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Efficacy of chlorine dosage recommendations on the microbiologic quality of turbid waters

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Bachelor of Science
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ABSTRACT

Efficacy of chlorine dosage recommendations on the microbiologic quality of turbid waters

By Shannon Oliver

Background: Recent figures indicate that 884 million people lack access to improved water supplies, and 2.6 billion people do not have access to improved sanitation. The World Health Organization and Centers for Disease Control and Prevention have therefore promoted the use of sodium hypochlorite (chlorine bleach) as a cheap and readily available point-of-use water treatment intervention in an effort to alleviate the disease burden associated with unclean water and unimproved sanitation. ***Objective:*** The goal of this research project was to understand the efficacy of sodium hypochlorite (NaOCl) treatment in turbid waters. Specifically, each of two chlorine dosages (1.875 and 3.75 mg/L NaOCl) were tested on their ability to achieve the following: 1) maintain recommended chlorine residual levels (0.2-2.0 mg/L free chlorine); and 2) reduce microbiologic contamination to <1 CFU. ***Methods:*** A field study to investigate these dosing recommendations was completed in which source waters were collected for the efficacy trial. Three jerry cans were filled to 10 liters each from sources identified as >10 nephelometric turbidity units (NTUs), and randomly assigned to treatment arms (control, 1.875 mg/L and 3.75 mg/L). Source water characteristics were collected at the source location and chlorine dosing occurred in the lab. Follow-up analyses were completed at one-hour (T1), eight-hour (T8) and twenty-four-hour (T24) increments to quantify follow-up contamination (*E. coli* MPN) and free chlorine residual. ***Results:*** No major differences were seen between the two dosing levels with regard to follow-up microbiologic water quality. The higher chlorine dose was more effective at meeting chlorine residual standards, but no significant differences were seen between the two dosages in meeting the *E. coli* standard or for meeting both standards concurrently. There were no consistent associations noted between source water characteristics and follow-up level of contamination or residual chlorine. ***Discussion:*** Point-of-use interventions relying on chlorination as the primary means of water treatment may be effective in lower turbidities. When turbidity is of concern (>10 NTUs) as presented here however, an increased chlorine dose was not shown to significantly improve the microbiologic quality of otherwise untreated source water.

RESUMEN

La eficacia de las recomendaciones para la dosis de cloro en la calidad microbiológica de las aguas turbias

Por Shannon Oliver

Fondo: Las cifras recientes indican que 884 millones de personas no tienen acceso a las fuentes de agua mejoradas, y 2,6 billones de personas no tienen el acceso a las condiciones mejoradas de salubridad. Por lo tanto, la Organización Mundial de la Salud y el Centro para el Control y la Prevención de las Enfermedades han fomentado el uso del hipoclorito de sodio (cloro común) como un método barato y disponible para tratar el agua al punto-del-uso para aliviar el peso de las enfermedades que están asociadas con el agua sucia y las condiciones rudimentarias de salubridad. ***Objetivo:*** La meta de esta investigación fue comprender la eficacia del uso de hipoclorito de sodio (NaOCl) en las aguas turbias. Específicamente, se examinó la capacidad de cada una de las dos dosis de cloro (1,875 y 3,75 mg/L NaOCl) para lograr lo siguiente: 1) mantener niveles recomendados (0.2-2.0 mg/L cloro libre) de cloro residual; y 2) reducir la contaminación microbiológica al <1 UFC. ***Métodos:*** Se completó un estudio de campo para investigar las dosis recomendadas y se recolectaron aguas de la fuente para las pruebas de eficacia. Se llenaron tres pomas con 10 litros cada una, de las fuentes identificados como >10 unidades de turbiedad nephelometric (UTNs), y asignadas al azar a los brazos del tratamiento (control, 1,875 mg/L y 3,75 mg/L). Se recogieron las características del agua de la fuente en su lugar de origen y se administro' la dosis del cloro en el laboratorio. Se recogió el análisis siguiente en incrementos de una hora (T1), ocho horas (T8) y veinticuatro horas (T24) después para cuantificar la contaminación en esos momentos (UFC de *E. coli*) y el residual del cloro libre. ***Resultados:*** No se observaron diferencias importantes entre los dos niveles de dosis con respecto a la calidad microbiológica inmediata del agua. El nivel elevado del cloro fue más eficaz al cumplir con el nivel estándar del cloro residual, pero no se observaron diferencias significativas entre las dos dosis con respecto a cumplir con el criterio de *E. coli* ni con respeto a cumplir con los dos criterios al mismo tiempo. No hubo asociaciones constantes entre las características del agua de la fuente y el nivel inmediato de contaminación ni el cloro residual. ***Discusión:*** ***Las*** intervenciones en el punto-del-uso que dependen de la cloración como el primer tratamiento del agua podrían sean efectivas en aguas de baja turbiedad. Sin embargo, cuando la preocupación es la turbiedad (>10 UTNs) tal como se presenta aquí, una dosis elevada de cloro no demostró una mejoría en la calidad microbiológica de aguas no tratadas.

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INTRODUCTION

Background

Recent figures indicate that 884 million people lack access to improved water supplies, and 2.6 billion people do not have access to improved sanitation (WHO & UNICEF, 2010). Disparities in access to safe water and sanitation infrastructure have resulted in 2.5 million deaths per year from diarrheal disease. This represents 21% of all mortality among children under five years of age in developing countries (Kosek, 2003). In emergency settings such as refugee camps and natural disasters, diarrheal diseases continue to be of primary concern in mortality rates (Doocy, 2006; Toole, 1990). At the close of the 20th century, the Centers for Disease Control and Prevention (CDC) noted the reduction of diarrheal diseases like typhoid and cholera as one of the ten great achievements in public health within the United States from 1900-1999 and clarified that these reductions were achieved through the promotion of clean drinking water and improved sanitation (CDC, 1999).

Impact of Turbidity

Historic efforts to improve the quality of drinking water were based on aesthetic properties of water such as taste and odor, and primarily incorporated filtration or settling of source waters. Stemming from aesthetic indicators of water quality, early municipal drinking water systems in the United States and Europe were focused on the reduction of turbidity (Cech, 2009; EPA, 1999a). Turbid waters appear dirty and/or cloudy when held up to a light source, and have an increased concentration of suspended solids and other organic matter (CDC, 2010; EPA, 1999c). While the cloudiness or clarity of drinking water was a primary indicator in early water development and treatment programs, it

wasn't until 1900 that Whipple and Jackson first proposed methods for the measurement and quantification of turbidity (Hofmann, 1995; Sadar, 1998). This methodology led to the eventual development of the current standard methods (APHA, 1995; EPA, 1999b) for the measurement of turbidity, utilizing light refracted at a 90° angle and quantified in nephelometric turbidity units (NTUs).

Standardization of analytical methods allowed for further investigation into the association between turbidity and microbiological water quality, which showed that a variety of infectious organisms may persist in turbid waters, despite efforts toward disinfection (LeChevallier, 1996). The mechanism for this persistence stems from the organic matter and other substances associated with turbidity, which may inactivate or interrupt the action of chlorine or other disinfection processes. Increased organic load has been shown to decrease disinfection efficacy through the protection of micro-organisms by providing physical protection from chlorine, as well as by interacting with the disinfection agents (EPA, 1999b; LeChevallier, 1981). Due to the interaction of turbidity with common disinfection practices, the United States Environmental Protection Agency (EPA) and the World Health Organization (WHO) have developed recommended turbidity levels for drinking water at <1 NTU and <5 NTUs (respectively) to ensure proper disinfection occurs (EPA, 2011; WHO, 2008). Recommended turbidity levels are commonly met in conventional water treatment practices through flocculation and/or filtration.

Chlorine as a Disinfectant

When combined with chlorine in point-of-use (POU) interventions, disinfection and filtration are effective methods for ensuring sustained water quality after treatment (Crump, 2004; Kotlarz, 2009). Where the ability to utilize flocculation or filtration is

limited due to remoteness, poor infrastructure or emergency settings however, disinfection alone remains the primary method for treating drinking water. In an effort to combat the negative health outcomes associated with unsafe water and unsanitary conditions, the WHO and CDC have promoted the use of sodium hypochlorite (NaOCl) as a drinking water disinfectant for POU interventions. Programs such as Safe Water Systems (CDC) and Household Water Treatment and Safe Storage (WHO) advocate the use of sodium hypochlorite (common household bleach) as an affordable and effective component of POU interventions (CDC, 2010; WHO, 2011). Appropriate dosage of sodium hypochlorite in such situations will facilitate the ability of water treatment interventions to ensure sustained water quality and can assist in the reduction of exposure to microbial contamination (Colindres, 2007; Fewtrell, 2005; Lantagne, 2008; Mintz, 1995). Dosing recommendations for sodium hypochlorite were therefore developed by the CDC, with the aim of protecting human health through the removal or inactivation of microbiologic pathogens from drinking water. Current dosing recommendations call for the application of 1.875 mg/L NaOCl in waters <10 NTUs and 3.75 mg/L NaOCl in waters 10-100 NTUs (CDC, 2010; Lantagne, 2008).

When chlorine is initially added to water, it reacts by binding with organic and inorganic materials. This is referred to as chlorine demand and depends on the concentration of inorganic and organic matter in the treated water. This process occurs over time and reduces the initial chlorine concentration (dose) to the Total Chlorine concentration. A portion of the Total Chlorine is also bound to nitrogen in water; which is known as combined chlorine. The remaining chlorine represents Free Chlorine, which is chlorine that is available for disinfection (AWWA, 1999; CDC, 2010).

Based on the ability of free chlorine to continue its action on micro-organisms, the US EPA has established a free chlorine residual range of 0.2-2.0 mg/L NaOCl as a recommended drinking water quality target (LeChevallier, 1996; Snead, 1980). Many pathogens, including those responsible for cholera, typhoid and Rotaviral infections, are generally susceptible to chlorination at this level (APHA, 2004; Harakeh, 1984; Sousa, 2001). Additionally, long term exposure to chlorinated water in the 0.2-2.0 mg/L free chlorine residual range is not thought to be immediately harmful to human health (Lubbers, 1982). Finally, behavioral aspects of chlorine use such as taste preference have been shown to be acceptable with the 0.2-2.0 mg/L range promoted by the EPA (Lantagne, 2008).

Microbiologic Water Quality Standards

Microbiological targets of the disinfection processes follow the fecal-oral route of exposure, and may enter the human body through the ingestion pathway, by drinking contaminated water (APHA, 2004; Gratacap-Cavallier et al., 2000). In order to disrupt this exposure pathway, drinking water treatment must sufficiently remove or inactivate harmful organisms. For this reason, WHO and EPA have promoted the use of Total Coliforms as an indicator of water quality due to the ability of properly applied disinfection processes to remove these bacteria. Presence of Total Coliforms is an indication of insufficient disinfection processes. Furthermore, the use of *Escherichia coli* as an indicator organism of drinking water quality testing has been promoted by the WHO and EPA. Detectable levels of *E. coli* (>1 CFUs) are an indication of recent fecal contamination of the water in question (AWWA, 1999; Borchardt & Walton, 1971; EPA, 1998).

Context

In a recent study examining WHO and CDC recommendations for POU water treatment, Lantagne (2008) compared free chlorine residuals in stored waters of POU projects in multiple settings. The goals of this study were to clarify dosage recommendations in a variety of turbidities, and the ability of those dosages to meet the chlorine residual criteria (<2.0 mg/L at 1 hour after dosing and >0.2 mg/L at 24 hours after dosing) set forth by WHO and EPA. Findings of this study indicated that waters with lower turbidity (<10 NTU) were more likely (71 of 82 samples or 86.6%) to meet chlorine residual criteria when treated with 1.875 mg/L dose than were waters of higher turbidity (10-100 NTU), in which 3 of 11 samples (27.3%) met chlorine residual criteria at the 1.875 mg/L dosage. Fewer samples (n=15) were analyzed at the 3.75 mg/L dosage, and findings did not indicate that chlorine residual criteria were well met in the 10-100 NTU range (5 of 12 samples, or 41.7%) or for waters >100 NTU (0 of 3 samples). These findings support current recommendations (1.875 mg/L NaOCl) for chlorine dosage in waters of <10 NTU to meet the WHO and EPA chlorine residual standard (Lantagne, 2008).

This study highlighted uncertainty as to the efficacy of current dosing recommendations (1.875 mg/L NaOCl) in waters of 10-100 NTUs, however. Findings on the ability of the 3.75 mg/L dosage to meet chlorine residual standards within waters of 10-100 NTUs are equally unclear. Furthermore, while Lantagne (2008) highlighted the ability of chlorine dosages to meet free chlorine residual standards, clarity on their efficacy in meeting contamination standards has not been well characterized. Given that contamination levels (*E. coli* levels) are an important aspect of drinking water standards,

data on the efficacy of chlorine dosages to meet these standards in waters of 10-100 NTUs is needed (Crump, 2004; Lantagne, 2008; McLaughlin, 2009).

Purpose

The goal of this research project was to understand the efficacy of sodium hypochlorite treatment to improve water quality in turbid waters. Field activities focused on monitoring the effect of sodium hypochlorite on *E. coli* counts and free chlorine residual in turbid waters of the 10-100 NTU range. Specifically, data was collected on the ability for each of two chlorine dosages (1.875 and 3.75 mg/L NaOCl) to achieve the following water quality standards:

1) Maintain recommended chlorine residual levels (0.2-2.0 mg/L free chlorine) over a 24-hour time period, and;
2) Reduce microbiologic contamination to <1 CFU, and maintain that level over a 24-hour period.

The ability of the sodium hypochlorite doses tested here to meet each of these standards individually, as well as to meet both concurrently was examined. It was anticipated that waters treated with a higher chlorine dose (3.75 mg/L NaOCl) will have improved water quality over waters treated with a lower chlorine dose (1.875 mg/L NaOCl). Additionally, the interaction of source contamination, source turbidity, pH, temperature and conductivity with follow-up contamination and chlorine residuals were examined. Physiochemical parameters of source water such as pH, temperature and conductivity impact the ability of chlorine to disinfect through a variety of actions. Primarily, these parameters affect chlorine chemistry, potentially reducing the free chlorine that is available for disinfection (White, 1999).

METHODS

Study Site

Field activities were completed in northern, rural Ecuador during the summer of 2010 and were coordinated through a larger project managed by field partners EcoDess. Eco-Dess is a multi-institution research project focused on diarrheal disease transmission in the communities surrounding Borbón in Esmeraldas Province. Since 2003, EcoDess researchers have worked among 24 villages within this area to monitor diarrheal disease as the community develops and expands as a function of road building in the region (EcoDess, 2010). Sampling and laboratory analysis was carried out during two field visits from June 5 – June 20 (Visit 1) and July 3 – July 20 (Visit 2), 2010. Visit 1 was completed within the communities of Punta de Piedra and San Agustín; while Visit 2 was completed within the communities of Zancudo, Santo Domingo and Colon Eloy. The villages of Punta de Piedra, San Agustín and Colon Eloy are accessible by road, while the villages of Zancudo and Santo Domigo are accessible only by boat. Source locations were selected from untreated surface water sources, resulting in a mix of stream, river and unprotected well locations. Numbers and locations of sources, as well as the number of samples analyzed for those sources can be seen in **Table 1.**

Table 1. Summary of source water locations and samples analyzed for each source location. Details total number of samples taken and number of duplicate samples ran, as well as where there were missing follow-up data.

	Efficacy	Total Samples (# of duplicates)				
Source Location	Trials	Source	Time 0	Time 1	Time 8	Time 24
Colon Eloy	3	3	9	11 (2)	9	10 (1)
Punta de Piedra	6	6	18	18	20 (2)	12^
San Agustin	5	6 (1)	15	18 (3)	16 (1)	16 (1)
Santo Domingo	4	4	13 (1)	13 (1)	13 (1)	12
Zancudo	7	7	21	24 (3)	21	18^
Total	**25**	**26 (1)**	**76 (1)**	**84 (9)**	**79 (4)**	**68 (2)**

^Incomplete trial

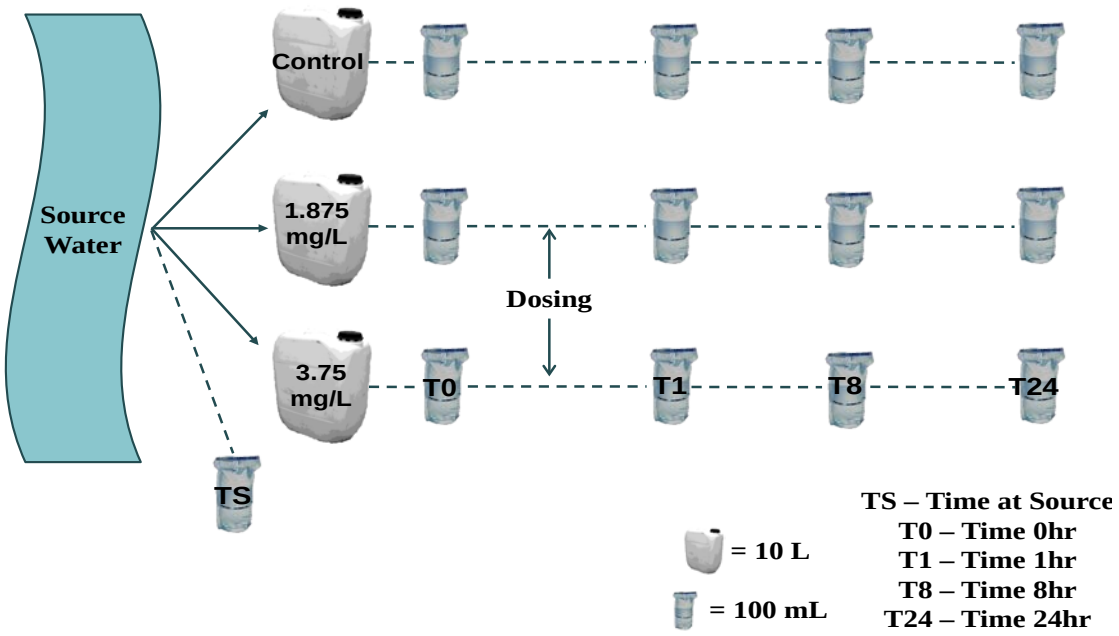
Study Design

Highly turbid source waters (>10 nephelometric turbidity units, or NTUs) were selected for analysis in order to determine the efficacy of sodium hypochlorite (NaOCl) in meeting chlorine residual and microbial contamination standards when turbidity is of concern. During the two 15-day field visits, the author and partners collected water for analysis in locally purchased (new and used) and freshly cleaned (using both detergent and chlorine) 20L jerry cans. These jerry cans are often used in developing settings for the collection, transport and storage of drinking waters in the home (Lantagne, 2008). For this study, source waters (10L) were collected in jerry cans for transport to the lab where they were then dosed with chlorine and stored over the 24-hour efficacy trial period. Each efficacy trial consisted of one jerry can for Lab Control (0.0 mg/L NaOCl), one for 1.875 mg/L NaOCl dosing and one for 3.75 mg/L NaOCl dosing.

Chlorine dosage treatment arms were selected based on prior investigations supporting the use of 1.875 mg/L NaOCl in waters <10 NTUs and lending evidence toward the use of 3.75 mg/L in waters 10-100 NTUs (Lantagne, 2008). Further support on the improved efficacy of 3.75 mg/L over the 1.875 mg/L dose is necessary in waters of >10 NTUs before these dosages can be promoted for point-of-use water treatment interventions.

To this end, baseline data was collected on microbiologic contamination and physiochemical properties for both the source location at the time of collection (Time at source, T_s) and each of the three jerry cans immediately before dosing (Time Zero, T_0). Subsequent analyses of microbiologic contamination and chlorine residual were completed on each jerry can at one-hour (T_1), eight-hour (T_8) and twenty-four-hour (T_{24})

Figure 1. Overview of sampling schematic



intervals after dosing. A sampling schematic can be found in **Figure 1**. Results of each laboratory analysis were hand-recorded in a field log for subsequent statistical analysis.

Sampling Methods

Turbid sources (defined for this study as >10 NTUs) were identified through questioning of the local health promoter as to the location of turbid water sources within his/her respective community, followed by direct measurement at the source to verify the source was of a desired turbidity. Once verified, source waters were collected for the efficacy trial, consisting of three jerry cans filled to 10 liters each. Jerry cans were labeled with source location and date/time of collection, randomly assigned to one treatment arm of the study (A=control, B=1.875 mg7L NaOCl and C= 3.75 mg/L NaOCl) and transferred to lab for storage during the 24-hr efficacy trial period. After the 24-hour period, jerry cans were emptied and washed with commercially available laundry detergent prior to collecting water for the next efficacy trial. Additionally, between each

new village (approximately once every seven days) all jerry cans were rinsed with chlorine bleach to ensure no residual contamination persisted.

A 100 mL sample was also pulled directly from the source location at the time of source water collection, stored on ice and returned to lab for analysis as a baseline of contamination for that efficacy trial. Source location descriptions were completed to identify the source type (river, stream, well, etc.), number of people in the source at time of collection and a general description of source location (low flow/high flow, where in the community the source was located, etc.). Source water characteristics were also recorded at the initiation of the efficacy trial, taken directly from the source waters. These included turbidity (NTU), temperature (°C), pH and conductivity (mV).

It should be noted that during Visit 1, source water characteristics were captured solely at the time of source water collection, pulled directly from the source itself. During Visit 2 however, turbidity values were pulled both from the source location (as during Visit 1) and again from each jerry can, directly before sampling for Time 0 analysis. Due to the recognition that flowing source waters may have highly variable turbidity values depending on time and location, jerry-can-specific turbidity data was collected to more accurately characterize source turbidity for each arm of the efficacy trial. This resulted in source-only data for turbidity during Visit 1, and source-only as well as jerry-can-specific data for turbidity data during Visit 2.

Once in the lab, three individual 100 mL samples were pulled, one from each jerry can, for the quantification of contamination at Time 0, or immediately before dosing. Jerry cans labeled ‘B’ and ‘C’ were subsequently dosed (1.875 mg/L and 3.75 mg/L NaOCl, accordingly), shaken to ensure complete mixing and stored covered for

follow-up sampling. One hour after dosing (Time 1), three 100 mL samples were again pulled, one from each jerry can, analyzed for chlorine residual (jerry cans B and C only) and processed for the quantification of microbial contamination. Jerry cans were thoroughly shaken prior to sampling to ensure no settling of contamination had occurred. All 100 mL samples were collected in a WhirlPak (Nasco, Fort Atkinson, WI), by dipping the bag (for source water sample collection) or tipping the jerry can and pouring water into the bag (for stored water sample collection), without brining hands into contact with sample water or the inside of the bag. Samples collected from jerry cans B and C (those dosed with NaOCl) were collected in WhirlPak bags containing sodium thiosulfate to neutralize the action of the chlorine at the time of collection, allowing for the growth and quantification of *E. coli* and Total Coliforms present in the water at the time of sample collection.

Laboratory Methods

Chlorine dosing was completed utilizing a volume-adjustable pipette with disposable tips. Commercially available bleach was chosen for dosing in all treatment arms and villages. The bleach (Ajax brand) was purchased in Quito prior to field visits, and was analyzed to verify exact concentration of sodium hypochlorite. Chlorine concentration was assessed with a Digital Titrator (HACH Chemical Company, Loveland, CO) according to standard methods, in order to determine the appropriate dosage (volume) to add to 10L of source water for each treatment arm.

Microbiologic analysis was performed following the IDEXX QuantiTray/2000 method, and each 100mL sample was prepared by adding one Colilert reagent pillow (IDEXX, Westbrook, ME) directly to each WhirlPak bag. Samples were shaken until reagent powder was completely dissolved, contents were poured into an unused

QuantiTray, and the tray was processed through the IDEXX sealer to create an air-tight seal on the tray. Processing date and time were recorded on the back of the tray as well as in the lab notebook, and the expected read time (24 hours after process time) was recorded on the tray along with a separate lab tracking sheet. Trays were stored in one of two incubators, that were maintained at a temperature of 37.5°C (+/- 3°C), for a period of at least 24 hours.

Results from microbial analysis detailed the Most Probable Number (MPN) of Colony Forming Units (CFUs) for both Total Coliform and *E. coli* contamination, recorded as the number of large and small cells that turned yellow or fluoresced under UV light (respectively) after a 24-hour incubation period. Results were quantified utilizing the MPN chart made available by IDEXX, indicating an upper detection limit of 1011.2 MPN and a lower detection limit of <1 MPN for both Total Coliform and *E. coli* counts. Due to limited resources, a UV light of differing specifications (25 watt fluorescent, unspecified wavelength) than that recommended by IDEXX (6 watt fluorescent, 365-nm long wave) was utilized for quantification of *E. coli* MPN during field trials. Following the field study however, 70 source water samples were processed following the same IDEXX methods as the field trials and analyzed for *E. coli* using both the UV bulb from the field trials and a UV bulb of the recommended wavelength. Results showed 100% agreement between the UV bulb from the field and the UV bulb of recommended specifications, indicating the UV bulb utilized in the field provided accurate results (Robb, 2011).

Turbidity was measured utilizing a HACH 2100P Turbidimeter, which was calibrated at the onset of each Visit as well as after moving to each new village utilizing

STABLCAL Stabilized Formazin Standards (HACH Chemical Company, Loveland, CO) and standard methods. Results of turbidity analysis were recorded in nephelometric turbidity units (NTUs). Chlorine residual was assessed using a LaMotte 1200 Colorimeter (LaMotte, Chestertown, MD) spectrophotometer, utilizing standard methods. Results were recorded as the Free and Total Chlorine (mg/L) for each time point. Temperature (°C), pH and conductivity (mV) were measured utilizing a sensION2 pH ISE Meter (HACH, Loveland, CO), which was calibrated at the onset of each Visit utilizing buffered standards of 10.0 pH and 7.0 pH per standard methods. The pH electrode meter was cleaned and stored between each source water collection, following standard practices as directed by the instrumentation manual.

It should also be noted that during the field visits, blank samples were run each day to ensure aseptic conditions in the field lab. These were 100mL samples of deionized water prepared with one Colilert reagent pillow and processed exactly as efficacy trial samples. On six occasions, there were detectable levels of total coliforms and on two occasions *E. coli* was present within the processed lab blank. In a review of all samples processed on each day of the efficacy trials on which a positive blank was recorded, at least one negative sample from the field study was recorded. This indicates aseptic technique was maintained as it would be expected that contamination would be pervasive if it were of concern. It is unclear whether the deionized water being utilized for the blanks was sufficiently prepared and protected during transport, which may have contributed to the positive blanks in the field study.

Data Analysis Methods

Data entry and cleaning – Hand-recorded data from field logs was initially entered into a Microsoft Excel spreadsheet for data cleaning and exploratory data analysis. The data

cleaning process included double entry of ten percent (10%) of field data to verify accuracy. Quality assurance of double entry yielded a 0.2% error rate and data were therefore considered accurate. Exploratory data analysis included quantifying numbers/types of samples from the various communities, descriptive statistics on source water characteristics and descriptive statistics on follow-up water quality outcomes. Water quality parameters were also compared across time points, for each treatment arm, and were summarized graphically in Excel. All statistical analyses were completed utilizing the SAS v9.2 (Cary, NC) statistical package.

Sample Population and Variables of Interest – Due to equipment malfunction in the field, one of the 25 initial efficacy trials was missing data on both source water characteristics (turbidity, temperature, pH and conductivity) and 24-hr follow-up analysis (both for contamination and chlorine residual), and was therefore excluded from all subsequent analysis. One efficacy trial was missing source water contamination data and was not included in the comparative analysis of source water characteristics with subsequent free chlorine and contamination levels. Finally, two efficacy trials were missing follow-up data for both contamination and free chlorine residual at the 24-hr time point and were not included in analyses for that time point. Mean, median and quartiles for all variables of interest were computed and histograms were generated in order to clarify the distribution of each variable.

Dependent variables of interest for this study are follow-up contamination (*E. coli* MPN), log reductions in contamination (ΔLog_{10}[*E. coli* MPN]) and free chlorine residual (mg/L). Follow-up data from the efficacy trials are spread across three time points (Time 1, Time 8 and Time 24) as well as three treatment arms (Control, 1.875 mg/L Dose

and 3.75 mg/L Dose). Independent variables of interest are source contamination (*E. coli* MPN), turbidity (NTU), conductivity (mV), pH, temperature (°C), follow-up free chlorine residual (mg/L), turbidity range (high/low) and source contamination range (high/low).

Log reductions in *E. coli* – In order to clarify the impact of each treatment arm (1.875 mg/L and 3.75 mg/L) on log reductions of contamination, a test for differences of mean log reductions between the two treatment arms was completed utilizing a student's t-test. Log reductions of contamination (ΔLog_{10}[*E. coli* CFU/100mL]) were determined within each treatment arm as the difference in contamination between time-0 (T0) and follow-up time points of time-1 (T1), time-8 (T8) and time-24 (T24). The paired t-test allows for examination of differences in mean values of paired samples such as are present in this study.

Impact of Turbidity – In order to clarify the impact of turbidity on the outcomes of interest, high and low turbidity ranges were established for comparison across turbidities, split at the median source turbidity for all source water samples. Median source turbidity (26.7 NTUs) was identified, utilizing the PROQ UNIVARIATE function in SAS. Samples were placed into low (coded as 1) and high (coded as 2) turbidity ranges, based on their source turbidity (taken from source location). Average log reductions were compared between the high and low turbidity range for each treatment arm and at each time point. Turbidity range was also included in simple and multiple linear regression, as well as in analysis of variance modeling (ANOVA), as will be explained shortly.

Due to the availability of source-only turbidity values as well as jerry-can-specific values for Visit 2 efficacy trials, univariate analysis was performed on both sets of data.

Analysis of intra-trial variability in turbidity for Visit 2 samples, containing both source turbidity and jerry-can-specific turbidity, was also completed by averaging the difference (source-only – jerry-can-specific turbidity) in each sample and computing the student's t-test on the differences.

Impact of Source Contamination – In order to test the impact of source contamination on follow-up parameters of interest, high and low source contamination ranges were established to test for differences within these ranges, split at median source contamination. Median source contamination (913.5 CFU/100mL) was identified, utilizing the PROQ UNIVARIATE function in SAS. Samples were placed into a low (coded as 1) and high (coded as 2) source contamination range. A test for differences was computed, comparing average log reductions between source contamination ranges, within each treatment arm and at each time point. Source contamination range was also included in simple and multiple linear regression, as well as in ANOVA modeling, as will be explained later in this section.

Comparison of Standards – A major question of this study is whether or not chlorine dose plays a role in achieving commonly accepted drinking water quality standards in highly turbid waters. Specifically, the ability to maintain 0.2-2.0 mg/L NaOCl over a 24-hr period and to reduce microbial contamination to <1 CFU *E. coli* was examined within each treatment arm and for each follow-up time point (EPA, 2011; WHO, 2008). To assess the ability of each treatment arm to achieve these water quality standards, percentages and numbers of samples meeting these standards within each turbidity range and time stamp were summarized. Dummy variables were created to code pass/fail (1/0, respectively) of the microbial contamination standard and chlorine residual standard

individually, as well as for those samples meeting both standards concurrently. Differences between the two treatment arms for each time point were compared using the student's paired t-test. Differences were examined under high/low turbidity and high/low source contamination stratifications as well.

To investigate the impact of source water characteristics (namely turbidity and contamination) and treatment arm on the ability to meet the water quality standards, ANOVA modeling was completed utilizing a general linear model in SAS. The variables created for high/low turbidity, high/low source contamination and the variable for treatment arm (1.875 mg/L coded as 1 and 3.75 mg/L coded as 2) were included in models to test their association with samples meeting or not meeting the water quality standards. As with the student's t-test above, samples were tested on their ability to meet the *E. coli* standard and chlorine standards individually, as well as their ability to meet both standards concurrently.

Linear Regression – Of primary interest in this study is the association between source water characteristics and follow-up water quality parameters. To clarify these associations, simple linear regression was performed comparing each source water characteristic (turbidity, *E. coli* CFUs at source location, conductivity, temperature and pH) to each outcome of interest (*E. coli* CFU/100mL, ΔLog_{10}[*E. coli* CFU/100mL] and free chlorine residual). Additionally, free chlorine residual was analyzed against log reductions in contamination and follow-up contamination to determine the association between chlorine dose and subsequent water quality parameters. Finally, the impact of turbidity on follow-up parameters is of interest in this study and data were therefore classified as falling in a high or low category for turbidity (as clarified in **Impact of**

Turbidity above) and this variable was included in regression analysis as an independent variable.

Similarly, the impact of source contamination on follow-up parameters is of interest and a variable was created to classify data into a high and low range of source contamination (as detailed in **Impact of Source Contamination** above), and this variable was included in regression analysis as an independent variable. The PROC REG function in SAS was utilized to perform these analyses, for each time point and stratifying by the Control, 1.875 mg/L and 3.75 mg/L NaOCl treatment arms.

Multiple Linear Regression – To further clarify the association of source water characteristics and the outcomes of interest, multiple linear regression was completed comparing all independent variables to each dependent variable of interest. Specifically, models were established to test the ability of turbidity, free chlorine residual, conductivity, temperature, pH and source contamination range to predict log reductions of contamination (ΔLog_{10}[*E. coli* CFU/100mL]). Source contamination and source turbidity range were excluded in this model due to their lack of association with log reductions in *E. coli* within simple linear regression.

Another model was constructed to predict the impact of turbidity, source contamination, free chlorine residual, conductivity, pH and temperature on follow-up levels of contamination (*E. coli* CFU/100mL). Source contamination range and turbidity range were excluded in this model as they did not show a strength of association within simple linear regression modeling.

Finally, water characteristics of turbidity source contamination, conductivity, pH and temperature were analyzed against free chlorine residual at each time point and for

both treatment arms, to test for associations. Again, source contamination range and turbidity range were not seen to be highly correlated with free chlorine residual under simple linear regression and were therefore excluded from the multiple regression model. As with the simple linear regression models, free chlorine residual was included in the multiple regression models for log reductions in contamination and follow-up contamination level. The PROC REG function was again utilized in SAS for these analyses.

RESULTS

Summary statistics

Source water characteristics are summarized in **Table 2**. Source water contamination (*E. coli*) ranged from 55.6-1011.2 Colony Forming Units (CFU) per 100 mL sample, with a mean value of 790.5 CFU/100 mL, and a geometric mean values of 703.6 CFU/100mL. Six of twenty-four efficacy trials (25%) had initial *E. coli* levels of 1011.2 MPN, which is the maximum detectable contamination level utilizing the IDEXX method. Source water turbidity ranged from 10.7-142.0 NTUs, with a mean value of 35.0 NTUs and a geometric mean value of 25.4 NTUs. Conductivity ranged from -32.0- 120.4 mV, with an average of 29.5 mV. Temperature ranged from 25.1-30.4°C, with an average of 26.7. Finally, pH ranged from 5.1-7.9 with an average of 6.7.

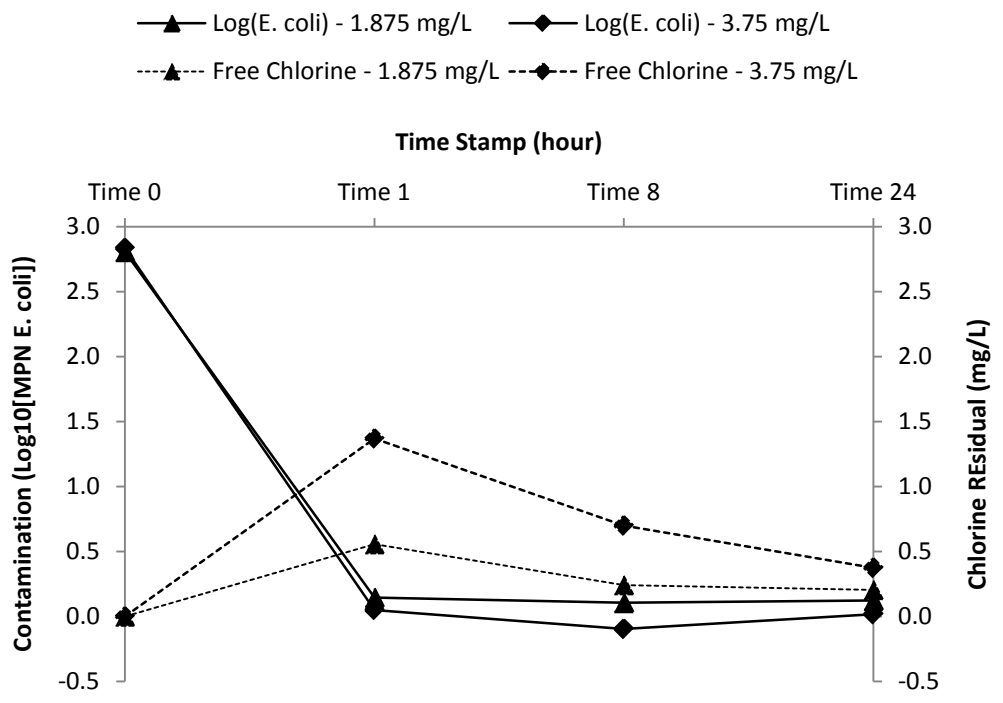
Table 2. Summary of source water characteristics. Includes arithmetic mean, geometric mean and standard deviation. (n=24)

Parameter	Arithmetic Mean	Geometric Mean	Std. Dev.
Turbidity (NTU)	35.0	27.3	29.2
E. coli (MPN)*	782.3	698.8	258.4
Conductivity (mV)	29.2	N/A	49.3
pH	6.7	N/A	0.9
Temperature (°C)	26.6	N/A	1.4

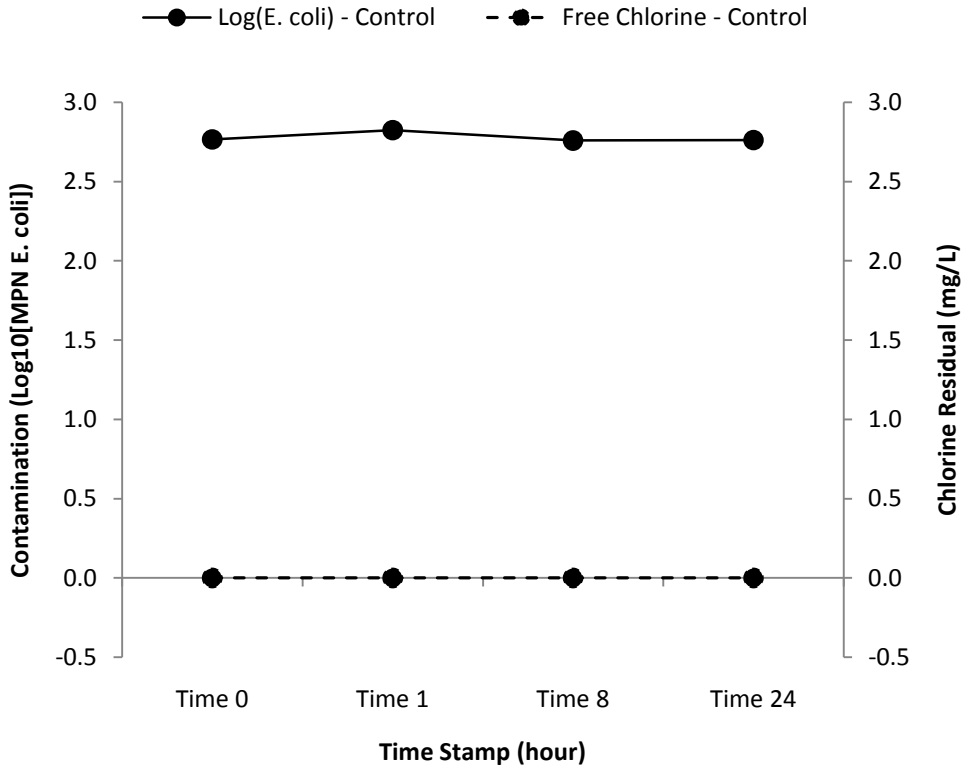
*n=23

Figure 2. Changes in log(*E. coli* MPN) and free chlorine residual (mg/L) over each time point for the 1.875 and 3/75 mg/L NaOCl treatment arms.

A. 1.875 mg/L and 3.75 mg/L NaOCl treatment arms



B. Control treatment arm



Changes over time in contamination levels (Log_{10}[*E. coli* MPN]) and chlorine residual (mg/L) are shown in **Figure 2-A,** for both treatment arms. For clarity in the figure, control arm values have been displayed separately in **Figure 2-B**. After a sharp decline in contamination between 0-hr and 1-hr time points, both the 1.875 mg/L and 3.75 mg/L dose experienced a slight increase in contamination on average (0.23 and 2.53 MPN, respectively) between the 8-hr and 24-hr follow-up. Free chlorine residuals 1 hour after dosing were, on average, within the 0.2-2.0 mg/L range for both the 1.875 mg/L and 3.75 mg/L treatment arms. These levels reduced over time to 0.21 mg/L and 0.34 mg/L at 24-hr follow-up, respectively.

Log reductions in E. coli

Reductions in contamination over time are an indication of disinfection efficacy. Reductions have been characterized here as the average log reduction of *E. coli* MPN for each treatment arm (control, 1.875 mg/L and 3.75 mg/L) seen for each time period. Average log reductions, as well as the variance (standard error) in these measures, can be seen in **Figure 3** for each treatment arm and for the time periods of T0 to T1, T0 to T8, and T0 to T24, respectively. The control arm had slight

Figure 3. Average log reductions in *E. coli* MPN by time point and treatment arm, with standard error bars.

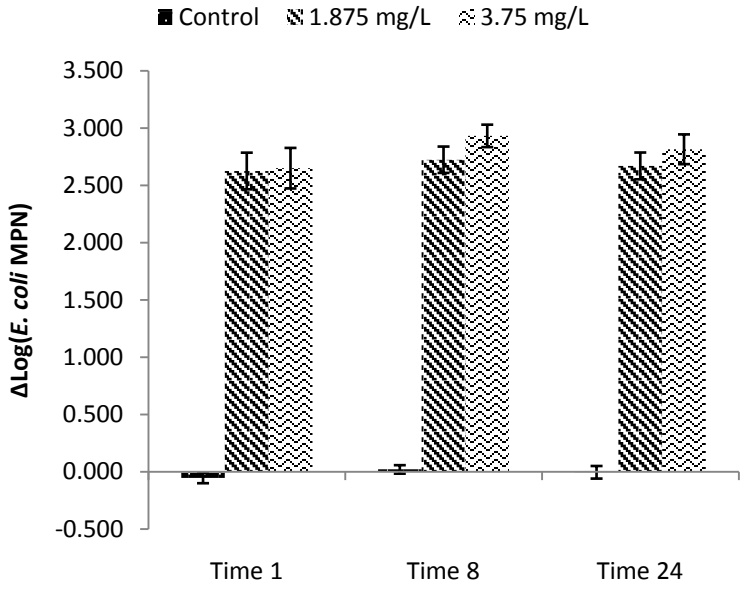


Table 3. **Comparison of log reductions in *E. coli* over time.** Data presented represent the mean change over time of log(*E. coli*) between control arm and each treatment arm, as well as between the two treatment arms for the time periods detailed. Student's t-test of the mean difference was utilized to test whether these differences were significant. Mean difference, p-values and 95% confidence intervals are presented

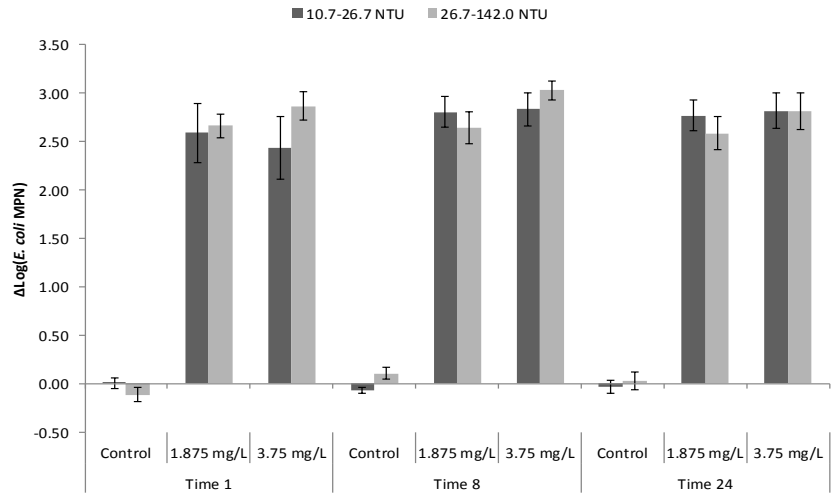
Comparison *Time Period*	**Mean Difference**	**95% CI**	**p-value**
Control - 1.875 mg/L			
T0-T1	2.68	2.35-3.01	**<.0001***
T0-T8	2.70	2.43-2.98	**<.0001***
T0-T24	2.71	2.40-3.03	**<.0001***
Control - 3.75 mg/L			
T0-T1	2.71	2.32-3.09	**<.0001***
T0-T8	2.91	2.68-3.14	**<.0001***
T0-T24	2.78	2.44-3.12	**<.0001***
1.875 mg/L - 3.75 mg/L			
T0-T1	0.03	-0.29-0.34	0.8748
T0-T8	0.21	-0.09-0.51	0.1660
T0-T24	0.15	-0.15-0.44	0.3185

increases in the average concentration of contamination over time periods T0-T1 and T0-T24, with slight decreases in time period T0-T8, indicating relatively stable contamination concentrations in the control arm. Both the 1.875 and 3.75 mg/L treatment arms had between 2.5 and 3.0 log reductions in *E. coli* for all time periods. When comparing log reductions between the control arm and each treatment arm individually, significant differences were seen for all time points (all p-values <0.001). However when comparing the 1.875 mg/L dose to the 3.75 mg/L dose across all time points, no significant differences were seen (all p-values >0.05). Mean differences, confidence intervals and p-values for these comparisons can be seen here in **Table 3**.

Impact of Turbidity

In order to determine the impact of turbidity, stratification of the dataset was completed, splitting samples at the median source turbidity value of 26.7 NTUs. Differences in log reductions of *E. coli* MPN between the two turbidity ranges were then compared within

Figure 4. Comparison of average log reductions in *E. coli* MPN between turbidity ranges and displayed for each time point and treatment arm.



	Control	1.875 mg/L	3.75 mg/L	Control	1.875 mg/L	3.75 mg/L	Control	1.875 mg/L	3.75 mg/L
	Time 1			Time 8			Time 24		
Difference Between Turbidity Ranges	-0.12	0.07	0.43	0.17	-0.16	0.20	0.06	-0.18	-0.01
p-value	0.2092	0.8183	0.2347	**0.0169***	0.4917	0.3279	0.6111	0.4502	0.9778
95% CI	-0.31 - 0.07	-0.60 - 0.74	-0.29 - 1.16	0.03 - 0.31	-0.65 - 0.32	-0.21 - 0.60	-0.17 - 0.29	-0.68 - 0.31	-0.57 - 0.55

treatment arms and for each time point. As detailed in **Figure 4,** one significant difference was noted within the control arm at Time 8. No other significant differences were noted for this comparison. Average log reductions and variance, as well as mean differences, p-values and confidence intervals can be seen in **Figure 4.**

Due to the availability of turbidity data during Visit 2 for source location as well as jerry-can specific turbidities, intra-trial variability was examined. To understand if there was significant variability in turbidity measures when sampling from source location only, a test for differences between jerry-can-specific turbidity data and source water turbidity data was completed for each sample collected. Intra-trial variability of turbidity ranged from 0-23.0 NTUs with an average difference between source turbidity and jerry-can-specific turbidity of 5.8 NTUs. This difference was significantly different from zero within each efficacy trial ($p<0.0001$), and are summarized in **Table 4.**

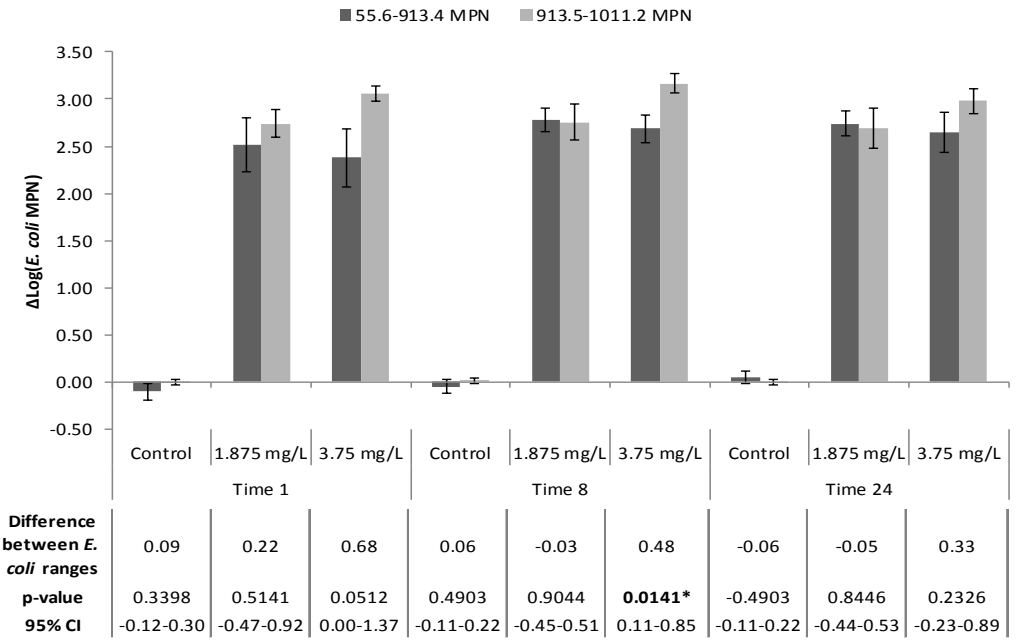
Table 4. Intra-trial variability in Turbidity data. Student's t-test comparing average difference between source-only turbidity and jerry-can-specific turbidity.

Mean Difference	Std. dev	Range	95% CI	p-value
5.8	7.1	0.0-23.0	3.5-8.0	<0.0001

Impact of Source Contamination

In order to test the impact of initial contamination on log reductions of *E. coli*, data were split at the median source contamination level (913.5 CFU/100mL). Stratification into high and low source contamination ranges yielded one significant difference between the 1.875 mg/L dose and the 3.75 mg/L dose, for the 8-hr time point. No other significant differences were noted in this comparison. Average log reductions

Figure 5. Comparison of average log reductions in *E. coli* MPN between source contamination ranges and displayed for each time point and treatment arm.



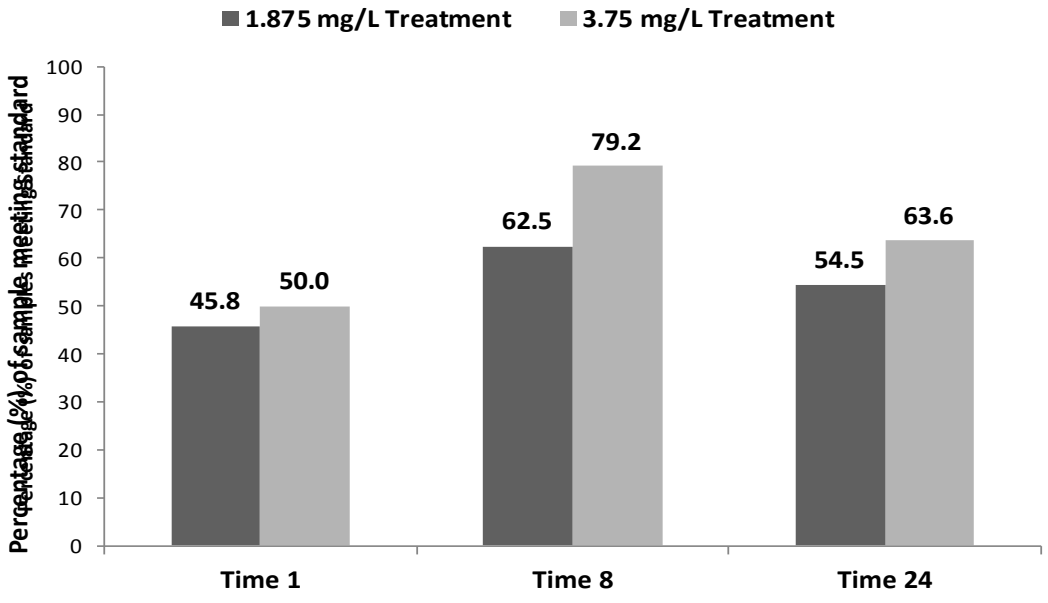
	Time 1			Time 8			Time 24		
Difference between *E. coli* ranges	0.09	0.22	0.68	0.06	-0.03	0.48	-0.06	-0.05	0.33
p-value	0.3398	0.5141	0.0512	0.4903	0.9044	**0.0141***	-0.4903	0.8446	0.2326
95% CI	-0.12-0.30	-0.47-0.92	0.00-1.37	-0.11-0.22	-0.45-0.51	0.11-0.85	-0.11-0.22	-0.44-0.53	-0.23-0.89

and variance, as well as mean differences, p-values and confidence intervals for the impact of source contamination on log reductions can be seen in **Figure 5.**

Comparison of Standards

The ability for each treatment arm (1.875 mg/L and 3.75 mg/L NaOCl) to satisfy the water quality standards set forth by the WHO and USEPA for chlorine residual (0.2-2.0 mg/L NaOCl) and contamination (<1 CFU *E. coli*) was examined to clarify the impact of an increased chlorine dose on the key outcomes of interest (EPA, 2010; WHO,

Figure 6. Comparison of treatment arms and their ability to meet the <1 CFU *E. coli* standard for each time period. Differences are presented between treatment arms for each time point, with associated p-values and confidence intervals.



	Time 1	Time 8	Time 24
Difference between treatment arms	4.2	16.7	9.1
p-value	0.7784	0.2123	0.5506
95% CI	-25.5-33.8	-9.9-43.2	-21.4-39.6

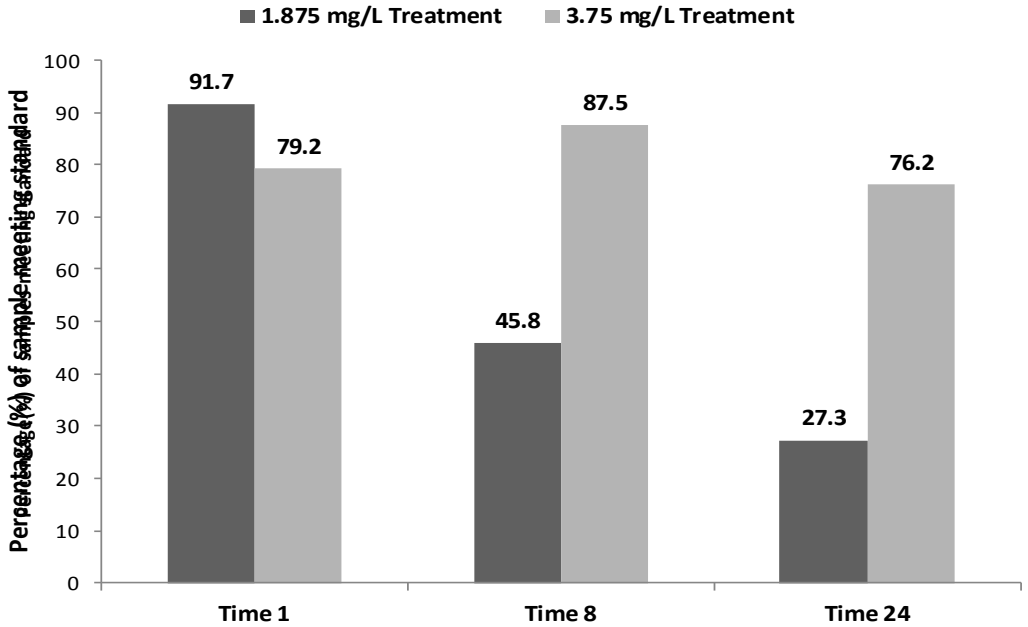
2008). Each sample was compared to the contamination and free chlorine residual standards individually, as well as for meeting both standards concurrently. A student's t-test was then performed to test the differences in the percentage of samples meeting each standard between the 1.875 mg/L and 3.75 mg/L treatment arms, for each time point. Data were first analyzed as a pooled sample, and later stratified by source turbidity and source contamination.

Data for the pooled results of these analyses are presented graphically in **Figure 6** for comparison to the *E. coli* standard, **Figure 7** for comparison to the chlorine residual standard, and **Figure 8** for those samples meeting both standards concurrently. It should be noted that of the samples not meeting the free chlorine residual standard, five samples within the 3.75 mg/L dose were above the 2.0 mg/L upper threshold for the 1-hr analysis.

Inclusion of these samples in the test for differences did not change the level or occurrence of significant differences however.

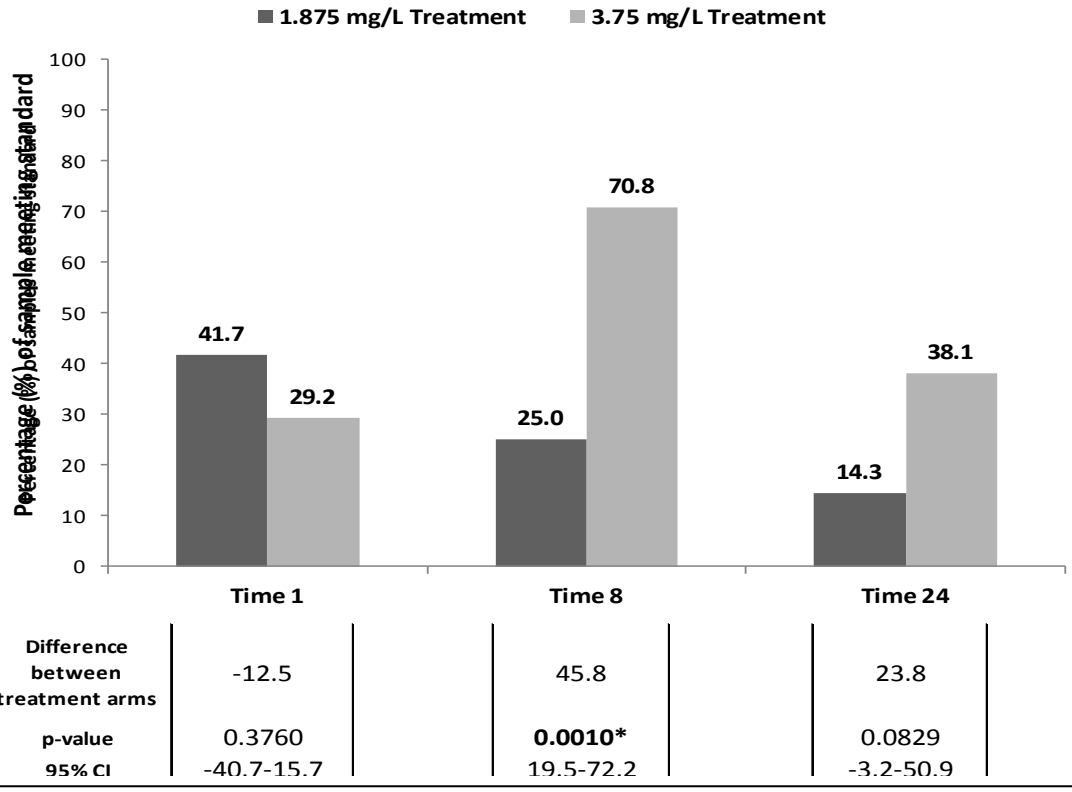
The pooled results presented here represent the samples within each time point and treatment arm for all turbidities and all source contamination levels. No significant differences were seen between the 1.875 mg/L and 3.75 mg/L dose when comparing pooled samples that met the *E. coli* standard. With regard to meeting the chlorine standard, the 3.75 mg/L dose had significantly more samples within the 0.2-2.0 mg/L NaOCl range than did the 1.875 mg/L dose for both the 8-hr and 24-hr time point. The 1.875 mg/L treatment arm had 2 samples (8.3%) below the standard 1 hour after dosing, 9 samples (37.5%) below the standard at the 8-hr follow-up and 11 samples (52.4%)

Figure 7. Comparison of treatment arms and their ability to meet the 0.2-2.0 mg/L chlorine residual standard for each time period. Differences are presented between treatment arms for each time point, with associated p-values and confidence intervals.



	Time 1	Time 8	Time 24
Difference between treatment arms	-12.5	41.7	38.1
p-value	0.2286	**0.0017***	**0.0127***
95% CI	-33.1-8.1	16.6-66.8	3.6-67.6

Figure 8. Comparison of treatment arms and their ability to meet both standards concurrently for each time period. Differences are presented between treatment arms for each time point, with associated p-values and confidence intervals.



below at the 24-hr follow-up. Within the 3.75 mg/L treatment arm, there were 5 samples (20.8%) above the standard at 1-hr follow-up, 1 sample (4.2%) below the standard at 8-hr follow-up and 4samples (19.0%) at the 24-hr follow-up. Finally, the higher chlorine dose (3.75 mg/L NaOCl) had significantly more samples meeting both standards (concurrently) than did the lower chlorine dose within the 8-hr time point.

In order to determine the impact of source turbidity on the ability for each treatment arm to meet the water quality standards, a similar comparison of standards analysis as above was completed, stratified by source turbidity (high/low) and the student's t-test was completed on the stratified data. Data were split into two groups at the median source turbidity (26.7 NTUs). Results for samples meeting the *E. coli* standard, the chlorine residual standard and both standards concurrently are shown in

Figures 9-A, 10-A and **11-A** (respectively), found in the **APPENDIX of TABLES and FIGURES**. When stratifying by turbidity, no significant differences were seen between the two treatment arms' ability to meet the *E. coli* standard for any time point. When examining the percent of samples that met the free chlorine standard within the two turbidity ranges, the 3.75 mg/L dose had significantly more passing samples during the 8-hr and 24-hr time points, within the lower turbidity range. Significant differences between the two treatment arms, when stratifying by turbidity, for samples meeting both standards concurrently occurred within the lower turbidity range during the 8-hr and 24-hr time point as well as within the higher turbidity range during the 24-hr time point.

In an effort to clarify how the level of source contamination impacts follow-up parameters of interest, a similar comparison of standards analysis was completed on the data, stratifying by source contamination range. A Student's t-test was again utilized to compare the differences in percent of samples meeting each standard, when stratifying by source contamination level. Results for these comparisons are shown graphically in **Figures 9-B** for the *E. coli* standard**, Figure 10-B** for the chlorine residual standard and **11-B** for those samples meeting both standards concurrently, and can be found in the **APPENDIX of TABLES and FIGURES**. When split at the median source contamination (913.5 CFU/100mL), no significant differences between the two treatment arms were seen for percentage of samples meeting the *E. coli* standard. Differences in the ability to meet the free chlorine standard occurred when stratifying by source contamination, with significantly more samples in the 3.75 mg/L treatment arm meeting the standard within the lower contamination range during the 8-hr time period and within the higher contamination range during the 24-hr time point. With regard to samples

meeting both standards concurrently, one significant difference was seen between the two treatment arms, occurring during the 8-hr time point within the higher source contamination range.

Table 5. Results from ANOVA model comparing source contamination range, turbidity range and treatment arm to samples meeting the *E. coli* standard.

Model		ANOVA Results		
Timepoint	n	R^2	p-value	β
Overall Model				
T1	46	0.0096	0.9382	N/A
T8	46	0.2266	0.7549	N/A
T24	42	0.1021	0.2464	N/A
Treatment Arm				
T1	46	N/A	0.7785	0.0433
T8	46	N/A	0.3466	0.1304
T24	42	N/A	0.7541	0.0476
Turbidity Range				
T1	46	N/A	0.9417	0.0114
T8	46	N/A	0.6063	-0.0718
T24	42	N/A	0.0901	-0.0271
Contamination Range				
T1	46	N/A	0.5707	-0.0886
T8	46	N/A	0.8395	0.0282
T24	42	N/A	0.5336	-0.0989

**Significant to 0.05*

Finally, to examine the impact of source contamination, turbidity and treatment arm on the outcomes of interest, ANOVA was completed utilizing a general linear model. Categorical variables for turbidity range, source contamination range and treatment arm were tested against each water quality standard individually as well as for samples meeting both standards at once. When comparing turbidity range, source contamination range and treatment arm to those samples meeting the *E. coli* standard, no significant associations were seen from ANOVA, as detailed in **Table 5.** For samples meeting the chlorine residual standard, significant associations were seen in the ANOVA model between treatment arm and ability to meet the chlorine standard for the 8-hr ($p=0.0012$) and 24-hr ($p=0.0064$) time points, as detailed in **Table 6**. These associations were positive ($\beta=0.4348$ and $\beta=0.4000$, respectively), indicating an increase in treatment arm (moving from 1.875 mg/L to 3.75 mg/L dose) as associated with a greater percent of samples meeting the standard. With regard to samples meeting both standards

Table 6. Results from ANOVA model comparing source contamination range, turbidity range and treatment arm to samples meeting the free chlorine residual standard.

Model	ANOVA Results			
Timepoint	n	R^2	p-value	β
Overall Model				
T1	46	0.1132	0.1619	N/A
T8	46	**0.2711**	**0.0038***	N/A
T24	40	**0.3126**	**0.0034***	N/A
Treatment Arm				
T1	46	N/A	0.2182	-0.1304
T8	46	N/A	**0.0012***	**0.4348**
T24	40	N/A	**0.0064***	**0.4000**
Turbidity Range				
T1	46	N/A	0.9697	0.0040
T8	46	N/A	0.0602	0.2389
T24	40	N/A	0.0876	0.2478
Contamination Range				
T1	46	N/A	0.0596	0.2040
T8	46	N/A	0.6322	-0.0611
T24	40	N/A	0.0786	0.2609

**Significant to 0.05*

Table 7. Results from ANOVA model comparing source contamination range, turbidity range and treatment arm to samples meeting both standards concurrently.

Model	ANOVA Results			
Timepoint	n	R^2	p-value	β
Overall Model				
T1	46	0.0249	0.7841	N/A
T8	46	**0.1938**	**0.0274***	N/A
T24	40	0.0849	0.3564	N/A
Treatment Arm				
T1	46	N/A	0.3803	-0.1304
T8	46	N/A	**0.0031***	**0.4348**
T24	40	N/A	0.0876	0.2500
Turbidity Range				
T1	46	N/A	0.9957	-0.0215
T8	46	N/A	0.7312	0.0483
T24	40	N/A	0.7894	0.0391
Contamination Range				
T1	46	N/A	0.5997	0.0785
T8	46	N/A	0.7133	-0.0517
T24	40	N/A	0.7164	0.1543

**Significant to 0.05*

concurrently, treatment arm was also significantly (p=0.0031) associated with meeting both standards for the 8-hr time point.

Again, a positive association (β=0.4348) was seen indicating increasing dosage was associated with increasing ability to meet both standards concurrently for that time point. Model results for analysis of samples meeting both standards concurrently can be found in **Table 7**.

Simple Linear Regression

In order to examine the association of source water characteristics and follow-up water quality measures, a series of simple linear regression models were analyzed comparing each source water characteristic (turbidity, source contamination, conductivity, temperature and pH) to each outcome of interest (*E. coli* CFU/100mL, ΔLog_{10}[*E. coli* CFU/100mL] and free chlorine residual). Additionally, free chlorine

residual was analyzed against log reductions in contamination and follow-up contamination to determine the association between chlorine dose and subsequent water quality parameters. Each follow-up time point (T1, T8 and T24), as well as each arm of the study (control, 1.875 mg/L and 3.75 mg/L) were examined within regression models. All results can be found in **Table 8** as the unadjusted values for each model run.

Log Reductions in E. coli – Simple linear regression models comparing source water characteristics and free chlorine residual to log reductions in *E. coli* were examined to determine the impact of these parameters on log reductions in *E. coli* across time

Table 8. All results from simple linear (unadjusted) and multiple linear (adjusted) regression models comparing source water characteristics to follow-up log reductions in *E. coli.*

Time 1		**1.875 mg/L**				**3.75 mg/L**			
	n	Unadjusted		Adjusted		Unadjusted		Adjusted	
Parameter		**β**	**p-value**	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**
Turbidity	24	-0.0019	0.7461	-0.0003	0.9671	-0.0073	0.2456	0.0054	0.3429
Source contamination	23	0.0002	0.7105	0.0002	0.7457	**0.0018**	**0.0076***	**0.0020**	**0.0045***
Free Chlorine Residual	24	0.7056	0.1954	0.6149	0.2988	0.2778	0.2725	0.2834	0.2316
Conductivity	24	-0.0018	0.5941	-0.0054	0.7231	-0.0017	0.6564	-0.0022	0.8597
Temperautre	24	0.0111	0.9265	0.0238	0.8749	0.1730	0.1917	-0.1074	0.3870
ph	24	0.0786	0.6813	-0.2183	0.7989	0.0892	0.6775	-0.0453	0.9477

Time 8		**1.875 mg/L**				**3.75 mg/L**			
	n	Unadjusted		Adjusted		Unadjusted		Adjusted	
Parameter		**β**	**p-value**	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**
Turbidity	24	<0.0001	0.9917	-0.0072	0.3295	-0.0028	0.4336	0.0010	0.7954
Source contamination	23	0.0003	0.5505	0.0002	0.6704	0.0007	0.0837	0.0007	0.0835
Free Chlorine Residual	24	0.7628	0.2927	1.6928	0.1893	0.1007	0.6478	0.1166	0.6204
Conductivity	24	0.0018	0.4532	-0.0002	0.9825	-0.0016	0.4421	0.0007	0.9383
Temperautre	24	-0.0217	0.8045	0.0072	0.9428	0.1167	0.1087	-0.0866	0.3124
ph	24	-0.0985	0.4785	-0.0274	0.9616	0.0889	0.4527	0.1057	0.8248

Time 24		**1.875 mg/L**				**3.75 mg/L**			
	n	Unadjusted		Adjusted		Unadjusted		Adjusted	
Parameter		**β**	**p-value**	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**
Turbidity	22	-0.0013	0.7512	0.0063	0.5888	-0.0028	0.5477	-0.0008	0.8521
Source contamination	21	0.0004	0.3446	0.0005	0.4008	0.0008	0.1561	0.0006	0.2264
Free Chlorine Residual	22	-0.0290	0.9701	-1.1782	0.5738	0.6086	0.1858	0.7209	0.1244
Conductivity	22	0.0017	0.4959	0.0106	0.3060	**-0.0055**	**0.0437***	-0.0043	0.6441
Temperautre	22	0.0142	0.8693	-0.0038	0.9702	0.0356	0.7111	0.1296	0.1920
ph	22	-0.0503	0.7254	0.6049	0.2999	**0.2922**	**0.0563***	0.2459	0.6341

**Significant to 0.05*

^Not Included in multiple regression model

points and for each treatment arm (1.875 mg/L and 3.75 mg/L NaOCl). All significant findings shown through simple linear regression with log reductions as the outcome of interest occurred within the 3.75 mg/L treatment arm. A significant (p=0.0076), slightly positive (β=0.0018) association was shown between source contamination and log reductions in *E. coli* during the 1-hr follow-up. Additionally, during the 1-hr follow-up there was a significant (p=0.0512), slightly positive (β=0.6822) association between source contamination range and log reductions in E. coli. There was a slightly positive (β=0.4794) and significant (p=0.0141) association between source contamination and log reductions at the 24-hr follow-up as well. When comparing conductivity to log reductions, a slightly negative (β=-0.0055) association was seen (p=0.0437) during the 24-hr follow-up period. Finally, pH was seen to be positively (β=0.2922) associated (p=0.0563) with log reductions during the 24-hr time point. No significant associations were seen for turbidity, temperature, for follow-up free chlorine residual at any time point of for either treatment arm.

As a secondary analysis, jerry-can-specific turbidity values from Visit 2 were examined under simple linear regression with the three outcomes of interest in an effort to determine if sample-specific turbidity was a more appropriate predictor of the outcomes than when utilizing turbidity from just the source location for all three treatment arms. Results from the simple linear regression models are shown in **Table 10**. No significant associations were noted between jerry-can-specific turbidity and log reductions in *E. coli* for any time point or treatment arm.

Free Chlorine Residual – To clarify the impact of source water characteristics on free chlorine residual, simple linear regression was completed comparing turbidity, source contamination, conductivity, temperature, pH, source contamination range and turbidity range to follow-up free chlorine residual within each time point and for the two dosages (1.875 mg/L and 3.75 mg/L). All results from simple linear regression are shown as the unadjusted values in **Table 9** for each treatment arm and for all three time points.

All significant findings within the simple linear regression models examined here occurred within the 1.875 mg/L treatment arm. Specifically, turbidity was significantly associated with free chlorine residual at the 8-hr (p=<0.0001) and 24-hr (p=<0.0001) time points, indicating a positive association for both time points (β=0.0044 and β=0.0051,

Table 9. All results from simple linear (unadjusted) and multiple linear (adjusted) regression models comparing source water characteristics to follow-up free chlorine residual.

Time 1		**1.875 mg/L**				**3.75 mg/L**			
	n	Unadjusted		Adjusted		Unadjusted		Adjusted	
Parameter		**β**	**p-value**	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**
Turbidity	24	0.0016	0.7655	0.0034	0.1958	0.0034	0.1148	0.0003	0.9619
Source contamination	23	<0.0001	0.8510	0.0001	0.8028	-0.0006	0.2808	-0.0006	0.3645
Conductivity	24	-0.0005	0.6812	-0.0022	0.6893	-0.0032	0.3052	-0.0056	0.6696
Temperautre	24	-0.0310	0.5053	-0.0023	0.9663	-0.0710	0.5298	-0.0563	0.6652
ph	24	0.0245	0.7421	-0.0968	0.7558	0.1765	0.3228	-0.1542	0.8337
Time 8		**1.875 mg/L**				**3.75 mg/L**			
	n	Unadjusted		Adjusted		Unadjusted		Adjusted	
Parameter		**β**	**p-value**	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**
Turbidity	24	**0.0044**	**<0.0001***	**0.0044**	**0.0001***	0.0018	0.6136	0.0005	0.8976
Source contamination	23	<0.0001	0.8994	<0.0001	0.8496	-0.0002	0.5904	-0.0002	0.6236
Conductivity	24	0.0005	0.4941	0.0006	0.7802	-0.0012	0.5737	-0.0069	0.4321
Temperautre	24	-0.0328	0.1955	-0.0024	0.9022	-0.0661	0.3621	-0.0625	0.4768
ph	24	-0.0277	0.4969	-0.0056	0.9598	0.0448	0.6997	-0.3585	0.4707
Time 24		**1.875 mg/L**				**3.75 mg/L**			
	n	Unadjusted		Adjusted		Unadjusted		Adjusted	
Parameter		**β**	**p-value**	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**
Turbidity	21	**0.0051**	**<0.0001***	**0.0051**	**<0.0001***	0.0031	0.1950	0.0020	0.4493
Source contamination	20	<0.0001	0.6787	<0.0001	0.2200	0.0002	0.5048	0.0002	0.4984
Conductivity	21	-0.0008	0.3515	0.0007	0.5920	-0.0008	0.5865	-0.0043	0.4404
Temperautre	21	-0.0357	0.1632	0.0004	0.9773	-0.0793	0.0933	-0.0713	0.2088
ph	21	0.0446	0.3370	0.0473	0.5230	0.0264	0.7632	-0.2566	0.4059

**Significant to 0.05*

^Not Included in multiple regression model

respectively). Turbidity range was also associated (p=0.0221) with free chlorine residual during the 24-hr time point, with a mildly positive relationship (β=0.1580).

As a secondary analysis, jerry-can-specific turbidity values from Visit 2 were examined under simple linear regression with the three outcomes of interest in an effort to determine if sample-specific turbidity was a more appropriate predictor of the outcomes than when utilizing turbidity from just the source location for all three treatment arms. Results from the simple linear regression models are shown in **Table 10**. When examining jerry-can-specific turbidity and free chlorine residual, one significant (p=0.0010), positive (β=0.0042) association was noted during the 8-hr follow=up within the 1.875 mg/L dose. Another positive (β=0.0050) association (p=<0.0001) was noted between the jerry-can-specific turbidity values and free chlorine residual for the 24-hr follow-up in the 1.875 mg/L dose.

Follow-up Contamination – Finally, simple linear regression was utilized to determine associations between source water characteristics (turbidity, source water contamination,

Table 10. Simple linear regression of jerry-can-specific turbidity values and outcomes of interests. Details findings for each model ran at each time point and within both treatment arms.

Model		1.875 mg/L Dose			3.75 mg/L Dose		
Timepoint	n	R^2	β	p-value	R^2	β	p-value
Log Reductions							
T1	14	0.0357	-0.0022	0.5177	0.0082	-0.0013	0.7588
T8	14	0.025	-0.0025	0.5895	0.0693	-0.0026	0.3630
T24	13	0.0003	0.0003	0.9521	0.0658	-0.0025	0.3977
Follo-up *E. coli*							
T1	14	0.0299	0.0164	0.5543	0.0001	-0.0032	0.9683
T8	14	0.0042	0.0124	0.8248	0.0454	0.0102	0.4644
T24	13	0.0067	-0.0512	0.7907	0.0407	0.0089	0.5087
Free Chlorine Residual							
T1	14	0.128	0.0032	0.2901	0.0002	0.0003	0.9597
T8	14	**0.6102**	**0.0042**	**0.0010***	0.0008	0.0015	0.7492
T24	13	**0.9171**	**0.0050**	**<0.0001***	0.0233	0.0015	0.6186

**Significant to 0.05*

temperature, conductivity, pH, turbidity range and source contamination range) to follow-up *E. coli* levels. All results from these models are shown in **Table 11**, as the unadjusted values for each model.

All significant findings within simple linear regression were seen within the 3.75 mg/L treatment arm. Specifically, conductivity predicted follow-up contamination (p=0.0031) during the 24-hr time point, with a slightly positive slope (β=0.1500). Temperature was significantly associated (p=0.0126) with follow-up contamination levels during the 8-hr time point, with a two-fold increase in contamination for each unit increase in temperature (β=2.04). Source contamination was significantly associated

Table 11. All results from simple linear (unadjusted) and multiple linear (adjusted) regression models comparing source water characteristics to follow-up contamination level (*E. coli*).

Time 1		**1.875 mg/L**				**3.75 mg/L**			
	n	Unadjusted		Adjusted		Unadjusted		Adjusted	
Parameter		**β**	**p-value**	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**
Turbidity	24	-0.5959	0.4473	-0.2144	0.8260	-0.2629	0.2867	-0.2365	0.3703
Source contamination	23	-0.0059	0.9492	-0.0158	0.8737	**-0.0534**	**0.0559***	**-0.0635**	**0.0384***
Free Chlorine Residual	24	-95.0377	0.2063	-81.7143	0.3710	-10.3476	0.2947	-11.7280	0.2834
Conductivity	24	0.6500	0.1575	0.4065	0.8407	0.1000	0.4836	0.2260	0.6918
Temperautre	24	12.0300	0.4665	2.4586	0.9027	4.2300	0.4198	0.8480	0.8815
ph	24	-35.3200	0.1721	-9.8645	0.9311	-5.0000	0.5485	7.7295	0.8095
Time 8		**1.875 mg/L**				**3.75 mg/L**			
	n	Unadjusted		Adjusted		Unadjusted		Adjusted	
Parameter		**β**	**p-value**	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**
Turbidity	24	0.0195	0.6356	0.0650	0.2782	-0.0322	0.4356	0.0045	0.9205
Source contamination	23	0.0061	0.1250	0.0067	0.1092	0.0006	0.9034	0.0008	0.8649
Free Chlorine Residual	24	-3.6797	0.6114	-12.8968	0.2167	0.6092	0.8133	2.1644	0.4337
Conductivity	24	-0.0300	0.2943	-0.0566	0.4921	0.0300	0.2749	0.0403	0.6842
Temperautre	24	-0.8600	0.3165	-0.5274	0.5207	**2.0400**	**0.0126***	**2.1035**	**0.0454***
ph	24	1.1300	0.4107	-2.5965	0.5755	-1.4300	0.2986	1.4985	0.7882
Time 24		**1.875 mg/L**				**3.75 mg/L**			
	n	Unadjusted		Adjusted		Unadjusted		Adjusted	
Parameter		**β**	**p-value**	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**
Turbidity	22	<0.0001	0.9993	-0.0247	0.7974	-0.0573	0.5332	0.0027	0.9738
Source contamination	21	0.0035	0.3383	0.0035	0.4439	-0.0072	0.5177	-0.0049	0.5800
Free Chlorine Residual	21	1.7310	0.8326	4.8943	0.7765	-9.5073	0.3131	-11.3381	0.1934
Conductivity	22	-0.0100	0.6568	-0.0645	0.4493	**0.1500**	**0.0031***	0.1096	0.5293
Temperautre	22	-0.3800	0.6719	-0.3494	0.6785	0.5800	0.7639	-2.8305	0.1345
ph	22	0.2600	0.8633	-3.9566	0.4085	**-8.3400**	**0.0041***	-6.1286	0.5302

**Significant to 0.05*

^Not Included in multiple regression model

with follow-up *E. coli* level (p=0.0559) during the 1-hr time point, showing a slightly negative association (β=-0.0534. Finally, pH was significantly associated (p=0.0041) with *E. coli* MPN at the 24-hr time point, with increases in pH showing decreases in contamination (β=-8.24). Source turbidity and follow-up chlorine residual were not associated with follow-up contamination at any time point or for either of the treatment arms.

As a secondary analysis, jerry-can-specific turbidity values from Visit 2 were examined under simple linear regression with the three outcomes of interest in an effort to determine if sample-specific turbidity was a more appropriate predictor of the outcomes than when utilizing turbidity from just the source location for all three treatment arms. Results from the simple linear regression models are shown in **Table 10**. No significant associations were noted between jerry-can-specific turbidity and follow-up *E. coli* concentration for any time point or treatment arm.

Multiple Linear Regression

To further characterize the associations seen in simple linear regression models, multiple regression models were developed to compare all independent variables of interest to each dependent variable of interest. Specifically, source water characteristics (turbidity, source contamination, conductivity, temperature, pH, turbidity range and source contamination range) were included in three models to test their association to log reductions of *E. coli* over time, follow-up free chlorine residual and follow-up contamination at teach time point and for each treatment arm.

Log reductions in E. coli – All independent variables of interest were included in a multiple regression model to clarify their association with log reductions in *E. coli*, as seen in simple regression. All results from multiple regression with log reductions as an

outcome are found in **Table 8**, as the adjusted values for each parameter. When controlling for all parameters of interest, one significant association was seen between source contamination range and log reductions in *E. coli* during the 1-hr (p=0.0045) follow-up period for the 3.75 mg/L treatment arm, with a slightly positive slope (β=0.0020).

Free Chlorine Residual – Findings from simple linear regression implicated multiple parameters as important in predicting free chlorine residual at follow-up time points. Source water characteristics were therefore compared to free chlorine residual in a multiple regression model in an effort to determine which parameters were indeed associated with free chlorine residual over time. Significant findings from these multiple regression models are found in **Table 10**, as indicated by the adjusted values for each parameter. Turbidity was significantly associated with free chlorine residual within multiple regression analysis at two follow-up time points (T8, p=0.0001; and T24, p=<0.0001) for the 1.875 mg/L treatment arm. These associations had minimally positive slopes (β=0.0044 and 0.0055, respectively), indicating slight increases in free chlorine with increases in turbidity. Other parameters were not found to be significantly associated with free chlorine residual within the multiple regression model. The model as a whole was seen as significant during the 8-hr (p=0.0022) and 24-hr (p=<0.0001) time points, for the 1.875 mg/L treatment arm.

Follow-up Contamination – Finally, a multiple regression model was built to compare source water characteristics to follow-up contamination, and clarify impacts from source water characteristics on *E. coli* as an outcome measure. All results for these models are detailed in **Table 11,** as the adjusted values for the parameters of interest. When

examining all parameters against follow-up contamination by time point and within treatment arms, one significant (p=0.0454), positive (β=2.1035) association was seen between temperature and follow-up contamination during the 8-hr follow-up for the 3.75 mg/L treatment arm. Additionally, source contamination level was significantly associated (p=0.0384) with follow-up contamination within the 3.75 mg/L treatment arm during the 1-hr follow-up, showing a slightly negative (β=-0.0635) association. The *E. coli* multiple regression model as a whole was not seen as significant for either time point however (T1, p=0.3272 and T8, p=0.3323).

DISCUSSION

The field study presented here was completed to clarify the efficacy of two chlorine dosages (1.875 mg/L and 3.75 mg/L NaOCl) in improving the microbiological water quality and maintaining chlorine residual in turbid waters, defined for this study as >10 NTUs. Environmental samples were collected, dosed accordingly and followed over time to measure the follow-up contamination levels and free chlorine residual. Data were examined to test for the relative log reductions of contamination over time between the two treatment arms, as well as each treatment arm's ability to meet commonly accepted water quality standards: 0.2-2.0 mg/L free chlorine residual and <1 CFU *E. coli* con (Borchardt & Walton, 1971; EPA, 1998; WHO, 2008). Finally, regression analysis was complete to determine what factors influence log reductions over time, follow-up contamination level and follow-up free chlorine residual.

Log Reductions in E. coli

Results of this study do not support increased efficacy of the 3.75 mg/L chlorine dosing to significantly improve microbiological water quality over 1.875 mg/L dosing

within waters of elevated turbidity. As would be expected from the addition of chlorine to source water (Zhao, 2001; Rice, 1999), significant reductions were seen in *E. coli* counts when comparing both treatment arms to the control arm for all time points, turbidities and source contamination levels (all p=<0.0001). When comparing the two dosing levels to each other however, no significant differences were seen for any time point, treatment arm, or for any stratification of the data. This would indicate that the 3.75 mg/L dosage was not more effective at reducing *E. coli* contamination than the 1.875 mg/L dosage under the circumstances presented here.

When examining factors that affect log reductions in *E. coli* over time through simple and multiple regression, findings were inconsistent. A significant (p=0.0076), slightly positive (β=0.0018) association was shown between source contamination and log reductions in *E. coli* during the 1-hr follow-up. Additionally, during the 1-hr follow-up there was a significant (p=0.0512), slightly positive (β=0.6822) association between source contamination range and log reductions in E. coli. There was a slightly positive (β=0.4794) and significant (p=0.0141) association between source contamination and log reductions at the 24-hr follow-up as well. When comparing conductivity to log reductions, a slightly negative (β=-0.0055) association was seen (p=0.0437) during the 24-hr follow-up period. Finally, pH was seen to be positively (β=0.2922) associated (p=0.0563) with log reductions during the 24-hr time point. No significant associations were seen for turbidity, temperature, for follow-up free chlorine residual at any time point of for either treatment arm.

While these associations were shown between source water characteristics under simple linear regression, they were generally no longer present when all parameters were

combined into a multiple regression model. When all parameters were combined under multiple regression, significant associations between water characteristics and follow-up log reductions in *E. coli* were limited to source contamination (p=0.0045) for only the 1-hr follow-up within the higher chlorine dosing (3.75 mg/L NaOCl). Furthermore, the association was characterized by a minimal slope (β=0.0020), indicating no distinct relationship between the two variables. The inconsistency of these findings across time points and treatment arms indicates the source water parameters of interest for this study were not strongly associated with log reductions in *E. coli* over time.

Free Chlorine Residual

One primary component of water treatment practice is to ensure residual chlorine levels in treated water of protection against future microbiological insults. Significant differences were seen within the 8-hr (41.7% difference, p=0.0017) and 24-hr (38.1% difference, p=0.0127) follow-up periods when examining the ability for each treatment arm to meet the chlorine residual standard proposed by the USEPA for 0.2-2.0 mg/L Free Chlorine (EPA, 2010; LeChevallier, 1996; Snead, 1980). This indicates the higher chlorine dose (3.75 mg/L NaOCl) as more efficacious in meeting the chlorine standard versus the lower dose, for waters of >10 NTUs. These findings are consistent with prior findings of 3.75 mg/L and 1.875 mg/L sodium hypochlorite dose in waters of elevated turbidity (Lantagne, 2008). Interestingly, the largest differences in this measure were seen when stratifying by turbidity, all significant findings were associated with the lower turbidity range. This may indicate the chlorine demand within the higher turbidity and higher source contamination ranges is great enough to impede the action of chlorine regardless of dosing.

Also of note, consistently (albeit not significantly) fewer samples within the higher chlorine dose met the chlorine standard during the 1-hr analysis versus the lower chlorine dose. This was due to the fact that four samples within the 3.75 mg/L dose were over the chlorine residual range (>2.0 mg/L free Cl_2) at one hour after dosing, indicating that higher dosing may require a longer period between dosing and consumption. Furthermore, the higher dose may not be well accepted by communities due to taste preferences, which are a partial basis for the upper threshold of the chlorine standard.

Analysis of variance modeling was completed to further characterize the association between meeting the free chlorine residual standard and source turbidity range, source contamination range and treatment arm. Findings detail a strength of association between a higher treatment (higher dose) and an improved ability to meet the free chlorine residual standard. This runs concordant with the findings of Lantagne (2008) regarding the abilities of the two treatment arms to meet the free chlorine residual standard.

Findings from the free chlorine regression models are some of the most interesting findings of this study. Specifically, turbidity was significantly associated with free chlorine residual at the 8-hr ($p=<0.0001$) and 24-hr ($p=<0.0001$) time points, indicating a positive association for both time points ($\beta=0.0044$ and $\beta=0.0051$, respectively). Turbidity range was also associated ($p=0.0221$) with free chlorine residual during the 24-hr time point, with a mildly positive relationship ($\beta=0.1580$). Under a secondary analysis of jerry-can-specific turbidity, findings were similar with associations being shown within the 1.875 mg/L dose for the 8-hr ($p=0.0010$) and 24-hr ($p=<0.0001$) follow-up time points.

The ability for turbidity to predict free chlorine, with a positive association, runs contrary to much of the published literature to date. LeChevallier (1981) characterized turbidity as creating an increased chlorine demand (due to organic matter), which would reduce the available free chlorine for disinfection. Results from this study indicate that as turbidity increase, so does free chlorine. One potential hypothesis for this relationship is that suspended solids in highly turbid waters are indeed providing protection to microbiological organisms from the action of chlorine as proposed by LeChevallier (1981), and the chlorine is there not used up through the action of disinfection.

Follow-up Contamination

Another outcome of interest within this study is the impact of source water characteristics and chlorine dose on overall levels of contamination over time. When comparing *E. coli* levels between the two treatment arms (1.875 and 3.75 mg/L NaOCl), we found no significant differences in the ability for either treatment arm to meet the United States Environmental Protection Agency's *E. coli* standard of <1 CFU (EPA, 2011) for any time point, or when stratifying by turbidity or source contamination. Again, it may be hypothesized that higher turbidity may be blocking the action of chlorine on microbial contamination, as proposed by LeChevallier (1981).

Another hypothesis implicates the high levels of source water contamination as overwhelming the action of the chlorine, preventing complete disinfection and resulting in lowered but detectable levels of *Escherichia coli*. This would result in breakthrough of contamination, which occurs when available chlorine is essentially used up to such a point that organisms are able to replicate and increase in numbers. This was indeed seen with the results presented here, as average increases in the number of *E. coli* between the

8-hr and 24-hr follow-up were seen for the 1.875 mg/L dose (3.36 to 3.59 MPN) as well as the 3.75 mg/L dose (2.20 to 4.74 MPN).

Analysis of variance modeling to investigate the impact of source turbidity range, source contamination range and treatment arm on the ability of samples to meet the *E. coli* standard do not support an increased chlorine dose as having greater efficacy to improve the microbiological quality of waters >10 NTUs. Findings are consistent with other finding of this study, no association we seen between treatment arm and percent of samples meeting the contamination standard for any time point.

When looking at source water parameters and *E. coli* as an outcome under simple and multiple regression, findings were not consistent across time points or within stratification arrays. Under simple linear regression associations were seen between conductivity and follow-up contamination ($p=0.0031$) during the 24-hr time point, with a slightly positive slope ($\beta=0.1500$). Temperature was significantly associated ($p=0.0126$) with follow-up contamination levels during the 8-hr time point, with a two-fold increase in contamination for each unit increase in temperature ($\beta=2.04$). Source contamination was significantly associated with follow-up *E. coli* level ($p=0.0559$) during the 1-hr time point, showing a slightly negative association ($\beta=-0.0534$. Finally, pH was significantly associated ($p=0.0041$) with *E. coli* MPN at the 24-hr time point, with increases in pH showing decreases in contamination ($\beta=-8.24$).

When these variables were examined under multiple regression however, most associations were no longer present. One significant ($p=0.0454$), positive ($\beta=2.1035$) association was seen between temperature and follow-up contamination during the 8-hr follow-up for the 3.75 mg/L treatment arm. Additionally, source contamination level was

significantly associated (p=0.0384) with follow-up contamination within the 3.75 mg/L treatment arm during the 1-hr follow-up, showing a slightly negative (β=-0.0635) association. The *E. coli* multiple regression model as a whole was not seen as significant for either time point however (T1, p=0.3272 and T8, p=0.3323).

The inability to predict follow-up contamination with the independent variables of interest within this study limits the associations that can be drawn between source water characteristics (such as turbidity and source contamination level) and improved microbiological water quality (log reductions in *E. coli* over time). Furthermore, no treatment affect was seen on follow-up contamination indicating the 3.75 mg/L NaOCl dose is not consistently better than the 1.875 mg/L NaOCl dose at improving water quality over a 24-hr period within waters >10 NTUs.

Implications for Point-of-use Interventions

The findings presented here do not indicate sodium hypochlorite (NaOCl) as an appropriate, stand-alone intervention for Point-of use (POU) water treatment in waters of elevated turbidity (>10 NTUS). Increasing chlorine dose did result in increased ability to meet the chlorine residual standard, although this might be expected from previous findings (Lantagne, 2008) as well as the fact that the chlorine residual standard (0.2-2.0 mg/L NaOCl) is simply based on chlorine concentration. Increasing the initial concentration of chlorine (dose), would understandably result in higher follow-up concentrations of chlorine.

Considering the importance of microbiologic quantity as a follow-up parameter in water quality measurements, chlorine residual should not be the sole determinant of water quality in POU interventions. When examining the ability of the two treatment arms to reduce microbiologic quantities within waters >10 NTUs, increased chlorine dose did not

show promise. No treatment affect was seen when examining the dosages with follow-up contamination and no significant differences were seen in log reductions of *E. coli* between the two treatment arms for any time point. This would indicate chlorine residual and follow-up contamination levels (or log reductions) should be considered concurrently when characterizing a water treatment intervention's ability to improve water quality.

It should be noted that follow-up water quality parameters were variable across time points, and the impact of source water characteristics and dosing regimen were variable as well. Data from the 1-hr time point would seem to indicate the lower dose (1.875 mg/L) as more efficacious in meeting chlorine residual levels, while the two does appeared similar at meeting *E. coli* levels within this time point. Guidelines promoting chlorine dosing indicate a half-hour to one-hour wait period after dosing and prior to consumption. Findings presented here indicate this may not be appropriate when applying 3.75 mg/L NaOCl to water intended for drinking as chlorine residual levels may be above the 2.0 mg/L NaOCl upper threshold for chlorine residual as indeed were seen in this study (5 of 22 samples, or 20.8% at 1-hr follow-up). Water quality from this study was best within the 8-hr follow-up time point with regard to meeting both the chlorine standard as well as overall reductions of *E. coli*. While contamination was not reduced to <1 CFU on average, regrowth occurred between the 8-hr and 24-hr time point. Considering much of the water might be consumed within 1-8 hours post collection, these data lend evidence to focusing outcome measures within this time frame.

Limitations

Limitations of this investigation include small sample size (n=24), significant variability in turbidity data and the frequency with which source water was at the upper limit of detection for contamination (*E. coli* MPN) under the methods utilized. While 24

source locations is an appreciable sample size, further stratification of these data into high/low categories yielded sample sizes as small as 11 for analysis. The ability to test for differences in outcomes based on an n of 11 is limited statistically, and may result in non-significant findings despite trends within the data.

Analysis of data on turbidity collected from both source location and from individual jerry cans also clarified there was significant variability within the source location as a whole and measurement of source water characteristics from each jerry can may be more representative of those characteristics impacting follow-up analysis of contamination and/or chlorine residual. Of the 24 initial efficacy trials, 18 (75%) had *E. coli* MPN values of 1011.2 CFU/100 mL at the source, which is the maximum detectable contamination level utilizing the IDEXX method. It is unknown within this study how much greater the contamination may have been, beyond the detection limit. Serial dilutions of source water would allow more accurate representation of starting contamination levels with which to compare log reductions and follow-up contamination levels. This is very much a limiting factor within this study as the presumption must be made from the data available that 75% of the samples were starting from the same place, at least with regard to contamination, and this is likely not an appropriate assumption.

Finally, conditions were often variable with regard to presence of non-lab personnel in the laboratory area, ability to properly clean both hands and the working areas of the lab during sample processing and the fact that four individuals were responsible for lab duties on a rotating schedule. These inconsistencies and the presence of *E. coli* in lab blanks highlight the complexities of field collection, processing and

analysis of fluid samples for the identification of micro-organisms that may be ubiquitous throughout the working environment.

CONCLUSION

It has been clearly shown in prior studies that turbidity interrupts the action of common disinfectants such as sodium hypochlorite (LeChevallier, 1981). The use of sodium hypochlorite in point-of-use interventions appears to be a promising approach to addressing the health disparities associated with diarrheal disease from consuming contaminated water in developing settings. Appropriate dosing recommendations are crucial however for success in POU application of sodium hypochlorite (Colindres, 2007; Fewtrell, 2005; Lantagne, 2008; Mintz, 1995). The use of chlorine bleach (sodium hypochlorite) in POU interventions should also consider the specific situation in which a particular community or population resides. Quantity and quality of source waters may impact the ability of POU interventions depending on the methods utilized to treat waters for drinking purposes.

Data presented here do not clearly indicate significantly greater efficacy of 3.75 mg/L sodium hypochlorite dosing in waters of >10 NTUs over the 1.875 mg/L dose recommended for waters of <10 NTUs. In effect, waters of increased turbidity (>10 NTUs) do not have sufficiently improved quality for the purposes of drinking when chlorine bleach is utilized as the sole method of water treatment. Within this study, water quality (as defined by the standards set forth by the WHO, USEPA and CDC) was not significantly improved over time for either chlorine dosage. It should be noted however, that specific limitations of this study need to be considered when interpreting this data: a small sample size (n-24) was available for analysis; methodological limitations yielded

variability in key independent variables of interest (namely in quantification of initial turbidity); and serial dilutions of water samples were not performed, preventing a range of source contaminations to be analyzed for both initial contamination and follow-up contamination data.

Future studies to clarify the impact of chlorine dose on follow-up water quality of waters >10 NTUs would benefit from increasing the number of samples and source water locations to analyze. Dilutions should be done in instances where contamination levels may be expected to exceed the maximum detection limit of the quantification methodology utilized. Source water characteristics, particularly turbidity and source contamination should be collected from all stored water containers at the time of source water collection, as opposed to pulling one sample from the source water location at the time of stored water collection at the source.

Ultimately, untreated source waters of >10 NTUs should not be consumed when chlorine treatment is the only available method for improving the water quality. In instances where other treatment is unavailable and waters of <10 NTUs may not be abundant, such as in emergency settings and certain development areas, increasing the chlorine dose to 3.75 mg/L is not consistently able to improve the water quality over the current dosing recommendation of 1.875 mg/L NaOCl. Considering taste preferences and other behavioral factors such as distrust for chemicals that may drive utility of household bleach as POU water treatment method, findings of this study indicate the 1.875 mg/L NaOCl dosing is preferable for water treatment, yielding similar improvements in the microbiological water quality of treated waters.

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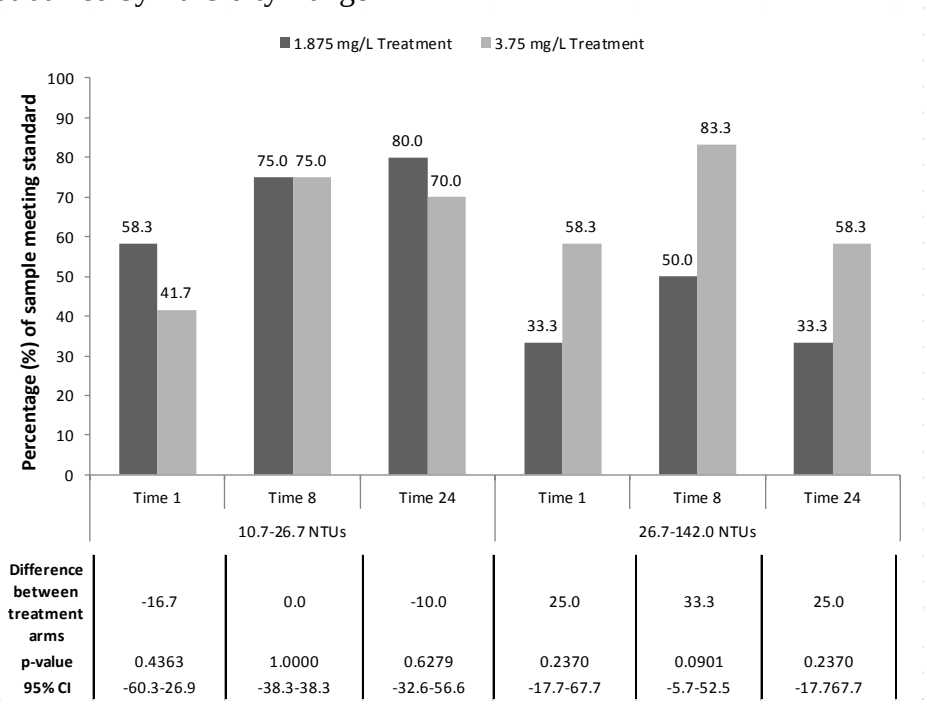
APPENDIX of TABLES and FIGURES

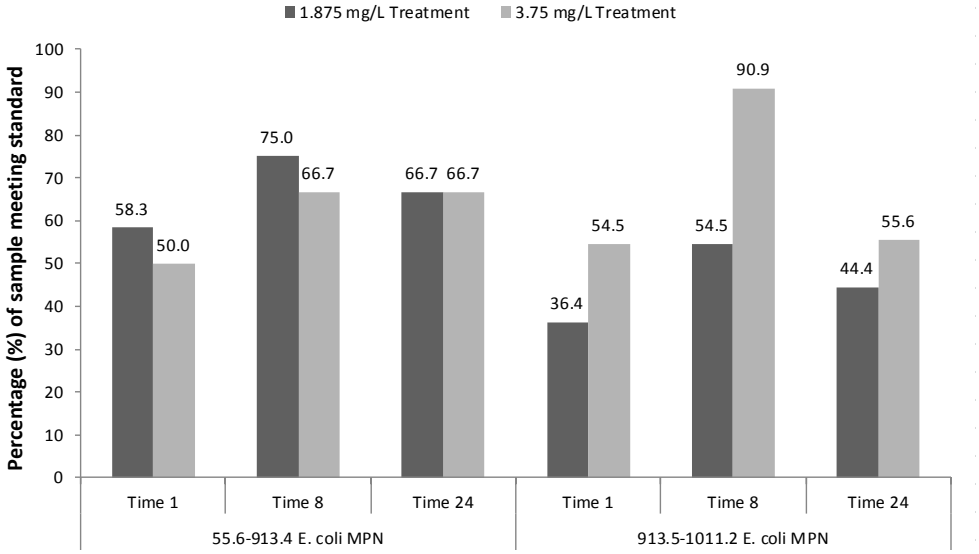
Figure 9. Comparison of treatment arms and their ability to meet the <1 CFU *E. coli* standard for each time period, stratified by Turbidity (A) and Source Contamination (B). Differences are presented between treatment arms for each time point, with associated p-values and confidence intervals.

A. Stratified by Turbidity Range



Difference between treatment arms	-16.7	0.0	-10.0	25.0	33.3	25.0
p-value	0.4363	1.0000	0.6279	0.2370	0.0901	0.2370
95% CI	-60.3-26.9	-38.3-38.3	-32.6-56.6	-17.7-67.7	-5.7-52.5	-17.767.7

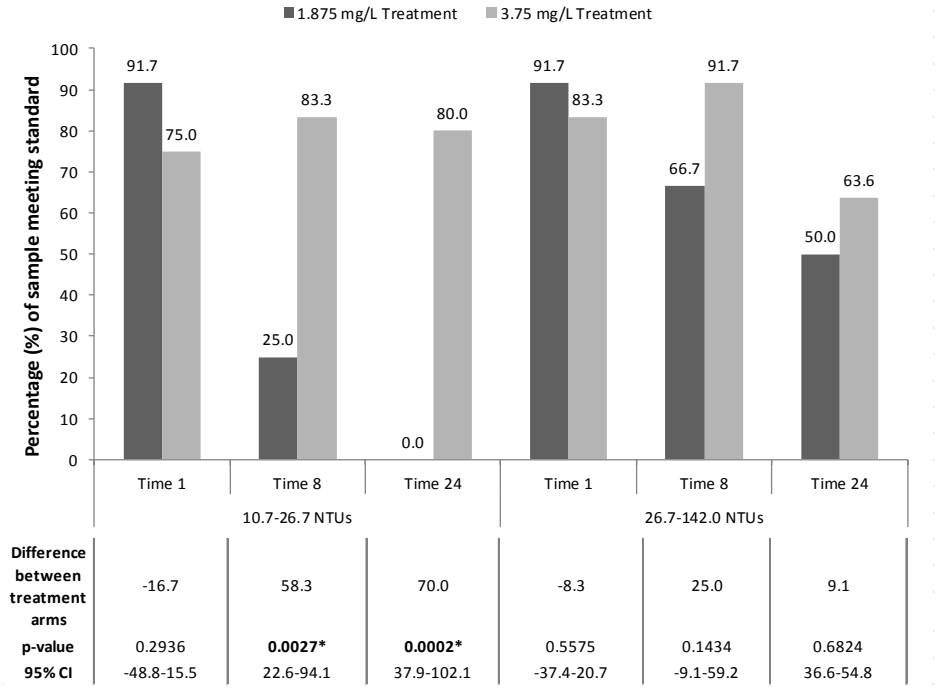
B. Stratified by Source Contamination Range



Difference between treatment arms	-8.3	-8.3	0.0	18.2	36.7	11.1
p-value	0.6977	0.6701	1.0000	0.4161	0.0593	0.6607
95% CI	-35.6-52.2	-48.4-31.7	-41.7-41.7	-27.5-63.9	-1.6-74.3	-41.6-63.8

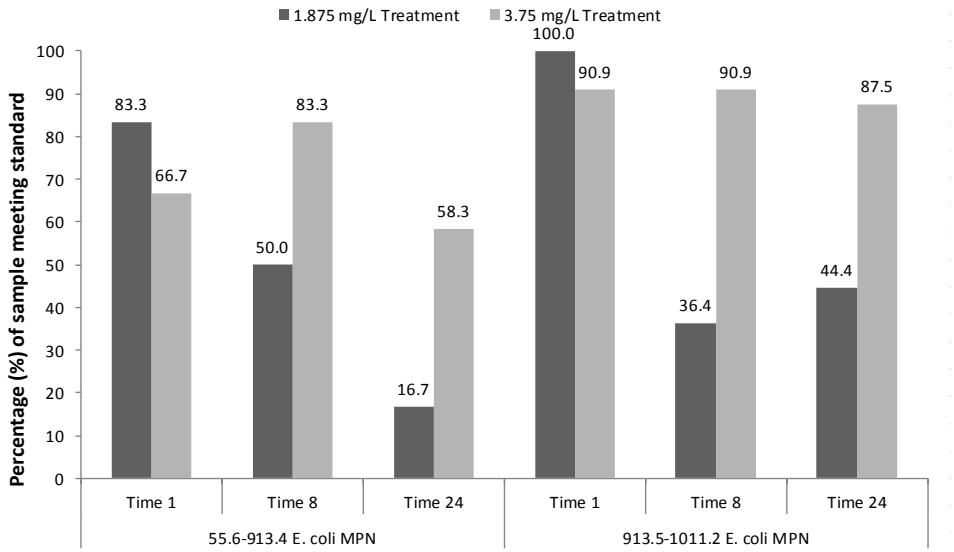
Figure 10. Comparison of treatment arms and their ability to meet the 0.2-2.0 mg/L NaOCl standard for each time period, stratified by Turbidity (A) and Source Contamination (B). Differences are presented between treatment arms for each time point, with associated p-values and confidence intervals.

A. Stratified by Turbidity Range



Difference between treatment arms	-16.7	58.3	70.0	-8.3	25.0	9.1
p-value	0.2936	**0.0027***	**0.0002***	0.5575	0.1434	0.6824
95% CI	-48.8-15.5	22.6-94.1	37.9-102.1	-37.4-20.7	-9.1-59.2	36.6-54.8

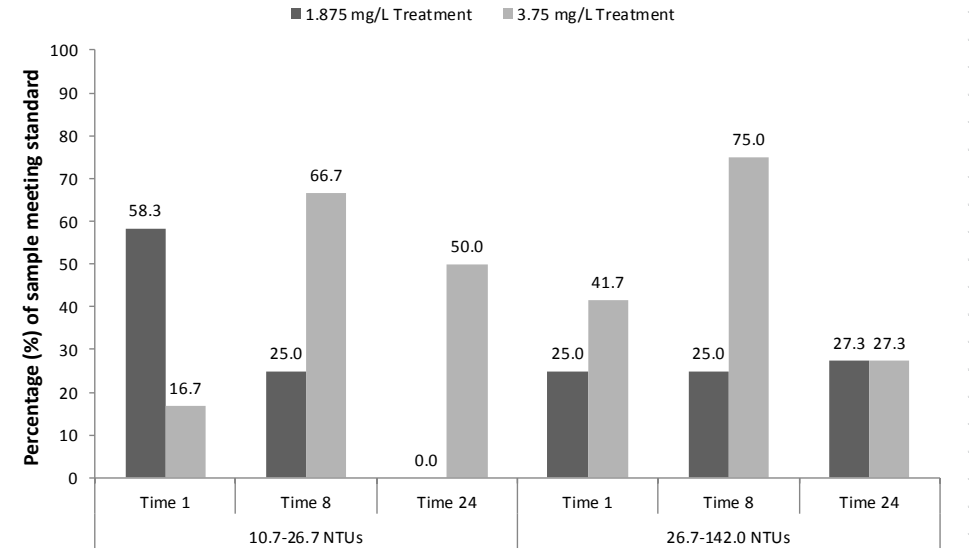
B. Stratified by Source Contamination Range



Difference between treatment arms	-16.7	33.3	41.7	-9.1	54.6	37.5
p-value	0.3676	0.0901	**0.0358***	0.3293	**0.0059***	0.1202
95% CI	-54.2-20.9	-5.7-72.3	3.0-80.3	-28.1-9.9	17.6-91.5	-11.1-86.1

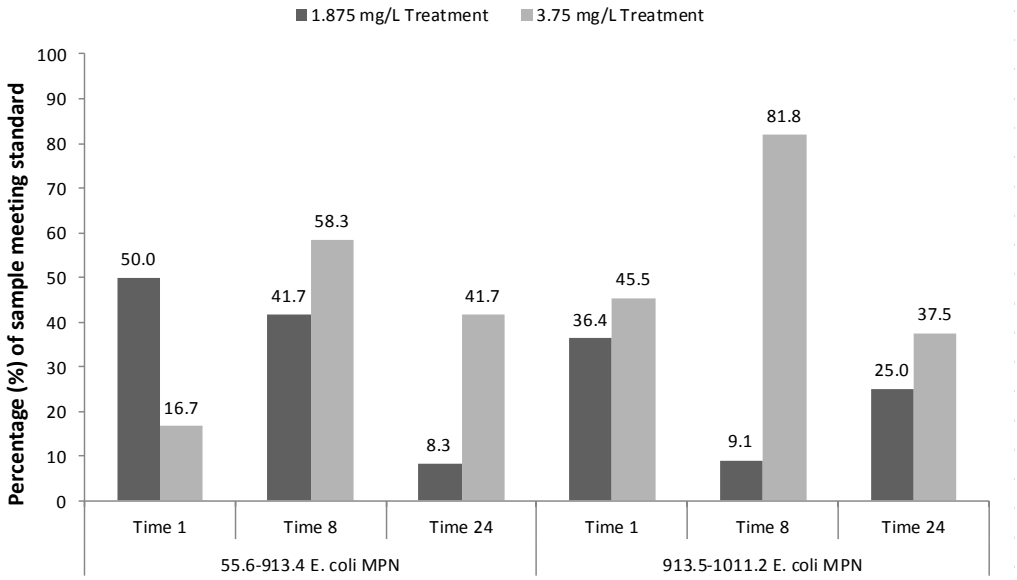
Figure 11. Comparison of treatment arms and their ability to meet the both standards concurrently for each time period, stratified by Turbidity (A) and Source Contamination (B). Differences are presented between treatment arms for each time point, with associated p-values and confidence intervals.

A. Stratified by Turbidity Range



Difference between treatment arms	-41.7	41.7	50.0	16.7	50.0	0.0
p-value	0.0358*	**0.0420***	**0.0077***	0.4086	**0.0128***	1.0000
95% CI	-(3.0-80.3)	1.6-81.7	15.0-85.0	-29.4-57.7	11.7-88.3	-41.6-41.6

B. Stratified by Source Contamination Range



Difference between treatment arms	-33.3	16.7	33.3	9.1	72.7	12.5
p-value	0.0901	0.4363	0.0633	0.6842	**0.0001***	0.6186
95% CI	-0.73-0.06	-0.27-0.60	-0.02-0.69	-0.37-0.05	0.41-1.04	-0.40-0.65