build local capacity and help change the population's mindset.

PRESENT STUDIES

This dissertation consists of two different studies: 1) Real-time detection of microorganisms in drinking water using intrinsic fluorescence, and 2) Well monitoring and design comparison in the Kilombero region, southeastern Tanzania.

The objectives of the first study were to improve the design for a prototype instrument which was previously designed to detect microbes on surfaces. The current prototype was modified to detect the microbial load in drinking water. The aim of the study was 1) to modify the design to improve the detection limit in water, and 2) to determine the detection limit for viable and non-viable bacterial cells as well as spores.

The objectives of the second study were 1) to compare three types of boreholes used as sources of drinking water: lined boreholes (Msabi rope pump), modified rope pumps (converted open well into closed well), and open wells by testing for *E. coli* and total coliforms (microbial water quality indicators) and turbidity, 2) to determine the occurrence of bacterial pathogens in these water sources, and 3) to assess the chemical water quality.

The methods, results and conclusion of these studies are presented in the manuscripts appended to this dissertation. The following is a summary of the most important findings in this paper:

The manuscript “Real-time detection of microbial contamination in water using intrinsic fluorescence” is in Appendix A. The prototype unit was modified by changing the

positions of the light emitting diode (LED) for the three different wavelengths used. The most sensitive LEDs (UV) were placed further from the photomultiplier tube (PMT) and the numbers were reduced to provide spaces for the least sensitive LEDs. Since amber and red light are absorbed by several orders of magnitude in particle-free water compared to UV light, amber and red LEDs were placed closer to the PMT and the numbers of these LEDs were increased to increase the sensitivity of the unit. The detection limit of live bacteria was determined to be 50 live bacterial cells/L. No fluorescence was observed with dead bacteria below 10^8 cells/L. The measurements for spores suggested that the spores had started germinating before or during measurements and thus the detection limit could not be accurately determined.

The manuscript “Well monitoring and characterization in the Kilombero region, southeastern Tanzania” is in Appendix B. Data was collected from three neighboring villages, where a total of 97 water points were sampled. The results of this study found that there was no statistical difference in HPC, *E. coli* and coliform numbers between Msabi lined boreholes and the modified rope pumps (converted open well into a closed well). There was more diversity in open wells than closed wells and Msabi rope pumps. Opportunistic pathogens were detected mostly from the open water sources. However, the Msabi design had lower water turbidity.

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APPENDIX A

CONTINUOUS REAL-TIME DETECTION OF MICROBIAL CONTAMINATION IN WATER USING INTRINSIC FLUORESCENCE

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Key words: intrinsic fluorescence of microbes, real-time monitoring, microbe detection, water monitoring

Abbreviations: DPA: Calcium Dipicolinic Acid, RPN: Reduced Pyridine Nucleotides, LED: Light Emitting Diode, PMT: Photomultiplier Tube, UV: Ultraviolet Light

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Abstract

Current monitoring methods for drinking water do not provide fast and reliable results. By using intrinsic fluorescence, microbial contamination in water can be monitored in real-time, continuously, without sample collection or contact and at low concentration. By monitoring the fluorescence of cellular components of microorganisms, their concentrations and metabolic states (live, dead) can be determined. These fluorophores include reduced pyridine nucleotides (RPNs), flavins, and cytochromes to distinguish live cells; cytochromes for dead cells; and calcium dipicolinic acid (DPA) for spores. Three types of LEDs emitting light at 362nm, 590nm and 635nm were used to excite cellular components within live cells (viable), dead cells (non-viable) and spores respectively. Emissions (intrinsic fluorescence of the live, dead bacteria or spores) were collected at 440nm, 675nm and 770 nm. Established detection limit of live bacteria was ~50 bacterial cells/L. No change is intrinsic fluorescence was observed below 10^{8} bacterial cells/L for dead bacteria. Results for spore measurements suggested that, spores had started germinating before or during measurements.

Introduction

Clean water is crucial for human health. With the growth of the global population, the scarcity of our most precious natural resource, clean water, has become an issue. However, this importance is at a very different magnitude and scale in different parts of the world. In the southwestern part of the United States, the problem of water scarcity is imminent. Over 30 million people in California, Arizona, and Nevada depend on the Colorado River for their water supply [1]. However, the lower Colorado River basin is facing a water shortage [2, 3]. One of the solutions to this problem is to treat and reuse water of impaired quality (e.g., wastewater) for potable use. One of the obstacles to direct potable reuse of wastewater is the need for the rapid assessment of microbial contamination of the treated water. Domestic wastewater contains a wide variety of waterborne pathogens (e.g., viruses, bacteria, and protozoa) including *Vibrio cholerae*, *Shigella dysenteriae, Escherichia coli* O157:H7, *Salmonella typhi*, Hepatitis A virus, *Cryptosporidium parvum* oocysts, and *Giardia lamblia* cysts [4]. Most analytical methods for the assessment of these pathogens require 18 hrs. or longer and none can be used continuously. More rapid methods for microbial load and pathogen detection are needed to ensure the quality of treated water if it is to be used for potable purposes.

While there is a wide range of methods that have been developed to detect microbes in water, they all suffer from a number of limitations. One of the most rapid methods available for the detection of microbial contamination in drinking water is polymerase chain reaction (PCR). PCR suffers from a number of limitations: 1) it does not determine the viability of the organism, 2) there is sometimes interference caused by solutes in the

water, 3) only small volumes can be assayed (10 to 100 microliters), 4) specific primers are required for each organism or groups of organisms, and 5) the recovery and purification of DNA from low numbers of microorganisms is problematic [5, 6, 7]. Another potential limitation is the sampling method used prior to the PCR. Currently, a blind sampling or subsample from high or flowing volumes are collected, which may not be representative of the water contamination.

Intrinsic Fluorescence

The use of intrinsic fluorescence to study cellular metabolic components has been explored since the 1950s [8-10]. Remote, real-time detection and quantification of live cells, dead cells, and spores in fluids (air, water) and on surfaces and sub-surfaces (like those of food, surgical theaters, soils, rock, ice) at low concentrations has been demonstrated [11,12,13]. Some microbes have demonstrated specific fluorescence signatures that depend on their environment or growth conditions [14] (e.g., *Escherichia coli, Enterococcus faecalis,* and *Staphylococcus aureus* causing otitis media and contamination source tracking of several *Pseudomonas* spp. in dairy products) [15]. Our approach makes use of the following metabolic intrinsic fluorescence signatures to detect the presence of microbes that are present under physiological conditions: reduced pyridine nucleotides (RPNs), flavins, cytochromes, calcium dipicolinic acid (DPA), and others [11-13, 15-17]. Metabolic signals, which are indicators of live cells, fluoresce in the blue-green region with 340-360 nm excitation. The fluorescence emitted is directly proportional to the concentration of metabolites and thus to the number of live cells [11]. Flavins and protoporphyrin IX, which also fluoresce in the red region upon 565-595 nm

excitations, are found in both live and dead cells. Cytochromes fluoresce in the near IR region upon 610-640 nm excitations. Excitations in the deep UV (e.g., 250-300 nm) produce fluorescence from a variety of biological molecules (e.g., aromatic amino acids), which are also found in dust, pollen, smog, and other environmental substances. While similar instruments using this approach have been used to demonstrate detection and quantification of microorganisms on surfaces [13], this paper will discuss the proof-of-concept for using this technology to continuously monitor drinking water in real-time as a public health measure.

Intrinsic fluorescence technology has several advantages over other rapid methods. It is less costly than PCR, no sample contact is required, and water monitoring can be done continuously and in real-time with high sensitivity. Based on our initial experiments with a prototype instrument reported here, the detection limit for viable bacteria is ~50 cells/L. While the method does not identify the microbes, it immediately identifies changes in total microbial load and hence the water quality caused by environmental conditions or a failure in the water distribution system. This system can also direct where and when to sample when such events occur. There are technologies that can be used simultaneously with the intrinsic fluorescence technology for the identification of microorganisms of interest. One such method that has been demonstrated is the use of cell-capture technology using surface-bound ligands [13, 18].

Methods

Intrinsic Fluorescence Technology

The fluid prototype instrument, shown in Figures 1 and 2, is similar in design to those reported previously [11, 12, 13] for surfaces except that the optical arrangement places the excitation light at right angles to the fluorescence collection for optimum signal-to-noise ratio. Light emitting diodes (LEDs) are used for the excitations and the intensity of each is modulated at a different frequency so that only a single photomultiplier tube is needed for the detection of fluorescence. Fourier transformation of the data isolates each component, separating the intrinsic fluorescence contributions resulting from the excitations. It is important to note that viable cells, dead cells, spores, and even media can be distinguished by these methods [11].

This instrument for fluids has a total of 10 LEDs: 4 amber, 4 red, and 2 UV (Figure 1). The number and position of these LEDs with respect to the photomultiplier tube (PMT) are designed to give similar intensities of each fluorescence emission at the photomultiplier tube for a sample containing an equal amount of live cells and dead cells. Red and amber LEDs are positioned closer to the PMT since the absorption of their light in water is considerably higher than that for UV. As a sample flows through the instrument, the fluorescence emission is measured by the PMT, which uses an excitation rejection filter [13] to discriminate the fluorescence emission from the excitation light produced by the LEDs. The output of the photomultiplier tube is sent to the electronics, which analyzes the data as described above and communicates it to the general user interface GUI [13].

Methods and Measurements

Preparation of live bacteria

Bacillus thuringiensis (ATCC10792) was used to demonstrate real-time continuous detection of live and dead bacteria, as well as spores. For the preparation of live bacteria, bacteria were grown in Luria–Bertani broth (LB; Sigma-Aldrich, St. Louis, MO) and sampled at the mid-log growth phase. Bacteria were centrifuged at 10,000 rpm for 5 minutes to obtain a pellet and subsequently washed three times in phosphate buffered saline solution (PBS) to remove any residual growth medium. To determine the total bacterial count, bacterial cells were suspended in PBS and counted using the Petroff-Hausser method [19] (Hausser Scientific, PA) as shown in Figure 3d. Serial dilutions (1:10) were performed to obtain different concentrations of bacteria. Bacterial measurements were taken by sampling 1 ml from each dilution into a water circulator containing 6 L of deionized water (DI water). A total of 25 measurements were averaged to obtain a single measurement for each dilution.

Preparation of dead bacteria

For the detection of dead bacteria, the same protocol for growth and sample preparation was used. Bacteria were killed by treatment with 10% bleach for 10 minutes after the three washes. Bacterial counts were performed as described above. However, instead of measuring 1 ml aliquots, 0.2 ml aliquots were dispensed into a water circulator. The measurements were performed as described above for live bacteria measurements. To

confirm dead cells, a 1:10 serial dilution was performed and the bacteria were plated onto LB agar plates with overnight incubation at 37°C.

Spore preparation

Bacillus thuringiensis was inoculated into 100 µl of Difco sporulation medium containing supplements (DSM+S), and this was spread on a DSM+S plate. The plate was incubated for 6 hours at 37°C. Following this, 5 ml of DSM + S was added to the light bacterial lawn, which was then scraped and recovered by pipetting. The absorbance was measured at 600 nm to determine the cell density, followed by inoculation of 0.1 ml into a 1 L flask and incubation overnight at 37°C at 150 rpm. The spores were collected by centrifuging as described previously and re-suspending in cold distilled water and by washing 6 times using cold distilled water. The spores were stored in PBS at 4°C. Crystal violet was used to stain the spores. Under the microscope, spores appeared to have a halo as shown in Figure 5d, and were also counted using the Petroff-Hausser method described above [19]. The fluorescence measurements were performed in the same manner as the dead cell measurements, except the water temperature in the circulator was held at 4°C. In order to determine viability, the spores were plated in triplicate following 1:10 serial dilution and incubated overnight on LB agar plates at 37°C.

Results

The results of the measurement of the intrinsic fluorescence as a function of concentration are shown below in figures. Note that the excitation wavelength is shown in blue and the dionized water results in red as annotated on the right of each graph. R^2 is the square of the Pearson product moment correlation coefficient and is a measure of the goodness of the fitted line to the data. One standard deviation is indicated by the associated error bar for each point.

Live Bacteria

UV excitation, which is used primarily to monitor the increase in total microbial load, showed a linear increase in the log of the intrinsic fluorescence with a corresponding increase in the log of the live bacterial concentration (Figure 3a). The detection limit for live bacteria was ~50 cells/L. There was no change in the intrinsic fluorescence produced by red excitation within the error with the addition of live bacteria (Figure 3b) because this channel detects only spores and dead bacteria (Table 1). Amber excitation produced fluorescence from both live and dead cells (Table 1) and the results (Figure 3c) showed a small increase in the intrinsic fluorescence with the increase in the concentration of live bacteria [the end points are outside of 3 standard deviations].

Dead Bacteria

The detection limit for dead bacteria was much higher than that for live bacteria. This is likely due to fact that the harsh treatment used to kill the cells has damaged some of the fluorescent molecules and that the cells may not be intact. No fluorescence below a concentration of ~10^8dead bacteria/L was observed (Figures 4a, b and c). The UV fluorescence contains ~15% dead cells (Table 1) while red and amber contain 50% dead cells. The observed changes in fluorescence with all three excitations are ~3 standard deviations of each other, indicating little change.

Bacterial Spores

The intrinsic fluorescence contribution from spores should be detected by the red excitation (50%); however, as shown in Figure 5b, no change larger than a single standard deviation was observed. Some change may be observed with UV excitation (only 15% of which is due to spores while 70% is due to live cells, Table I) as the first and final points are greater than 3 standard deviations (Figures 5a). In the amber excitation (50% live), the fluorescence of the beginning and end points is greater than 3 standard deviations (Figure 5c). Taken together, these would support a conclusion that the spores had largely begun to germinate before and/or during the measurements, since Figure 5d clearly shows over 90% of the sample were spores.

Discussion

The results for live and dead bacterial measurements demonstrate the capability of the prototype instrument to detect live and dead cells in flowing water in real-time with a detection limit of ~50 live bacterial cells and 10^8 dead bacterial cells/L. There was a significant and reproducible increase in the UV excited intrinsic fluorescence (70% of which was due to live cells) with the increase in the concentration of live bacteria. A small increase was observed in the amber-excited fluorescence (50% of which was due to live cells). Killed bacteria may have had damaged fluorophores as little fluorescence change within the error was observed with changing concentration. While changes outside the error were observed in the fluorescence for spores, they were observed for the UV excitation (15% of which was due to spores, 70% to live cells) and not for the red excited fluorescence (50% of which was due to spores). This suggests that the spores had largely begun to germinate before and/or during the measurements.

There are two main reasons why the detection of spores as well as dead bacteria in water may be difficult. First, the absorption coefficient of red light in pure, particle-free water is ~1000 times higher than the absorption coefficient of UV/blue light [20]. This means that most of the red light used for excitation and emitted as intrinsic fluorescence is absorbed by the water. Second, spores change morphology slowly compared to their metabolism. In experiments in cold water (4°C), we have observed spores to change their metabolism to more resemble that of a vegetative cell even though the morphology still superficially resembled that of a spore.

Even though amber excitation produces fluorescence from both live and dead bacteria (50% each), this fluorescence is in the red and reduced by a factor >100 by water absorption. While the intensities of the amber and red LEDs are significantly higher than that of the UV LEDs, this was not enough to compensate for the effects of the water absorption in this prototype.

The use of intrinsic fluorescence has been demonstrated for the presence of live bacteria in water with a detection limit of ~50 cells/L. We were able to quantify the total microbial load and differentiate between dead and live bacteria for water-monitoring purposes. This technology, used together with direct microbial cell capture onto a ligand-coated surface, may allow us to monitor our drinking water along the distribution water system continuously and to determine the presence of pathogenic microorganisms (11, 13, and 18) in real-time.

Tables and Figures

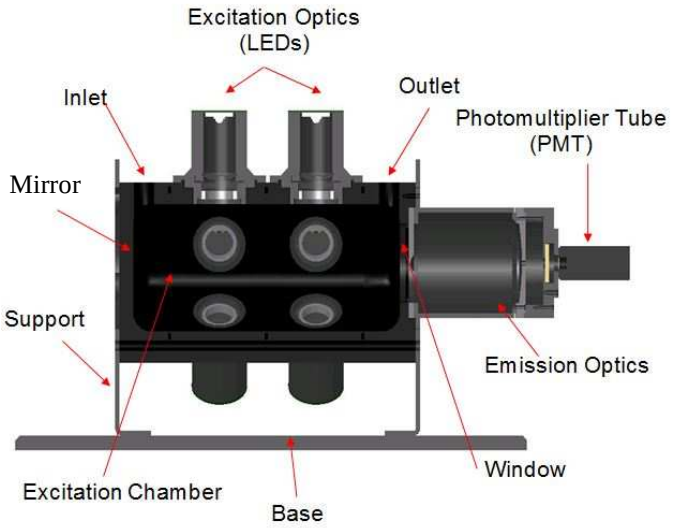

Figure 1: Intrinsic Fluorescence Instrument for Water Monitoring

Figure 2: Intrinsic Fluorescence Instrument for Water Monitoring (shown schematically in Figure 1)

Live bacteria detection

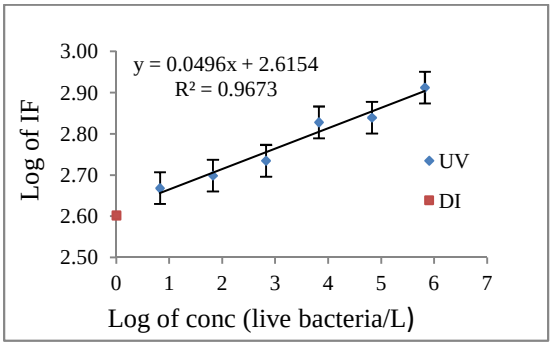

Figure 3a: Intrinsic Fluorescence Detection of Live Bacteria [Ex 365 nm/Em 440 nm].

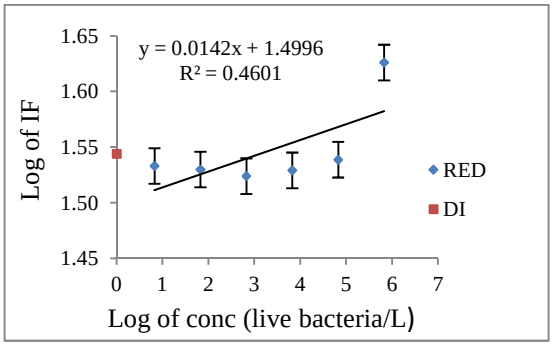

Figure 3b: Intrinsic Fluorescence Detection of Live Bacteria [Ex635 nm/Em 770 nm].

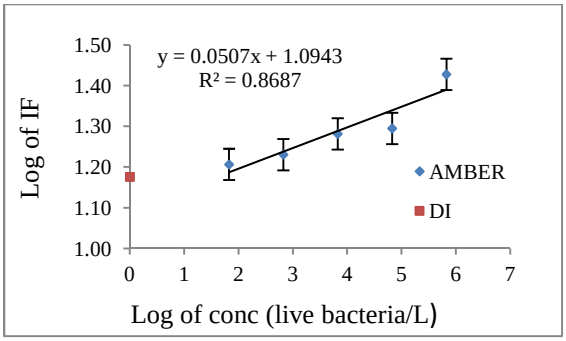

Figure 3c: Intrinsic Fluorescence Detection of Live Bacteria [Ex 590 nm/Em 675 nm].

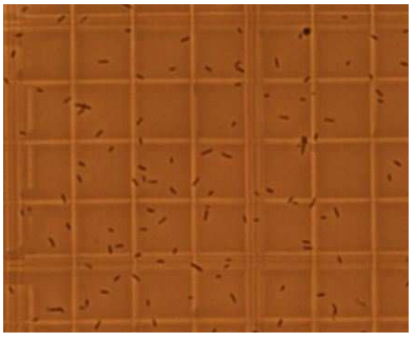

Figure 3d: Live *Bacillus thuringiensis* as seen under the microscope, using a Petroff-Hausser cell.

Dead bacteria detection

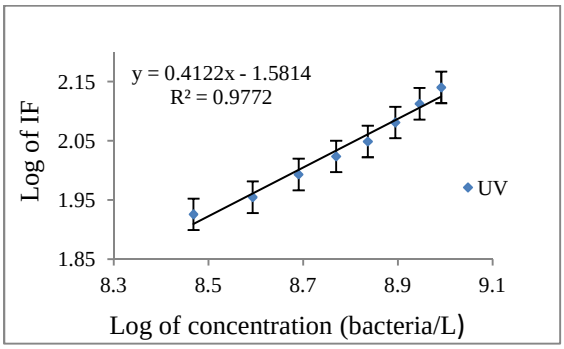

Figure 4a: Intrinsic Fluorescence Detection of Dead Bacteria [Ex 365 nm/Em 440 nm]. (DI reading 0, 1.95)

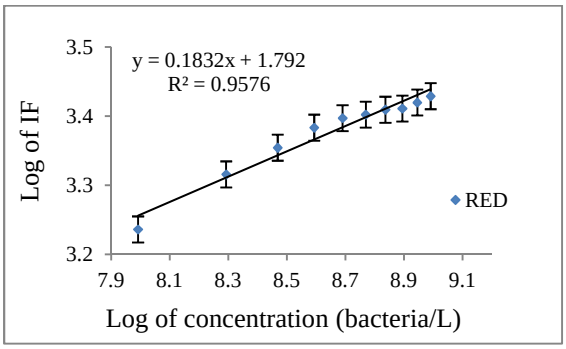

Figure 4b: Intrinsic Fluorescence Detection of Dead Bacteria [Ex635 nm/Em 770 nm]. (DI reading 0, 3.2)

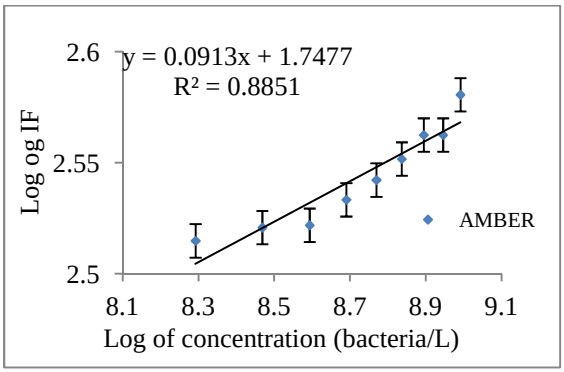

Figure 4c: Intrinsic Fluorescence Detection of Dead Bacteria [Ex 590 nm/Em 675 nm]. (DI reading 0, 2.5)

Bacterial spore detection

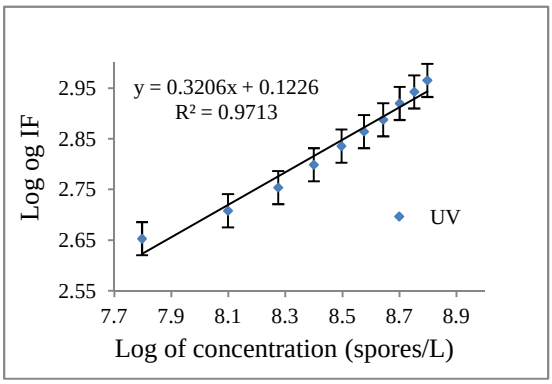

Figure 5a: Intrinsic Fluorescence Detection of Spores. [Ex 365 nm/Em 440 nm]. (DI reading 0, 2.6)

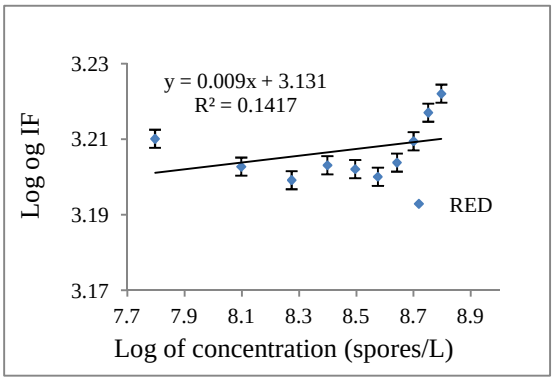

Figure 5b: Intrinsic Fluorescence Detection of Spores. [Ex635 nm/Em 770 nm]. (DI reading 0, 3.2)

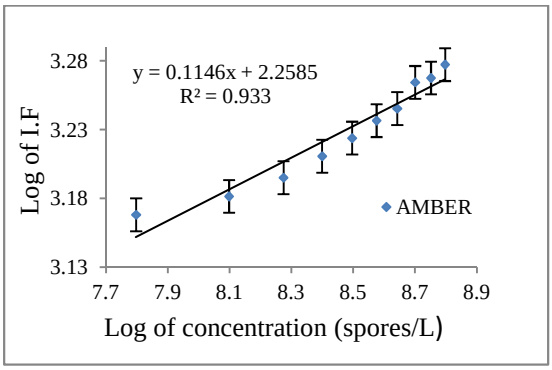

Figure 5c: Intrinsic Fluorescence Detection of Spores [Ex 590 nm/Em 675 nm]. (DI reading 0, 3.15)

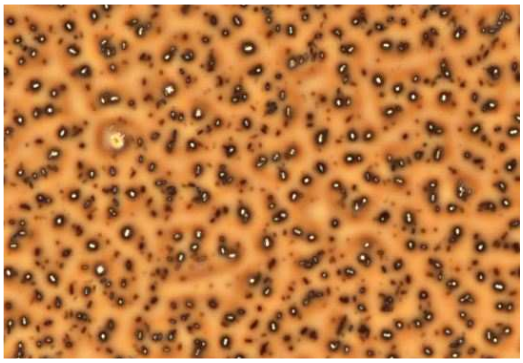

Figure 5d: *Bacillus thuringiensis* spores stained with crystal violet dye and viewed with a light microscope.

Table 1: Sources of Contributions to Intrinsic Fluorescence Signatures [13]

Excitation/Emission	**Contribution**
UV [365 nm/440 nm]	70% [live cells] + 15% [dead cells] + 15% [spores]
amber [590 nm/675 nm]	50% [live cells] + 50% [dead cells]
red [635 nm/770 nm]	50% [dead cells] + 50% [spores]

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APPENDIX B

WELL MONITORING AND DESIGN COMPARISON IN THE KILOMBERO REGION, SOUTHEASTERN TANZANIA

Paper was prepared to be submitted to Journal of Water Sanitation and Hygiene for Development

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Key word: *Escherichia coli*, coliforms, rope pump, well design, water quality, well comparison, groundwater, Ifakara, Tanzania
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Abstract

People in the Kilombero region of Tanzania rely on groundwater and the Kilombero River, and its major tributaries for drinking water. These water sources are shared by livestock and also used for recreation. In 2008, Clean and Safe Water for Ifakara (Msabi), a non-profit began to install lined and covered boreholes and modified open wells so as to provide better and safer drinking water in this region. The number of water extraction points used for drinking water in five villages is estimated at over 750, with more than 75% being wells and surface water. The purpose of this study was to compare three types of boreholes: closed and lined boreholes (Msabi rope pump), open well converted into a closed well, and open wells in terms of chemical, microbial quality and turbidity. Heterotrophic plate count bacteria (HPC), total coliforms (TC) and *Escherichia coli* were determined to assess microbial quality. Samples were collected from a total of 97 water point; 94 well and three rivers. Additional analyses were done for Ifakara village only; fifteen samples were further analyzed for microflora and to compare diversity within the three well designs by using cell culture for isolation followed by PCR and genome sequencing Ten samples were analyzed for calcium (water hardiness), potassium, nitrates, nitrite, chloride, fluoride, bromide, sulfate, phosphate, iron, arsenic, total organic carbon and total nitrogen. Two water points that appeared to have an oil-like layer were also analyzed for organic compounds (gasoline components). Only two Msabi rope pumps (lined borehole) and four closed well (improved well designs; open well modified into closed and lined well) tested positive for *E. coli*. All open wells and open water sources (i.e. rivers) tested positive for *E. coli* with an average concentration of 154 MPN/

mL. The average *E. coli* concentration was < 22 CFU/ 100mL for closed wells and Msabi rope pumps. All the samples that were analyzed using Colilert reagent method tested positive for total coliform with an average of > 160 MPN/100mL, 102 MPN/100mL and 88 MPN/100 ml, for open wells, closed wells and Msabi rope pumps respectively. The average total HPC for the open wells was 7×10^3 CFU/mL and that of closed wells and Msabi rope pumps were 8×10^2 and 6×10^2 CFU/ml respectively. Open wells had one order of magnitude higher compared to closed wells and Msabi rope pumps. There was a statistically difference in terms of HPC, total coliform and *E. coli* between the open wells, closed wells and Msabi rope pumps (p=<0.001). However, there was no statistical difference between the closed wells and Msabi rope pumps for HPC (p=0.18), total coliforms (0.2) and *E. coli*. There was more microbial diversity in the open wells comparing to the closed wells and Msabi rope pumps. Opportunistic bacterial pathogens were detected mostly open wells. The wells that tested positive were; one Msabi rope pump, one closed well and the rest were from open water sources. No traces of gasoline components were detected. One well out of ten had high amounts on nitrates-nitrogen (> 10 mg/L).The average turbidity were 49 NTU, 28 NTU and 11 NTU for open wells, closed wells and Msabi rope pumps respectively. There was a statistical difference in turbidity between the three well designs (p=<0.001). The results showed that Msabi rope pumps and closed wells performed similarly in terms of water microbial quality. Msabi rope pump performed better in turbidity followed by closed wells. The open wells performed poorly in water microbial quality and turbidity.

Introduction

The Kilombero region has a population of approximately 75,000 and is situated in the southeastern part of Tanzania in the east African rift valley on the river delta flood plains. The average annual rainfall ranges from 1200 – 1800 mm, and is characterized by a short rainy season period from October to December and a long rainy season from February to June (Hetzel et al., 2008). The annual average temperatures range from 20°C to 30°C (Masuki et al., 2009). Due to this favorable tropical climate, the region serves as a source for many tropical diseases, particularly malaria and frequent diarrhea. As a result, the Kilombero region has been a center for tropical disease studies in East Africa since the 1960's. In 1992 and 1997, outbreaks of cholera occurred in the village of Ifakara. There have since been cyclic cholera outbreaks (Acosta et al. 2001), the most recent in 2007 (Msabi, unpublished). Half of the population use pit latrines, which often are unusable during heavy rainfalls (Tanner et al.1987). These latrines are usually up to 5 m deep and can be a source of groundwater contamination.

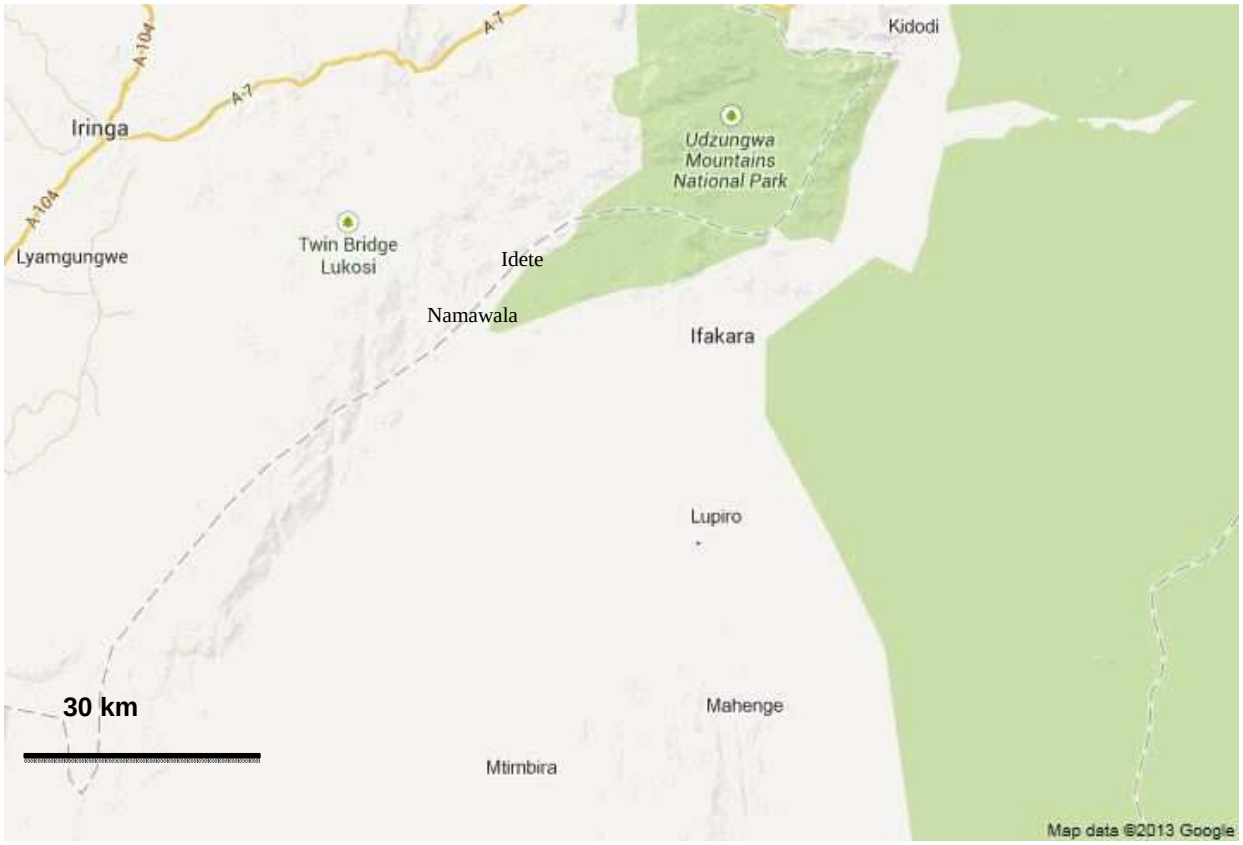
In 2008, in order to provide safer drinking water, Msabi, a non-profit organization, began to install lined and covered boreholes in the region in addition to modifying open wells to closed wells. Each borehole costs $700 AUS (~$ 690 U.S.) (Msabi, unpublished). As of 2011, 150 such Msabi boreholes had been installed in the region. These Msabi boreholes average 20-30 m in depth and 0.15 m in diameter. A concrete sanitary seal is matched to the soil clay layer between 4 - 10 m in depth and the remainder of the hole is lined by a polyvinyl chloride (PVC) pipe. Water enters at the bottom of the borehole through a cut

slot covered by a filter screen (Msabi, unpublished). The open wells that have been converted to closed wells average > 5 m in depth and are lined by a concrete plaster which starts from the bottom of the well upwards to a depth of about 3 - 5 m. The plaster is applied to the locally made bricks (lean cement /sand mix 1:15) that line the well. The infiltration of water into the well occurs at depths below the water table even in the lined portions of the well. The goal is to avoid infiltration near the surface by the use of a cover so as to avoid murky water entering the well, especially during rainfall. Other open wells in the region tend to be shallow with an average of 3 m in depth, some of which are lined with bricks. Approximately 75% of the people in this region rely on such shallow open water sources for their drinking water (Msabi, unpublished). To date, no comprehensive water quality data is available for the various types of water sources available in the Kilombero region.

The current study was conducted in the summer of 2011. Three out of the 28 villages in the Kilombero region were selected. The first of these was the village of Ifakara. The villages of Namawala and Idete were also included based on their proximity to Ifakara and their ease of access. The purpose of this study was to compare the turbidity and microbial water quality of three types of water sources: closed and lined boreholes (Msabi rope pumps), closed and lined modified rope pumps (open wells converted into closed wells by Msabi), and open wells. Additional water samples only from the village of Ifakara were assessed for chemical water quality so as to identify any potential chemical contamination. The overall goals of this study were to provide the first comprehensive water quality data in the region, to identify some of the factors

contributing to gastrointestinal diseases in the region, and to assist the community and stakeholders in determining where to focus their resources for further improvements.

Figure 1: Map of the Kilombero region, Tanzania showing the villages of Ifakara, Idete and Namawala



Materials and Methods

A total of 97 samples were collected with an average of 32 wells from each village (Ifakara, Namawala, and Idete); approximately 11 Msabi rope pumps, closed wells, and open wells were sampled from each village. Additionally, a river water point was also sampled in each village for comparison purposes. Due to limited resources, 15 wells from Ifakara only were analyzed for the presence of potential pathogens and the water from 10 wells analyzed for their chemical composition.

Microbial Analysis

Water samples were collected using plastic sterile whirl packs and transported to the laboratory using an ice chest. The sampling took place from early morning until late afternoon and the water samples were processed within 8 hours of collection. For heterotrophic plate count and total coliforms, one mL of each sample was cultured onto a 3M petrifilm (3M, St. Paul MN) via spread plating after dilution in phosphate buffered saline. Heterotrophs are microorganisms that require organic carbon for growth. There is no evidence from epidemiological studies to suggest any correlation with occurrence of waterborne pathogens and any health risks (WHO, 2003). Heterotrophic plate count was performed for well comparison purposes only. Coliforms are a group of bacteria which are aerobic and facultative anaerobic, gram negative, non-spore formers, rod shaped and ferment lactose with gas formation within 48 hours at 35°C. Total coliforms are naturally present in the environment, and at high concentration levels may indicate potential presence of pathogens (US EPA, 2009). Even though total coliforms are no longer considered as indicators of fecal contamination, they are indicators of water microbial

quality (WHO, 2003). The number of *E. coli* was determined using Colilert Test Kits (IDEXX,Westbrook ME). *E. coli* is a fecal coliform. Fecal coliform bacteria are bacteria whose presence indicates water contamination from human or animal waste. The test kit used pre-dispensed substrate which was added to 10 mL volumes of the water samples. This method results in a Most Probable Number. Colilert is a designed substrate technology with two nutrient indicators, O-nitrophenyl (ONPG) and 4-methyl-umbelliferyl (MUG). The β-galactosidase enzyme is present in all coliforms. This enzyme utilizes ONPG, resulting in a yellow product, indicating the presence of coliforms. *E. coli* has an additional enzyme, β-glucuronidase, which cleaves MUG, resulting in a product that fluoresces under UV light (IDEXX). The Colilert method allows for the detection of as little as 1 MPN/100mL. All samples were incubated at 35°C for 24 hours. The results were multiplied by 10, to determine the MPN per 100 mL.

Turbidity Measurements

Turbidity measurements were taken using a turbidity meter (Hach, Loveland CO). All measurements were determined on site at the time of sample collection.

Microbial Analysis

In addition to the detection of microbial water quality indicators, diversity microflora within the three wells designed was also of interest for comparison purposes. To compare microflora, different type of media were used to facilitate growth of different types of bacteria, including *vibrio cholerae,* to periodic outbreaks of cholerae in the region. Hemolytic bacteria were also of interested since they are associated with pathogens.

Hemolytic bacteria produce hemolysins which are associated with virulences (Welch et al. 1981). In some microorganisms such as *E. coli*, the ability to lyse red blood cells is common in strains isolated from infections and not from normal feces (Cooke et al. 1975, Minshew et al. 1978). Hemolytic bacteria produce an exotoxin that can lyse red blood cells. Therefore, an attempt was made to also detect *Vibrio cholerae* and hemolytic bacteria so as to have an idea of the types of potential pathogens present in wells with different designs. Due to some of the limitation of polymerase chain reaction (PCR) such as interferences by environmental contaminants (Toze, 1999) that inhibit Taq polymerase, especially in the presence of organic and inorganic compounds (Queiroz et al., 2001), the samples were cultured to isolate bacterial colonies prior to performing the PCR. The water samples were collected in 15 ml polypropylene sterile tubes and placed in on ice in a cooler and shipped to the United States, where they were immediately processed upon their arrival, four days after collection.

To culture *Vibrio cholerae,* of one mL of water sample was inoculated in alkaline peptone water (BBL Alkaline Peptone Water), an enrichment medium (Becton, Dickinson and Company, Sparks MD), with incubation at 35°C for 8 hours, followed by inoculation onto Thiosulfate Citrate Bile Salts agar (TCBS) plates with incubation at 35°C for 24 hours.

To culture hemolytic bacteria, 0.1, 0.01, and 0.001 mL of each water sample was inoculated on blood agar plates (Trypstic Soy Agar with 5% sheep blood). The blood

agar plates were incubated both aerobically and anaerobically at 35°C for 1-4 days (or until colonies appeared) so as to increase the recovery of diverse hemolytic bacteria.

To further increase microbial diversity, different types of medium were used. In order to recover stressed and slow-growing bacteria, water volumes of 01, 0.01 and 0.001 mL were cultured on R2A agar plates and incubated at 35°C for 5 days. To culture other rapidly growing bacteria, 0.1, 0.01, and 0.001 mL of each water sample was cultured on Luria Bertani (LB) agar plates, a nutrient rich media, with incubation at 35°C for 24 hours.

A total of 93 colonies were selected for further species identification tests. These included the α and β hemolytic colonies, totaling 50 colonies from the blood agar plates, all 12 colonies TCBS agar plates, and selected 22 representative colonies from the LB and 9 colonies from R2A plates. The PCR DNA template was prepared using QIAquick PCR purification kits following the manufacturer's protocol (QIAGEN, Valencia CA). The PCR reaction was performed using universal V3 primers for both forward and reverse direction. The V3 primers amplify the 16s rRNA, a highly conserved region found in all bacteria. They were selected because of their ability to provide sufficient phylogenic information on bacteria (Huse et al., 2008, Liu et al., 2007) as the V3 region tends to be longer and provides a better resolution than many other genes, and can be unambiguously mapped to the genus level approximately 97% to 99% of the time (Huse et al., 2008). The PCR was performed with the following cycle conditions: 95°C (denaturation) for 30 seconds, 60°C (annealing) for 1 minute, and 72°C (extension) for 45

seconds for a total of 40 cycles (BioRad MJ Mini Personal Thermocycler, Hercules CA). The PCR mix without any added DNA was included as a negative control. The sequencing was performed at the University of Arizona Genetic Core laboratory (Tucson, AZ). Forward and reverse strands were aligned and only the aligned sequences were compared to sequences published under the NCBI database (Blast search).

Water Chemical Analysis

A subset of ten water samples from the village of Ifakara (included samples collected from two Msabi rope pumps, three closed wells, three open wells, one rope pump of a different design, and the Lumemo River) were analyzed for anions (fluoride, chloride, bromide nitrate, nitrite, and sulfate), cations (calcium, potassium, arsenic, and iron), total organic carbon (TOC), total inorganic carbon (TIC), and total nitrogen (TN). \The water samples for cation analysis were collected using 50 mL polypropylene tubes and acidified to pH 2 with ultrapure nitric acid. For the anion analysis, ion chromatography was performed using a Dionex ICS-1000 with AS-22 and AG-22 anion exchange and guard columns (Dionex Corp., Sunnyvale, CA). For cation analysis, the ELA-DRC-II inductively-coupled plasma spectrometer (ICP-MS) (Perkin Elmer, Shelton CT) was used.

The Lumemo River and the additional rope pump were further screened for the presence of polycyclic aromatic compound (PAHs). The water samples were collected in amber vials and stored in the dark. All samples were kept on ice and shipped to the United States where they were submitted for analysis immediately upon receipt, four days

following their collection. The samples were analyzed using full scan Gas Chromatography Mass Spectrometry (GC/MS) with a J & W DB-5ms Column (Agilent Technologies, Santa Clara CA) by the Arizona Laboratory of Emerging Contaminants (University of Arizona, Tucson, AZ).

Results and Discussion

HPC is used to measure bacteria that are present in water. Even though there is no health effects associated with HPC, the lower the concentration the better (US EPA, 2009; WHO, 2003). The average total HPC for the open wells was 7 x 10^3 CFU/mL and that of closed wells and Msabi rope pumps were 8 x 10^2 and 6 x 10^2 CFU/ml respectively (Table 1). Open wells had one order of magnitude higher compared to closed wells and Msabi rope pumps. There was a statistical difference for HPC within the three wells design ($p=<0.001$). The average HPC for closed wells and Msabi rope pumps was 876 CFU/ ml and 636 CFU/ml respectively (Table 2). There was no statistical difference for HPC between closed wells and Msabi rope pumps ($p=0.18$).

Coliforms are used as indicators of fecal contamination in drinking water. It is recommended that no more than 5% of drinking water samples test positive for the presence of coliforms in 100 mL (US EPA: Drinking Water Contaminants, 2012; WHO, 1997). However, coliforms are sometimes naturally present in the environment, and in tropical climates, coliforms tend to occur in higher numbers (Carrillo et al. 1985, Lavoie 1983). Hence, their presence does not always provide a true indication of the presence of human or animal waste in water. All the samples tested positive for total coliform using the Colilert reagent methods, with an average of > 160 MPN/100 mL, 102MPN/100 mL and 88 MPN/100 ml, for open wells, closed wells and Msabi rope pumps respectively. There was a statistical difference between the open wells, closed wells and Msabi rope pumps ($p=<0.001$). The average coliforms in closed wells and Msabi rope pumps was 2.8 x10^3 CFU/100mL and 1.5 x 10^3 CFU/mL respectively, using the 3M petrifilm method

with p=0.04, indicating there is a statistical difference between the two wells designs However, the total coliforms test using the Colilert method indicated that there was no statistical difference between closed wells and Msabi rope pumps, with average concentrations of 22 CFU/100 ml for both wells designs (Table 2)

E. coli is more specific to fecal contamination than the non-fecal coliforms. *E. coli* was detected in all of the open water sources, with concentrations of > 154 MPN/100 mL. Only four closed wells out of eleven and two closed wells out of ten Msabi rope pumps tested positive for the presence of *E. coli* with an average concentration of < 22 / 100mL (Table 1). There was a statistical difference in E. coli numbers between open wells, closed wells and Msabi rope pumps (p=<0.001).

Turbidity is another general indicator of water quality. The Msabi rope pump wells had the lowest turbidity levels, followed by the converted closed wells (Table 3). The turbidity was higher in open wells. There are no requirements for turbidity for groundwater; however, The USEPA recommends that the turbidity not exceed 5 NTU for drinking water treated using slow sand filtration (USEPA, 2009).More than half of Msabi rope pump wells had turbidity levels below 5 NTU. The average turbidity for open wells was 49.2 NTU. The average turbidity for closed and Msabi rope pumps was 27.7 NTU and 10.9 NTU respectively (Table 3). There was a statistical difference in turbidity between the three wells designs (p=<0.001). Some of the Msabi rope pumps had turbidity as low as 5 NTU, as recommended for drinking water treated using slow sand filtration.

Table 1: **Comparison for water microbial quality between open well, closed well and Msabi rope pump**

	Msabi Rope Pump		Closed Well		Open Well		
	Average	positives (n)	Average	positives (n)	Average	positives (n)	p -value
Total HPC (CFU/1mL)	656 ±310	30 (30)	876 ±820	29 (29)	7033 ±2000	23(23)	<0.001
Total Coliform[1] (CFU/100mL)	1506 ±1000	23(34)	2836±2800	18(30)	42743±25000	29 (29)	<0.001
Coliform[2] (MPN/100 mL)	>88±29	10 (10)	>102±19	11(11)	>160	14 (14)	<0.001
***E. coli* (MPN /100 mL)**	<22±6	2(10)	<22±6	4(11)	>154±10	14(14)	<0.001

Table 2: **Comparison for water microbial quality between Msabi rope pump and closed well**

	Msabi Rope Pump		Closed Well		
	Average	positives (n)	Average	positives (n)	p-value
Total HPC (CFU/1mL)	656 ±310	30 (30)	876 ±820	29 (29)	0.175
Total Coliform[1] (CFU/100mL)	1506 ±1000	23(34)	2836±2800	18(30)	0.041
Coliform[2] (MPN/100 mL)	>88±29	10 (10)	>102±19	11(11)	0.202
***E. coli* (MPN/100 mL)**	<22±6	2(10)	<22±6	4(11)	NAN*

* Not applicable

Table 3: **Comparison for water turbidity between open well, closed well and Msabi rope pump**

	Msabi Rope Pump		Closed Well		Open Well		
	Average	No. of samples	Average	No. of samples	Average	No. of samples	p-value
Turbidity (NTU)	10.9±2.2	34	27.7±13.1	31	49.2±14.9	29	<0.001

[1] Detection of total coliform using 3M Petrifilm Coliform Count Plates
[2] Detection of total coliform using Colilert test kit

The following are the results of the PCR and sequencing, where only sequences with 100% -94% match are reported in this paper. Of the 93 colonies selected for species identification via PCR/sequencing, no *V. cholerae* isolates were found, rather *Enterococcus faecalis, Enterococcus canistenii,* and *Acinetobacter haemolyticus* were detected using the enrichment/TCBS method. The majority of these isolates came from open water sources; however, one converted closed well was also positive for *Enterococcus canistenii* (Table 4). *Gordonia polyisoprenivorans* and *Microbacterium oleivorans* were detected from a converted closed well and a Msabi rope pump well using blood agar under aerobic growth conditions (Table 5). *Escherichia fergusonii, Shigella flexneri,* and *Shigella dysenteriae,* were identified (100% match) in one open well (Table 8) by culture on blood agar incubated under anaerobic conditions. Additionally, a *Clostridium irregulare*, a *Clostridium bartlettii* (94% match), and a *Sphingobacterium composti* were detected in another open well on blood agar under anaerobic conditions (Table 6). Only one potential pathogen, *Rhodococcus corynebacterioides*, was isolated from a converted closed well using R2A agar (Table 7). *R. corynebacterioides* has recently been discovered to cause sepsis in immunocompromised persons (Kitamura et al., 2012). Additionally, *Gordonia otitidis* and *Mycobacterium llatzerense* were detected from a well with a local rope pump (design unknown) (Table 8) using LB growth medium.

Table 4: Bacteria identified from open wells growing on TCBS.

Sample ID	Identified bacteria species
LumemoTc	*Micrococcus lylae*
OW03Ta	**Enterrococcus canintestini*
OW03Tb	**Enterococcus faecalis*
OW03Tc	**Enterococcus faecalis*
OW04Ta	*Exiguobacterium estuarii*
OW04Tb	**Enterococcus faecalis*
OW07Ta	*Exiguobacterium aestuarii*
OW07Tb	**Enterococcus faecalis*
OW07Tc	**Acinetobacter haemolyticus*
OW07Td	**Enterococcus faecalis*
CW07Ta	**Enterococcus canintestenii*
CW07Tb	**Enterococcus canintestenii*

*Documented cases of infection or potential opportunistic pathogen

Table 5: Bacteria identified growing on blood agar incubated in aerobic conditions.

Sample ID	Identified bacteria species
MRP020BAa	*Deinococcus ficus*
MRP020BAb	**Gordonia polyisoprenivorans*
MRP020BAc	*Aeromicrobium flavum*
MRP020BAe	*Rhodococcus kroppenstedtii*
RPIBAa	*Streptomyces species*
RPIBAa	*Microbacterium insulae* or *Microbacterium trichothecenolyticum*
RPIBAd	*Kitasatospora saccharophila*
RPIBAe	*Bacillus aerophilus*
RPIBaf	*Bacillus aerophilus*
LumemoBAa	**Bacillus anthracis* strain ATCC 14578
LumemoBAb	**Bacillus anthracis* strain ATCC 14578
OW03BAa	**Bacillus anthracis* strain ATCC 14578
OW03BAb	*Halotolerans*
OW03 BAc	*Cellumonas aerilata*
OW03bBAd	*Exiguiobacterium acetylicum*
OW03 BAe	*Cellumonas aerilata*
OW04 BAa	**Bacillus anthracis* strain ATCC 14578
OW04BAb	*Bacillus aerophilus*
OW04BAc	**Bacillus anthracis* strain ATCC 14578
OW07BAa	No matches
OW07BAc	*Streptopmyces thermoalcalitolerans*
OW07BAd	*Phycicoccus aerophilus*
OW07BAe	*Halotolerans*
OW07 Baf	*Pseudomonas panipatensis*
CW01BAa	*Pseudonocardia xinjiangensis*
CW01BAb	*Bacillus thermoamylovorans*
CW01BAc	*Phycicoccus dokdonensis*
CW01BAd	*Serinibacter salmoneus*
CW07BAa	**Microbacterium oleivorans*

*Documented cases of infection or a potential opportunistic pathogen

Table 6**:** Bacteria identified growing on blood agar incubated in anaerobic conditions resulted in isolation.

Sample ID	Identified bacteria species
LumemoBNa	**Bacillus anthracis* strain ATCC 14578
LumemoBNb	*Enterrococcus mundtii* strain ATCC 43186
LumemoBNe	*Paeibacillus borealis*
OW03BNa	**Bacillus anthracis* strain ATCC 14578
OW03BNd	*Bacillus aerius*
	**Escherichia fergusonii* strain ATCC 35469 or **Escherichia albertinii*or **Shigella flexneri* strain ATCC 29903 or **Shigella dysenteriae*
OW03BNe	strain ATCC 13313
OW03BNf	*Clostridium frigidicarnis* or *Clostridium carnis*
	**Clostridium irregulare* or **Clostridium*
OW03BNb	*bartlettii*
OW03BNc	*Clostridium gasigenes*
OW04BNb	*Lactococcus Lactis subsp. hordniae*
OW04BNc	No match
OW04BNd	*Planobacterium taklimakanense*
OW07BNe	*Bacillus aerophilus*
OW07BNb	*Bacillus aerius*
OW07BNd	*Bacillus aerophilus*
OW07BNf	*Bacillus aerophilus*
OW07BNh	*Bacillus firmus*
OW07BNi	*Bacillus aerius*
OW07BNc	*Bacillus aerius*
OW07BNa	**Sphingobacterium composti*
CW01BNa	*Cellumonas aerilata*

*Documented cases of infection or potential opportunistic pathogen

Table 7: Bacteria identified growing on R2A Agar.

Sample ID	Identified bacteria species
MRP020Ra	No match
MRP020Rc	*Aeromicrobium flavum*
MRP120Ra	*Bacillus solisalsi* or *Bacillus macauensis*
CW01Ra	*Phycicoccus dokdonensis*
CW01Rc	*Micrococcus endophyticus*
CW01Re	*Phycicoccus dokdonensis*
CW01Rf	*Rhodococcus kroppenstedtii*
CW07Rc	*Microbacterium kitamiense*
CW07Rd	**Rhodococcus corynebacterioides*
CW07Re	*Micromonospora pattaloongensis*

*Documented cases of infection or a potential opportunistic pathogen

Table 8: Bacteria identified growing on LB agar.

Sample ID	Identified bacteria species
MRP020La	*Rhodococcus kroppenstedtii*
MRP020Lb	*Microbacterium insulae*
RPILc	*Arthrobacter niigatensis*
RPILf	**Gordonia otitidis*
RPILj	*Rhodococcus kroppenstedtii*
RPILk	*Microbacterium insulae*
RPI Lg	**Mycobacterium llatzerense*
LumemoLa	*Exiguobacterium acetylicum*
LumemoLc	*Bacillus bataviensis*
Lumemo Lg	*Micrococcus lylae*
OW04La	*Rhodococcus pyridinivorans*
OW04Lb	*Streptomyceses spec.*
OW04Ld	*Bacillus firmus*
OW04Le	*Kocuria marina*
OW07Lg	*Halotolerans*
OW07Le	No match
CW01La	*Pseudonocardia xinjiangensis*
CW01Lb	*Arsenicicoccus bolidensis*
CW01Lc	*Kocuria marina*
CW07La	*Streptomyces* species
CW07Lb	*Aeromicrobium* species

*Known documented cases or a potential opportunistic pathogen

Due to cost limitations, 10 wells from the Ifakara village only were tested for fluoride, chloride, bromide, nitrate, nitrite, phosphate, and sulfate. These wells were selected to represent the surrounding areas. The maximum contaminant levels (MCL) of nitrates is 10 mg/L of nitrates measured as only the nitrogen portion (USEPA, 2009). Only one open well (OW03) had levels higher than the MCL value, with 11.21 mg/L of nitrate-nitrogen (Table 11). The MCL for nitrite-nitrogen is 1 mg/L. None of the wells exceeded this level. Bromide is not regulated by the USEPA, but other forms such as ethylene dibromide are regulated; however, no bromide was detected.. Phosphorus (elemental form) is considered a non-priority pollutant and therefore is not regulated by the USEPA (USEPA, 2009). However, the USEPA recommends that phosphate levels not exceed 0.1 mg/L for streams to control algal growth (USEPA, 1988). The level found in the Lumemo River was 1.54 mg/L and in a converted closed well (CW03) was 1.44 mg/L; both were thus higher than the recommended value (Table 11). Low concentrations, below the MCL levels of fluoride, chloride, and sulfate were detected. These are considered secondary contaminants with MCL's of 4 mg/L, 250 mg/L, and 250 mg/L, respectively (USEPA, 2009).

Iron and arsenic were below their respective MCL (Table 13). The MCL for iron, a secondary contaminant, is 0.3 mg/L (USEPA, 2009). Arsenic is a carcinogen that can cause skin damage or increase the chances of developing cancer. Arsenic is monitored under primary drinking water regulations. The MCL for arsenic is 0.010 mg/L (USEPA, 2009). There is no MCL for calcium or potassium. Dissolved calcium results in water hardiness.

Table 9[3]: Fluoride, chloride, and sulfate concentrations in well water and Lumemo River in Ifakara Village.

Well ID	Fluoride (mg/L)	Chloride (mg/L)	Nitrates (mg/L)	Nitrite (mg/L)	Sulfate (mg/L)	Phosphate (mg/L)	Bromide (mg/L)
IF MRP120	0.73	33.35	3.32	nd	15.91	nd	Nd
IF MRP020	0.50	15.45	0.10	nd	11.49	nd	Nd
IF CW07	0.4	16.34	0.07	nd	1.66	nd	Nd
IF CW01	0.3	14.15	3.64	0.54	4.23	nd	Nd
IF CW03	0.3	3.14	0.51	nd	58.12	1.44	Nd
IF OW03	0.26	16.98	*11.21	nd	7.46	nd	Nd
IF OW04	0.25	36.72	0.44	nd	7.31	nd	Nd
IF OW07	0.31	107.34	0.35	nd	5.14	nd	Nd
IF RPI	0.19	0.9	1.12	nd	1.4	nd	Nd
LUMEMO	0.11	2.71	0.12	nd	9.9	1.54	Nd

* Exceeds MCL
nd – none detected

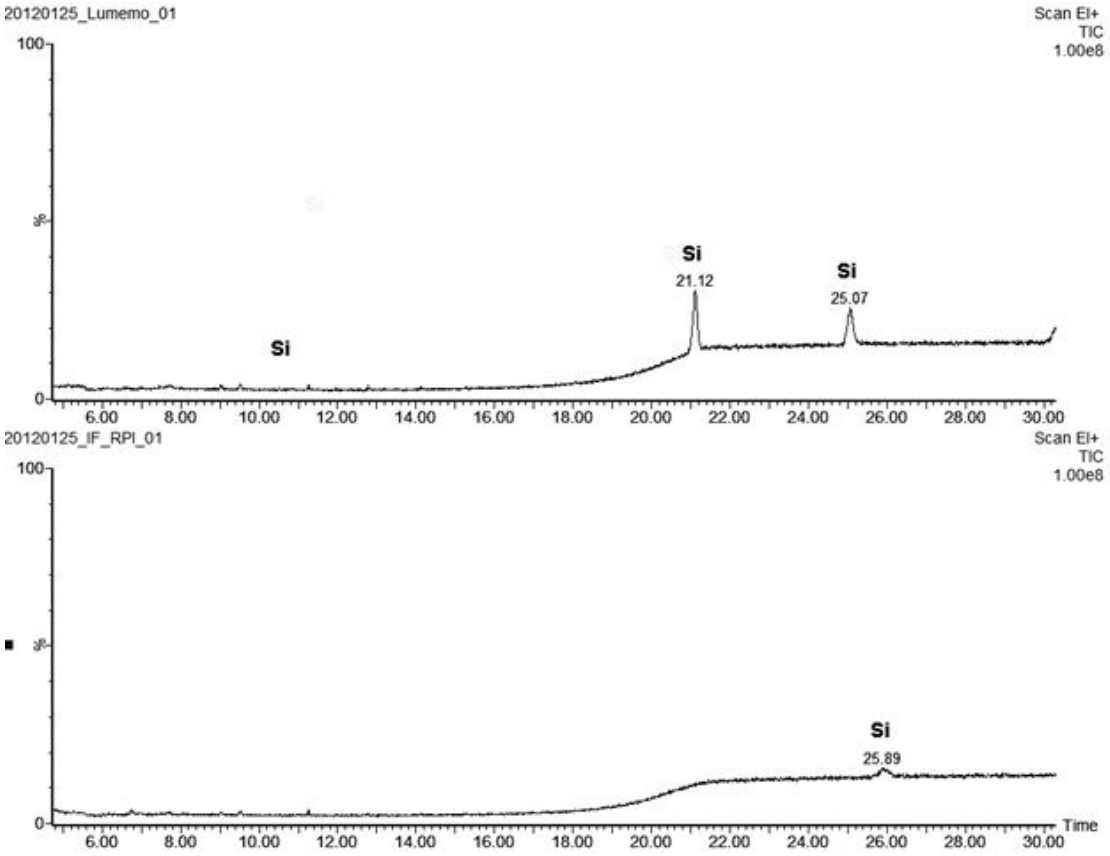
Table 10[4]: Cations concentration detected in well water and River in Ifakara Village.

Well ID	Potassium (mg/L)	Calcium (mg/L)	Iron (mg/L)	Arsenic (mg/L)
IF OW07	23.71	47.23	0.04	0.00
IF OW03	3.78	13.28	0.08	0.00
IF MRP020	1.90	16.83	0.02	0.00
LUMEMO	1.64	4.17	0.01	0.00
IF OW04	1.14	21.48	0.04	0.00
IF MRP120	1.04	21.13	0.02	0.00
IF CW01	0.64	13.92	0.02	0.00
IF CW03	0.52	9.22	0.01	0.00
IF CW07	0.23	16.66	0.01	0.00
IF RPI	0.12	3.51	0.02	0.00

[3] MCL for anions on table 12: Fluoride (4.0 mg/L), Chloride (250mg/L), Nitrate-nitrogen (10mg/L), nitrite-nitrogen (1 mg/L), Sulphate (250mg/L), Phosphate (non-priority contaminants) and Bromide (no MCL).
[4]MCL for cations on table 13; Arsenic (0.010 mg/L); Iron (0.3 mg/L); Potassium (no MCL); Calcium (no MCL).

Two water samples, the Lumemo River and a non-Msabi rope pump well, were selected for aromatic polycyclic compounds (PAHs) analysis. This rope pump well had a layer that appeared to be oil-lik material that was also observed in a few other wells. The Lumemo River is the main aquifer in Ifakara, close to the only gas station in the village. PAHs (gasoline components) were considered as one of the possible causes for the oil-like appearance; however, no gasoline components were detected (Figure 2) in these samples.

Figure 2: Figure shows stacked Total Ion Current (TIC) mass chromatograms for two samples; the Lumemo River (Lumemo) and an individual rope pump (RPI). Each sample run in full scan mode from m/z = 50 – 600. This mass range is generally sufficient to observe many PAHs of interest or other small molecule environmental contaminants. No gasoline components were detected. The chromatogram in this figure shows the only peaks are those of Silica, indicated as Si. The Si peak is typically produced by the gas chromatography column.



Conclusions

Closed wells performed comparably with the Msabi rope pumps in terms of microbial water quality, but Msabi rope pumps had less diversity. There was no statistical difference in HPC, total coliforms and *E. coli* between Msabi rope pumps and closed wells. Closed wells did not perform as well as Msabi rope pumps in terms of turbidity. Msabi rope pumps are deeper (~20-30 m) and have a PVC lining, providing additional protection to the water. The converted closed wells averaged ~5 m in depth. The Msabi rope pumps are thus three to four times deeper than most converted closed wells. One would expect Msabi rope pump wells to perform better than the closed wells in terms of microbial water.

The closed wells are lined with a mix of lean cement/sand that have been fired into a brick and are lined up to 5m from below. Infiltration of water occurs at all levels. A mix of cement and sand also act as filter, in addition to the natural soil filtration processes. This may offer an advantage to the closed wells. Covering the top of the well offers a second advantage. Thus, covering the open wells so as to prevent contamination from the top and surface runoff, and providing a well lining may offer sufficient protection from groundwater contamination. The findings of this study indicate that the closed wells design (open wells, converted into closed wells) may also offer protection and should also be considered as a source of safe water. The results also suggest that the Msabi borehole design had low bacteria diversity and turbidity which may also indicate less contamination in the groundwater or surface water.

Opportunistic pathogens were isolated mostly from the open water sources rather than from the closed wells or Msabi rope pumps. The presence of bacteria specific to domestic animals such as dogs and cats in closed wells indicates poor maintenance and inadequate protection of the well to avoid water contamination, and is not necessarily indicative of poor well design. In this particular case, in the converted closed well CW07, *E. canintestenii* was isolated; the well was heavily used and was close to a road. In one Msabi rope pump (well MRP020) in which *Gordonia polyisoprenivorans* was isolated, the well had evidence of algae on the ground nearby and the latrine was approximately 150 m distant and the surrounding grounds were wet. All open wells performed poorly in terms of water microbial quality and turbidity. Open wells had more bacteria diversity and most of the opportunistic pathogens were detected from the open wells.

One open well had a nitrate-nitrogen concentration above the USEPA MCL of 10 mg/L. The presence of nitrates in water indicates contamination and potential leaching of nitrates into groundwater. There were multiple potential sources of nitrates in the area such as the application of fertilizers, and the presence of livestock and latrines. Since the majority of the wells tested did not have higher nitrate concentrations, this problem may be localized to a very small area. Consuming higher levels of nitrates in drinking water may cause death in infants in which it is converted into nitrite and reacts with oxyhemoglobin to form metheglobin and results in oxygen deprivation (Addiscott, 1996). This phenomenon is known as blue baby syndrome (methemoglobinemia). However, no evidence of blue baby syndrome has been documented in the region.

Well water contamination occurs through 1) subsurface transport of contaminants, 2) near proximity of a latrine to the well and a gradient in the ground between the well and the latrine, and 3) inadequate maintenance of the well. Closed wells performed comparably to the wells with Msabi rope pumps. Thus, the conversion of an open well into a closed well may still offer better water quality, particularly to those who cannot afford to install a Msabi rope pump or had a well prior to Msabi. Furthermore, taking into account the physical location and well design such as distancing the well from sources of contamination (latrines, poultry, livestock etc.), accounting for the soil type (e.g., permeable soils), area topography, and locating the well on a higher gradient would provide additional safety. These considerations, along with proper well maintenance, may provide adequate protection for open wells converted into closed wells. The closed well designed should be considered as an option if there is a significant cost reduction in providing this type of water source to the community.

Recommendations

It is not an easy task, to provide safe water in these remote areas, let alone conduct frequent water monitoring. Taking into account the cost and other difficulties such as access to the water, Msabi should include a water monitoring plan as part of the initiative to provide safe and clean water. The water monitoring could be conducted yearly or seasonally, especially in areas determined as high risk during rainy seasons. The number of samples and the frequency of their collection could be determined based on the population in the area. The World Health Organization (WHO) recommends water monitoring of a water supply from a point source such as a borehole to be conducted twice a year (during wet and dry seasons). The WHO also recommends sanitary inspection, which can be carried out by trained community members (WHO: Water sanitation and health, unpublished). The USEPA Total Coliform Rule can also be used as a tool or as a basis of the decision making process (USEPA: Total Coliform Rule, 1989) for Msabi. By implementing these strategies, organizations such as Msabi, can set standards and provide examples to lead to the next steps towards providing clean and safe water to reduce diarrheal diseases in the region.

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